Behavioural Genetic Analysis of Biorhythms in the *melanogaster* Subgroup of *Drosophila*

Thesis submitted for the degree of
Doctor of Philosophy
at the University of Leicester

Matheos Christaki Demetriades

JULY 1997

UMI Number: U096268

UMI

Dissertation Publishing

UMI U096268

ProQuest

"... nothing in biology makes sense, except in the light of evolution."

- Theodosius Dobzhansky

ACKNOWLEDGEMENTS

Abbreviations

BD	Burst Duration
bp	base pairs
cm	centimetres
^{0}C	degrees centigrades
CT	Circadian Time
D:D	dark/dark
DNA	deoxyribonucleic acid
g	grams
hrs	hours
IBI	Interburst Interval
IPF	Intrapulse Frequency
IPI	Interpulse Interval
kb	kilobase pairs
l	litre
L:D	light/dark
mm	millimetres
mins	minutes
mRNA	messenger RNA
ms	milliseconds
o/n	overnight
RNA	ribonucleic acid
s	seconds
SSF	Sine Song Frequency
ZT	Zeitgeber Time

ABSTRACT

Differences in *Drosophila* courtship song components are considered to play an important role in species sexual isolation, because of the observed lack of variation within individuals of a species, and the relatively large differences between closely related species. When a male courts a female, a song is produced, usually consisting of a hum song and trains of pulses. Song recordings from the 8 members of the *D. melanogaster* subgroup, as well as from several types of interspecific hybrid crosses reveal that the Interpulse Intervals (IPIs) oscillate rhythmically about their mean, in a species-specific fashion, as courtship progresses. Various other song components, such as Intrapulse Frequency (IPF), Sine Song Frequency (SSF), Cycles per Pulses (CPP), Mean Burst Duration (MBD), and Mean Interburst Interval (MIBI) which are also thought to contribute to the maintainance of species-specific differences, thus sustaining species barriers were also studied in different species. Hybridisation between species gave evidence for possible X-linked factors in song rhythms, but generally, autosomal factors appear to be involved in controlling the other song characters. Another behavioural trait that may contribute to the species isolation of the members of the *melanogaster* subgroup is the circadian locomotor activity patterns. Locomotor activity profiles in constant darkness conditions revealed species-specific differences between the species in the period of their circadian oscillator, while locomotor activity profiles in light/dark cycles demonstrated phenotypic differences between the various species of the *melanogaster* subgroup. Hybridisations were used to assess the relative contribution of maternal and paternal factors. The overall species pattern of activity appeared to be determined by the sex-chromosomes, whereas other characteristics were primarily autosomally controlled. The implication of these findings on song and circadian behavioural cycles with respect to the current molecular analysis of circadian clock genes is discussed.

CONTENTS

INTRODUCTION

CHAPTER 1

INTRODUCTION:

The goal of communication amongst most animals, like the ultimate aim of animal behaviour on the whole, is reproduction (Gould, 1982). Most animals are solitary, implying that they must actively seek their mating partners; and since most animals have no chance to learn where to look for the opposite sex of their species or how to recognise a suitable mate once there, much of the behaviour associated with mating is innate (Bonner, 1969). A good understanding of the manner in which mating associations between individuals develop is essential for generating a comprehensive model of social interactions (Bonner, 1969). Male-female patterns of interactions are generally based on the males' interest in maximizing the number of matings, they achieve with females (Bonner, 1969).

Darwin proposed his theory of 'Sexual Selection' to explain certain characteristics of organisms not explicable in terms of survival or natural selection (Darwin, 1859). However, Galton (1865) was probably the first to affirm that behaviour might be subject to the 'Sexual Selection'. Sexually dimorphic attributes in insects play a prominent role in the development of Darwin's ideas on sexual selection. Sexual reproduction is a ubiquitous feature in the life cycle of almost every higher animal, so finding a suitable mating partner is of utmost importance. The female of a species, is the one mostly involved in reproduction, and caring for her offspring (Halliday, 1978). It has been argued that this preoccupation of the female leads to selection for males to mate without taking care of partners or offsprings, whereas females are much more particular about choosing their mating partners (Bateman, 1948). Parental investment patterns in insects vary; in the vast majority of insects, there is no paternal contribution beyond what is placed in the gametes. As the number of gametes and subsequent progeny that a female can produce decreases as a result of increased maternal investment per gamete or offspring, the female should be selected to show greater discrimination of mating partners, because her potential reproductive success becomes more and more dependent on the genetic contribution of the male and/or on male care.

Genetic dissection of *Drosophila* courtship behaviour

Males of many species go to inordinate lengths in order to persuade females to mate with them, often performing complex rituals and displays. Elaborate courtship behaviours which constitute sexual reproduction are experienced from fruit flies to humans. Courtship, in *Drosophila*, is generally considered as a stimulus-response chain of events which leads to the sexual act, with each action step by one sex releasing the next action by the other sex. Various signals or cues have been developed by either or both sexes, in order to attract potential conspecific mates. These include sexually dimorphic 'olfactory signatures', produced mainly by females (Vernard and Jallon, 1980; Tompkins, 1980; Anthony and Jallon, 1982), contact pheromones, or tactile stimuli (Tompkins, 1984), or visual cues such as wing vibration (Tompkins *et al.*, 1982). These various modalities have been dissected with the use of single gene mutations in *D. melanogaster*.

Evidence that visual stimuli are important in courtship is provided by blind *no-receptor-potentialA* (lacking light-elicited photoreceptor potentials) and *glass* (lacking photoreceptors and having reduced compound eyes) mutants, which are seen to be unable to orient themselves in relation to the female, thus spending less time courting than normal males (Tompkins, 1984). *Optomotor-blind* (defective optomotor responses) males are not sensitive to horizontal moving stimuli, and behave as if they are totally blind during courtship, implying that movement of the female is also a visual stimulus to the courting male (Tompkins *et al.*, 1982). *Smellblind* (poor responses to a variety of volatile compounds) and *olfactoryC* (poor responses to acetates and some alcohols) mutants also court poorly, suggesting that they do not respond to female pheromones, implying that female flies are producing volatile substances that stimulate males to court (Tompkins *et al.*, 1980; Tompkins and Hall, 1981). If only visual and olfactory cues, provided by the females, were responsible for triggering courtship by the males, then males that can neither see or smell would not court females. Yet, doubly mutant *smellblind;glass* males are observed to court females, if they touch them, implying that males also respond to tactile cues and begin to court (Tompkins, 1984). From the preceeding observations, it would not be unreasonable to infer that the visual, olfactory and tactile stimuli that females

provide are sex-specific, since females evoke vigorous courtship and mature males do not (Tompkins, 1984).

Wing display forms a habitual element in the courtship behaviour within the genus of *Drosophila* (Spieth, 1952). In many species, it involves the production of acoustic stimuli, collectively known as courtship song, generated by the male fly vibrating its wings (Bennet-Clark and Ewing, 1969; Burnet and Connolly, 1974; Schilcher, 1976a). The complete mating ritual typically involves circling of the female by the male, the orientation of the male in relation to the female (Cook, 1980), the male tapping the female with his foretarsi, and vibrating either/or both his wings producing a species-specific courtship love song. The male proceeds then to lick the genitalia of the female, followed by numerous attempts to copulate (Bastock and Manning, 1955). If the female is sexually mature and she has been sufficiently stimulated by the male's courtship, she opens her vaginal plates in response to one of the male's copulation attempts and coital engagement ensues (see Figure 1.1).

In the *D. virilis* group of species, Suvanto *et al.* (1994) discovered that the males generate a stimulatory courtship song for females, as well as an inhibitory song to discourage other males, which has different acoustic characteristics to the former song. Satokangas *et al.* (1994) discovered that not only males, but also females vibrate their wings during courtship, generating either pulses or sine song. The males' reactions to female songs range from licking and singing (eliciting courtship) to arresting courtship altogether. Furthermore, Boarke and Hoikkala (1995) reported that rather than being the passive observers to the male displays, females of *D. silvestris* are actively involved in courtship by flying away from the male, thereby arresting courtship momentarily, but often returning back to the male, while waving their wings (females). Moreover, females of many *Drosophila* species are seen to spread their wings apart prior to copulation, which has special significance in species of the *D. virilis* group of species; by spreading her wings the female provokes as well as making the male aware of her readiness (acceptance signal) to copulate (Spieth, 1952; Vuoristo *et al.*, 1996). Grossfield (1966) argued that in *D. occidentalis* and *D. guttifera* (species of the *D. guinaria*-group), the failure to mate in the dark was due to the fact that the males could not perceive these visual acceptance cues.

Figure 1.1: The principal elements of courtship in *D. melanogaster*.
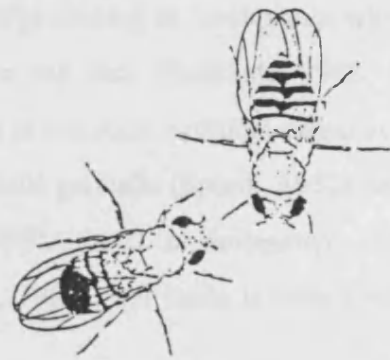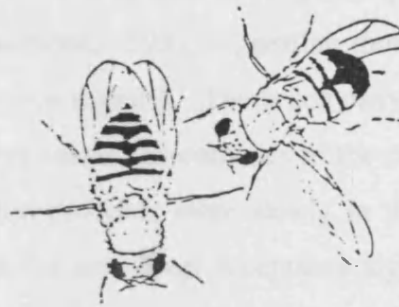
ORIENTATION   VIBRATION

LICKING   ATTEMPTED COPULATION

COPULATION

Original drawings by Dr. Barrie Burnet.

Similarly, *D. auraria* (*D. auraria*-group) males were observed to court wingless females (Grossfield, 1968), but the courtship ceased short of copulation due to the impossibility of the female producing an 'acceptance signal'. Males of the *D. virilis*-group species are able to mate in the dark (Hoikkala, 1988; Liimatainen, 1993), suggesting that female wing spreading is not such a critical visual acceptance stimulus. The accompanying spreading of the female genitalia (Spieth, 1952), conveys contact pheromones to the male (Anthony *et al.*, 1985). In *D. melanogaster*, courtship proceeds more slowly in the dark (e.g., Kyriacou, 1981), but there is little evidence for any visual acceptance signals from the females.

The *D. melanogaster* courtship song consists of two elements, hum or sine song and a pulse song (see Figure 1.2). The former is comprised of a sinusoidal hum with a frequency (SSF) of about 160 Hz (von Schilcher, 1976a), while the latter consists of a train of pulses, with an interpulse interval (IPI) ranging from between ~30-40ms (Ewing and Bennet-Clark, 1968), in *D. melanogaster*. Tomaru and Oguma (1994) reported that the males of the species of the *D. auraria* complex only produce a pulse song which consists of bi- and tricyclic pulses, whose wave form remains invariant throughout the courtship. Even though, the wave pattern of courtship song changes together with male behaviour in other species (Ikeda *et al.*, 1980; Crossley, 1986; Cobb *et al.*, 1989), this was not observed in species of the *D. auraria* complex (Tomaru and Oguma, 1994). Furthermore, it was observed that males produced more courtship song during copulation rather than before copulation (Tomaru and Oguma, 1994), whereas in the *D. melanogaster* subgroup, males produce the bulk of their courtship prior to copulation (Clark-Bennet and Ewing, 1968; Schilcher, 1975; Kyriacou and Hall, 1980; Cowling and Burnet, 1981; Cobb *et al.*, 1989). Moreover, males of species of the *D. repleta* group of species are observed to produce a short and long song type, with no sine song being produced during courtship (Ewing and Miyan, 1986).

The need to know more about what constitutes sexual behaviour and to identify the various genes that govern it, led various experimenters using single-gene mutations and other genetic techniques to disrupt the fly's ability to court or elicit courtship, and determine the reasons why these behaviours are sex-specific, if indeed they

Figure 1.2: Burst of *D. melanogaster* courtship song, including hum and pulse components

PULSE SONG

HUM SONG

interpulse-interval   50 ms.
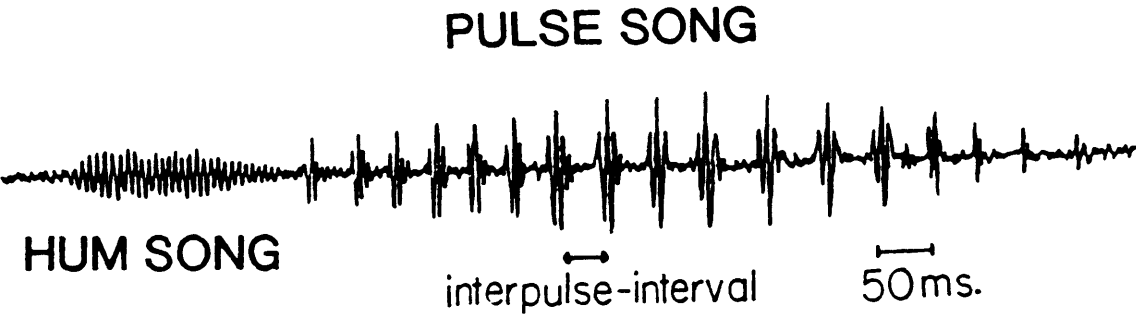
are. Both very young males and females behave similarly, in that they elicit courtship, but they do not themselves court. Yet, when they are 1-2 days old, the males' behaviour changes, in that they begin to court females, and they themselves become relatively less attractive to other males (Tompkins, 1984). Therefore, mature females become more attractive to males, i.e., able to evoke courtship while being unable to court themselves, whilst mature males become less attractive to other males and are able to court females (Tompkins, 1984).

Like other aspects of sexual differentiation, sexual behaviour is dictated by a cascade of sex-determining regulatory genes (reviewed McKeown, 1994-see Figure 1.3). In summary, the presence of two X chromosomes in diploid individuals indoctrinates female development by activating the *Sex-lethal* (*Sxl*) gene (Sinchez, 1982; Cline, 1984; Tompkins,1984; 1985). Using temperature sensitive diplo-X mutants (*Sxl*$^{M\#1,fm\#3}$/ *Sxl*$^{fm\#7,M\#1}$), which externally looked like males, it was shown that the *Sxl* gene must function normally during the pupal period or the first few days of adult life for a fly to elicit vigorous courtship (Tompkins, 1984; 1985), suggesting that at least one female-specific *Sxl* transcripts is essential for the production of the female pheromone, 7,11-heptacosadiene (HCD) (Tompkins *et al.*, 1980; Tompkins *et al.*, 1981; Jallon *et al.*, 1987; Ferveur and Sureau, 1996; Ferveur *et al.*, 1996), and the repression of the male inhibitory pheromone, 7-tricosene (7T) synthesis (Jallon, 1984; Jallon *et al.*, 1987; Ferveur and Sureau, 1996; Ferveur *et al.*, 1996-see Figure 1.3). In the males the converse holds true (Baker and Ridge, 1980).

The answer to how female-specific *Sxl* transcripts control the production of pheromones in diplo-X flies, or conversely, how the lack of *Sxl* activity regulates pheromone production in haplo-X flies, comes from analysing mutations in the *intersex* (*ix*), *transformer* (*tra*), *transformer-2* (*tra-2*) and *doublesex* (*dsx*) genes, which are under the control of *Sxl* (Baker and Ridge, 1980) (see Figure 1.3). The *Sxl* gene by autoregulating its own pre-mRNA splicing (several transcripts) in turn, activates the *tra* genes by regulating their mRNA splicing. The *tra* and the *tra-2* gene products, in turn, regulate the splicing of the *dsx* gene, leading to the production of a female-specific dsx protein (McRobert and Tompkins, 1985). In contrast, *tra* and *tra-2* mutant diplo-X flies,

FEMALE DEVELOPMENT   **MALE DEVELOPMENT**

Sxl

Sex-lethal (Sxl)-
Activation

Non-functional *Sxl, tra & tra-2*
gene products

fru gene
Activation

tra & tra-2-
Activation

Female pheromone
production:
7,11 HCD

7-tricosene
repression

fru gene product

No Sine
Song

dsx & *ix-*
Activation

ix pre-mRNA
dsx pre-mRNA
by default splicing

dsx -mutant

Muscle of
Lawrence

Reduced
courtship
vigour

Male pheromone
production:
7-tricosene

7,11 HCD
repression

Production of female-specific
Dsx protein

Production of male-specific
Dsx protein with a different
Carboxy-terminus to the
female counterpart

Male courtship
behaviour

Figure 1.3: Genetic control of sex pheromone synthesis and courtship
behaviour in *Drosophila* females and males.

which look and behave like males (Baker and Ridge, 1980), are unattractive to normal males (Baker and Ridge, 1980; McRobert and Tompkins, 1985), suggesting that the normal production of the female pheromone (HCD) has been arrested (Tompkins *et al.*, 1980; Tompkins *et al.*, 1981; Jallon *et al.*, 1986; 1987) and in its place, these mutant flies are producing the male pheromone (7T) instead (Jallon,1984; Scott, 1986; Scott *et al.*, 1988; Scott and Jackson, 1988). *ix* mutations transform females into flies which look like intersexes (hermaphrodites, Baker and Ridge, 1980), and have a slight effect on the flies' sex- appeal (Jallon and Hotta, 1979; Hall, 1979; Tompkins, 1984). Diplo-X *dsx* mutant flies are phenotypically similar to intersexes (Baker and Ridge, 1980), being not as attractive as normal females, although they are able to elicit more courtship than males (McRobert and Tompkins,1985). As is the case for *Sxl*, it is reasonable to assume that these genes (*tra*, *dsx* and *fru*) exercise their effects on the synthesis of pheromones in the tissues that should be diplo-X for sex-appeal (Jallon and Hotta, 1979; Tompkins, 1984), since mutations in any of these genes affect the fly's appearance in a cell-autonomous manner (Baker and Ridge, 1980).

The sexual orientation of *Drosophila* also has a complex neural basis. Using the feminizing transgene *UAS-tra* under the control of various P-GAL4 enhancer-trap lines (Brand and Perrimon, 1993), Ferveur *et al.* (1995) produced transformed male flies that exhibited a bisexual orientation. These males were shown to express the *tra* gene in areas of the antennal lobes, or mushroom bodies, or both, regions which are known to receive or process olfactory input (Barinaga, 1995). Extending this work, Ferveur *et al.* (1997) were able to express the *UAS-tra* gene (under the control of a heat-shock promoter) at different developmental stages, and more particularly, in a group of subcuticular abdominal cells known as the oenocytes (Miller, 1950; Romer, 1991). These transgenic male flies had an unaltered sexual orientation, but they now expressed a predominantly female pheromonal profile, causing other males to respond to them as though they were female. Mosaic studies had previously restricted the source of pheromonal sexual dimorphism to the fly abdomen (Nissani, 1977; Jallon and Hotta, 1979; Coyne and Oyama, 1995).

In males, where the *Sxl* and *tra* gene products are non-fuctional, splicing the *dsx* pre-mRNA in its default pattern, results in a male-specific dsx protein, which differs

from the female form at its carboxy-terminus (Burtis and Baker, 1989-see Figure 1.3); these proteins take up the role of sex-specific transcription factors that coordinate many aspects of sexual differentiation, including the external morphologies of both sexes (see Figure 1.3). Mutations in the *ix* gene, in haplo-X flies, have no effect on the flies. Therefore one might expect these males to be unattractive to other males (Baker and Ridge, 1980). Surprisingly, these mutants are able to evoke more courtship than normal males, even though they are not as attractive as females, implying there might either be some HCD production in these flies or not enough 7-tricosene synthesis (inhibitory pheromone) as in normal males (McRobert and Tompkins, 1985; Jallon,1984; Scott, 1986; Scott *et al.*, 1988; Scott and Jackson, 1988). Nevertheless, *dsx* haplo-X mutant flies, which look like intersexes, do not evoke much courtship, i.e., have a reduced willingness to court females and have certain elements of the courtship song, e.g., sine song absent (Villela and Hall, 1996). *tra* haplo-X mutants, phenotypically normal males, are also unattractive to other males. The above observations suggest that the *ix* gene is in control of some aspect of pheromone synthesis in normal males, perhaps by acting in tissues that should be haplo-X for a fly to be unattractive.

dsx does not control all aspects of somatic sexual differentiation, as *Sxl*, *tra* and *tra-2* genes do (reviewed by Burtis, 1993); for example, the *tra* and *tra-2* genes, but not *dsx*, control the development of a male-specific abdominal muscle known as Muscle of Lawrence (MOL; Lawrence and Johnston,1986; Taylor, 1992-see Figure 1.3). Lawrence and Johnston (1986), using mosaics, were able to suggest that the development of the MOL depended on the sex of the neurons that connect to it. Furthermore, constitutive expression of the male form of the dsx protein transforms females into morphologically wild-type males, but these flies were found to be unable to court (Taylor *et al.*, 1994), suggesting that there exists some other route downstream of *tra* and *tra-2*, which controls MOL development and other aspects of male sexual behaviour.

Even though, many genes are seen to affect the courtship of *Drosophila* males, *fruitless* (*fru*) is one of the very few that appears to affect male courtship specifically (reviewed by Hall, 1994; Taylor *et al.*, 1994-see Figure 1.3). *fru* mutant males are observed to be unable to perform normally the later stages of the courtship ritual, from

7

singing through to copulation (Hall, 1978; Gailey and Hall, 1989), and are sterile. The most dramatic reproductive anomaly associated with *fruitless* is that the *fru* mutant male courts other males just as vigorously and indiscriminately as he does a female. Furthermore, groups of *fru* males are also observed to form "courtship chains" in which most individuals are simultaneously courting and being courted (Gill, 1963; Gailey and Hall, 1989). An additional *fru* phenotype is that the male-specific MOL is either incompletely formed or completely absent (Gailey *et al.*, 1991; Taylor and Knittel, 1995). Since no phenotypic effects of *fru* have been observed in females (reviewed by Hall, 1994), *fru* is a promising candidate gene functioning in the new proposed route downstream of the *tra* genes. This notion has being validated by Ryner *et al.* (1996) and Ito *et al.* (1996), whose findings suggest the function of *fru* is to specify the sex-specific fate and activities of neurons, including cells in the antennal lobe, that command the coordination of the complex array of steps that comprise male courtship behavioural rituals. Thus, sexual orientation in flies is controlled by the same hierarchy of genes that governs all other aspects of sex.

The fact that the FRU protein shows similarities to BTB-ZF family of transcription factors (Albagli *et al.*, 1995), raises the possibility that *fru*, like *dsx*, is the final regulatory gene in its branch of its cascade, and therefore it would be directly controlling the expression of downstream genes, which might be responsible for dictating the sex-specific Muscle of Lawrence (MOL) development, sexual orientation and the behaviours that constitute male courtship. The neurons that are expressing sex-specific *fru* transcripts are not only found in the Central Nervous System (CNS)-where cells responsible for particular male courtship steps are situated (reviewed by Greenspan, 1995), but in addition are associated with higher order neuropils (Heisenberg, 1994).

Various regions of the central nervous system (CNS) of the fly have been identified that play a role in performing specific steps of the male courtship ritual (reviewed by Greenspan, 1995). In brief, using gynandromorphs, various researchers were able to roughly define the various parts of the brain that are required to be either male or female in order for a particular step in the courtship ritual to be achieved (Hotta and Benzer, 1976; von Schilcher, 1977; Nissani, 1977; Hall, 1977 and 1978a; von

Schilcher and Hall, 1979; Tompkins and Hall, 1983). It was found that in order to produce the early steps of courtship behaviour, such as tapping, following the females and wing extension, the posterior dorsal brain, on at least one side, must be haplo-X (Hall, 1979). Fate-mapping was also used to discover the control site of the courtship songs in *Drosophila*. Some male-female mosaics, with male brain tissue, followed females and extended their wings at them (Schilcher and Hall, 1979). Normal pulse song production has been found to be closely associated with male tissue in the ventral thoracic ganglia, and more specifically the mesothoracic neuromere (Schilcher and Hall, 1979). Mapping of the sine song did not lead to the location of a definite focus, but particular regions of the brain and thoracic ganglia needed to be of a certain genotype for the sine song to be produced. Licking (proboscis extension) is also found to be the same focus, but is submissive, i.e., male tissue has to be present in both the right and left dorsal brain (Hall, 1979). Attempted copulation, especially coital engagement, has its focus in the thoracic ganglia, but not in any specific region (Hall, 1979); copulation attempts, in mosaics with gravid abdomens, can be observed and may be correlated with the presence of sex combs (Hall,1979).

Hall and Tompkins (1983), using sex mosaics were able to show that a group of cells in the dorsal anterior brain had to be female, so that females could become sufficiently receptive to copulation. Yet, the neuronal connections that underlie the courtship behaviour and the developmental processes needed to organize these circuits, still remain an enigma. Modified sexual orientation has been correlated with feminising of certain brain regions (see above) and, strangely the misexpression of the *white* (*w*)gene in the brain, also leads to homosexual behaviour, because these atypical males ectopically express the *w* gene (Zhang and Odenwald, 1995; Hing and Carlson,1996). Behavioural analysis of *Drosophila* mutants has shown genetic lesions that knock out single genes often have pleiotropic effects that disturb normal courtship behaviours (Hall, 1994). The apparent male-specific behavioural change imposed on the males by the *w* misexpression resembles that exhibited by the autosomal recessive *fru* mutants (Gill, 1963; Hall, 1978; Ito *et al.*, 1996; Ryner *et al.*, 1996).

Several defective song mutants have been isolated, which could eventually lead to a deeper insight into the neurogenetic basis of acoustic stimuli in mating. *cacophony* (*cac*) shows a mutant phenotype with respect to the pattern of individual pulses as well as IPIs (Schilcher, 1977; Kulkarni and Hall, 1987). The *dissonance* (*diss*) mutant, now known as $nonA^{diss}$, mutant shows errant pulses within song phrases (Kulkarni *et al.*, 1988; Stanewsky *et al.*, 1996), *croaker* (*cro*) mutants also show pulse song defects as well as an apparent decrement in male-mating success accompanied with subnormal flight patterns (Yamamoto *et al.*, 1993- reviewed in Hall, 1994), while *fruitless* (*fru*) exhibits several courtship behavioural defects, including abnormal song pulses (Hall, 1978; Gailey and Hall,1989). Wheeler *et al.* (1989) showed that *cac* and $nonA^{diss}$ mutants produce polycyclic pulses, with IPF and SSF in the wild-type range in the former, and a defect in sine wave of the 'hum' song for the latter. Eventually, it will be possible to clarify the actions of genes, such as *cac* (Peixoto *et al.*, 1997;) and $nonA^{diss}$ (Kulkarni *et al.*, 1988; Stanewsky *et al.*, 1996), which have both been isolated and cloned, and specify products that influence the development or physiology of the song-controlling 'circuit'. Furthermore, Villella and Hall (1996) reported that *dsx* mutants lack the sine song component from their courtship song repertoire, making this the first mutation that affects the male acoustic output, but leaves the pulse song unaffected.

Species-specific differences in courtship songs

Interspecific differences are considered to be of great importance, for recognition of conspecific flies and maintainance of sexual isolation between the mating partners of the various species. Cowling and Burnet (1981) studied the courtship songs of six sibling species in the *D. melanogaster* subgroup, namely, *D. melanogaster*, *D. mauritiana*, *D. simulans*, *D. yakuba*, *D. teissieri* and *D. erecta*. They also were able to produce a few interspecific hybrids, as well as reciprocal interspecific hybrids between these species, where it was feasible, and analyse the lovesongs of these hybrids. Their results suggested that the genes controlling IPI variation were located on autosomes and that the "hum" or sine song was controlled by one or more sex-linked genes. Moreover,

they found clearcut differences between the songs of five of the species, with the only exception being that of *D. mauritiana* which showed ambivalent song characteristics, in that its mean SSF and intrapulse frequency (IPF) are closer to *D. simulans*, while its modal IPI lies much closer to *D. melanogaster* (Cowling and Burnet, 1981).

It is clear, that sexual isolation mechanisms should be strongest, when two species have the potential to interbreed, i.e., when they live in sympatry (Welbergen *et al.*, 1987). Being endemic to the island of Mauritius, where no other members of the *D. melanogaster* subgroup are found, the chance of *D. mauritiana* interbreeding with other species of the *D. melanogaster* subgroup is highly unlikely. Thus, the rules for sexual isolation for this species are more relaxed. On the contrary, sympatric species such as *D. melanogaster* and *D. simulans* produce more distinct songs. Cowling and Burnet (1981) found that *D. simulans* produces a pulse song with a mean IPI of ~55ms, whereas other workers (Ewing and Bennet-Clark, 1968) reported that a strain of *D. simulans* showed a mean IPI value of ~48ms. Kawanishi and Watanabe (1979) found that the IPIs of strains of *D. simulans* gave a much more variable pattern than strains of *D. melanogaster*. This was confirmed by Kyriacou and Hall (1986). The same variability in mean IPI is observed for *D. mauritiana* strains, Robertson (1983) reporting mean IPI values of ~45-55ms. Cobb *et al.* (1989) characterised *D. sechellia* song and reported that it only consists of phrases of pulses with no audible sine song, and a mean IPI of ~85ms.

Cowling and Burnet (1981) also discovered that the *D. yakuba* males produce only trains of pulses, with a mean IPI of ~96ms, but no hum song. Thackeray (1989), extending the characterisation of the *D. yakuba* song, observed that the *D. yakuba* pulses consisted of two distinctly different sound types. By using wingless ('mute') *D. yakuba* virgin females, and simultaneous observation and acoustic monitoring of these songs, Thackeray (1989) confirmed that both types of pulses were produced by the males only. It was clear from simple visual inspection, that the two pulse types had very different waveforms, and were given the names of "clack" and "thud" according to the auditory impression these pulses projected (Thackeray,1989).

Further audio-visual examination of the *D. yakuba* courtship song (Thackeray, 1989), revealed that each pulse type had a distinct part to play during different behavioural phases of the courtship itself. Moreover, Thackeray (1989) observed that each pulse type was produced by a different physical mode of wing vibration, e.g., clack pulses are produced by the wings only slightly outstretched from the abdomen and by apparent simultaneous vibration of both wings, which might explain its double-pulse nature seen in Figure 3.4.2a. The Thud pulses (see Figure 3.4.2b), on the other hand, are produced by one wing only, usually the wing closest to the female's head, at an angle of between 60^0 and 90^0 to the male's midline. Furthermore, Thackeray (1989) observed that Clack occurred when the male was oriented towards the female, but at a distance from her, whereas Thud was found to be most frequent when the male is attempting to lick the female genitalia or orientated quite near the female. The two types of *D. yakuba* are reminiscent of von Schilcher's (1976b) suggestion, that the hum and pulse song types of *D. melanogaster* might be acting as a prestimulator of females, and a "trigger" to mating, respectively.

Cowling and Burnet (1981) also reported that the songs of *D. teissieri* and *D. erecta* consisted of both a 'hum' and a pulse song. *D. teissieri* was shown to have the shortest IPI (~20ms) of the subgroup, and was composed of primary and secondary pulses which were 180^0 out of phase with each other. *D. teissieri* also showed the lowest SSF (~105Hz) of all the six species that were included in the study. *D. erecta* gave a mean IPI pulse song of ~40ms, which was close to that of *D. melanogaster*, and Ewing (1977) pointed out the pulse song of this species was polycyclic. Cowling and Burnet (1981) also found that the *D. erecta* sine song had a higher SSF (~245Hz) than the corresponding IPF. Cobb *et al.* (1989) reported that in *D. orena* the sine song constituted the majority of the song. The mean IPI of this species was found to be ~40ms, which is very similarly close to the value of both *D. melanogaster* and *D. erecta* pulse songs (see above), but the SSF was ~320Hz, representing the highest sine song frequency seen amongst the members of the *D. melanogaster* subgroup.

Kyriacou and Hall (1980), discovered a novel feature of *D. melanogaster* and *D. simulans* songs. The interpulse intervals (IPIs) oscillated sinusoidally around

values of about ~30-40ms, giving an ultradian rhythm with a period of between 50-60s, in

D. melanogaster, and 35-40s in *D. simulans* (Kyriacou and Hall, 1980; 1986) . This was

shown by dividing the song into 10s bins and calculating a mean IPI for each 10s-bin.

When these values were plotted against time, the means followed a typical rhythmic

pattern (see Figure 1.4). Konopka and Benzer (1971) induced three mutations in a screen,

involving circadian 24 h eclosion and locomotor activity rhythms in *D. melanogaster*. The

mutations shortened the cycle to 19 h, increased it to 29 h, or obliterated rhythmicity

altogether. The mutations were found to be alleles of the same gene, situated on the X-

chromosome, and given the name *period* (*per*). The mutants individually became known

as per^S (short), per^L (long) and per^{01} (arrhythmic) (Konopka and Benzer, 1971). The

courtship love song of *D. melanogaster* *per*-mutants was also found to be affected

(Kyriacou and Hall, 1980). The per^{L1} mutant was found to have a song rhythm period of

~80s, the per^S mutant was found to have a song rhythm period of ~40s, whereas the per^{01}

male seemed to be arrhythmic. These effects on the ultradian courtship song rhythm seem

to be in parallel to the effects shown on the eclosion and locomotor activity rhythms.

Kyriacou and Hall also found that the sibling species *D. simulans* had a shorter ~35-40s

cycle (Kyriacou and Hall, 1980; 1986). *D. yakuba* has been reported to have a song cycle

of ~70-80s (Thackeray,1989).

Kyriacou and Hall's finding of song cycles in *D. melanogaster* and *D.*

simulans (1980) sparked off a contentious debate. Crossley (1988) and Ewing (1988)

claimed they could neither replicate the original finding of song cycles in wild-type, nor of

altered cycles in *per* mutants and contested the effects of *per* mutations on the song

rhythm. They concluded that the statistical methods used by Kyriacou and Hall (1980) to

detect song cycles were inappropriate. Kyriacou and colleagues (1988, 1989, 1990a and

1990b) reexamined both their own original data as well as Crossley's and Ewing's data in

great detail, and scrutinized the statistical methods.

It was clear from the onset that rhythm detection, in *Drosophila* songs,

requires experience, diligence, skill and common sense. Moreover, there are a few

parameters that are needed to be handled with care before and during recording of flies,

such as not rearing male flies in solitude-which may influence the flies' courtship vigour

Figure 1.4: Diagrammatic representation of how a courtship song rhythm is obtained. The mean IPIs of every 10s of courtship song are computed and plotted, giving the "song rhythm".
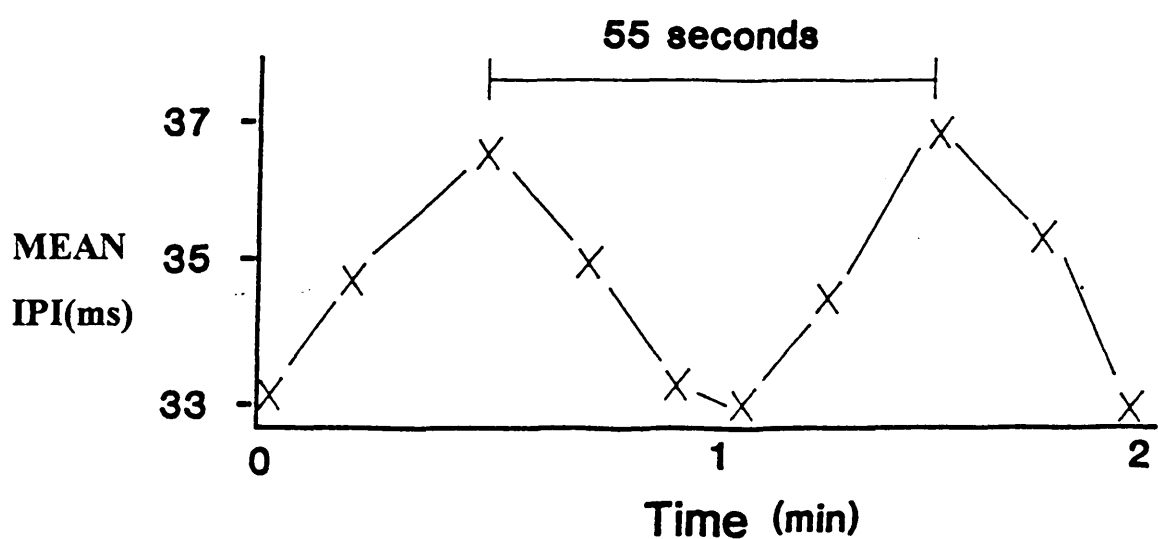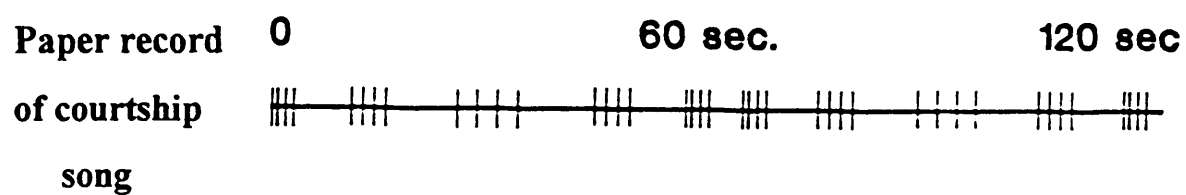
(Hall and Kyriacou, 1990b), or using recording equipment of sufficient quality, which could minimize background noises-which was one of the problem encountered in Ewing's recordings (1988). Kyriacou and colleagues pinpointed several key differences between the different experiments, which began to shed some light at the sources of disagreement (Kyriacou and Hall, 1989 and 1990). These were several, namely:

1) Ewing (1988) had used smaller than usual mating chambers, which might have been the source for obscuring any innate rhythmicity of the male's song (Kyriacou and Hall, 1988). When Kyriacou and Hall (1989) used the tiny chambers that Ewing had used (1988), they observed a number of unusual behaviours that had not been observed when recording flies in more roomy chambers. In addition, there was an increase in the extraneous noise levels due to aggressive interactions between the flies, and the male not being able to fully extend its wings during vibration.

2) Ewing (1988) and Crossley (1988) had filled 'empty' time bins with mean IPI values averaged from the preceeding and subsequent bin. This was unnecessary, as there exist spectral methods that deal with missing data points (Roberts et al., 1987; Van den Berg, 1989).

Kyriacou and Hall (1989) were able to show how Crossley's use of inserting missing data points could prejudice the outcome of song analysis. To press home the point, Kyriacou and Hall (1989) showed how in those songs that did not have missing data, Crossley found $\approx$ 60s cycles in almost all of them. The probability of rhythmicity resulting by chance in Crossley's data was 40 in 10^{-6}. The debate was finally put to rest by Alt et al. (1997), where by employing related spectral methods, they showed that $\approx$ 60s song rhythmicity is present in the wild type and is altered in per-mutants, as claimed by Kyriacou and Hall (1980). They also observed that per^{01} flies had periods in the range of 20-30s, as claimed by Kyriacou and colleagues (1989, 1990). Thus the song rhythm controversy seems to have finally been resolved.

The physiological pathways mediating the song cycle were investigated by using two temperature-sensitive mutations of the nervous system: nap^{ts} (non-action

potential) and *para*[ts] (paralytic) which were immobilised at >35° and 29°, respectively. This defect is due to the blocking of neural membrane sodium channels at restrictive temperatures (Kyriacou and Hall, 1985). Courting *nap*[ts] or *para*[ts] males were subjected to short heat pulses which temporarily 'shut-down' the males' nervous system. The males were then allowed to continue courting females, after recovery, and any phase-shifts in the song after the heat shock treatment were recorded. Wild-type males given brief heat pulses, or *nap*[ts] or *para*[ts] flies which were simply removed from the females and then replaced, sung in virtually identical phases before and after these control treatments. However, *nap*[ts] and *para*[ts] flies treated with the heat pulse, gave delays in the phase of the song, approximately corresponding to the length of the shut-down in their nervous system (Kyriacou ang Hall, 1985). These results demonstrate clearly that the clock producing the song rhythm is neurally mediated. Konopka *et al.* (1997) produced genetic mosaics for the *per*[s] and *per*[+] alleles. Those which had a *per*[s] brain and a *per*[+] thoracic ganglion showed short circadian activity cycles and a normal 55s song rhythm, whereas flies with a *per*[+] brain and *per*[s] thoracic ganglion showed a normal 24h activity rhythm and a short song cycle, implying the existence of two separate pacemakers, one for the courtship song rhythm in the thorax, and one for the locomotor activity rhythm in the head.

In the wild, courtships may occur over a brief period of time. Ewing and Ewing (1987) argued that it is highly questionable that female flies would have enough time to summate the IPI cycles. However, Kyriacou and Hall (1982), suggested that perhaps the rate of change of IPI that may be important, or even that males might 'scan' female IPI preferences. It is not inconceivable then that females may recognise the first derivative changes of IPI about a certain value and that the oscillation period is a way in which male flies could accomplish a continuous IPI 'gradient'. Indeed, Alt *et* al. (1997) have recently suggested that females may recognise the 'nature' of a cycle from the IPIs generated in less than a complete 60s rhythm. Alternatively, under the scanning hypothesis, individual female flies may have their own preferred IPI value, and thus consequently, males singing with a constant IPI may stimulate fewer females than males which generate many different IPIs. By singing rhythmically, a male fly can vary the IPI repertoire it produces; therefore by increasing his repertoire is able to stimulate a larger number of females.

A character that has an important role in sexual isolation should be species-specific. In the *D. auraria*-group species for example, females of the different species use IPIs as a species-discriminator (Tomaru and Oguma, 1994). Not surprisingly, amongst the *D. auraria* complex, a strong premating isolation is observed, indicating perhaps that no two species share gene pools (Tomaru and Oguma, 1994). Species' differences in courtship songs can be characterised by one or more components, such as IPI, fluctuation of the interpulse intervals, intrapulse frequency (IPF), sine song frequency (SSF), number of pulses per burst (PB), burst duration (BD) and number of cycles per pulse (CPP) (Bennet-Clark and Ewing, 1969; Kyriacou and Hall, 1980; Crossley, 1986; Hoikkala and Lumme, 1987; Wheeler *et al.*, 1988). One or more song parameters may exhibit species-specificity depending on the species, but playback experiments are required to examine whether any species differences are used as disciminating cues by females.

Schilcher (1976a & b) artificially pre-stimulated females with "hum" song before introducing them to males. The time to copulation of these pre-stimulated females was significantly shorter compared to females which had not been pre-stimulated. However, when female flies were pre-stimulated with a constant 34ms IPI pulse song, the effect was not observed. Females also mated faster with wingless ('mute') males in the presence of the conspecific 34ms song than with the 48ms song (Bennet-Clark and Ewing, 1969). Further experiments by von Schilcher (1977) indicated that females showed some preferences to conspecific IPI values, but the effects were marginal at best (Hall and Kyriacou, 1990). Further playback experiments by Tomaru *et al.* (1995), using female flies from the *D. auraria* group in the presence of wingless males, revealed that females would more readily mate with the wingless males, after having been stimulated by conspecific artificially generated song, rather than being stimulated by either heterospecific or intermediate songs, which further supports the notion that females discriminate against non-conspecific song.

In von Schilcher's experiments, the IPIs were produced at a constant 34 or 48ms. However, IPIs fluctuate rhythmically throughout the entirety of a courtship song, with a period of ~55s in *D. melanogaster* and ~35s in *D. simulans* (Kyriacou and Hall,

1980; 1989). Having discovered this new dimension in the the courtship song, Kyriacou and Hall (1982) investigated the function of the song rhythm by performing playback experiments. They played artificially generated songs, which incorporated all possible combinations of a 35ms IPI, a 48ms IPI, a 55s and a 35s oscillation periods to *D. melanogaster* and *D. simulans* females. They found that *D. melanogaster* females showed preferences to the song with '*melanogaster*-like' characteristics- a 55s rhythm with a superimposed 34ms IPI. Likewise, *D. simulans* females "preferred" a '*simulans*-like' song, with a 35s rhythm and a 48ms IPI.

Greenacre *et al.* (1993) extended these observations to show that wild-type females did not prefer the 55s song cycle to the 35s '*simulans*-like' cycles, but they discriminated against the heterospecific song, in that a song with an 80s cycle was just as effective as 55s. Thus females seemed to be actively rejecting the *D. simulans* song. Greenacre (1990) also artificially selected females that preferred either constant 40ms or 30ms IPI pulse song in the presence of wingless males, and used the females that mated fastest to produce both 'high' and 'low' IPI preference lines. After 2 generations the different lines were observed to respond better to their selected pulse song. Unfortunately, this result could not be upheld, and by generation 6 females from both lines mated so quickly that any effect of the different IPIs were completely overshadowed. Even when 'indiscriminate' females, females that mated extremely rapidly, were removed in a rerun of the selection procedure the early positive results could still not be sustained (Greenacre, 1990). Therefore, whether an individual female matches up the cycle to some internal rhythm template or whether she simply prefers some IPIs over others is unknown.

The IPI length has been argued to be involved in sexual selection, as it tends to be highly variable among species, thus potentially contributing to the maintenance of species barriers (Ewing and Bennet-Clark, 1968; Cowling and Burnet, 1981; Ritchie and Gleason, 1995). Yet, Noor and Aquadro (submitted) suggest that ecological pressures may potentially modify the mean IPI, rather than sexual selection, and could account for the differences observed in the courtship song of different *D. pseudoobscura* and *D. persimilis* populations. Occasional matings between *D. pseudoobscura* males and *D. persimilis* females revealed no differences in song characteristics between those *D.*

pseudoobscura males that did hybridize and those that did not (Noor and Aquadro, submitted). Moreover, Ritchie *et al.* (1994) investigating the genetic variability across European *D. melanogaster* populations, reported that there were no significant differences in the mean IPI, yet the differences in mean IPI across the populations were similar in magnitude, as those reported by Noor and Aquadro (submitted). An explanation that could account for the lack of significant differences within a species or among populations for the male IPI song, is stabilizing selection, possibly through female discrimination. An alternative explanation could be that variation does exist within species, but remains undetected due to the high levels of IPI variation within and between an individual song, and which could partially be explained by the periodicity in IPI. Furthermore, Noor and Aquadro (submitted) suggest that these differences, even between closely related species, may be just by-products of adaptation to different environments coupled with an absence of the homogenizing effect of gene flow, which is also observed in at least some *Drosophila* species (Coyne and Milstead, 1987; Schaeffer and Miller, 1992).

Experiments that measured the female's receptivity to copulation attempts by wingless ('mute') males were shown to be enhanced, when the females had been exposed to simulated artificial songs (Kyriacou and Hall, 1984). Genetically normal females, which had been pre-stimulated for two minutes with artificially-generated cycling pulse song, are observed to show enhanced mating kinetics, when mixed with intact males (Kyriacou and Hall, 1984). The 'memory' of such sensitization is 'short-term', lasting a few minutes in normal females (Kyriacou and Hall, 1984). When such experiments were performed on *dunce*, *rutabaga* and *amnesiac* females (e.g., Tully and Quinn, 1985), very brief or even non-existent after-effects of prestimulation are exhibited (Kyriacou and Hall, 1984). Consequently, it appears that 'acoustic priming' (pre-stimulation) of the mating behaviour involves complex interactions between information, storage and retrieving mechanisms. Experiments by Tully and Quinn (1985), and Dudai (1985) suggest that the fly's abilities to learn and remember in a sexual context, is of adaptive significance. Sensitization to certain auditory stimuli produced by courting males seems to be a 'simple' learning process. Griffith *et al.* (1993), using both cycling IPI and hum song as a prestimulator, were able to show stronger after-effects in wild-type females than when using either pulse or sine song alone (Kyriacou and Hall, 1984). Females carrying a calcium/calmodulin-

dependent protein kinase (CamK) peptide inhibitor transgene under the control of a heat-shock promoter, which produce learning and memory defects in proportion to the amount of transgene inhibition in sexual situations (Siegel and Hall, 1979), also showed defective acoustic priming (Griffiths *et al.*, 1993). Thus, playback experiments on song function have served both evolutionary and neurogenetic purposes.

Signals that function to bring potential mates together are components of a sexual signal-receiver system. Since both sexes must be able to recognize each other, the characters of this system must be co-adapted, and it is possible that they would be likely candidates for stabilizing selection. Even though co-adaptation suggests that the sexual signal-receiver system is resistant to change (Paterson, 1985), it does not imply that it can not evolve. Therefore the evolution of this sort of system would depend on the extent of genetic variation in male and female components and their genetic control. Charalambous *et al.* (1994) used artificial selection to test for the presence of genetic variation in the male signal (syllable length), and in the female preference for this particular signal in the common field grasshopper, *Chorthippus brunneus*. In just one generation of selection, of a two generation selection experiment on the male calling song, a response occurred, resulting in a significant difference between the two divergent lines. Selection on the female preference to artificially-generated male calling song, resulted in an immediate and significant response to selection (Charalambous *et al.*, 1994), which, unlike the experiments of Greenacre *et al.* (1991), was stabilized in later generations. These results suggest the presence of additive genetic variation in the female preference for the syllable length of the male song; however, the female preference to differing syllable lengths has not yet been tested.

Piñeiro *et al.* (1993) selected for high and low receptivity (mating speeds) in *D. melanogaster* females collected from the wild. Selection was achieved in both directions, which is in agreement with Carracedo *et al.* (1991), whereby a large amount of genetic variation for female receptivity in the base population, as well as a large proportion of additive genetic variation, was observed. Yet, only a slight dominance component in another *D. melanogaster* population was detected. Their results suggest that female receptivity in *D. melanogaster* was not under any directional selection pressure

in the wild. The lack of directional dominance for female receptivity was also apparent in selected lines after selection was relaxed for 42 generations. Piñeiro *et al.* (1993) also yielded a line with diminished receptivity from selection of unmated females. Furthermore, it was found that lines selected for high receptivity had an increased probability of hybridising with *D. simulans* males, whereas in the case of the low receptivity line the converse was true. This is in line with the results of Izquierdo *et al.* (1992), in which selection for increasing hybridisation, between *D. melanogaster* and *D. simulans*, was associated with an increase in receptivity. Bearing all this in mind, a fine balance between natural selection for increasing (risking hybridisation with subsequent fitness impairment for females that hybridise), and diminishing (risking individual genetic extinction) receptivity, could explain the large additive variation for receptivity observed in this study and that of Casares *et al.* (1992).

Kyriacou and Hall (1986) also examined the mating preferences of hybrid F_1 *melanogaster/simulans* females, by artificially stimulating these females with various combinations of parental or intermediate IPI means and rhythm periods. They found that the latency to mating was shortest with a hybrid song with a 45s rhythm superimposed on an intermediate mean IPI of 41ms, suggesting that the genes controlling the species-specific receptivity to male songs, acted additively in these hybrid females. It was impossible to carry the analysis beyond the F_1. Kyriacou and Hall (1986) also analysed courtship songs in reciprocal *D. melanogaster/D. simulans* interspecific hybrid males. Even though, the mean IPI of such hybrids is intermediate between the means of the parent species, indicating autosomal control of this song character, the period of their IPI oscillation was dependent upon the origin of their X chromosome. In other words, hybrids with an X^{sim}/Y^{mel} genotype produce a '*simulans*-like' cycle of ~40s, whereas an X^{mel}/Y^{sim} hybrid produces IPIs of ~55s oscillation period, typical of *D. melanogaster*. Since *per* is carried on the X chromosome, their finding is consistent with *per* being the cause of this species-specific difference in the song period between *D. melanogaster* and *D. simulans*. These experiments supported the notion that genes generating the species-specificity of the male song may include the sex-linked *per* locus, but say nothing about the genes involved in the female preference.

Greenacre *et al.* (1993) examined the mating speed of females, carrying homozygous *per* mutant alleles (*per*^{L} and *per*^{s}), when these flies were artificially stimulated with mutant song rhythms (80s and 40s, respectively), and compared them to congenic *per*$^+$ females. It was observed that these mutant females did not show any preference for their corresponding mutant song cycles, but instead showed the usual wild-type female discrimination against a ~40s song cycle (Kyriacou and Hall, 1980; 1986). Clearly the *per* gene is not involved in influencing female preferences. However, *per*^{01} female flies which lack a functional Per protein (Yu *et al.*, 1987a; Baylies *et al.*, 1987), mated very rapidly and were less discrimatory against the 40s cycle. Greenacre *et al.* (1993) extended this line of experimentation by testing the female preferences of a *per*^{s} mutant line, from an established laboratory stock maintained for over 20 years. The females, from this 'old stock', showed an enhanced response to the mutant 40s song cycle. As congenic *per*$^+$ and *per*^{s} females both show enhanced discrimination against a 40s cycle, it is probable that this preference for the 40s cycle had 'evolved' by a secondary selection at other loci. (e.g., see Bastock, 1956). Such coevolution was proposed by von Helversen and von Helversen (1975) with the signalling system of *Chorthippus biggutulus* and *Ch. mollis*, which in essence means that if a genetic change in a male character occurs, this change would instigate the selection at other loci for an appropriate change in the female character, or vice versa.

It is clear that genetic coupling as described by Alexander (1962) is not operating in this case, in that *per* affects the male courtship signals, but not the female receptivity to these signals. Thus, the species-specific correlation between male and female preferences must be achieved by the co-evolution of different genes controlling each characteristic. Genetic coupling is a tantalizing hypothesis in that a new mutation that would affect male output and female input characters simultaneously could bring about rapid speciation. Conversely, the coevolution hypothesis furnishes a much slower alternative, whereby a change in one sex brings about a change in the other. Most experiments carried out in order to dichotomize between the coevolution and genetic coupling hypotheses, e.g., in crickets (Hoy *et al.*, 1977; Doherty and Hoy, 1985), and tree frogs (Doherty and Gerhardt, 1984), have been unsuccessful because of the difficulties in

obtaining F_2 hybrid female progeny, and thereby looking for segregation of the male and female characters.

As mentioned earlier Konopka and Benzer (1971) identified the *per* mutations which affect eclosion and locomotor cycles. *per* was cloned by two separate groups, idependently (reviewed in Hall, 1995; Kyriacou *et al.*, 1996). All three *per* mutations have been mapped to single amino acid substitutions (Baylies *et al.*, 1987; Yu *et al.*, 1987a). The most striking feature of the *per* gene is the central coding region which encodes for a run of alternating threonine-glycine (Thr-Gly) residues (Jackson *et al.*, 1986; Citri *et al.*, 1987), which is found to show length polymorphism, in both natural and laboratory populations of *D. melanogaster* (Yu *et al.*, 1987b; Costa *et al.*, 1991), with the number of Thr-Gly repeats ranging from 14 to 24 (Rosato *et* al., 1996).

Deletion of the Thr-Gly region, together with a few flanking amino acids, to create the ΔTG transgene (Yu *et al.*, 1987b), produced a ~ 40s male courtship song rhythm. The circadian locomotor activity pattern was observed to be unaffected, at 25^0C (Ewer *et al.*, 1990). Hence, the courtship song cycle appeared to be more sensitive to Thr-Gly alterations than the circadian cycle. As the species-specific differences between *D. melanogaster* and *D. simulans per* genes mapped to the X chromosome (Kyriacou and Hall, 1986), could *per* be a reservoir for species-specific song cycles?

In follow-up experiments performed by Wheeler *et al.* (1991), the molecular transfer of a species-specific behaviour was demonstrated using *D. simulans* and *D. melanogaster per* genes. A number of transgenic constructs, where the Thr-Gly repeats together with some flanking sequences of *per*, were interchanged between *D. simulans* and *D. melanogaster*, were created, and these chimeric constructs were used to transform *per*[01] flies. The resultant transgenic flies carried the *D. melanogaster per* gene, but a *D. simulans* Thr-Gly region. The flies were found to sing with a *simulans*-like (~40s) song cycle. Transformed *per*[01] host flies carrying the reciprocal construct (*D. simulans per* with *D. melanogaster* Thr-Gly repeat), sang with a *melanogaster*-like (~60s) song cycle (Wheeler *et al.*, 1991). Therefore, it was revealed that the observed species-specific difference in behaviour mapped to the small 700 bp fragment of DNA in *per* which had

been swapped between the two species. This 700 bp DNA fragment encodes for eight species-specific amino-acid differences (Wheeler *et al.*, 1991) and thus any one (or all) of these species-specific differences could account for the differences observed in the species rhythms. If we disregard the small difference in Thr-Gly length between the two swapped fragments (a difference of four Thr-Gly pairs), four species-specific amino acid substitutions are found downstream of the repeat, and thus by deduction, one or more of these changes could most likely account for the species-specific song rhythm (Wheeler *et al.*, 1991).

The *per* gene has been cloned and sequenced in a number of *Drosophila* species (reviewed by Hall, 1995; Kyriacou *et al.*, 1996). Strains of the sibling species of *D. simulans* shows a similar length polymorphism, ranging from 23 to 25 Thr-Gly pairs (Wheeler *et al.*, 1991; Peixoto *et al.*, 1993, Rosato, *et al.*, 1994). One strain of *D. yakuba* revealed the presence of 15 Thr-Gly pairs (Thackeray and Kyriacou, 1990). *D. mauritiana* was shown to have 25 Thr-Gly pairs, while *D. sechellia* had 20 Thr-Gly pairs (Peixoto *et al.*, 1992). *D. teissieri* appeared to encode for 14 Thr-Gly pairs, while *D. erecta* had 14 Thr-Gly pairs plus two more that had the glycine residue changed into aspartic acid and arginine, respectively (Peixoto *et al.*, 1992). *D. orena* was shown to encode for 19 Thr-Gly repeats plus three more pairs that had their glycine residue changed into aspartic acid, glutamine and arginine (Peixoto *et al.*, 1992). This specific fragment could potentially control song rhythmicity in each of the other species of the *D. melanogaster* subgroup. Moreover, the different amino acids substitutions, downstream the Thr-Gly repeat, which were apportioned by Wheeler *et al.* (1991) as playing a critical role in the song rhythmicity of *D. melanogaster* and *D. simulans* are observed to be different among the different species of the *D. melanogaster* subgroup. Specifically, *D. melanogaster* has an alanine and lysine residues at positions 102 and 107 (Peixoto *et al.*, 1992), as compared to *D. simulans* that has threonine and arginine residues at these positions, respectively. The other species in the subgroup have various amino acid substitutions in these positions (Peixoto *et al.*, 1992), and we might speculate that if these sustitutions are the crucial ones for song rhythmicity (as opposed to the two found further downstream- Wheeler *et al.*, 1991), then these could indicate species-specificity of song cycles in the other member species of the *D. melanogaster* subgroup.

Ritchie and Kyriacou (1994) carried out a series of female preference experiments, allowing *D. melanogaster* females to be courted by *D. melanogaster* transformant males, generated by Wheeler *et al.* (1991), which carried either the *melanogaster-* or *simulans-per* gene. Surprisingly, transformants expressing *D. simulans per*, were at least as successful as the conspecific *per* transformants, implying that the females did not discriminate between the different song cycles in these males, which seem to be at odds with the results of Kyriacou and Hall (1982; 1986). However, the song rhythms in these male transformants are not as robust as in the wild-type *D. melanogaster* and *D. simulans* males (Wheeler *et al.*, 1991). Thus, in order to properly assess whether song rhythms in male transformants are differentially detected by *D. melanogaster* and *D. simulans* females, a large number of independent transgenic constructs must be made and tested in order to alleviate the 'position effects', that are consistently observed with transformation experiments (Ashburner, 1989). Despite their shortcomings, future larger scale experiments using many transformed lines for each *per* transgene could provide a more definite answer to the question of whether *per* is indeed a 'speciation gene' by way of its effects on mating behaviour.

The *per* orthologues from *D. pseudoobscura* and *D. virilis*, distantly related species to *D. melanogaster*, have also been cloned and sequenced by Colot *et al.* (1988). Petersen *et al.* (1988) reported that species-specific circadian locomotor activity instructions are transferred by the *D. pseudoobscura per* coding region, when this was used to transform per^{01} *D. melanogaster* mutants. The resultant transformants showed one predominant evening peak, characteristic of *D. pseudoobscura* in light:dark (LD) conditions. This species-specific circadian locomotor activity pattern has been mapped to the N-terminal half of *per* using interspecific chimeric genes between *D. melanogaster* and *D. pseudoobscura* (J.M. Hennessey and C.P. Kyriacou, pers. comm.). If a species is active in the mornings, it will be behaviourally isolated from another species which may be active in the evenings, and so locomotor activity patterns could be significant in sustaining sexual isolation. This type of isolating mechanism could potentially play an even more potent role in maintaining barriers to interspecific matings than sexual selection for

species-specific courtship attributes. In this regard, Coyne (1992) has called *per* a potential 'speciation gene'.

If *per* is a 'speciation gene', it may show the signature of past selective events, in its putative role in sexual isolation. Kliman and Hey (1993) sequenced a number of *per* alleles in *D. melanogaster* and three of its sibling species. They performed various neutrality tests (Hudson *et al.*, 1987; Tajima, 1989) on a 1.9kb region, upstream of the Thr-Gly region, and found no evidence of any excess or dearth of nucleotide variation that could be taken as evidence for balancing selection or selective sweeps. This finding was in agreement with Begun and Aquadro's (1991) study, which also provded no evidence for selection at the *per* locus in *D. melanogaster* and *D. simulans*. Nonetheless, Rosato *et al.* (1994; 1997) extended the analysis to the Thr-Gly region, in *D. simulans* and *D. melanogaster* respectively. Using various statistical analyses, a pattern of nucleotide variation consistent with weak selection was observed in both species. These differences between the two sets of studies may be due to the fact that the Thr-Gly domain, because of its repetitive nature, does not obey the same evolutionary rules as the upstream non-repetitive sequences analysed by Kliman and Hey (1993) and Begun and Aquadro (1991).

The evolutionary dynamics that play on the Thr-Gly domain could be due either to the fact that this region may control the species-specific song cycle, or to the recent observation that the domain plays a pivotal role in temperature compensation of the *Drosophila* clock (Sawyer *et al.*, submitted; Peixoto *et al.*, submitted; Peixoto *et al.*, 1993; Nielsen *et al.*, 1994). Phylogenetic relationships between semispecies of *D. athabasca*, based on mitochondrial DNA data, suggest that the patterns of sympatry and allopatry strongly implicate the action of selection in the rapid evolution of behavioural isolation for this complex (Yoon, 1991; Yoon and Aquadro, 1994). However, Ford *et al.* (1994), studying the nucleotide variation at the *per* locus of the species in the *D. athabasca* complex, suggested that *per* may not play a role in the mating isolation between these groups (Yoon and Aquadro (1994). Consequently, the evolutionary analyses of nucleotide sequences within *per* appear to center on the Thr-Gly domain, as a focus for natural selection, whereas other regions of the *per* molecule do not reveal such intriguing patterns.

Aim of the project

The male courtship song and locomotor activity patterns are of interest to speciation biologists, because changes in either could lead to sexual isolation. In light of the controversy about the existence of song rhythms in the *D. melanogaster* courtship song (Kyriacou and Hall, 1980; 1989; Crossley, 1988; Ewing, 1988; Alt *et al.*, 1997), and the recent evidence furnished by Noor and Aquadro (submitted) for the existence of short song cycles in *D. persimilis* (distantly related to the *D. melanogaster* subgroup) Chapter 3 attempts to confirm the original findings of Kyriacou and Hall (1980), and extend the song rhythm analysis to *D. yakuba* rhythms. Chapter 4 investigates the possible existence of song rhythms in the courtship songs of the other members of the *melanogaster* subgroup. Chapter 5 offers a further insight into the inheritance of song components such as the presence or absence of sine song (SSF), intrapulse frequency (IPF), interpulse interval (IPI), and song rhythms. Byrne and Kyriacou (pers. comm.) have reported, in simulator experiments, that the song burst duration, may play an important role in the conspecific recognition between *D. mojavensis* and *D. arizonae*. Chapter 6 attempts to shed some light on any short-term temporal changes that may occur during the duration of a song burst, and tries to identify any species-specific differences in the *D. melanogaster* subgroup of species. In addition, chapter 6 investigates the grosser temporal changes such as the burst duration and interburst interval, and hybrids are generated to examine the inheritance of any species-specific song components.

As mentioned earlier, *per* has also been found to change the species-specific pattern of locomotor activity (Petersen *et al.*, 1988). Chapter 7 investigates the freerunning activity patterns of the members of the *melanogaster* subgroup and their interspecific hybrids in constant darkness (D:D). Chapter 8 examines the activity patterns during 12:12 LD cycles for both the *melanogaster* subgroup members and their interspecific hybrids. Are there any species-specific aspects to these phenotypes? If so, could these be relevant to sexual selection between the *D. melanogaster* subgroup species? In endeavouring to answer these questions, further insights might be furnished, not only into biological rhythmicity, but also into the possible impact of behavioural rhythms on evolution.

MATERIALS & METHODS

CHAPTER 2

2.1 STOCKS

D. melanogaster (Brighton) was provided by Prof. Linda Partridge. D. simulans (Florida) and D. yakuba (Lamto3) were provided by Dr Barrie Burnet (Sheffield University,UK), and had been maintained in the laboratory for at least six years. D. erecta Sheffield (both light and dark body females), D.orena Sheffield and D. mauritiana (MG-71 and MG-17) were also provided by Dr Barrie Burnet. D. teissieri (Sweden-UMEÅ), D. yakuba (Ivory Coast) and D. mauritiana (Sweden-UMEÅ) were provided by the Drosophila Stock Center, Umeå, Sweden. D. mauritiana 1014, together with all the Isofemale lines were all provided by the Drosophila Stock Center, Bowling Green (Indiana State University, Indiana, USA). D. orena France and D. erecta France were provided by Dr Matthew Cobb (France) and D. sechellia was provided by Prof. M. Ashburner's group (Cambridge-U.K.).

2.2 STOCK MAINTENANCE

Fly stocks were reared on sugar/agar medium (65 g of sugar, 115 g of yeast, 10 g of agar and 2 g Nipagen in 1 l of water), in either one-third pint milk bottles or glass vials (10 cm x 2.2 cm). The flies were kept in controlled temperature rooms at either 18 or 25 ($\pm$ 1)°C and entrained in light-dark cycles of 12 hours (LD 12:12) with lights on/off at 09:00/21:00 h. Parents of flies which were used for experiments were removed from the bottle after 5-6 days to avoid overcrowding the progeny. The fly strains and species used in this work are described in the relevant chapters.

2.3.1 FLY COLLECTION

For all song recordings and genetic crosses, virgin females up to 10 hours old were collected. Flies eclosing overnight were discarded and collections were made every 2 h thereafter until late in the afternoon. Flies were collected under CO_2 anaesthesia and kept in vials at a density of 3 female flies per vial and one male fly per vial. Vials containing female flies were carefully checked before either any recording experiment or

setting up crosses, to guarantee that no larvae were present, and hence that the flies were indeed virgins. If any females were found not to be virgins, the vials were discarded. All the flies that were collected for the subsequent experiments were stored at 25°C, unless stated otherwise.

2.3.2 INTERSPECIFIC CROSSES

As stated above, male flies were reared in solitude, which increases their sexual vigour, giving a higher possibility for a successful mating (von Schilcher, 1976b; Hall & Kyriacou, 1990). However, *D. mauritiana* males were kept, not only in solitude at 25°C, but also under constant light conditions (LL), as the male mating capability was discovered to be maximised under constant light conditions, since its courtship behaviour is very light-dependent (Robertson, 1983). Two-day old female flies and three-day old males were then placed in a new food vial and left for five days. If no larvae were observed, the vials were discarded. If, on the other hand, larvae were visible, then males and females were placed over into fresh vials, thus perpetuating the cross.

2.3.3 FEMALE WING AMPUTATION

During song recording, it was observed that *D. mauritiana* females could violently reject males, by pushing them away forcibly, using both their wings. I decided to amputate the wings from the female flies in order to see whether this 'amputation' might increase the chances of producing hybrid progeny (see Chapter 5). Furthermore, as *D. mauritiana* mating behaviour is light-dependent (Robertson, 1983) and since light increases sexual vigour (Robertson, 1983), I decided to reverse the *mauritiana* rearing conditions, described by Robertson. Instead of keeping *D. mauritiana* female virgins under constant light conditions, as in the regime for rearing male flies, the females were kept under a 12:12 LD light regime, in an attempt to decrease their aggressiveness; the removal of their wings rendered the female flies incapable of violently rejecting any courting males. After many unsuccessful attempts to generate hybrid larvae with *D. mauritiana* females and *D yakuba* males, hybrid larvae were eventually produced, when *D. mauritiana* females (with amputated wings) were used. To my knowledge, it is the first time, that hybrid flies have been produced in this cross. *D. mauritiana* has only previously

been used as the paternal participant in interspecific crosses (Cowling and Burnet, 1981). The same procedure was used when *D. mauritiana* females were crossed with *D. simulans* and *D. teissieri* males.

Removing the wings of female flies involved anaesthetizing the flies under CO_2. A pair of tweezers was used to pin down the fly and spread the wings of the fly flat onto a substrate dish, which was constantly supplied with CO_2. Using a sharp needle, the wing was carefully removed, and the whole operation was executed under a dissecting microscope.

2.4.1 SONG RECORDING

Male flies whose courtship song was to be analysed were collected within one day of eclosion and stored in vials at 25°C, in a 12:12 LD cycle with the exception of the *D. mauritiana* strains (see above). All males were stored singly in vials, as this can increase the amount of song production, except for *D. melanogaster* males which were kept at a density of two per vial (Hall & Kyriacou, 1990). Females were kept five to a vial. The song was recorded when the male was 4 or 5 days old.

The recording equipment consisted of a small, rectangular perspex cell, with a mesh underside into which one male and two females were introduced without anaesthesia, using a mouth-sucking device. The cell used for recording songs had dimensions of 19mm length x 12mm width x 8mm height. The females used in these recordings were one or two days old. The cell was placed in a foam lined box with its mesh underside over a condenser microphone-(an Insectavox from Brandeis University, U.S.A- Gorzyca and Hall, 1987)-see Figure 2.1. Courtship songs were recorded on a Revox reel-to-reel tape recorder. Depending on the species, the duration of the songs ranges from ~180s (*D. melanogaster*) to 1200s (*D. yakuba*). A graphic representation of the song was obtained by tracing the recording through a band pass filter (EF5-20 Power Unit and EF5-03 LP/HP Filter, Barr and Stroud, Cambridge, U.K.) (high pass, 100Hz, low pass 1000Hz- to remove extraneous noise) and in a CED 1401 A-to-D converter. The song was displayed on the monitor of Tandon 486 computer, using Spike 2 software,

Figure 2.1: Schematic diagram cross-section through Insectavox

(after Thackeray, 1989)

Magnifying view lens

Mating cell

Entry-hole
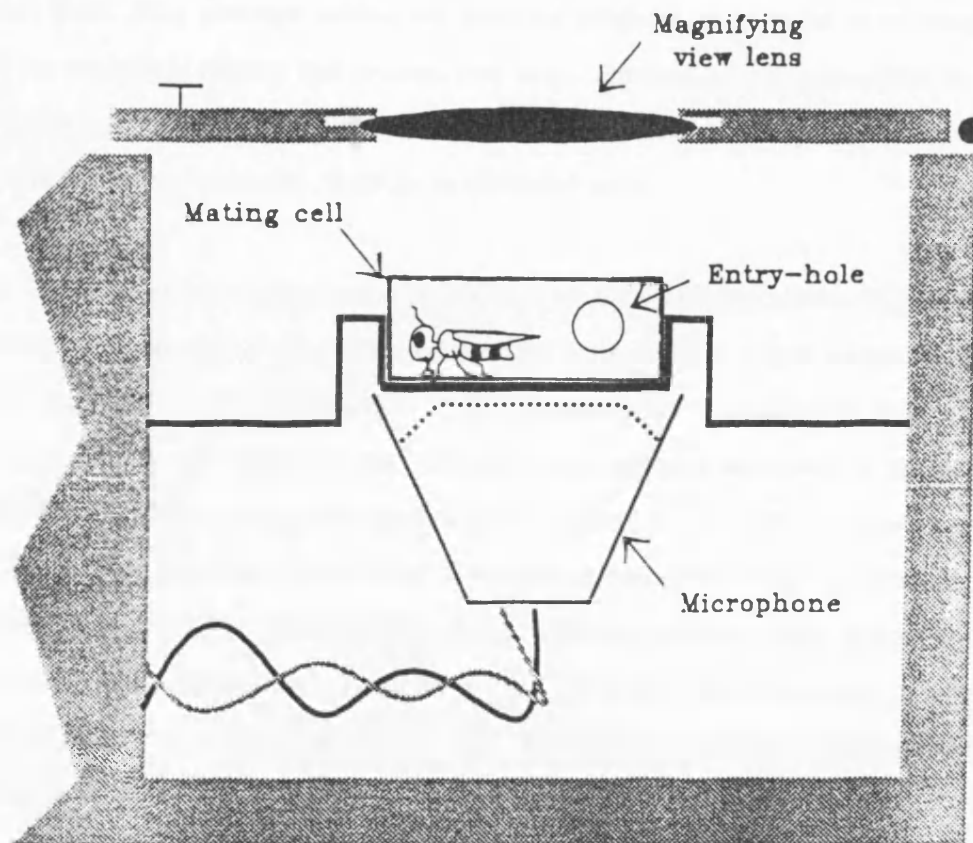
Microphone

The mating cell used for courtship song recording is shown within the Insectavox unit (Gorczyca and Hall, 1987), postioned over a condenser microphone.

Version 4.70 (C.E.D. Ltd-Cambridge, U.K.). The song patterns were then printed onto dot matrix printer paper using the 'oscillograph' program, written by Dr Mike Ritchie.

2.4.2 SONG ANALYSIS

The Spike 2 Version 4.70 software is a suite of programs for the CED 1401-IBM PC compatible-Converter, designed to capture and process both events and waveform data. The concept behind the analysis program is to make it as simple as possible for anyone to display and process raw data. Analysis of data can either be done manually from the monitor or automatically. In the course of this present study song editing was performed manually, in order to minimize error.

In order to investigate the presence or absence of songs cycles (Kyriacou and Hall, 1980), after obtaining a printed profile of the songs on dot matrix computer paper, songs were divided into 10s 'bins' and the IPI's measured within each 10s time window with a ruler. The upper and lower cut-off values were adjusted according to the value of the mean IPI of individual courtship songs and according to the different species, under investigation. Bins with less than 10 IPIs were not included in the analysis, unless eight or nine pulses constituted a burst. Courtship songs with more than one third of the mean IPI points missing were discarded. To analyse overall mean IPI, and other song parameters, the Spike 2 programmes Events.Txt and IPI.TXT were used to compute the appropriate statistics.

2.4.3 STATISTICAL ANALYSIS OF SONG CYCLES

Two methods of assessing the statistical significance of song rhythms were employed; one which uses the CLEAN algorith (Roberts et al., 1987; Kyriacou and Hall, 1989) and another which was a modification of a standard Fourier analysis written by Marc van den Berg (Van den Berg, 1989; Kyriacou and Hall, 1989 and Kyriacou et al., 1990).

The CLEAN spectral analysis estimates missing data points by a procedure approximating to a least squares interpolation. The highest peak or frequency from the

spectral analysis (or spectrogram) is taken as the cycle length or period; sometimes the second highest peak of the spectrogram is used, when the first peak gives a value of more than half the duration of a song recording. This occurs most frequently when the mean IPI of a song rises systematically during the course of the courtship. Songs with this characteristic are known as 'climbers'. This long cycle represents the upward trend in the data, which may be considered to be part of a very slow oscillating sine wave. The second peak value is also used when a period of between 20-22s is obtained. This is the 'Nyquist' value, i.e., twice the value of a bin length of 10s. An artefact of spectral analyses is that they will occasionally find the highest frequency in the spectrogram to be around twice the sampling frequency.

The Van den Berg (VdB) program is a basic Fourier analysis, but differs from CLEAN in that any missing data points are omitted from the analysis and are not estimated. However, the phase of the data points is maintained (Kyriacou *et al.*, 1990). As with CLEAN, the value of the second peak of the spectrogram is taken as the true cycle length, when the first peak gives a value of $\approx$ 20s or a value of more than half the duration of the song recording.

The VdB analysis also takes the highest peak in the spectrogram and uses this in a least squares curve fitting procedure, to find a new period via regression. An associated F-ratio for goodness of fit is given. Using Monte-Carlo simulations, Kyriacou and Hall (1989) found that these F-ratios are, under certain circumstances, useful statistics for describing the 'signal-to-noise' ratio. F-tables were also used by Kyriacou and Hall (1980) to operationally define "weaker" rhythms, i.e, songs with a small F-ratio, from "stronger" ones. This allowed them to separate per^{01} songs which have much weaker cycles from the other *per-* variants (Kyriacou and Hall, 1989). Consequently, F-tables give a reasonable indication of the strength of a rhythm, but are only occasionally used in this work.

2.4.4 OTHER SONG CHARACTERISTICS

For examining the Cycles per Pulse (CPP), Intrapulse Frequency (IPF) and Interpulse Interval (IPI) song components, a minimum of the ten longest bursts were

chosen from each song. The IPI, CPP, and IPF was computed for each sequential pulse position in the burst, in order that regressions could be carried out to look for trends in the data. IPI's were calculated from paper records of song traces, CPP, IPF and Sine Song Frequency (SSF) were calculated from the Spike 2 facilities on the monitor. The FLY.TXT program in the Spike 2 package was used to count CPP in a pulse. They were then scored manually for further statistical analysis. This was done as follows:

The song baseline was used as the reference start line for counting the cycles within the pulse under consideration. One cycle was considered to be one complete oscillation (see Figure 2.4.3, below).
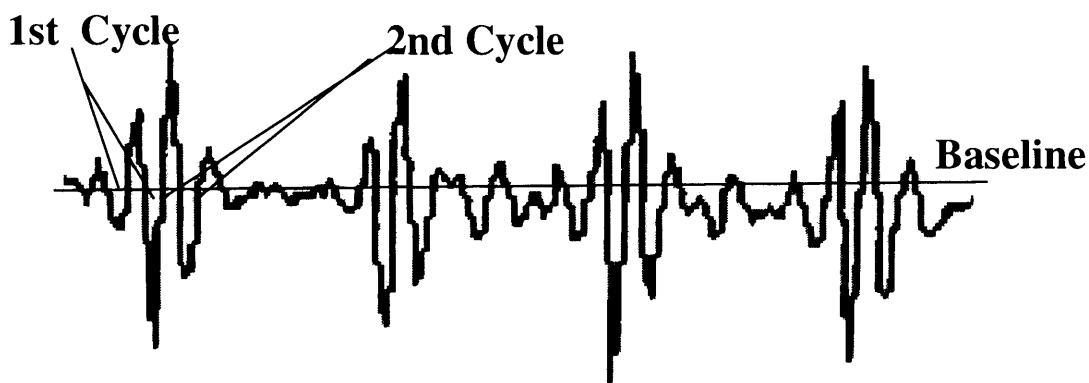
Figure 2.4.4.1: Cycles per Pulse, (CPP)

It was possible to record the IPF by taking the reciprocal of the time duration for a complete pulse. In addition, the SSF was determined, by placing the cursors from the beginning to the end of a sine burst song, calculating the number of complete cycles, dividing the total time of the burst by the number of cycles and using the reciprocal as the SSF.

2.4.5 Determination of the Mean Burst Duration and Mean Interburst Interval

The burst duration (bd) and mean interburst interval (ibi) were also calculated manually from dot-matrix paper. Different cut-off points were used to describe a 'burst', depending on the species (see Chapters 3, 4 and 5). Burst duration was measured from the beginning of the first pulse in a burst to the end of the last pulse of the burst in question. Then, a mean was calculated for the entire song, and for each of the

two separate halves of the song. The ibi was measured from the end of the last pulse of the previous burst, until the beginning of the first pulse of the following burst, taking into account the maximum IPI that was accepted for each species. A mean ibi was determined for the entire song, and for the two separate halves.

2.4.6 Clack versus Thud pulses in *D. yakuba* songs

Thackeray (1989) discovered that within a D. *yakuba* song two distinctly different types of pulses were present. These two different types of pulses were marked on the dot-matrix oscillograph paper, prior to analysis. This was done by simultaneously listening to the recorded songs, while observing the waveform of each burst of pulse song on the oscilloscope. The song was then analysed by dividing the analysis into 'clack' and 'thud' to give two independent sets of data. In the majority of songs examined, thud pulse-type predominates with the clack pulse-type making little or no difference to combined overall mean IPI (see Chapter 3-Tables: 3.4.2a & b).

2.4.7 Statistical Analysis

All formal statistical analyses were performed by using the Minitab Statistical Software, Release 8.2-(Minitab Inc., 3081, Enterprise Drive, State College, PA 16801-3008), and the Statistica Microsoft for Windows- (StatSoft, Inc., 2325 East 13th Street, Tulsa, Oklahoma 74104, U.S.A.). The former statistical package was used primarily for further statistical analysis of courtship songs, and locomotor activity data, while the latter package was principally used for the two-way ANOVA.

2.5.1 LOCOMOTOR ACTIVITY EXPERIMENTS

The locomotor activity experiments were carried out using a locomotor activity event recorder produced by BIODATA Ltd. (Manchester, U.K.). Each fly was loaded into a cylindrical glass tube (8 x 0.3 cm) containing 2 to 3 cm of sugar/agar medium at one end, sealed with cling film, so that the food would not dry out. Some cotton wool was used to seal the other end. Each tube was placed between an infra-red light emitter and detector, which recorded an event every time the fly crossed the light

33

beam. Infra-red wavelengths were used because flies are insensitive to this region of spectrum (Bertholf, 1932; Kyriacou and Burnet, 1979). The number of events registered in every half-hour time window (bin) was then fed directly into Tandon PC-386 computer.

Before analysis, the data was edited in the following ways: Flies which died during the experiment before the end of the 5th day of data collection were excluded from analysis. Furthermore, flies from channels which were considered to be hypersensitive, that is, which exhibited from 500 to 1000 events in many half-hour bins, were excluded from the analysis. Flies which died within the last two days of data collection were included in the analysis after deleting from the activity record the last 12 hours of data before the fly died. Occasionally an otherwise 'normal' record showed an isolated bin with very high counts. For example, in one case a bin with 834 events was observed for a fly which showed for its entire record less than 70 events per bin during its peak activity phase. This apparently 'deceptive' data point was corrected by simple interpolation, that is, by replacing the value of that bin with the average value of its two neighbouring bins. As a rule any bin with more than 150 events, which also gave 10 times more events than its interpolated estimate was corrected in this way. Similar problems have been described for other activity monitors by Hamblen-Coyle et al., (1992).

The periodicity in the locomotor activity is sometimes evident in the raw data or can be visualised by inspection of the 'actogram'. In an actogram, the data is double-plotted so that the events of day one and day 2 are plotted in real time on the first horizontal line. Below this line are plotted the events from day 2 and 3, and below this the data from days 3 and 4 and so on. A rough estimate of the period can be obtained by tracing a straight line to connect the times of activity 'onset' or 'offset'. However this is quite often misleading and more precise ways to detect rhythmicity use statistical techniques such as autocorrelation or spectral analysis (Dowse and Ringo, 1989). In this present work, the periodicity of locomotor activity, in DD conditions, for each fly was determined by using autocorrelation analysis (Diggle, 1990) available in the SPSS/PC+ Version 5.0 software package (SPSS Inc., 444 N. Michigan Avenue, Chicago, Illinois 60611, USA).

Briefly, the number of events obtained at two time points separated by a certain time lag are correlated. Thus with a lag of one time bin, bin 1 data is correlated with bin 2, bin 2 with bin 3, etc. If there is a 24 h cycle in the data, bin 1 and bin 49, bin 2 and bin 50, bin 3 and bin 51, etc, should produce the highest correlation coefficient, as the data in each time bin represents the total activity from a half-hour segment. The correlation coefficients are then plotted against each 'lag' (1,2,3... etc.) in a correlogram which extends to 116 lags (58 h). This represents an extremely conservative and robust estimation of rhythmicity (Chatfield, 1980). The 95% confidence limits ($2\sqrt{N}$; where N is the number of bins) were used to assess whether a given record was rhythmic. The highest peak in the correlogram within the range of 15-40h was taken as the period, so long as its peak was greater than the 95% confidence limit. The decision to consider a fly rhythmic or not was also made by considering the overall pattern of its correlogram, which in a rhythmic fly will be sinusoidal.

A spectral analysis on locomotor activity data was also carried out using the CLEAN algorith (Roberts *et al.*, 1987; Kyriacou and Hall, 1989). The spectrogram gives the frequencies which best describe the signal, and their power plotted is on the Y-axis (modulus). The highest relevant peak was taken as the period. However, if a large peak was found in the 12h domain this period was doubled to give the period, which nearly always agreed with the circadian period observed in the correlogram.

The data for each fly was randomised and the spectral analysis repeated. This was originally done 1000 times, and the modulus values for each run at each frequency were ordered from lowest to highest. The 950th and 990th values in ascending order represent the approximate 95 and 99% confidence limits, based on this Monte Carlo simulation of random data (see also song analysis). To be judged 'significant' a peak had to be greater than the 99% confidence limit. This procedure was very expensive on computer time, so the data were randomised 100 times only. The 95 and 99% confidence limits were a little more liberal than those produced by a 1000 random trials and so that is why the 99% confidence limit is used. A similar procedure to one employed for assessing the significance of song cycles, for both the CLEAN and VdB analyses (see chapter 3), was also used to assess the significance of the spectral analyses. The circadian periods of the flies were entered in a Statistica Microsoft or Minitab speadsheet for ensuing statistical

analysis. Only flies which were rhythmic for both their spectral and autocorrelation analyses were considered as rhythmic.

2.5.2 Standardisation of the data in DD conditions.

Males used in the locomotor activity experiments were raised at 25^0C entrained for 3 days in light/dark cycles (LD conditions), with lights on/off at 09:00/21:00. Data collection began at circadian time CT21, where the last lights-off was at CT12. The standardisation procedure that was followed, manipulated the data into the same number of bins for all flies. Briefly, after the data from the individual flies have been 'wrapped', using the autocorrelation-determined periods, 48 equidistant points are interpolated over the data. So, each bin no longer represents a fixed amount of time. For example, if a dataset has been wrapped over 49 bins (corresponding to a period of 24.5 h), the 49 points are plotted on a graph, and these points are joined by straight lines. 48 new values are then interpolated.

2.5.3 Collection of Lococomotor activity data in LD conditions.

Entrainment for flies used in the light/dark cycles (LD) experiments was the same as above. Virgin females were occasionally used. The experiments were run for five to seven days using the following procedure: Two days before data collection was to begin, flies one to three days old were loaded in the tubes and placed in an incubator which was set to the desired temperature (25^oC) and the same light/dark cycle in which the flies had been raised. On the following day the incubator was set automatically to darkness, beginning from 21:00 h and 9 hours later (at 06:00h), data collection began for a period of 5-7 days. The data were 'wrapped', that is, a mean activity for each of the 48, 30 min bins across the seven days was determined by superimposing the data for each day, giving a representation of an average days' mean activity for each fly. The mean activity was then calculated for each bin. Data recording commenced at 6am (ZT21), with lights-on at ZT0 and lights-off at ZT12.

A number of behavioural indices were calculated in chapter 8, which reflect various aspects of the locomotor activity profile. Specifically, 2h (4 bins, ZT0-

ZT2) after lights-on (Startle-On, STON) and 2h (4 bins, ZT10-ZT12) just before lights-off (Anticipation-Off, ANTOFF), and 2h (4 bins, ZT22-ZT24) just before lights-on (Anticipation-On, ANTON) and 2h (4 bins, ZT12-ZT14) just after lights-off (Startle-Off, STOFF) were divided by the total amount of day activity (DA), and total amount of night activity (NA) respectively (see Appendix 8.0). Moreover, the night activity (NA) was divided by the day activity (DA), and the resultant proportion was then transformed into arcsine (see Appendix 8.0). Furthermore, night and day activities were divided by the overall total activity (TA), thus converting these values into a proportion of the total activity and then transformed into arcsine. These latter transformed proportions are no longer absolutely independent since they represent a proportion of the total activity.

General characteristics of *D. melanogaster, D. simulans* and *D. yakuba* songs. Can song rhythms be detected?

CHAPTER 3

3. General characteristics of *D. melanogaster, D. simulans* and *D. yakuba* songs. Can song rhythms be detected?

The courtship songs of all the eight members in the *D. melanogaster* subgroup have been previously studied (Manning, 1959; Bennet-Clark and Ewing, 1968; Ewing, 1970; von Schilcher, 1976b; Cowling and Burnet, 1981; Cobb et al., 1988). The genetic control of particular song parameters was also investigated, by analysing interspecific hybrids (Cowling and Burnet, 1981). Kyriacou and Hall (1980; 1989), used a time-series analysis to describe the rhythmic variation of *Drosophila* courtship song interpulse intervals (IPIs); this analysis was carried out only for two members of the subgroup, namely, *D. melanogaster* and *D. simulans*. The existence of song rhythms sparked a contentious debate, some years after Kyriacou and Hall (1980) published their initial paper. Crossley (1988) and Ewing (1988) attacked these findings, because they claimed they could not replicate Kyriacou and Hall's results. Kyriacou and Hall (1989) and Kyriacou *et* al. (1990) re-examined both their own original data as well as those of Crossley's (1988) and Ewing's (1988), and managed to show that there were several sources of discrepancies between the investigations. More details about this debate can be read in chapter 1. The matter has finally been laid to rest by an independent American group, who, by using completely different behavioural and statistical procedures, did show, nevertheless, the existence of *per*-determined song rhythms and thus fully supported Kyriacou and Hall's work (Alt *et al.*, 1997).

Although the pulse component was observed in all the songs of all the members of the *D. melanogaster* subgroup (Cowling and Burnet, 1981), the pattern of rhythmic (IPI) variation over an extended period of time, has not been explored and evaluated outside *D. melanogaster* and *D. simulans*. Consequently, a key question that needs to be addressed is whether there is any rhythmic variation of IPI in the songs of the other member species of the *melanogaster* subgroup, and if so, are the periods species-specific, as in *D. melanogaster* and *D. simulans* ? Addressing this question would provide useful information on the general evolutionary and functional significance of such behavioural rhythms in *Drosophila*.

This chapter introduces the analysis of song cycles in the *melanogaster* subgroup. As mentioned in Chapter 1, Thackeray (1989) discovered that the *D. yakuba* song contains two different and audibly distinguishable types of pulses, with a mean IPI ranging from ~100-150ms, representing the longest IPI in the *melanogaster* subgroup (Cowling and Burnet, 1981). The problem that Thackeray (1989) encountered was that when the *yakuba* songs were divided up into adjacent real time 10s-bins (Kyriacou and Hall, 1980) they did not contain enough individual IPIs in any 10s segment of time, to generate reliable IPI means for extensive rhythm analysis.

Bearing the controversy in mind and the possible problems that might arise in trying to detect the existence of song rhythms in species of the *D. melanogaster* subgroup, I initially set out to determine the existence of song cycles in *D. melanogaster* (Brighton-strain) and *D. simulans* (Florida-strain), and in several *D. yakuba* strains (see section 3.5). The aim of the ensuing work was an attempt to reproduce the preliminary results of Thackeray (1989) using several strains of *D. yakuba*, and try to find any evidence for song cycles. As a comparison, I examined song cycles in *D. melanogaster* (Brighton) and *D. simulans* (Florida) strains, and re-analysed the same *per*⁺- and *per*-mutant songs originally examined by Kyriacou and Hall (1980, Kyriacou and Hall, 1989 and 1990), using a new method for detecting significance in individual song cycles.

3.1 Reanalysis of Kyriacou and Hall's original data

In order to validate the Monte Carlo simulation applied to both the CLEAN and VdB spectral algoriths (see Methods- Chapter 2), I reanalysed Kyriacou and Hall's (1980) data as well as a few songs that had not been published before. Kyriacou and colleagues (1989; 1990) reanalysed their 1980 data, which included songs from the three original *per* mutants (Konopka and Benzer, 1971). In brief, they examined several songs of each *per* genotype, whose duration ranged from 2-5mins long (depending on whether copulation had occurred or not). They then divided each song into 10s-time bins and determined the mean IPI of each 10s-bin (see Introduction-Figure 1.4). The periods were then determined by employing CLEAN and Van den Berg (VdB) spectral analyses and the original curvilinear regression used by Kyriacou and Hall (1980).

Using a Monte Carlo analysis, Kyriacou and Hall (1989) simulated 1000 songs composed of 30 random IPI's, representing the 30 mean IPI's (in 10s bins), for a song of 300s duration. They used realistic IPI means and standard deviations and calculated the "*signal-to-noise*" (SN- or F-) ratio for each random song by fitting the best sine wave through the data, after the VdB spectral analysis had initially provided the best frequency. In this way, the best fit through the data was assured. They then arranged these F-values in an ascending order and took the 51st highest value out of the 1000, to indicate the 5% significance level. Thus, the significance of a song rhythm depends on a Monte Carlo simulation of 1000 random songs. Nearly all of the per^+, per^s and per^{L1} had an F-value greater than the critical 5% significance level value, whereas nearly all the per^{01} songs had smaller F-ratios. The F-ratios required for significance in each range-per^+ (50-65s), per^s (35-45s) and per^{L1} (65-105s) (Kyriacou and Hall, 1989), were very similar to the critical F-values Kyriacou and Hall had used to assess significance in their original data (1980). Under more stringent criteria, where no *a priori* assumptions were made about the expected periods for each genotype, a higher critical F-value was required, but even under such conditions, the majority of the per^+, per^s and per^{L1} songs were still significantly rhythmic (Kyriacou and Hall,1989), whereas the F-ratios from per^{01} derived songs were, in their majority, non-significant. This confirmed the authors' opinion that the short-cycle rhythms seen in this *per*- (arrhythmic) mutation were weak at best (Kyriacou and Hall, 1980, 1989; Kyriacou *et al.*, 1990; Zehring *et al.*, 1984; Hamblen *et al.*, 1986).

My task was to re-analyse Kyriacou and Hall's original data (1980), but the difference, this time, was to assess the significance of each individual song rather than base significance on 1000 random simulations as above. In short, I did this by randomising each song's binned mean IPI values 100 times, thereby generating 100 random songs, and then employing both CLEAN and VdB spectral analyses on each random song. I then found the 95% and 99% confidence limits, by taking the 5th and the 2nd highest spectral values at each frequency, respectively. F-ratios are also obtained from the VdB analysis, from an automatic fitting of the best sine wave through the data, after fitting the best VdB-generated spectral period. These F-ratios are based on a simple 'horizontal sine wave' and cannot be fitted to a 'climber' (see chapter 2). I reanalysed only those of Kyriacou and Hall's (1980) songs, which met the 50% full bin criterion (see table 3.1.1). In addition, I ignored the

Nyquist frequency of approximately twice the bin size, i.e., ~20s (an artefact of spectral analysis), and long 'climbing' frequencies (see chapter 2).

Figures 3.1.1-3.1.4 illustrate examples of songs, from the different genotypes, originally analysed by Kyriacou and Hall (1980), analysed with both CLEAN and VdB and with Monte Carlo simulation applied. Figure 3.1.5 also shows a representative *D. simulans* song. *D. simulans* songs were not reanalysed spectrally by Kyriacou and colleagues (1989; 1990). In each figure the 95% and 99% confidence limits are shown. Each figure includes three graphs, Graph (A) represents a plot of the mean IPI against time, with the most significant cycle through the data being defined by the VdB spectral analysis, and Graphs (B) and (C) depict the CLEAN and VdB spectrograms, with their corresponding periods, for each particular song, respectively. The 95 and 99% confidence limits, defined by Monte Carlo simulations are also shown (see chapter 2). For the results of the individual songs reanalysed from the original Kyriacou and Hall' data (1980), see Appendix 3.1.

Table 3.1.1 and Figure 3.1.6 (below) shows the periods of those songs which gave significant rhythms (p<0.05, defined by Monte Carlo) with both VdB and CLEAN spectral analyses. This new method gives very similar results in terms of significance to Kyriacou and Hall (1989) and Kyriacou *et al.* (1990), but has the additional advantage that each song acts as its own control. Thus significance can be gauged for each individual song, representing a considerable improvement on the analysis of song rhythms, and avoiding the use of S/N- and F-ratios.

Table 3.1.1: The song periods for different genotypes calculated with CLEAN and VdB spectral Analyses for data reanalysed by Kyriacou and Hall (1980,1989).

Genotype	Number of songs analysed	Clean (s) ± sem	Significance (Monte Carlo/ Clean)			VdB (s) ± sem	Significance (Monte Carlo/ VdB)		
			n.s	5%	1%		n.s	5%	1%
simulans	7	38.09 ± 4.17	-	1	6	39.28 ± 4.70	1	-	6
Oregon	12	57.51 ± 2.73	-	3	9	55.74 ± 1.51	-	1	11
Canton-S	18	57.13 ± 1.63	-	3	15	56.47 ± 1.16	-	-	18
per[s]	11	42.73 ± 1.23	3	2	6	41.80 ± 1.19	-	-	11
per[l]	11	83.42 ± 3.43	-	2	9	83.82 ± 3.92	-	1	10
per[01]	10	35.23 ± 9.96	4	5	1	43.64 ± 7.55	1	-	9

(A) period=53.8s, F=3.057, df=3,14

(B) 53.30s   CLEAN

Frequency
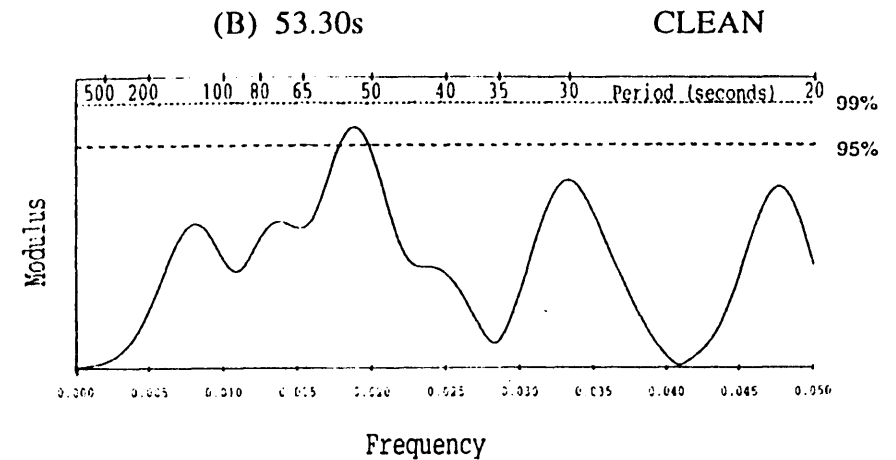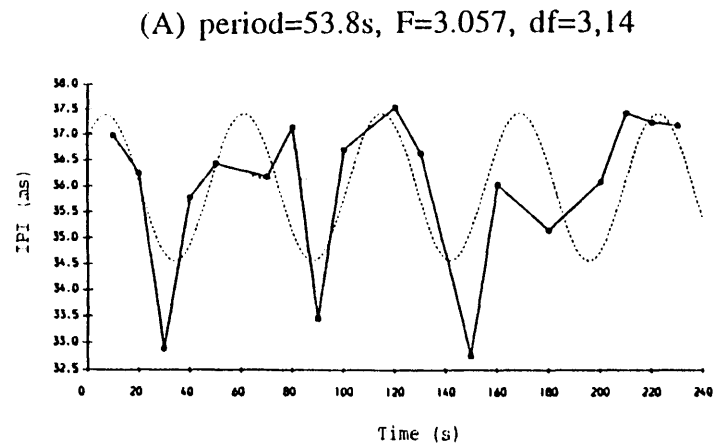
Figure 3.1.1:

D. melanogaster (Canton-S) courtship song (csy4) reanalysed-see Appendix
3.1-(Kyriacou and Hall, 1980), using the 65 & 15 ms cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the data, initially defined by VdB spectral analysis, and
   then fitted to the data by non-linear regression, using a least squares
   procedure. Note even though a robust period is observed, the F-ratio is
   non-signicant.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods song. The significant periods are
   shown. Note that the CLEAN analysis shows a 53.30s primary period
   (p<0.05). Furthermore, note that the VdB analysis shows a 54.42s primary
   period (p<0.01), as well as a weaker secondary 30.19s period (p<0.05).
   The 95 & 99% confidence limits are defined by Monte Carlo simulations
   (see Chapter 2).

(C) 54.42s. 30.19s   VdB

Frequency

(A) period= 20.9s, F=5.061, df= 3, 20

(B) 20.83s   CLEAN

(C) 21.00   VdB
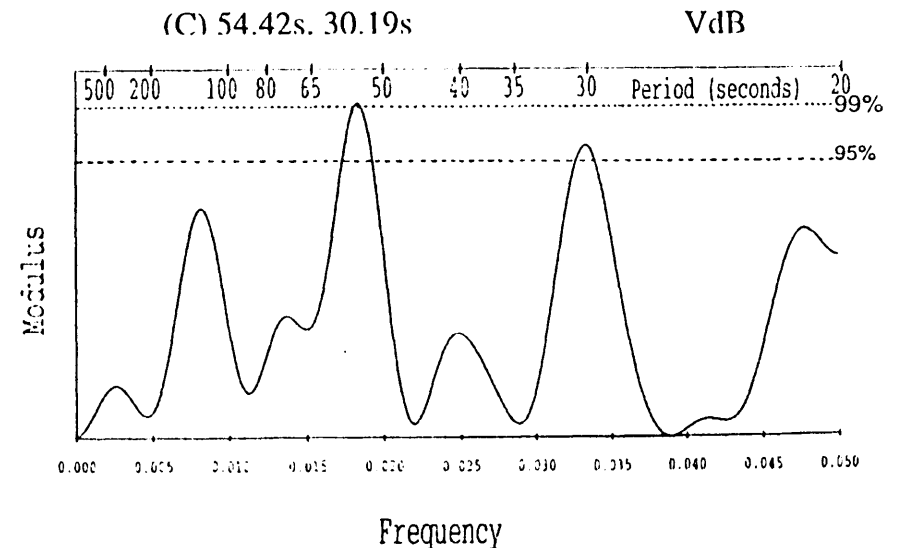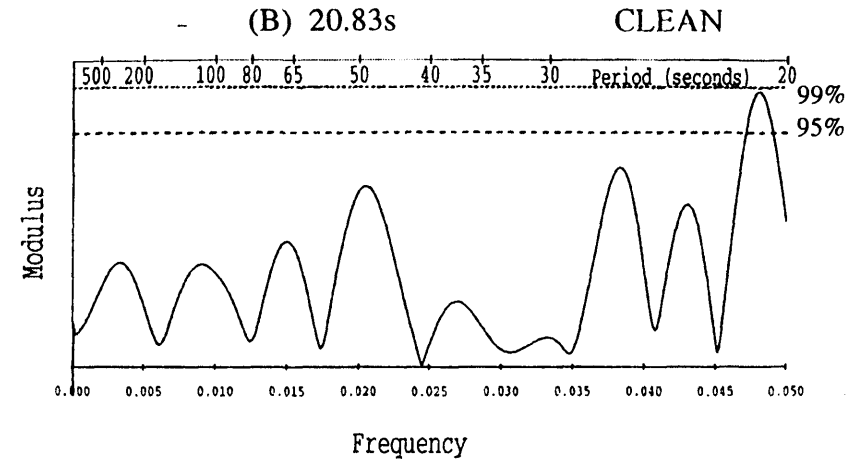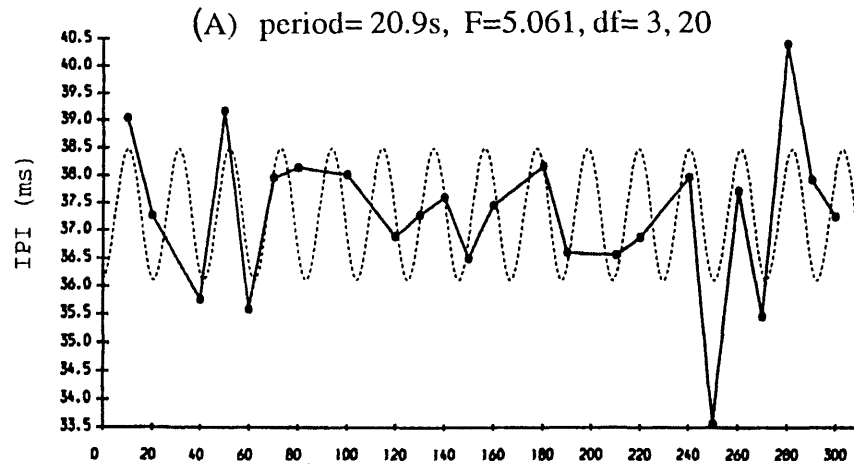
Figure 3.1.2:

D. melanogaster (*per*[01]) courtship song (arrb3) reanalysed-see Appendix
3.1-(Kyriacou and Hall, 1980), using the 65 & 15 ms cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the data, initially defined by VdB spectral analysis, and
   then fitted to the data by non-linear regression, using a least squares
   procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods song. The 95 & 99% confidence
   limits are defined by Monte Carlo simulations (see Chapter 2). Note the
   significant period near the Nyquist frequency.

(A) period= 43.5s, F=7.722, df= 3,24

(B) 43.91   CLEAN

(C) 43.96s   VdB
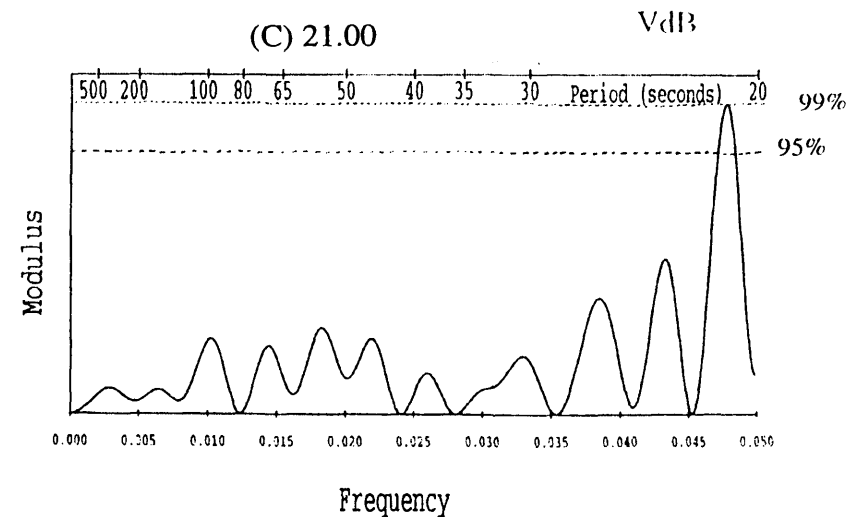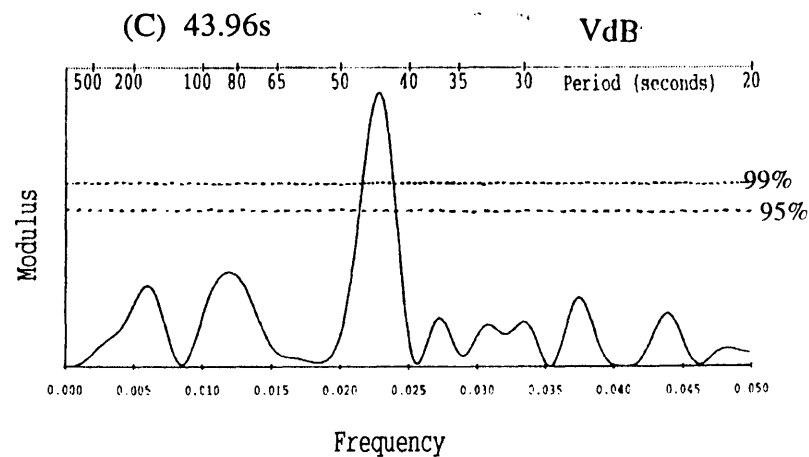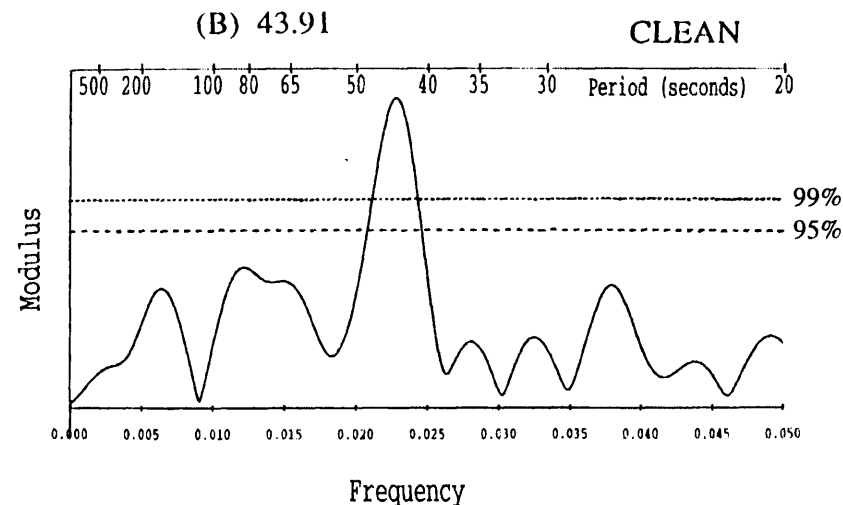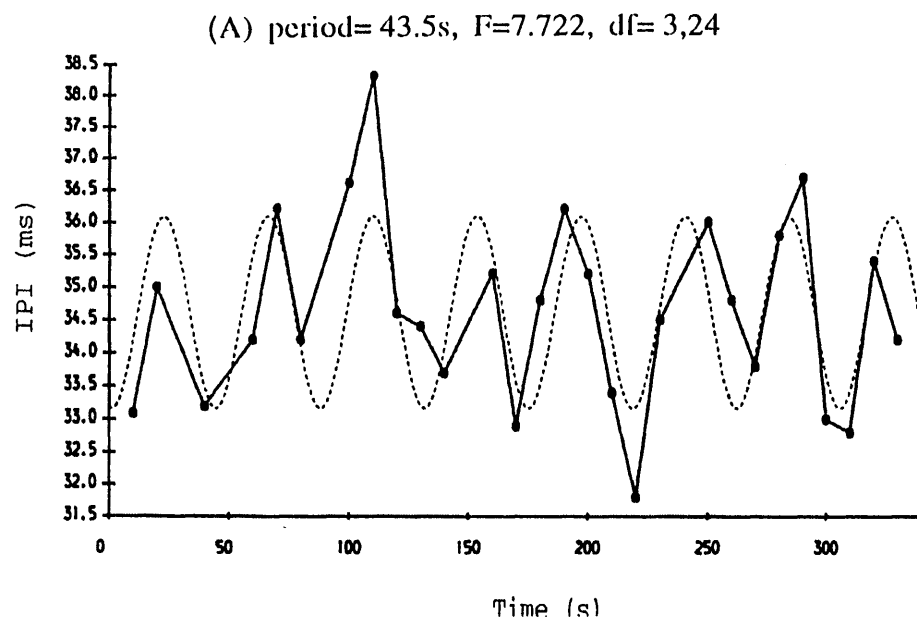
Figure 3.1.3:

D. melanogaster (*per*S) courtship song (shortb3) reanalysed-see Appendix 3.1-(Kyriacou and Hall, 1980), using the 65 & 15 ms cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods song. The 95 & 99% confidence limits are defined by Monte Carlo simulations (see Chapter 2).

(A) period= 93.1s, F=16.080, df=3, 28

(B) 93.75s   CLEAN

(C) 94,12   VdB
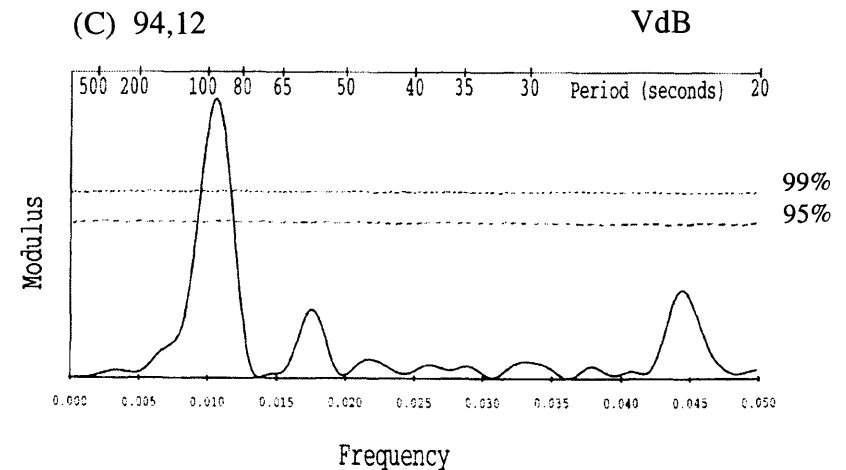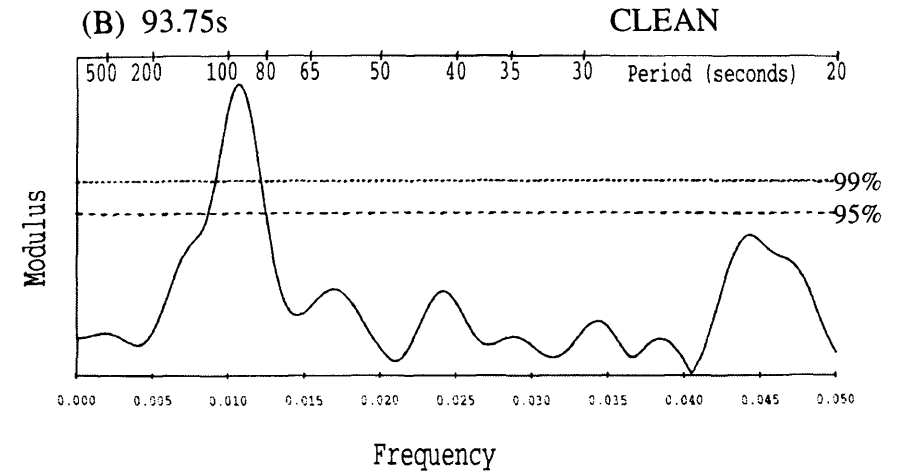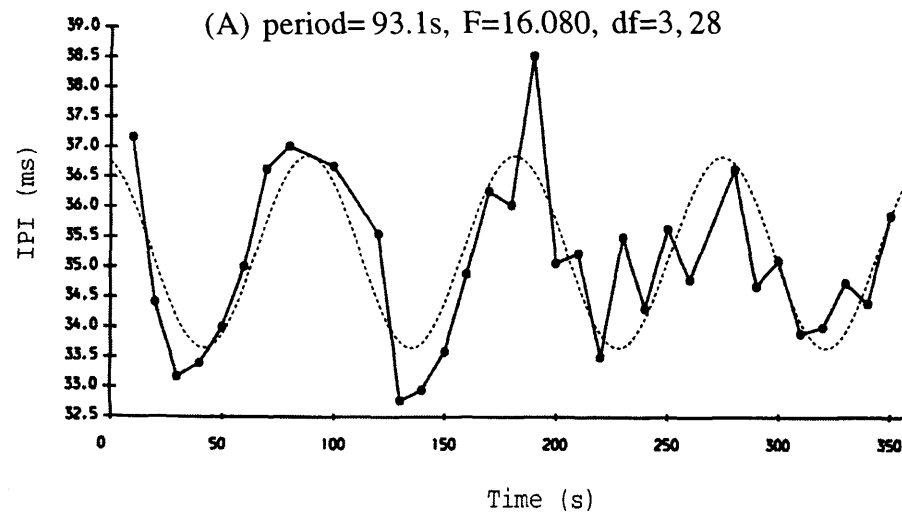
Figure 3.1.4:

D. melanogaster (*per^L*) courtship song (long9) reanalysed-see Appendix
3.1-(Kyriacou and Hall, 1980), using the 65 & 15 ms cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the
 strongest cycle in the data, initially defined by VdB spectral analysis, and
 then fitted to the data by non-linear regression, using a least squares
 procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
 each indicating the corresponding periods song. The 95 & 99% confidence
 limits are defined by Monte Carlo simulations (see Chapter 2).

(A) period= 33.8s, F=13.916, df= 3,22

Time (s)
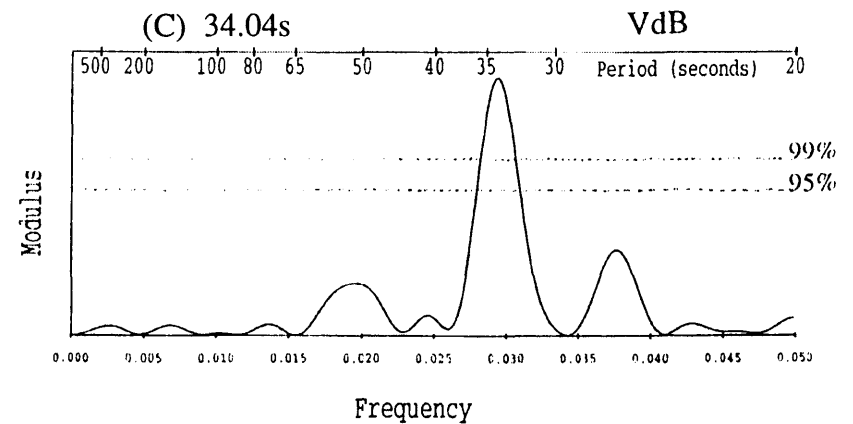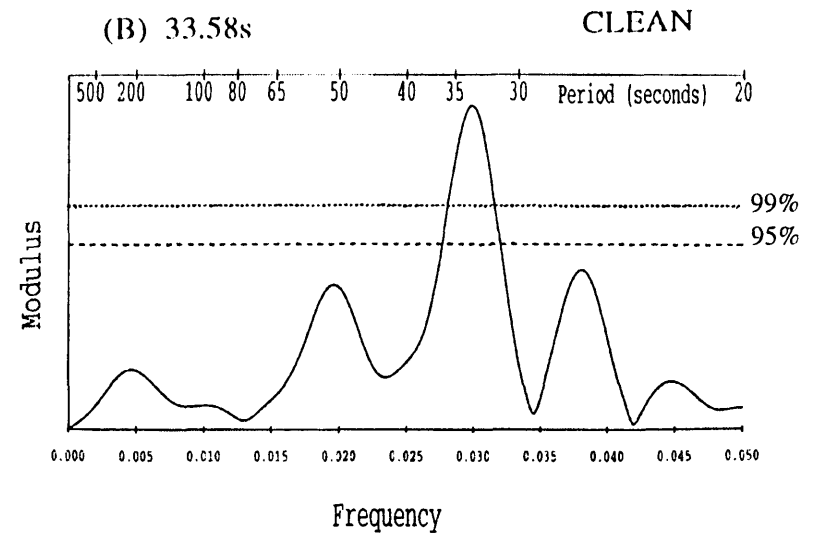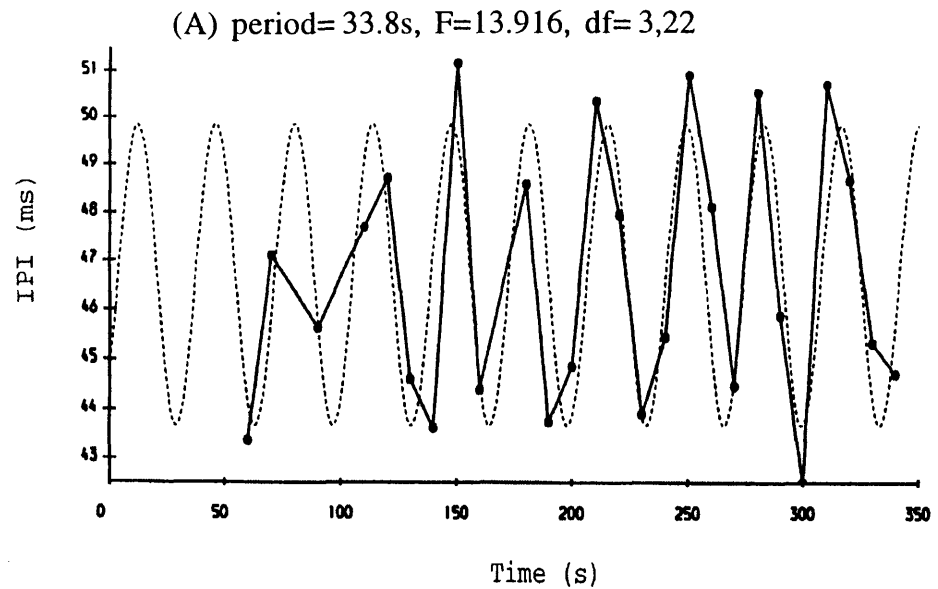
Figure 3.1.5:

D. simulans courtship song (sim8) reanalysed-see Appendix
3.1-(Kyriacou and Hall, 1980), using the 65 & 15 ms cut-off points.
a) A plot of the mean IPI against time. The dotted line represents the
strongest cycle in the data, initially defined by VdB spectral analysis, and
then fitted to the data by non-linear regression, using a least squares
procedure.
b) and c) show the CLEAN and VdB spectrograms of this song respectively,
each indicating the corresponding periods song. The 95 & 99% confidence
limits are defined by Monte Carlo simulations (see Chapter 2).

(B) 33.58s   CLEAN

Frequency

(C) 34.04s   VdB

Frequency

Out of 12 per^{01} songs, 2 did not satisfy the 50% bins filled criterion, 4 were non-significant, 5 were significant at the 5% and 1 was significant at the 1% significance levels, respectively, using CLEAN. The period, in table 3.1.1, represents the mean of the 6 significant songs. The VdB analysis gave 9 significant per^{01} songs, by my analysis, but their periods, like those from CLEAN, vary from 20-84s, giving them large sem's; the great majority of the periods of these songs lie between 20-30s (see Figure 3.1.6), which is in agreement with the original Kyriacou and Hall observations (1980,1989) and more recently those of Alt *et al.* (1997). The VdB analysis gives similar results to CLEAN for the other genotypes.

Figure 3.1.6: The distribution of the periods of songs (CLEAN) from Kyriacou and Hall's (1980) original data, reanalysed.
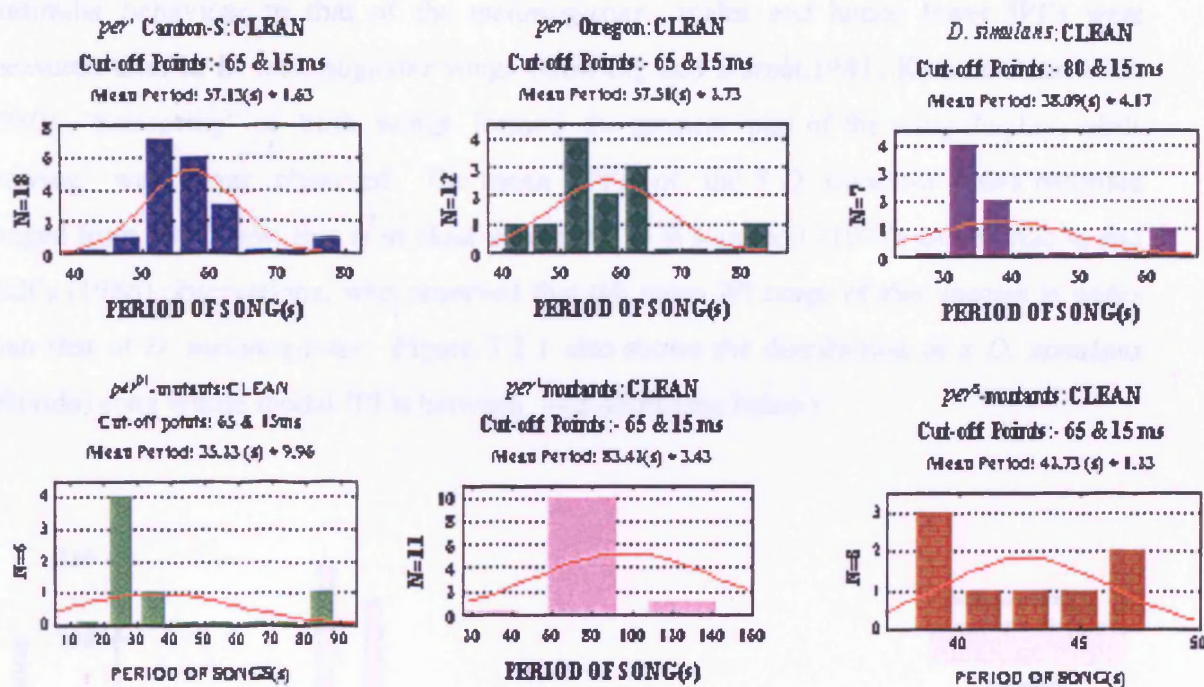
N= Number of Observations, Red Line= Expected Normal Distribution

3.2 *D. melanogaster* (Brighton) and *D. simulans* (Florida) song:

The courtship songs of 5 *D. melanogaster* (Brighton) and 5 *D. simulans* (Florida) males were recorded (as described in chapter 2) and are considered in this section below.

The wing display of the *D. melanogaster* males was quite restricted in that 'rowing' was never observed during recording, while 'scissoring' was infrequently seen (Cowling and Burnet, 1981). The majority of the wing display consisted of extending one wing at an angle of about 90^0 and vibrating it (Cowling and Burnet, 1981). The IPIs appear to be normally distributed in all the Brighton songs analysed with a mean IPI ranging from ~32-38ms. Figure 3.2.1 (see below), shows the distribution of IPIs in one of the *D. melanogaster* Brighton song, whose modal IPI lies between 27-30ms.

The *D. simulans* (Florida) males produced courtship songs where, at the beginning of a burst, the pulses had long IPI's, which progressively become shorter and more constant in value, which is in agreement with Cowling and Burnet's observations (1981). The males did not readily court their conspecific females, which is a quite dissimilar behaviour to that of the *melanogaster* males and hence fewer IPI's were measured than in *D. melanogaster* songs (Cowling and Burnet,1981; Kyriacou and Hall, 1980). 'Scissoring' of both wings formed the greatest part of the wing display, while 'rowing' was never observed. The mean IPI's of the 5 *D. simulans* males recorded ranged from ~39-57ms; this is in close agreement to Watanabe's (1977) and Kyriacou and Hall's (1986) observations, who observed that the mean IPI range of this species is wider than that of *D. melanogaster*. Figure 3.2.1 also shows the distribution of a *D. simulans* (Florida) song whose modal IPI is between ~42-45ms (see below):
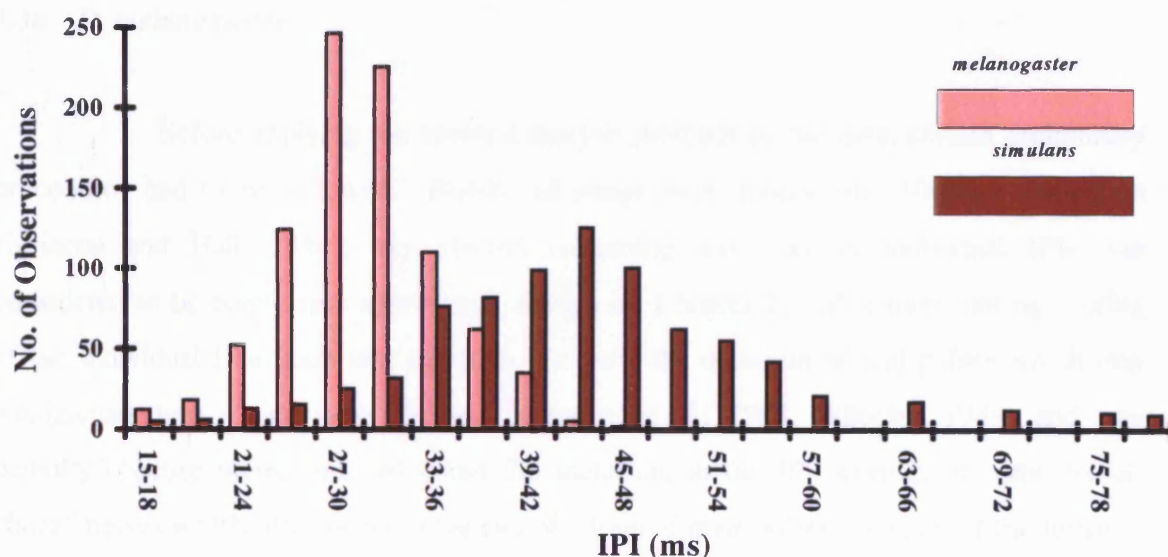
Figure 3.2.1: Distribution of IPIs of a *melanogaster* (Brighton) and *simulans* (Florida) song.

43

The mean and modal IPI of each song, were scored, summed and averaged in order to determine the overall species values. Furthermore, the mean Intrapulse Frequency (IPF) and Sine Song Frequency (SSF) were determined, as described in the appropriate section of Chapter 2, and the overall species (strain) mean IPF and mean SSF are shown in table 3.2.1, which for comparison includes the results from Cowling and Burnet's (1981) study.

Table 3.2.1: Song Characters for *D. melanogaster* and *D. simulans*.

SPECIES/ STRAIN	Number of songs recorded	IPI(ms) MEAN ± sem	IPI (ms) MODE	SSF(Hz) MEAN ± sem	IPF(Hz) MEAN ± sem
D. melanogaster(Brighton)	5	**35.60±0.71** *35.80±0.40*	**33.00** *30.00*	**162.75±4.35** *169.90±2.10*	**286.70±2.53** *282.20±6.20*
D. simulans(Florida)	5	**53.34±5.74** *55.20±1.70*	**36.17** *47.50*	**205.75±6.21** *196.80±2.30*	**408.01 ±3.55** *483.00±7.20*

KEY : Bold: Results from Present Study   Italics : Results from Colwing & Burnet(1981)

As it can be observed from the above table 3.2.1, the results of this present study and those of Cowling and Burnet (1981) are quite similar; any differences may stem from the fact that different strains were used in the two studies and/or from the different ways of scoring the values of the different parameters.

3.3 Do IPI rhythms exist in *D. melanogaster* (Brighton) and *D. simulans* (Florida) songs?

3.3a *D. melanogaster*

Before applying the spectral analysis methods to the data, certain preliminary procedures had to be followed. Briefly, all songs were divided into 10s bins (based on Kyriacou and Hall, 1980); any 10s-bin containing less than 10 individual IPIs was considered to be empty (see appropriate section in Chapter 2). Moreover, during scoring of the individual IPIs, care was taken to (a) avoid the omission of real pulses which may amalgamate into a sine song 'phrase' (Wheeler *et al.*, 1988; Wheeler, 1989) and may partially obscure them, and, (b) avoid the inclusion, in the IPI scoring, of some female "buzz" noises which often occur at the end of a train of male pulses, as a part of the female's "rejection" repertoire (Connolly and Cook,1973). With a little practise, by simultaneous

acoustic and visual monitoring, one becomes familiar with the sounds that should be excluded from the analysis (see Chapter 2). A song was only considered for rhythm analysis if 50% or more of its 10s time bins were 'filled'. The IPI means were analysed with the CLEAN and VdB spectral algorith (see Chapter 2).

Table 3.3.1 (Top half) shows the distribution of IPI periods using CLEAN and VdB analyses of *D. melanogaster* Brighton songs, using 65ms (approximately twice the value of mean IPI, Kyriacou and Hall, 1980; 1986), as the upper IPI cut-off point and 15ms, as the lower cut-off point. When the upper and lower cut-off points were changed to 80 and 15ms, respectively, a completely different picture of the distribution of the song periods emerged (Table 3.3.1-bottom half).

Table 3.3.1: Spectral analysis of *D. melanogaster* Brighton individual songs using lower and higher cut-off points.

SPECIES	Clean		Van den Berg		
	1ST PEAK	2ND PEAK	1ST PEAK	2ND PEAK	BINS FILLED
melanogaster	CUT-Off POINTS : 65 & 15 ms				
Mb1	55.56	x	21.62	54.79	17/17
Mb2	43.72	x	30.53	42.78	18/18
Mb3	65.69	x	65.57	24.17	20/20
Mb4	54.60	x	57.55	x	21/21
Mb5	52.29	109.59	51.95	112.68	18/18
CUT-Off POINTS : 80 & 15 ms					
Mb1	x	x	222.22	x	17/17
Mb2	40.61	x	40.40	21.86	18/18
Mb3	x	x	29.96	x	20/20
Mb4	x	x	119.40	x	21/21
Mb5	89.89	30.08	94.12	x	18/18

The most striking difference in Table 3.3.1 (Lower half) is that when using 80 and 15ms cut-off points, the rhythmicity of several songs is completely 'masked', e.g., Mb1, Mb3 and Mb4. The period values in table 3.3.1-above represent only those significant at least at the 95% confidence level. Only the primary (the most significant period-99%) and the secondary (the next most significant period-95%) peaks are shown, respectively (see for example Figure 3.1.1c). An 'X' in the table means that there are neither primary nor secondary significant peaks (>95%). It can be clearly seen that usually the CLEAN and the corresponding VdB values for each individual song, are similar. Any period of 30s or less is assumed to represent arrhythmicity, unless a second significant peak is found in the spectrogram above the 95% confidence limit. This is because arrhythmic *per*-songs have

cycles ranging predominantly from ~20-30s cycles (Kyriacou and Hall, 1989; Alt *et al.*, 1997). For example, in Mb1 and Mb2 songs, the second significant peak value, in the VdB analysis, was taken to be the period because this value is similar to its corresponding primary one in the CLEAN analysis. The shaded-in values on the table are those have been used to determine the rhythm periods of each species/strain (see Table 3.3.1-above).

Figure 3.3.1a shows the appropriate results from a particular *D. melanogaster* Brighton song (mb4), when the 'higher' cut-off points (80 & 15ms) are used. As it can be seen from the Figure 3.3.1a, the rhythm is 'masked'. In the VdB spectrogam (Figure 3.3.1a), there is a significant primary peak of 119.40s (p<0.05) and a weaker secondary peak at ~50s which does reach the 95% confidence level. These two peaks are seen to have corresponding counterparts in the CLEAN spectrogram (Figure 3.3.1a), which do not reach the 95% confidence level and consequently no peaks are registered for the CLEAN analysis. If these graphs are compared with those in Figure 3.3.2b, where the 'lower' cut-off points (65 & 15ms) are utilised from the same song, the period is revealed to be ~54.60s and ~57.6 s from CLEAN and VdB analyses, respectively. The best fitting sine wave is generated by the VdB algorith and which is shown to the left-hand part of each figure. The iterative procedure 'smooths' the observed 57.6s period to 57.4s by a least squares procedure (see Chapter 2).

3.3b *D. simulans*

Table 3.3.2 (see below) shows the results of a similar analysis for song periods using *D. simulans* Florida songs, using the IPI cut-offs of 80ms and 15ms and 100 and 15ms (see Table 3.3.2-upper and lower halves, respectively), for CLEAN and VdB analyses. Even though 5 songs were originally recorded, only 4 were analysed further, as one of these songs did not satisfy the ≥50% bins filled criterion. Note that the Si1 song (see table 3.3.2-Top half) has changed from being rhythmic when using 80 and 15ms as cut-off points, to becoming arrhythmic when using the higher 100 and 15ms cut-offs. Since the 'long-term' trends or 'climbers' are ignored (see chapter 2), the primary peak, in the CLEAN and VdB analyses, are disregarded; therefore the secondary peak, in the CLEAN analysis, which is

(A) period=120.8s F= 2.585, df= 3,17

Time (s)

(B)   **CLEAN**

Frequency
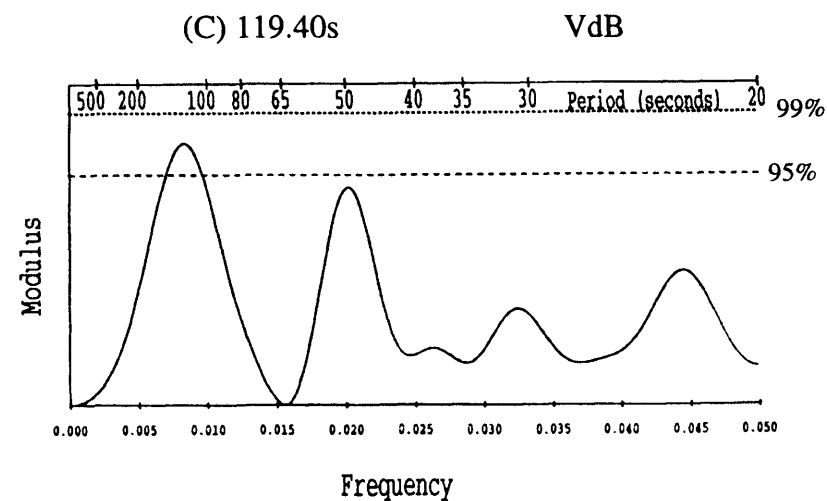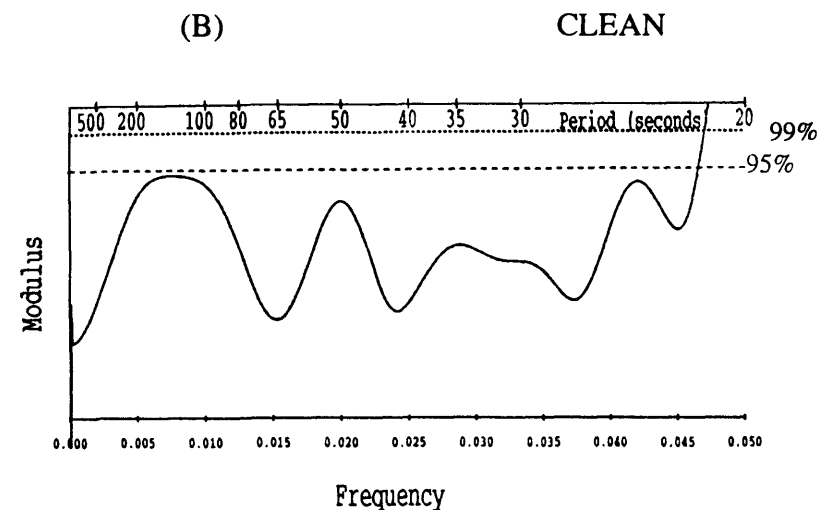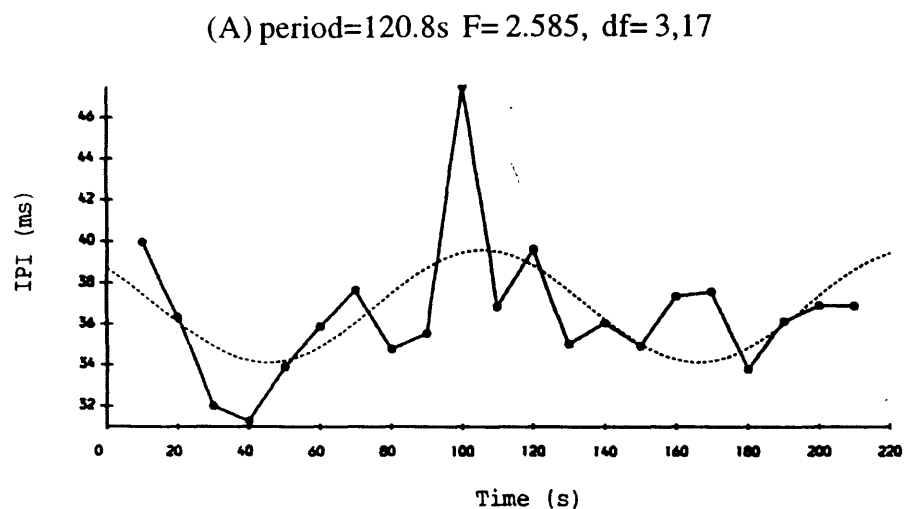
Figure 3.3.1a:

D. melanogaster courtship song (mb4)-see table 3.3.1a-lower half
and spectral analysis, using thehigher 80 & 15 ms cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the
    strongest cycle in the data, initially defined by VdB spectral analysis,
    and then fitted to the data by non-linear regression, using a least squares
    procedure.

b) and c) show the CLEAN and VdB spectrograms of this song
    respectively, each indicating the corresponding periods. The 95 & 99%
    confidence limits are defined by Monte Carlo simulations (see chapter 2).

(C) 119.40s   VdB

Frequency

(A) period=57.4 F= 3.830,   df= 3,17

(B) 54.60s   CLEAN

(C) 57.55s   VdB
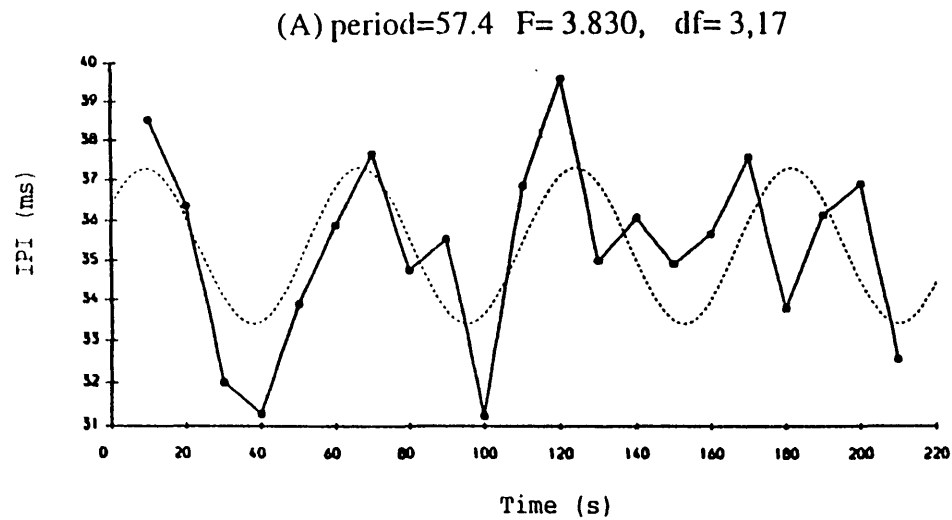
Figure 3.3.1b: The same *D. melanogaster* courtship song (mb4) table 3.3.1a
-top half, using the lower 65 & 15 m cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the strongest
   cycle in the data, initially defined by VdB spectral analysis, and then fitted
   to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods song. The 95 & 99% confidence
   limits are defined by Monte Carlo simulations (see Chapter 2).
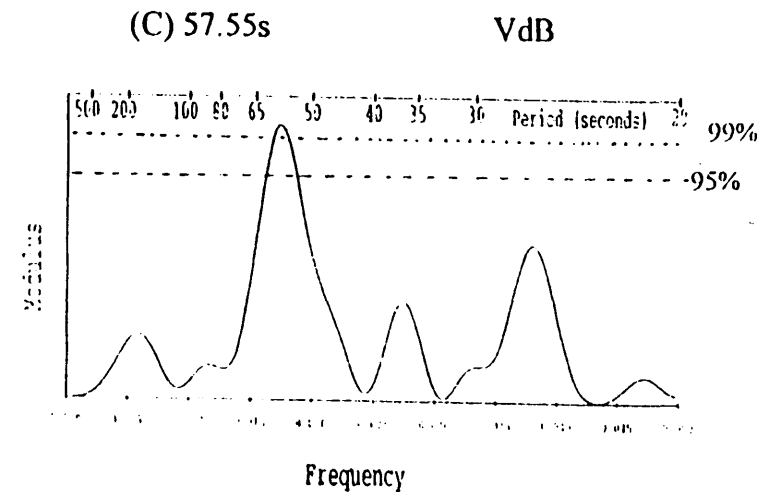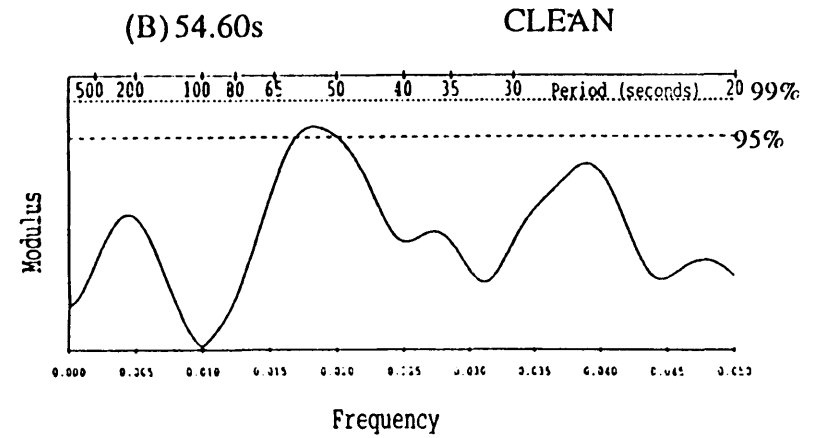
significant, is taken to the period of the song, even though there is no significant secondary peak in the VdB analysis.

Table 3.3.2: Spectral analysis of *D. simulans* Florida individual songs using lower and higher cut-off points.

SPECTRAL ANALYSIS					
SPECIES	Clean		Van den Berg		
	1ST PEAK	2ND PEAK	1ST PEAK	2ND PEAK	BINS FILLED
simulans	CUT-Off POINTS : 80 & 15 ms				
Si1	339.6	59.21	347.8	x	32/39
Si2	42.55	x	42.11	x	12/22
Si3	34.62	48.42	34.63	x	18/36
Si5	41.77	54.01	x	x	20/37
CUT-Off POINTS : 100 & 15 ms					
Si1	x	x	666.67	20.00	32/39
Si2	42.55	x	42.11	x	12/22
Si3	34.55	x	34.63	571.43	18/36
Si5	41.77	54.01	390.5	x	20/37

Figure 3.3.2a shows the spectral analyses graphs of a particular *D. simulans* Florida song (Si1), when the 'higher' cut-off points (100 & 15ms) are used. The raw data shows an almost significant periodicity of 35s (peak slightly below the 95% confidence limit-Figure 3.3.2a-Graph B). The VdB spectrogram shows the 20s Nyquist frequency plus the 'climbing' frequency of ~667s. The VdB-derived regression period gives a nyquist frequency of 20s. When the 'lower' cut-off points (80 & 15ms) are used, the song period is revealed to be ~59.21s for CLEAN analysis, but VdB analysis gives the climbing period of ~348s, significant at the 99% confidence limit level; in addition, there is a second peak in the region of ~60s period that just misses the 95% confidence limit level. Therefore, this song (Si1) was considered to be rhythmic. Even though this particular *D. simulans* Florida song does not have the representative period for the *D. simulans* species (~40s-Kyriacou and Hall, 1980, 1989), it was chosen because it was the *D. simulans* song containing the most IPI data, and furthermore, it was the only song to show most graphically the effects on the song period, of using the 'different' cut-off points.

In general the different cut-offs of 80 & 100ms do not make as much of a difference in these *D. simulans* songs, as in the *D.melanogaster* songs. Table 3.3.3 shows a synopsis of the song periods, based on the results shown in Table 3.3.1 and 3.3.2.

(A) period= 20.0s   F=2.178,   df=3,28

(B)   **CLEAN**

Frequency
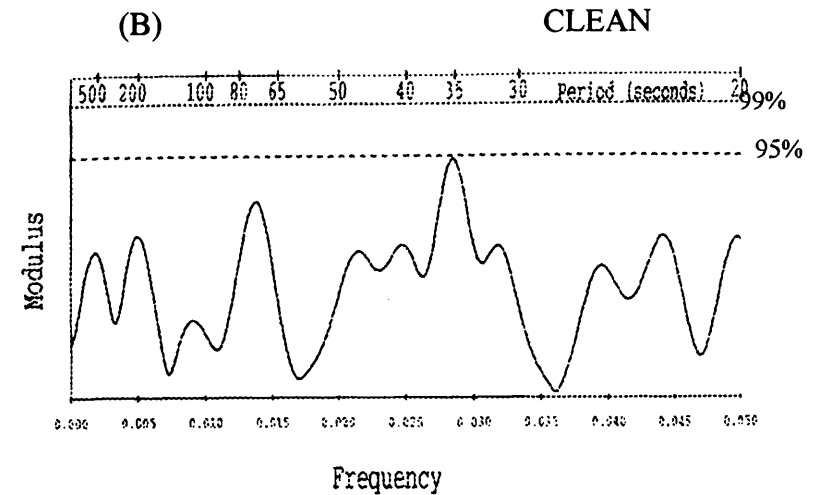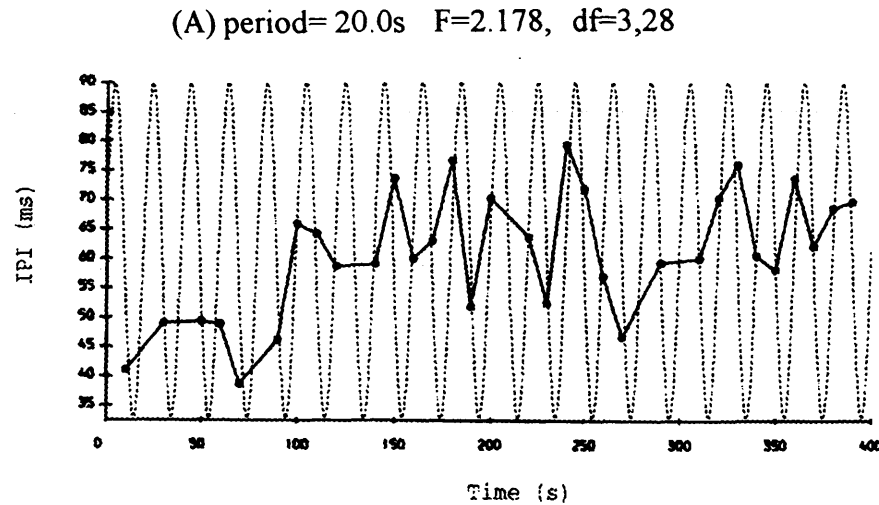
Figure 3.3.2a:

D. simulans courtship song (Si1) analysed-see table 3.3.1b-lower half,
using the higher 100 & 15 ms as the higher cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the strongest
   cycle in the data, initially defined by VdB spectral analysis, and then fitted
   to the data by non-linear regression,using a least squares procedure. However,
   one can observe a 35-40s periodicity between 100-260s.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods. Note that the highest primary peak
   is at 500+s (p<0.01) with VdB, with a secondary peak at 20s (p<0.01)- the
   'Nyquist' frequency. This is an artefact of spectral analysis, where occassionally
   the period given is exactly 2 x bin size. The regression uses the VdB value in
   the curve fitting. The 95 & 99% confidence limits are defined by Monte
   Carlo simulations (see Chapter 2).

(C) 666.67s   **VdB**

Frequency

(A) period=316.0s f=4.005,3,27

(B) 339.62s, 59.21s   CLEAN

(C) 347.83s   VdB
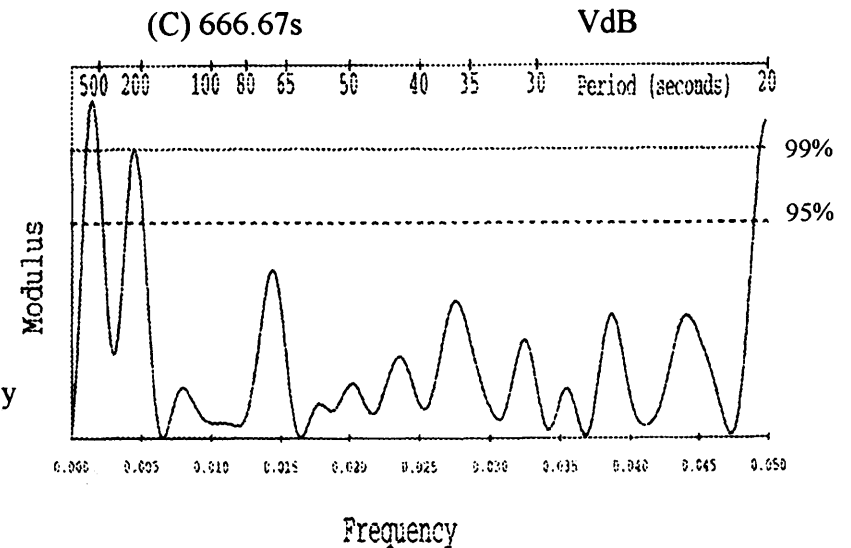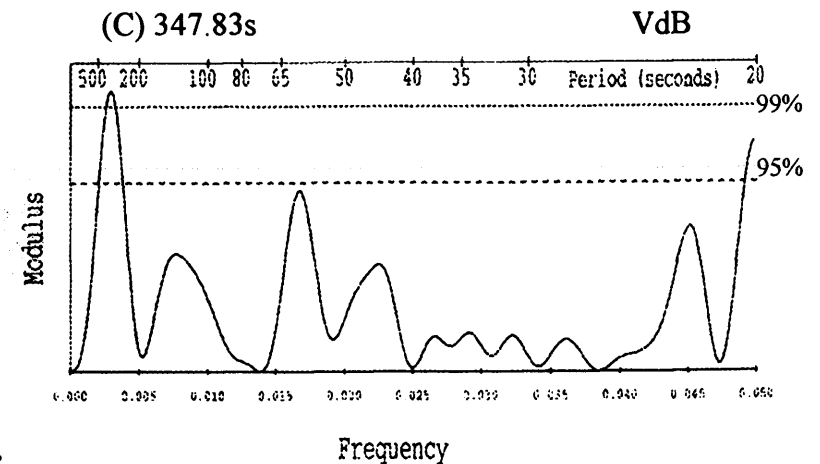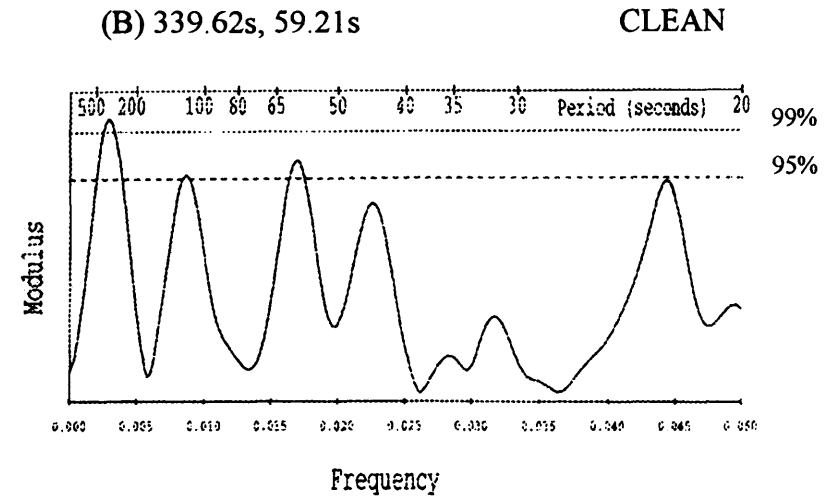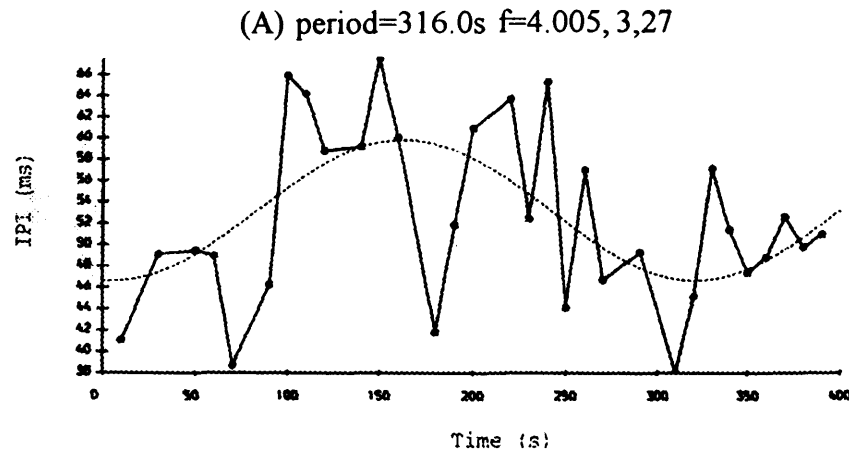
Figure 3.3.2b:
The same *D. simulans* courtship song (Si1) analysed-see table 3.3.1b
-upper half, using the lower 80 & 15 ms cut-off points.
a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the data, initially defined by VdB spectral analysis,
   and then fitted to the data by non-linear regression, using a least squares
   procedue.
b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods. Note the 300+ period (p<0.01)
   in both spectrograms is indicating 'climbing' IPI's; the CLEAN analysis also
   shows a significant secondary peak at ~59s (p<0.05).The regression uses the
   highest peak in the VdB spectrogram to initiate the curve fitting. Consequently,
   a period of 316s is observed. The 95 & 99% confidence limits are defined
   by Monte Carlo simulations (see Chapter 2).

Table 3.3.3: Synopsis of song rhythm data.

Species/Strain	Cut-off points (ms)	Number of songs analysed	Number of rhythmic songs	Period (s) ± sem
D. melanogaster	65-15	5	5	54.37±3.52
	80-15	5	2	65.25±24.64
D. simulans	80-15	4	4	47.99±4.02
	100-15	4	3	39.62±2.55

The results in Table 3.3.3 confirm those of Kyriacou and Hall (1980,1985, 1986 and 1989) and those of Wheeler *et al.* (1991). Furthermore, these results reinforce the existence of song rhythms and the precautions that have to be taken in order to detect them, particularly in the use of the appropriate cut-off points in *D. melanogaster* (Kyriacou and Hall, 1989; Kyriacou *et al.*, 1990).

Since the existence of song cycles was re-affirmed (see also Alt *et al.*, 1997), the next logical step was to examine *D. yakuba* songs recorded from several different strains, in an attempt to replicate Thackeray's (1989) preliminary findings.

3.4 *D. yakuba* song:

The *D. yakuba* courtship song has been described as a relatively simple song, consisting of trains of pulses only and completely missing the "hum" component of the song (Cowling and Burnet, 1981; Thackeray, 1989). Courtship songs of various strains of *D. yakuba*- 6 different strains (France, Edinburgh, Malawi, Lamto3, S.T. and Ivory Coast) and 4 different intraspecific hybrid crosses (Malawi(f) x S.T.(m), S.T.(f) x Lamto3(m), Lamto3(f) x S.T.(m) and Lamto3(f) x Ivory Coast(m)) were recorded, for a minimum of 300s to a maximum of 1200s, depending on the vigor of each particular song. New females were only introduced in the recordings, when copulation had occurred. The method used to discriminate between the two pulse types, during a *D. yakuba* courtship song, is described in detail in the corresponding section of Chapter 2. Briefly, three datasets were created, one

for Thud plus (+) Clack, one for Thud and another for the Clack pulse types. IPI's were measured, after the different types of pulses had been marked on dot matrix paper, while simultaneously listening and examining their waveform using the oscilloscope.

In these present recordings, the songs produced, from both parental strains and intraspecific hybrid crosses, have individual mean IPIs between ~85-150ms, making them the longest in the subgroup. Cowling and Burnet (1981), found the *D. yakuba* species mean IPI to be ~100ms, while Thackeray (1989) calculated individual song mean IPIs ranging from ~90-150ms. However, he discovered that the *D. yakuba* song consisted of two distinct types of pulses, Clack and Thud (see Figure 3.4.1, a & b), which he named after the auditory impression they produced. This impression is supported by the waveform produced by each type of song (see Figure 3.4.1 a & b). Figure 3.4.1 shows the different pulse types produced during a *yakuba* courtship song. Figure 3.4.1a shows a train of Clack pulses, noting that the minimum distance between consecutive Clack pulses, within a burst, is ~50ms. Figure 3.4.1b shows a train of Thud pulses, where the minimum distance between consecutive Thud pulses, within a burst, is ~50ms. Furthermore, the Clack waveform is different from the Thud, as a Clack pulse has 1.5-2.5 cycles per pulse (CPP), whereas the CPP for Thud pulse range from 1-1.5 cycles (Figure 3.4.1b and see also Chapter 6). An interesting point worth mentioning is that the Thud pulse-type of *D. yakuba*, and *D. melanogaster* normal pulse waveforms, are very similar, but have different mean IPFs (see Table 3.4.1-see below and Table 3.2.1, respectively).

In the *D. yakuba* strains recorded, I observed anecdotally that the clack pulse-type predominates while the male is trying to orientate himself in relation to the female. Thud pulse-type is primarily audible, when the male has placed himself in the correct position to start courting, by rowing one wing, which verifies Thackeray's (1989) original data and observations. Figure 3.4.2 (below) shows the distribution of Thud + Clack IPIs to be unimodal, similar in shape to the *D. melanogaster* IPI distribution, but much broader with a modal IPI ranging between 95-100ms. The distribution of Thud IPIs is unimodal, but broader in shape than the Thud + Clack IPIs distribution, with a modal IPI ranging from 95-100ms. The distribution of Clack IPIs looks to be unimodal, but even broader in shape than the former two distributions, with a modal IPI ranging between ~ 90-95ms, in this particular song:

CLACK PULSE TYPE

(a)

~120 ms

THUD PULSE TYPE

(b)
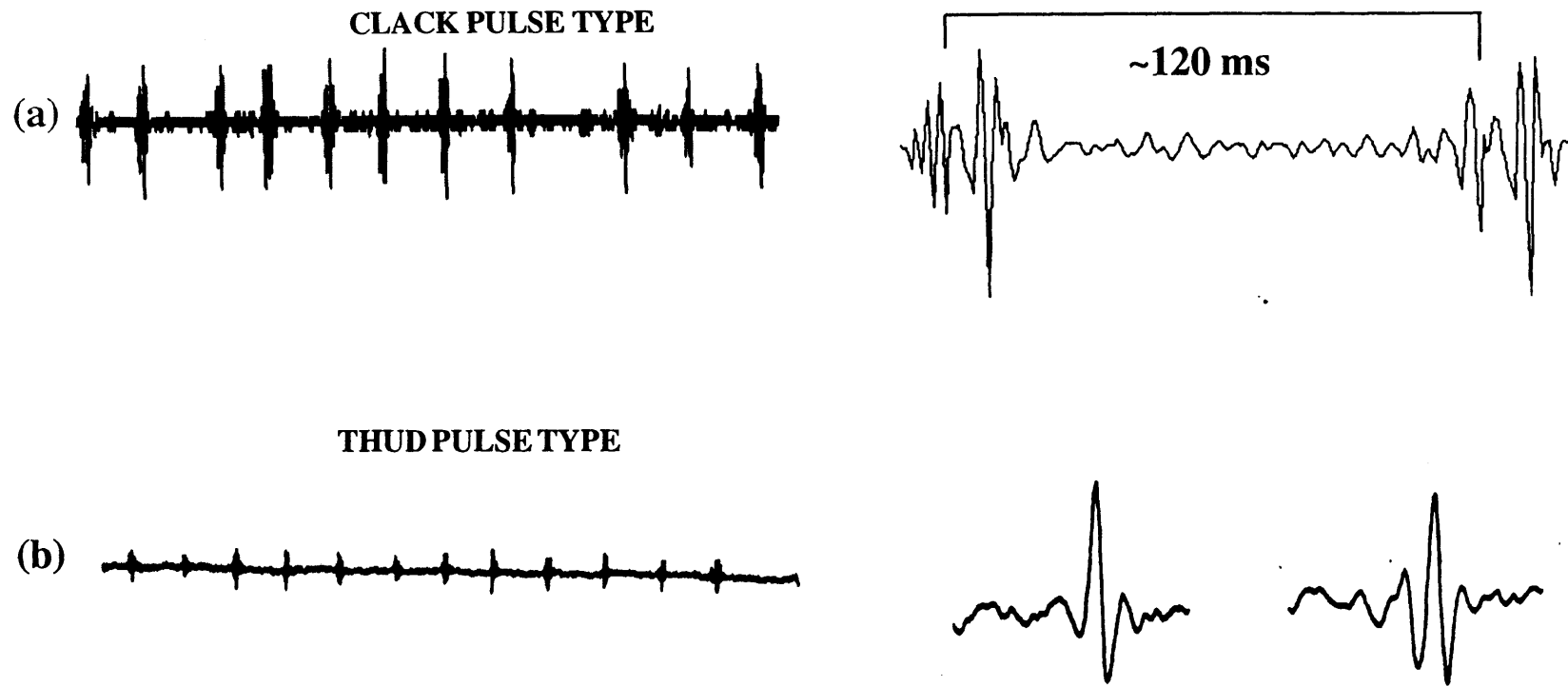
Figure 3.4.1 : Oscillographs of *D. yakuba* courtship song
a) Two "clack" pulses.
b) Two "thud" pulses, showing their simpler waveform
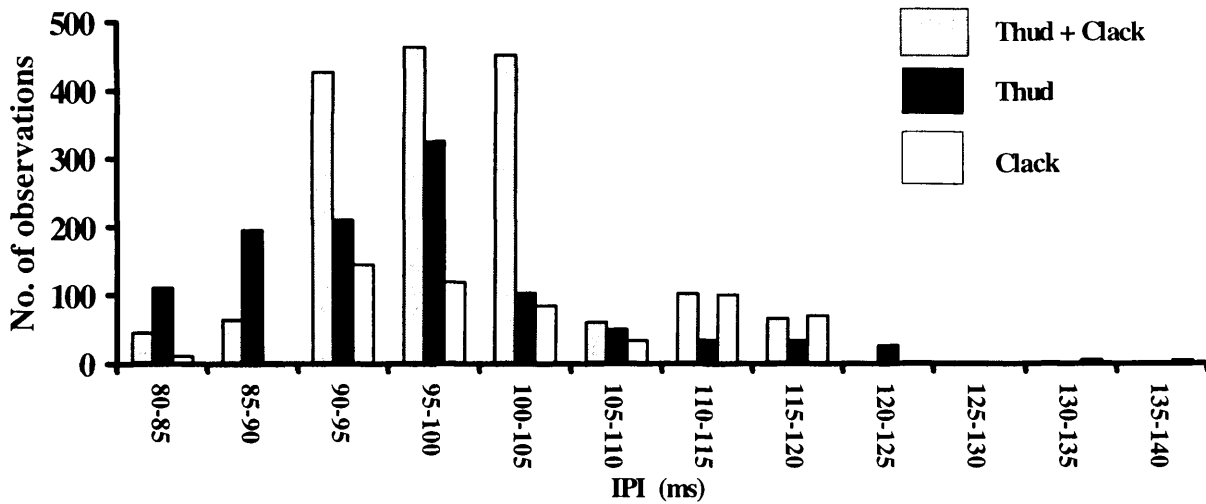
Figure 3.4.2 : Distribution of IPIs of a *D. yakuba* song (Yy5), showing the IPI distributions of the different pulse types.

Table 3.4.1 (see below) shows the mean IPF and IPI ± sem for the three types of song present within a *D. yakuba* courtship, for the six different strains and four intraspecific crosses, under investigation, in this study. The means refer to the mean of the means.

Table 3.4.1: Thud and Clack song Characteristics in various *D. yakuba* lines.

Species/Strains/ Intraspecific Hybrids	OVERALL MEAN IPI ± sem (ms)	CLACK MEAN IPI ± sem(ms)	THUD MEAN IPI ± sem(ms)	OVERALL MEAN IPF ± sem(Hz)	CLACK MEAN IPF ± sem(Hz)	THUD MEAN IPF ± sem(Hz)
SPECIES:YAKUBA						
STRAIN:						
FRANCE	124.61±2.71	128.74±3.66	122.79±3.02	302.22±3.17	330.81±5.42	280.72±3.43
EDINBURGH	126.01±4.44	127.91±4.46	126.13±4.58	302.12±3.09	328.44±5.27	282.32±3.37
MALAWI	124.80±14.7	131.10±16.2	103.79±1.61	309.84±3.34	364.58±6.47	280.36±2.82
IVORY COAST	109.15±8.60	114.22±4.87	106.49±6.83	365.99±2.80	415.92±4.92	338.71±2.75
LAMTO3	127.60±10.6	135.00±10.6	119.17±9.55	311.12±2.58	323.15±3.69	301.39±3.52
S.T.	137.86±5.88	142.07±5.49	136.57±6.03	327.18±5.08	419.90±8.00	281.44±3.74
Parental Means	125.01±3.76	129.84±3.77	119.96±5.04	319.74±9.97	363.80±18.1	294.16±9.50
INTRASPECIFIC HYBRIDS						
TYPE:						
Malawi(f)xS.T.(m)	114.66±4.95	128.76±5.67	104.99±8.75	402.58±3.53	443.06±4.66	372.36±4.46
S.T.(f)xLamto3(m)	101.54±0.90	115.16±1.77	96.94±1.06	384.25±4.44	468.10±5.43	314.65±3.16
Lamto3(f)xS.T.(m)	120.06±7.01	127.67±7.76	113.91±9.76	383.96±4.35	464.63±5.51	317.01±3.09
Lamto3(f)xI.C.(m)	120.17±3.44	127.92±3.01	112.24±3.81	360.58±3.80	436.28±4.40	292.82±2.52
Hybrid Means	114.18±4.38	124.88±3.25	107.02±3.88	382.84±8.60	453.02±7.86	324.21±16.95

Figure 3.4.3a depicts the mean IPF ± sem's of the various *D. yakuba* strains and intraspecific crosses examined in this study and the number songs analysed for each strain/intraspecific hybrid song for this characteristic.
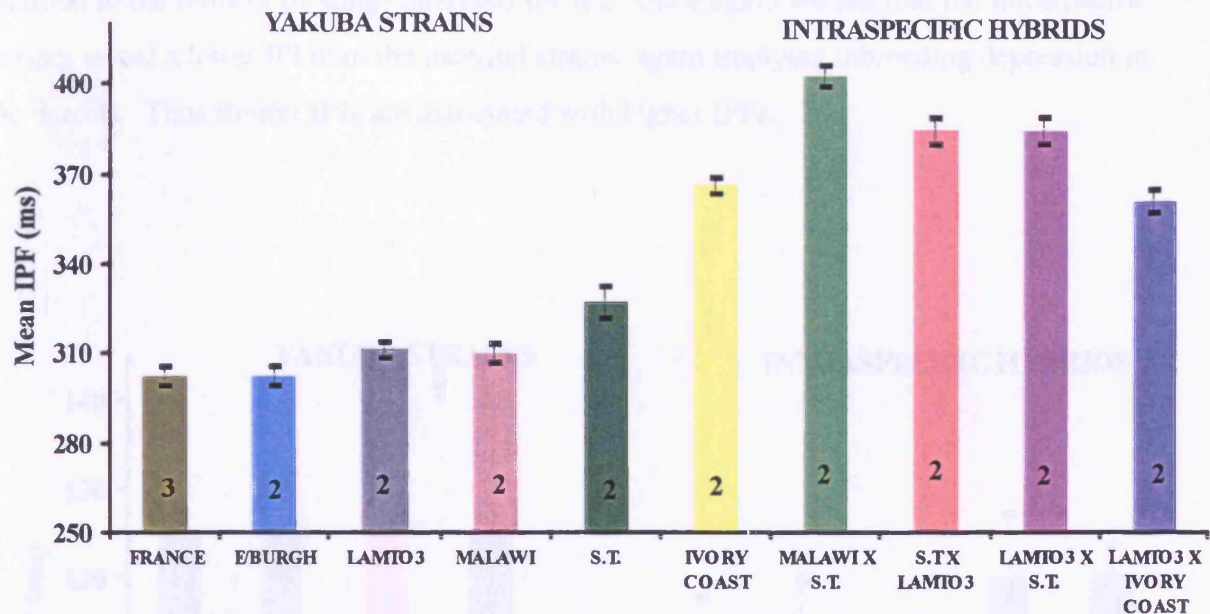
Figure 3.4.3a: The overall mean ± sem's for IPFs (Clack + Thud) of the songs of *D. yakuba*. The numbers inside each column represents the number of flies examined for each strain/ intraspecific cross.

From both table 3.4.1 and Figure 3.4.3a, it can be observed that Overall mean IPF's (Thud & Clack) for the various *D. yakuba* strains range from between ~300-370 Hz. These results are similar to those found by Cowling and Burnet (1981). The Thud + Clack mean IPF's, for each individual strain/intraspecific cross (table 3.4.1-above) are also shown, with the Clack mean IPF being higher than its Thud counterpart, within each individual strain. This fits in nicely with and supports Thackeray's (1989) distinction of Thud and Clack pulse-types. When inspecting the Thud and Clack mean IPF's from the intraspecific crosses, it is observed that these have values higher than the parental strains. Furthermore, Clack has a higher IPF than Thud, in all genotypes.

Since the differences between the IPF means of the intraspecific hybrids and their parental counterparts are so obvious, no ANOVA analysis was required. The observed increase in IPF in the hybrids suggests hybrid vigour and implies that the parentals have undergone inbreeding depression. Moreover, it suggests that superior fitness could be associated with higher IPF (see discussion).

Figure 3.4.3b (below) depicts the overall (Clack + Thud) mean IPIs of the various *D. yakuba* strains and intraspecific crosses recorded and examined in this study, in addition to the number of songs analysed for IPI. Once again we see that the intraspecific crosses reveal a lower IPI than the parental strains, again implying inbreeding depression in the parents. Thus shorter IPIs are associated with higher IPFs.
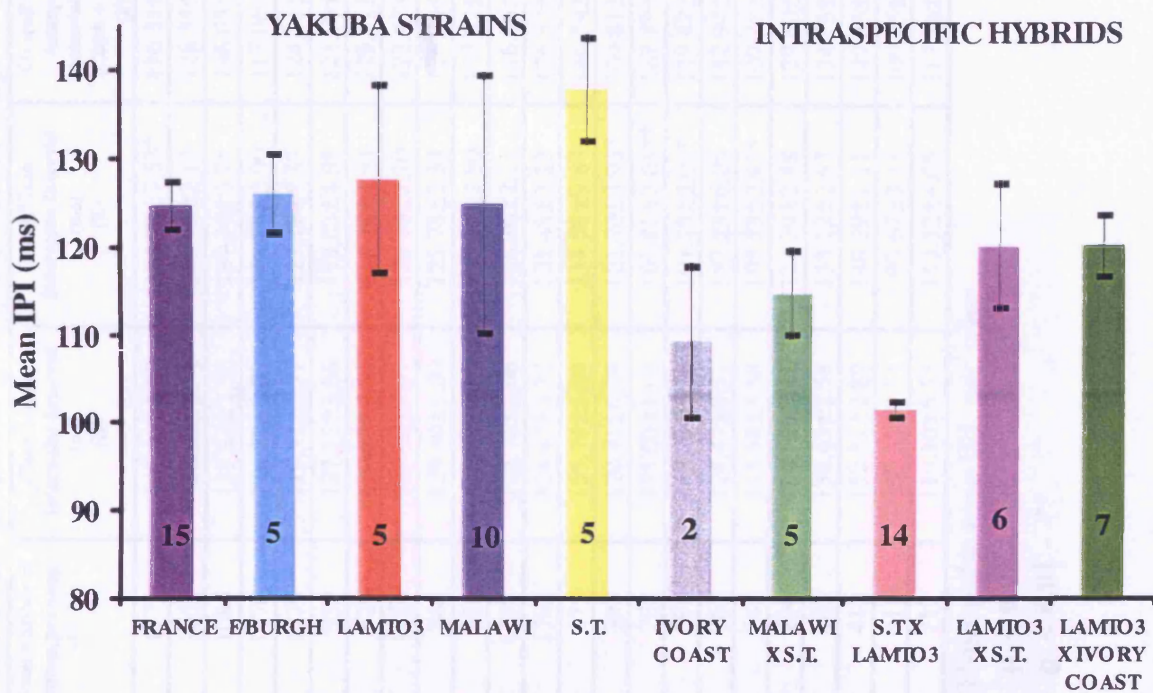
Figure 3.4.3b: The overall mean ± sem's for IPI songs of *D. yakuba*. The numbers inside each column represents the number of flies recorded and examined for each strain/intraspecific cross.

Table 3.4.2a reveals that in general the Thud-pulse type constitutes the majority of pulses in a song; out of the 23 different songs examined from six different parental strains, only five songs showed the Clack-pulse type to be the prevalent pulse type. One-way ANOVA was carried out on the Clack and Thud IPI, per song. Only 5 out of the 23 songs examined revealed a significant difference in IPI between the Thud- and Clack-pulse types (these 5 songs are marked with an asterisk in table 3.4.2a). Thus, in general the IPIs of Clack and Thud are similar within an individual courtship.

TABLE 3.4.2a: Clack and Thud Pulse Types in various *Drosophila yakuba* songs

Drosophila yakuba Strain	Song No.	Number of Clack pulses per song (A)	Number of Thud pulses per song (B)	Total number of pulses per song (C)	Clack Mean Interpulse Interval (ms) (D)	Thud Mean Interpulse Interval (ms) (E)	Overall Mean Interpulse Interval(ms)- (Clack + Thud) (F)	% of Clack pulse-type per song (G)
FRANCE	1	477	694	1171	145.41±6.97	114.24±7.53*	116.31±5.46	40.73
FRANCE	2	643	1008	1651	126.83±3.74	126.25±2.12	126.35±4.21	38.95
FRANCE	3	487	599	1086	135.23±3.50	137.34±3.71	136.65±3.14	44.84
FRANCE	4	772	898	1670	116.77±2.63	118.79±3.00	117.06±2.65	46.23
FRANCE	5	682	597	1279	119.32±3.77	127.18±5.37	124.31±1.59	53.32
FRANCE	6	703	256	959	127.15±3.66	119.03±4.98	121.90±3.57	73.31
FRANCE	7	470	159	629	130.49±4.52	116.72±4.53	129.71±3.54	74.72
EDINBURGH	1	203	1123	1326	129.30±5.07	120.78±2.20	122.47±1.93	15.31
EDINBURGH	2	133	735	868	129.30±5.00	125.78±2.33	126.70±2.59	15.32
EDINBURGH	3	265	715	980	136.18±3.45	139.12±2.90	137.92±2.18	27.04
EDINBURGH	4	458	936	1394	115.76±2.06	118.70±2.21	116.96±1.74	32.86
LAMTO3	1	1002	791	1793	128.47±3.72	121.48±3.23	126.52±2.68	55.88
LAMTO3	2	464	209	673	155.74±5.88	134.58±9.87	146.54±6.32	68.95
LAMTO3	3	100	298	398	120.91±6.79	101.70±3.93*	109.81±4.53	25.13
MALAWI	1	32	676	708	173.00±13.9	100.81±2.05**	167.88±6.83	4.52
MALAWI	2	75	619	694	138.79±2.26	101.28±5.03*	119.62±4.21	10.81
MALAWI	3	66	196	262	124.47±6.51	107.23±6.05	112.94±3.92	25.19
MALAWI	4	123	619	742	113.58±3.58	105.83±2.83*	107.88±2.51	16.58
S.T.	1	195	298	496	134.74±3.11	128.29±2.88	129.60±3.14	39.31
S.T.	2	153	309	462	138.65±2.58	133.12±2.67	134.75±3.01	33.17
S.T.	3	161	249	410	152.81±2.89	148.29±2.11	149.23±2.31	39.27
IVORY COAST	1	235	576	811	109.35±3.54	99.67±3.15	100.55±3.23	28.98
IVORY COAST	2	234	483	717	119.10±5.53	113.32±4.05	117.76±3.84	32.64

KEY: One-way analysis of variance of Clack- vs Thud-pulse type IPI per song

$p < 0.05$ - *

$p < 0.01$ - **

TABLE 3.4.2b: Clack and Thud Pulse Types in various *D. yakuba* intraspcific genotypes

Intraspecific Hybrids Genotype	Song No.	Number of Clack pulses per song (A)	Number of Thud Pulses per song (B)	Total number of pulses per song (C)	Clack Mean Interpulse Interval (ms)(D)	Thud Mean Interpulse Interval (ms) (E)	Overall Mean Interpulse Interval(ms) (Clack + Thud) (F)	% of Clack pulse-type per song (G)
MALAWI(f)xS.T.(m)	1	528	576	1104	123.08±2.89	113.74±3.00*	119.61±2.27	47.83
MALAWI(f)xS .T(m)	2	275	635	910	134.43±4.07	96.23±2.54**	109.71±3.26	30.22
S.T.(f)xLAMTO3(m)	1	496	921	1417	106.17±2.75	94.62±2.36**	98.22±1.98	35.00
S.T.(f)xLAMTO3(m)	2	160	655	815	116.51±6.10	101.65±2.72*	105.19±2.91	19.63
S.T.(f)xLAMTO3(m)	3	649	1154	1803	114.47±2.18	97.97±1.94**	103.21±1.50	36.00
S.T.(f)xLAMTO3(m)	4	368	735	1103	115.01±2.79	94.16±3.07**	102.85±2.07	33.36
S.T.(f)xLAMTO3(m)	5	96	337	433	115.19±7.25	91.13±1.84**	97.72±2.64	22.17
S.T.(f)xLAMTO3(m)	6	372	707	1079	115.19±3.81	96.50±3.03**	102.85±2.07	34.48
S.T.(f)xLAMTO3(m)	7	6	654	660	112.57±0.00	105.70±3.41*	105.62±3.40	0.009
S.T.(f)xLAMTO3(m)	8	112	330	442	111.19±6.05	93.90±2.15**	99.76±3.34	25.34
S.T.(f)xLAMTO3(m)	9	437	703	1140	111.43±3.23	100.23±2.64**	103.35±2.23	38.33
S.T.(f)xLAMTO3(m)	10	165	302	467	107.41±6.09	91.64±2.63**	95.56±2.84	35.33
S.T.(f)xLAMTO3(m)	11	90	459	549	128.29±11.8	98.28±4.02**	101.51±3.77	16.39
S.T.(f)xLAMTO3(m)	12	112	330	442	122.88±3.00	95.94±3.48**	99.76±3.74	25.34
S.T.(f)xLAMTO3(m)	13	1123	476	1599	117.03±4.80	99.04±1.56**	107.25±1.37	70.23
S.T.(f)xLAMTO3(m)	14	143	736	879	118.85±7.85	96.42±2.13**	98.78±2.34	16.27
LAMTO3(f)xS.T.(m)	1	250	653	903	131.82±7.48	118.04±2.45**	121.17±2.09	27.69
LAMTO3(f)xS.T.(m)	2	117	280	397	112.65±4.57	95.32±1.93**	107.40±2.15	29.47
LAMTO3(f)xS.T.(m)	3	195	317	512	138.54±2.74	128.36±2.45**	131.62±1.94	38.09
LAMTO3(f)xI.C.(m)	1	276	427	703	113.75±4.62	100.57±2.63*	106.14±2.76	25.47
LAMTO3(f)xI.C.(m)	2	163	569	732	127.30±4.43	112.93±3.70*	118.80±3.36	44.91
LAMTO3(f)xI.C.(m)	3	619	818	1437	138.16±3.84	126.78±2.86*	130.96±2.16	43.08
LAMTO3(f)xI.C.(m)	4	1095	199	1294	134.50±2.30	117.64±3.99**	132.08±2.33	84.60
LAMTO3(f)xI.C.(m)	5	319	319	638	124.71±4.66	98.16±3.31**	114.66±2.90	50.00
LAMTO3(f)xI.C.(m)	6	250	660	910	131.61±7.18	118.08±2.51*	121.17±2.09	27.47
LAMTO3(f)xI.C.(m)	7	536	689	1225	125.40±3.51	111.54±2.49**	117.31±2.41	43.75

KEY: One-way analysis of variance of Clack- vs Thud-pulse type IPI per song

$p < 0.05$ -   *

$p < 0.01$ -   **

Table 3.4.2b reveals that in general the Thud-pulse type constitutes the majority of pulses in a song, for the intraspecific genotypes; out of the 26 different songs examined from the various intraspecific genotypes, only two songs showed the Clack-pulse type to be the prevelant pulse type and one song to have equal numbers of the two pulse-types. Inspecting the p-values of each individual intraspecific hybrid song, after one-way ANOVA was applied to the IPIs for Clack and Thud per hybrid genotype, all of the 26 songs examined show significant differences between the Thud- and Clack-pulse types (marked by an asterisk (*) in Table 3.4.2b). In all the songs, the Clack and Thud IPI means are significantly different, and always the Clack mean IPI is greater than the Thud mean. Two-way ANOVA for mean IPI per song was performed on the pulse types (Clack and Thud) and strains (Parental and Intraspecific crosses).

Table 3.4.3: Two-way ANOVA of Clack and Thud IPI for the parental strains and their intraspecific genotypes

GENERAL MANOVA	1-STRAIN; 2-PULSE TYPES					
Effect	df Effect	MS Effect	df Error	Ms Error	F	p-level
Genotypes (10)	9	1007.355	78	102.3303	9.84415	0.0000
Pulse Type (2)	1	3509.751	78	102.3303	34.29825	0.0000
Strain x Pulse Type	9	187.168	78	102.3303	1.82906	0.076007

Significant differences between different genotypes and pulse types are observed. The Clack IPIs are higher than those of Thud (see Figure 3.4.4), with no significant interaction between pulse type and genotype (see Table:-3.4.3 and Figure 3.4.4-below).

Figure 3.4.4: Two-way ANOVA: CLACK vs THUD IPI

SPECIES: *Drosophila yakuba*
STRAIN Main Effect
F(9,78)=9,84; p<,0000

PULSE TYPE: Main Effect
F(1,78)=34,30; p<,0000
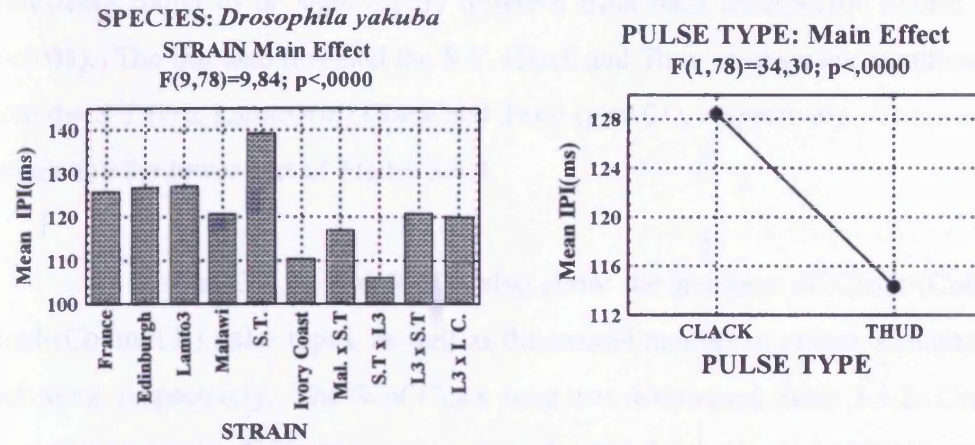
2-way interaction
F(9,78)=1,83; p<,0760

FRANCE
EDINBURGH
LAMTO3
MALAWI
S.T.
Ivory Coast
M x S.T.
S.T. x L3
L3 x S.T.
L3 x I.C.
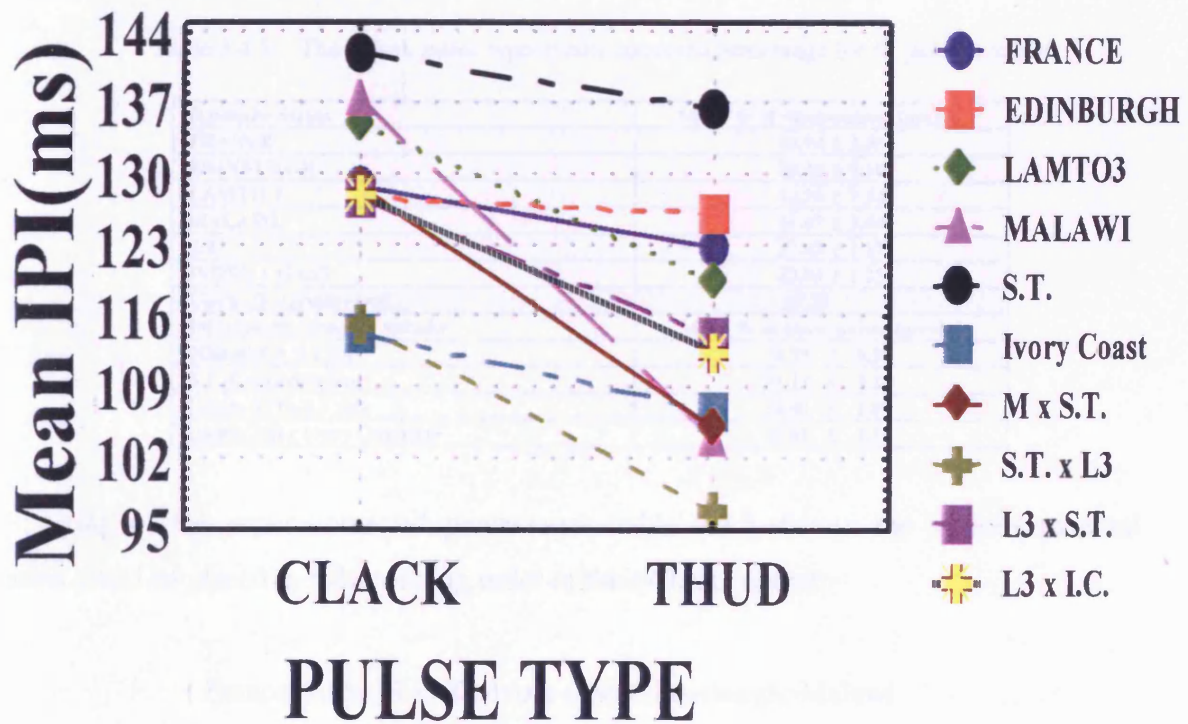
Newman-Keuls' *a posteriori* test revealed that within intraspecific crosses, the Malawi Clack IPI is different from Malawi Thud (p<0.01), the S.T. Thud and Clack were significantly different from their Malawi counterparts (p<0.01). The Malawi Clack and S.T. Thud were found to be significantly different from their intraspecific hybrid counterparts (p<0.01). The test also revealed the S.T. Clack and Thud means were significantly different from the S.T.(f) x Lamto3(m) Clack and Thud (p<0.01), respectively. These results can be revealed in the lower part of Figure 3.4.4.

Tables 3.4.2a and 3.4.2b also show the numbers of Clack-(Column A) and Thud-(Column B) pulse types, as well as the overall number of pulses (Column C) found in each song, respectively. The % of Clack song was determined (table 3.4.2a Column G) and converted to arcsin. Using these arcsin-transformed data, one-way ANOVA was employed, in order to see whether there were any differences in the Clack % content between any of the parental and intraspecific lines. A significant F-ratio (F=2.93, p=0.001, df=9,37) revealed that indeed there were significant differences. Table 3.4.5 (below) shows the mean % of Clack ± s.e.m. present in any song. Clack pulse-type represents only ~30% of all the pulses present in the parental *D. yakuba* songs.

Table 3.4.5: The Clack pulse-type arcsin-corrected percentage for *D. yakuba* strains

D.yakuba Strain	Mean % of clack-pulses (arcsin)
FRANCE	46.94 ± 3.35
EDINBURGH	28.00 ± 3.10
LAMTO 3	45.10 ± 7.54
MALAWI	21.67 ± 3.64
S.T.	37.43 ± 1.17
IVORY COAST	33.85 ± 1.25
Clack % (arcsin)-real	27.32
Intraspecific cross-*D. yakuba*	Mean % of clack-pulses (arcsin)
Malawi(f) x S.T.(m)	38.55 ± 5.35
S.T.(f) x Lamto 3(m)	31.13 ± 3.26
Lamto 3(f) x S.T.(m)	34.40 ± 1.89
Lamto 3(f) x Ivory Coast(m)	42.61 ± 4.59

Inspecting of the arcsin-corrected percentages (table 3.4.5-above), the various parental strains could be placed in a descending order of their Clack content:

France>Lamto3>S.T.>Ivory Coast>Edinburgh>Malawi

3.5 Do song rhythms exist in *D. yakuba*?

Both *D. yakuba* parental strains and intraspecific hybrids were investigated for the possible existence of song rhythms. The analysis of *D. yakuba* courtship song data proceeded as follows:

The two spectral methods (CLEAN and VdB) were applied to each of the 37 songs and the peak of the spectrogram was taken as the song period. If the two analyses disagreed as to the peak period, then if the same period was the peak in one analysis, but the second highest spectrogram peak in the second analysis, then the 'consensus' value was taken as the song period, e.g., see song Yk1t1l2 in table 3.5.1a. An example of song rhythms is illustrated in Figures 3.5.1a, b and c, which give the overall Clack + Thud, Thud and Clack cycles. The highlighted CLEAN analysis values in table 3.5.1a are those that have been used to determine the rhythm periods of each species/strain songs (see Table 3.5.1a - below). The corresponding VdB values are also highlighted. As it can be seen in song Yk1, in table 3.5.1a, the highlighted value in the 'Overall' song, is the second highest peak in the CLEAN which has a corresponding primary peak in the VdB spectrogram. Once again, I used two sets of upper IPI cut-off points:

a) 250 and 50 ms- see Figure 3.5.1, a, b & c ;

b) 350 and 50 ms.

Because the data is so extensive for each song, I have taken a sample of 5 songs out of the 37 songs that were analysed, to present in the chapter as tables 3.5.1a & b; for the entire tables see Appendix 3.2. As it can be seen from these two tables (3.5.1a & b), the different cut-off points do not make much difference to the detection of rhythms; the only thing that is apparent is that in some instances the number of empty bins (bins containing <10 IPI) increases, when the more stringent cut-off points (250 & 50 ms) were used.

(A) period=87.0s F=4.65, df=3,32

IPI (ms)

Time (s)

(B) 34.92s, 85.77s   CLEAN

500 200   100 80 65   50   40   35   30   Period (seconds)   20
99%
95%

Modulus

Frequency

(C) 87.91s   VdB

500 200   100 80 65   50   40   35   30   Period (seconds)   20
99%
95%

Modulus

Frequency
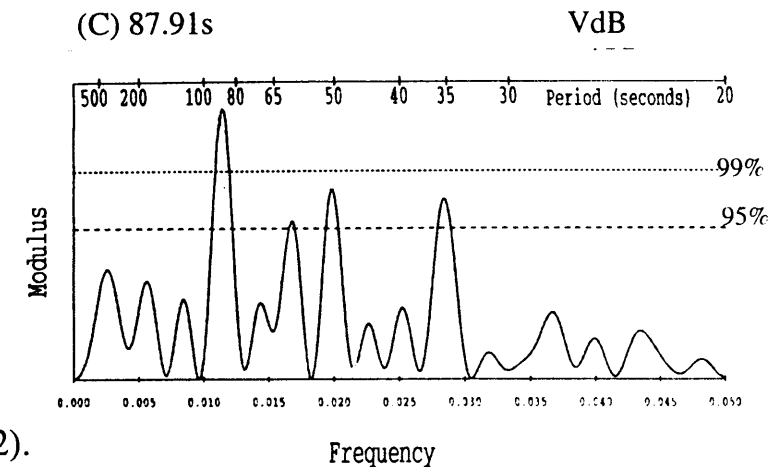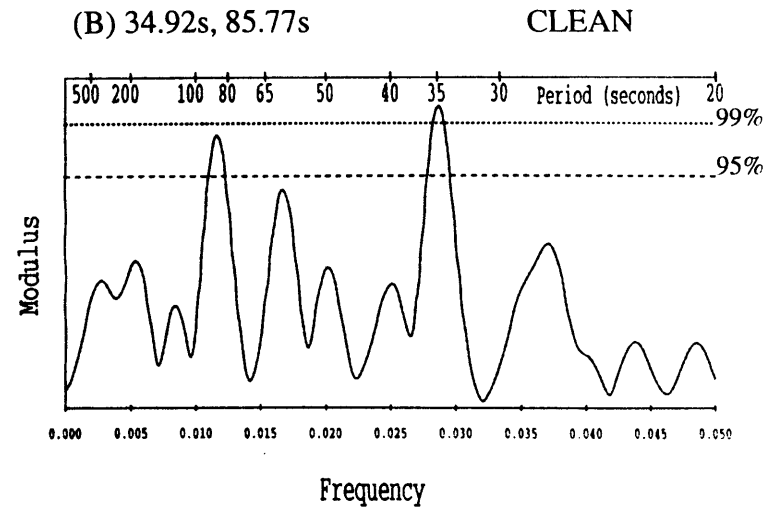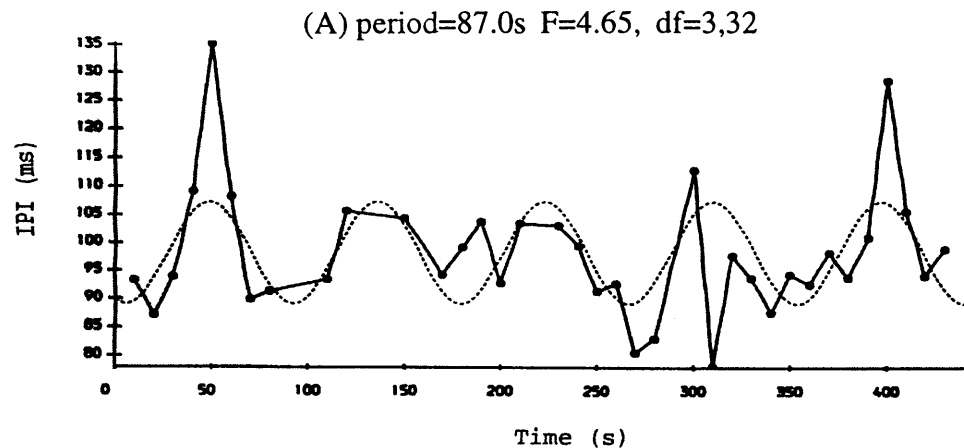
Figure 3.5.1a:

A *D. yakuba* courtship song (**Clack & Thud pulse-types**)(yk1t1l2)
-see table 3.5.1a, using 250 & 50 ms as cut-off points.

a) A plot of the mean IPI against time. The dotted line represents
   the strongest cycle in the the data, initially defined by VdB spectral
   analysis and then fitted to the data by non-linear regression,using a
   least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song
   respectively, each indicating the corresponding periods. The 95 & 99%
   confidence limits are defined by Monte Carlo simulations (see chapter 2).

(A) period=64.6s F=2.374, df=3,18

Time (s)

(B) 66.12s   CLEAN

Frequency

(C) 66.12s   VdB

Frequency
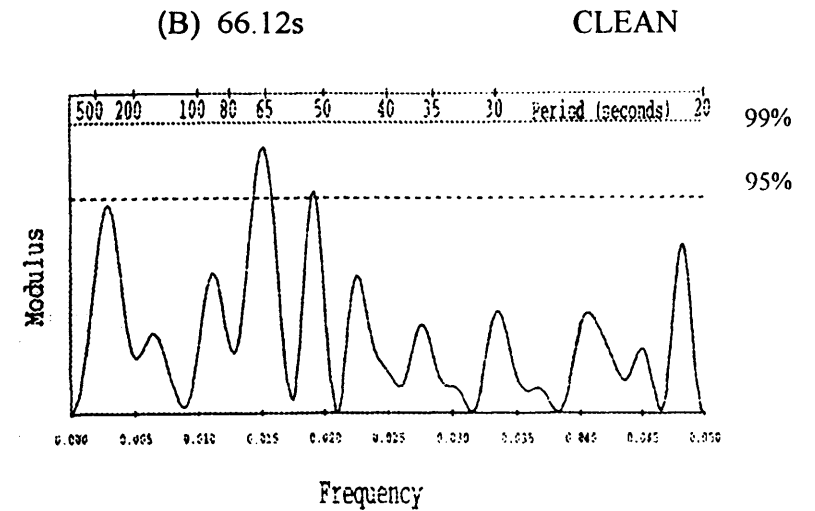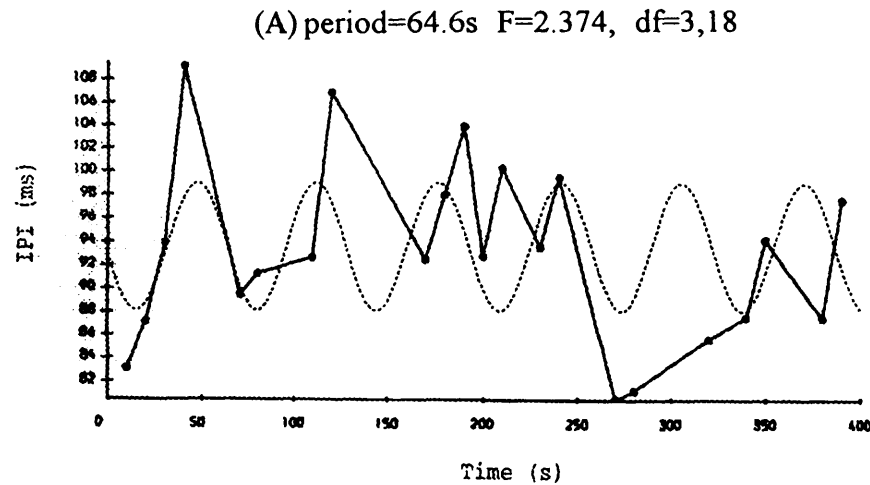
Figure 3.5.1b:

The same *D. yakuba* courtship song (Thud pulse type)(yk1t1l2)-see table 3.5.1a, using 250 & 50 ms as cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the the data, initially defined by VdB spectral analysis,and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,each indicating the corresponding periods. The 95 & 99% confidence limits are defined by Monte Carlo simulations (see chapter2).

(A) period=57.6s F=7.155, df=3,18

IPI (ms)

Time (s)

(B) 57.58s   CLEAN

Modulus

Frequency

(C) 57.97s   VdB

Modulus

Frequency
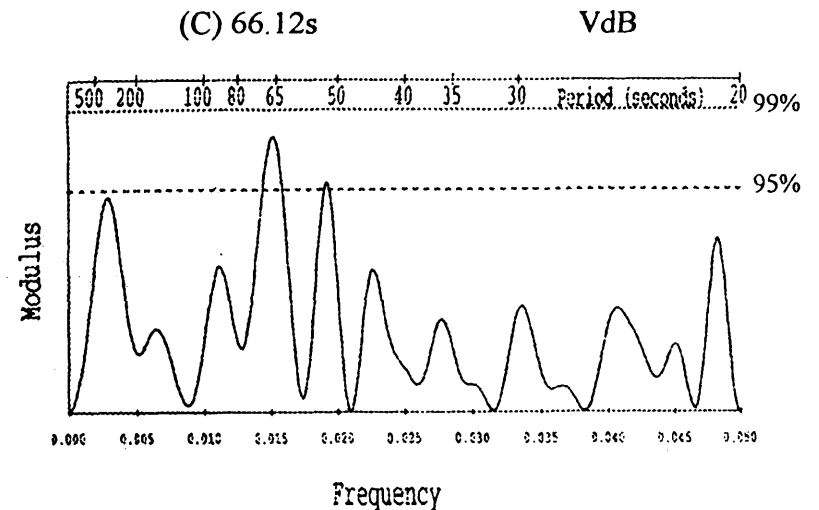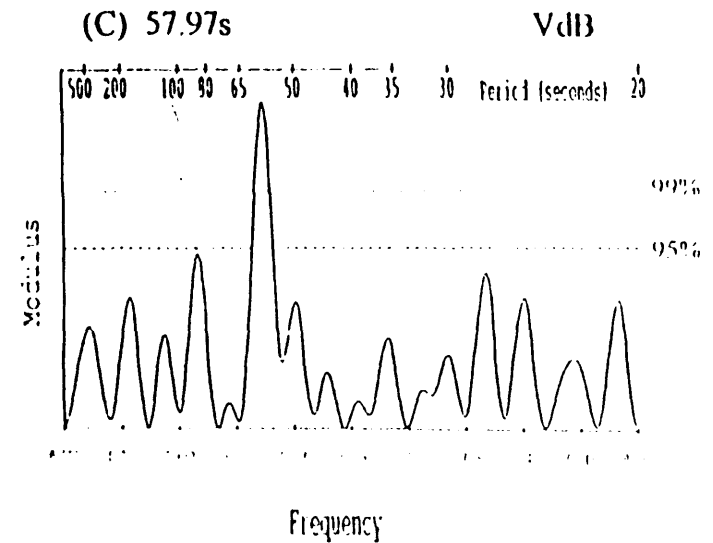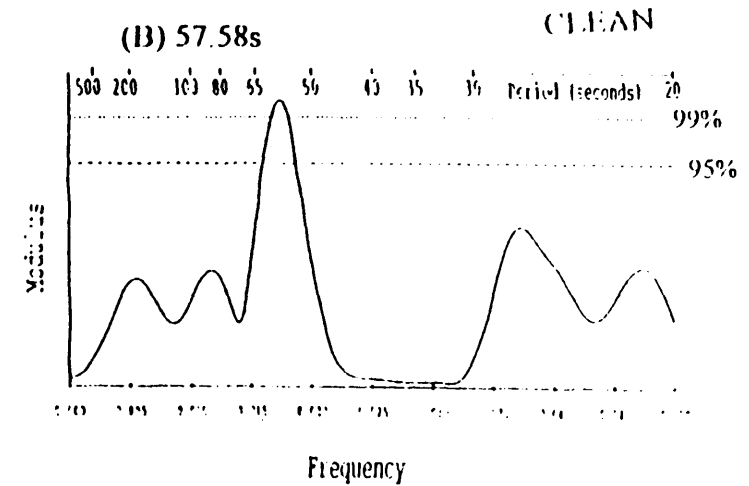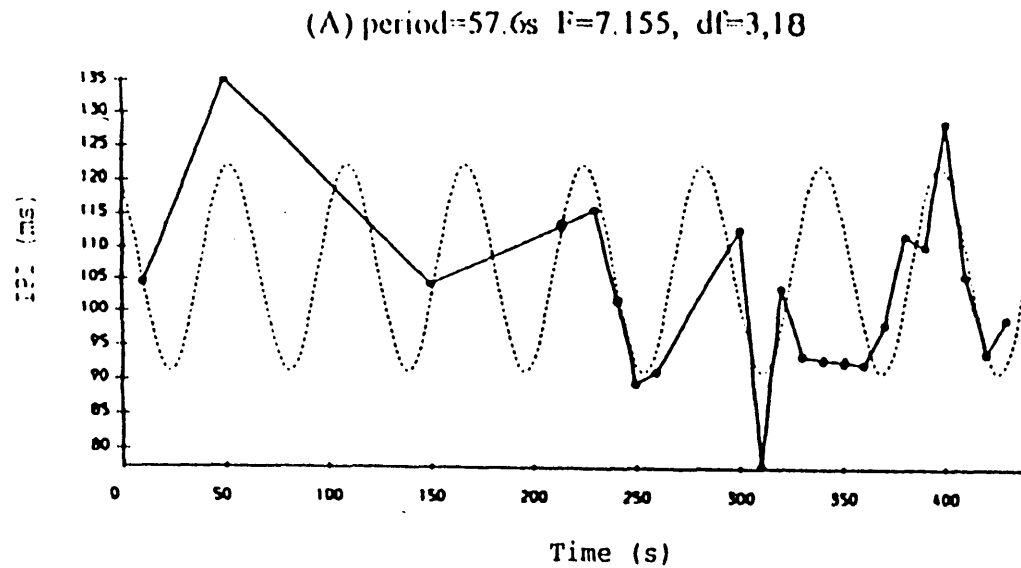
Figure 3.5.1c:

The same *D. yakuba* courtship song (Clack pulse type)(yk11112)
-see table 3.5.1a, using 250 & 50 ms as cut-off points.

a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the the data, initiallydefined by VdB spectral analysis
   and then fitted to the data by non-linear regression, using a least squares
   procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods. The 95 & 99% confidence
   limits are defined by Monte Carlo simulations (see chapter 2).

Table 3.5.1a: Spectral analysis of *D. yakuba* songs using lower IPI cut-off points.
'Overall' refers to Clack + Thud song.

(a) Lower cut-off points

SPECTRAL ANALYSIS					
CUT Off POINTS : 250 & 50 ms					
YAKUBA		Clean		VdB		Observations
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
Yk1	Overall	34.92	85.77	87.91	50.63	36/43
	Thud	66.12	x	66.12	x	22/39
	Clack	57.58	x	57.97	x	22/43
Yk2	Overall	75.42	45.15	76.92	x	43/56
	Thud	31.18	70.13	31.25	x	28/56
	Clack	23.52	21.82	23.67	21.86	26/51
Yk3	Overall	33.01	54.31	33.20	x	35/36
	Thud	34.07	21.17	34.04	21.16	20/36
	Clack	53.50	26.37	54.79	37.21	18/35
Y9	Overall	90.91	30.98	91.95	28.37	37/38
	Thud	95.74	23.87	97.56	x	36/36
	Clack	22.73	62.50	22.60	37.56	24/38
Yy10	Overall	102.89	24.26	24.24	103.90	50/59
	Thud	92.59	24.20	24.46	93.02	28/57
	Clack	30.82	24.11	31.01	x	22/52

Table 3.5.1b: Spectral analysis of *D. yakuba* songs using higher IPI cut-off points.

(b) Higher cut-off points:

SPECTRAL ANALYSIS					
CUT Off POINTS : 350 & 50 ms					
YAKUBA		Clean		VdB		Observations
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
Yk1	Overall	34.92	85.77	87.91	50.63	36/43
	Thud	66.12	x	66.12	52.29	22/39
	Clack	57.58	x	57.97	x	22/43
Yk2	Overall	75.42	45.08	76.92	22.22	43/56
	Thud	31.18	69.77	31.25	x	25/51
	Clack	23.52	21.82	23.67	21.86	26/56
Yk3	Overall	33.01	54.31	33.20	x	34/37
	Thud	34.07	21.17	34.04	21.16	20/37
	Clack	53.50	26.37	54.79	37.21	16/35
Y9	Overall	91.03	28.08	91.95	x	37/38
	Thud	96.26	23.87	23.87	97.56	36/38
	Clack	22.73	62.50	22.66	37.56	24/38
Yy10	Overall	102.89	24.26	24.24	103.90	50/59
	Thud	92.59	24.20	24.46	93.02	28/57
	Clack	30.82	24.11	31.01	x	22/52

It can be clearly seen that the CLEAN/Monte Carlo and the corresponding VdB values, for each individual song, are approximately similar. Any period of 30s or less was assumed to reflect arrhythmicity, if a second significant peak in the spectrogram was not found. This is because arrhythmic per^{01}-songs have cycles ranging predominantly from ~20-30s (Kyriacou and Hall, 1989; Alt *et al.*, 1997).

Thackeray (1989) scored only two sets of data for a *D. yakuba* song, one set for the Thud pulse-type and one set of data for the Clack pulse-type, but he did not calculate a third dataset where Thud and Clack pulse types were amalgamated. Hence, Thud was

Thackeray (1989) scored only two sets of data for a *D. yakuba* song, one set for the Thud pulse-type and one set of data for the Clack pulse-type, but he did not calculate a third dataset where Thud and Clack pulse types were amalgamated. Hence, Thud was found to give a rhythm with a period of ~78.1s ± 5.3 and Clack was found to be ~79.2s ± 4.2 (± sem) (Thackeray, 1989).

In this present study, all three datasets were calculated for parental strains plus F_1 intraspecific hybrids, and the periods are shown in Figure 3.5.2 which gives the distribution of the CLEAN-derived periods of *D. yakuba* songs from 250 & 50ms cut-off points, with the short periods (>30s) included. The mean periods were found to be ~ 63.2 ± 4.85 for Clack & Thud, ~67.0 ± 5.81 for Thud and ~57.3 ± 2.82 for Clack (± sem).
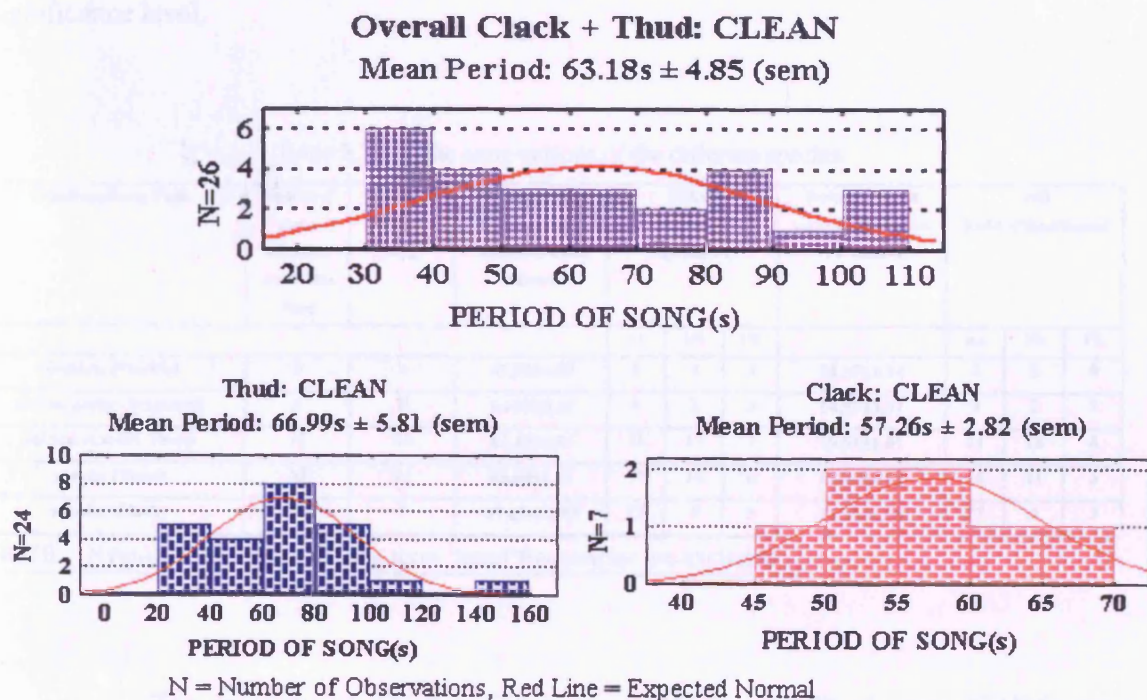
Figure 3.5.2: The distribution of the periods of *D. yakuba* songs.

Note that in the distributions above, the 'shoulder' in the Overall Clack + Thud song (Top Graph) and Thud song (Bottom left) of the periods between 20-40s are included. If I remove these short periods, which probably reflect arrhythmic songs, the mean period increases to 72.02(s) ± 4.73 and 75.95(s) ± 5.37, for Thud + Clack and Thud songs, respectively, which are very similar to those found by Thackeray (1989). Unfortunately,

ANOVA between the various strains and intraspecific hybrids could not be carried out, since a number of these genotype groups only had one or two valid song representatives for rhythm analysis. Moreover, several of the parental strains, did not have long enough songs with adequate number of data points. Thus, ANOVAs are restricted to a species group analysis.

Table 3.5.2 (see below) shows the periods of the different genotypes determined by CLEAN and VdB spectral analyses from this present study. Out of the 5 *D. melanogaster* Brighton songs examined, all of them show significant spectral rhythms. From the five *D. simulans* Florida songs examined, one did not satisfy the 50% bins filled criterion, whereas the other 4 showed significant spectral peaks at least at the 5% significance level.

Table 3.5.2:- The song periods of the different species.

Genotype/Song Type	Number of songs examined >50% bins filled	Number of rhythmic songs	Periods obtained using the significant Clean values(s)	CLEAN (Level of Significance)			Periods obtained using the significant VdB values(s)	VdB (Level of Significance)		
				n.s	5%	1%		n.s	5%	1%
simulans (Florida)	4	4	47.99±4.07	0	4	0	38.37±3.74	2	2	0
melanogaster (Brighton)	5	5	54.37±3.52	0	2	3	54.53±3.71	0	2	3
yakuba (Clack+ Thud)	37	26	63.18±4.85	11	19	7	68.55±5.01	11	18	8
yakuba (Thud)	35	24	66.99±5.81	11	18	6	64.92±4.85	11	21	3
yakuba (Clack)	24	7	57.256±2.82	17	4	3	59.13±5.70	17	4	3

NOTE:- Nyquist Frequencies or long term 'trend' frequencies are excluded

The majority of Thud + Clack song (26/37) and Thud song (24/35) gave significant primary or secondary peaks, in their respective CLEAN and VdB spectrograms (see chapter 2), but only a minority (7/24) of the Clack songs revealed rhythmic profiles (see table 3.5.2-above). Figure 3.5.3 (see below) depicts the distribution of the periods of songs for *D. melanogaster* Brighton, *D. simulans* Florida and *D. yakuba* (various strains & intraspecific crosses), recorded for this study.
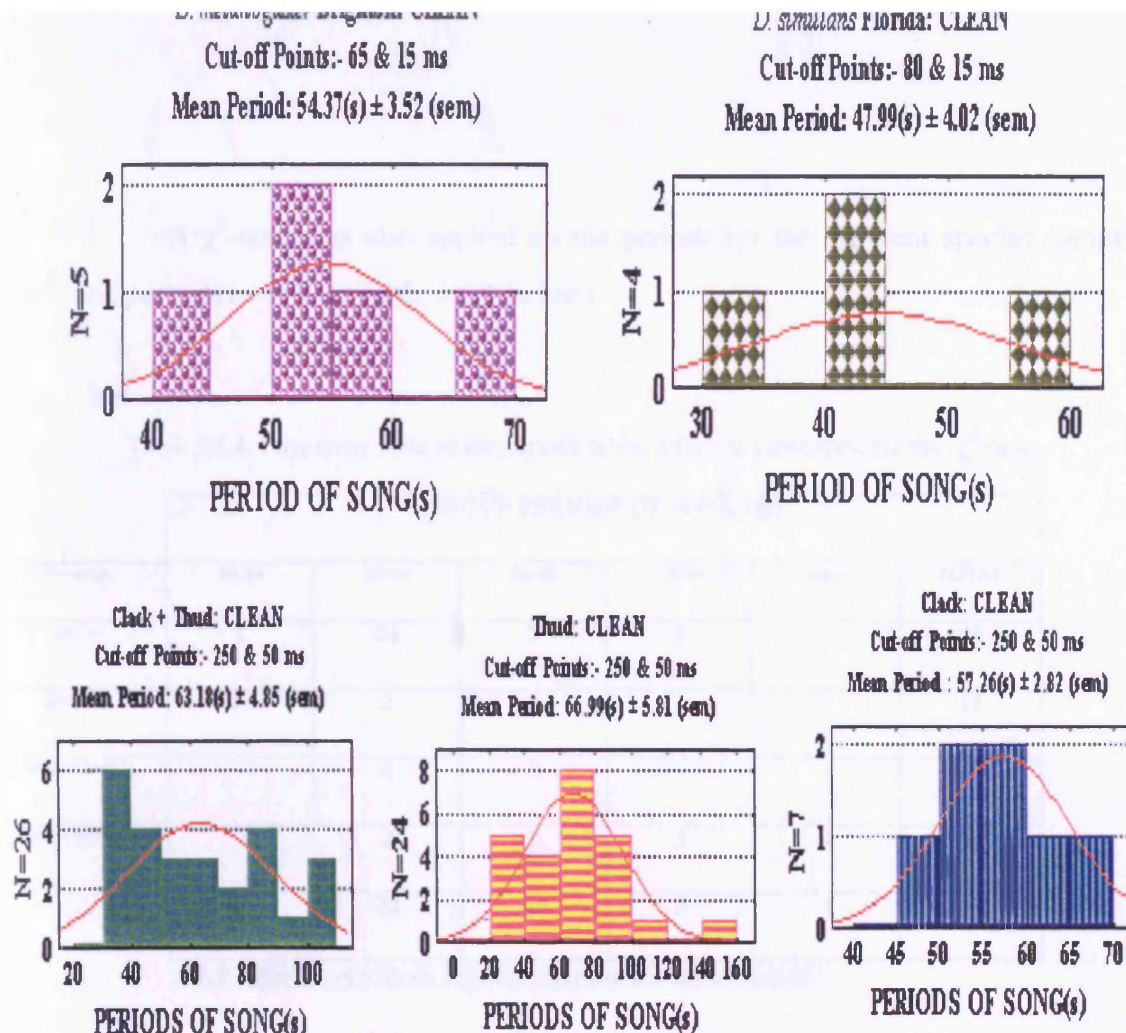
Figure 3.5.3: The distribution of the *periods* of songs the various species examined in this present study.

One-way ANOVA was employed on the CLEAN periods in order to test whether there were any differences between the periods of the various species. *D. melanogaster per*[+]-periods (see table 3.1.1- Oregon, Canton-S and Brighton, together), *per*[S], *per*[L] were compared to *D. simulans* and the two *D. yakuba* songs (Clack and Thud). A significant F-ratio (F=12.49, df=6, 97, p=0.000) was detected. The Newman-Keuls *a posteriori* test was employed and revealed that the *D. melanogaster* periods are significantly different, at least at p<0.05, from the periods of *D. simulans* and *D. yakuba* Thud song, but showed no significant differences to *D. yakuba* Clack song. In addition, the test revealed that there are significant differences between the different *per* genotypes, which is in agreement with Kyriacou and Hall's (1989), Kyriacou *et al.* (1990) and most recently with Alt *et al*'s (1997) observations.

60

A χ^2-test, was also applied on the periods for the different species (omitting per^S- and per^L-mutants- see Table 3.5.4-below).

Table 3.5.4: Frequency table of the periods of the different genotypes, for the χ^2-test.

Genotype	GROUP PERIODS OF SONGS (s)					
	30-45	45-60	60-75	75-90	90+	TOTAL
per +	2	24	7	2	-	35
simulans	8	2	1	-	-	11
yakuba (Clack)	-	4	3	-	-	7
yakuba (Thud)	7	2	8	3	4	24
	17	32	19	5	4	77

KEY : The short periods (nyquist frequencies) are excluded

The χ^2-value is 39.424 (p<0.01), again revealing species differences in song rhythms.

3.6 Discussion:

The *D. melanogaster* song is composed of hum (sine) and pulse song, with the sine song having a low carrier frequency (von Schilcher, 1976b), and constituting the majority of a courtship song (S. Campesan, pers. comm.). It has been suggested that the sine song may act as a prestimulator to the female (von Schilcher, 1976b), whereas the pulse song is a species-specific trigger for mating. However, Kyriacou and Hall (1984) showed that a cycling IPI also acts as a prestimulator. *D. yakuba* song has been found to consist of two types of pulses (Thackeray, 1989), a low frequency Thud song, which generally constitutes the majority of pulses present in a courtship, and a high frequency Clack (see tables 3.4.2a & b). It is therefore feasible that Thud is homologous to 'sine' song and Clack may represent *melanogaster* pulse song. Without any simulator experiments, such as those experiments performed by Kyriacou and Hall (1982, 1986) and Greenacre *et al.* (1993), to test the effects of the different types of signal on *D. yakuba* females, this remains just speculation.

The mean IPIs and IPFs, and mode of IPI for the different species are found to be in close agreement with Cowling and Burnet's observations (1981). Furthermore, their IPI distributions (see Figures 3.2.1 and 3.4.2) are unimodal in shape, but varying in the broadness of their respective distributions, as opposed to *D. mojavensis* and *D. arizonae* songs, for example, where the IPI distributions are bimodal (C. Byrne and C.P. Kyriacou, pers. comm.).

Inspecting the results for the different pulse-types within a *D. yakuba* courtship, it was confirmed that they are two distinctly different types of pulses (Thackeray, 1989), with different mean IPI's; the Clack song has a higher IPI than Thud. The mean IPF's for Clack song of the various *D. yakuba* strains are higher than those for Thud song. In addition, as mentioned above, in the majority of the songs, Thud constitutes the majority pulse-type present.

When comparing the song characters of a particular intraspecific genotype and their corresponding parental counterparts, it can be observed that Thud & Clack IPF's

and IPI's of the intraspecific genotypes are higher and lower, respectively, than the parental ones. This increase in the mean IPFs and decrease in the mean IPIs in the intraspecific hybrids (Figure 3.4.3 a and b, respectively, and table 3.4.1) are suggestive of hybrid vigour (Crow, 1948; Robertson, 1952; Sheridan, 1981), implying that the parental strains had undergone inbreeding depression (Reeve, 1955a; Roberts,1960). Moreover, it suggests that superior fitness could be associated with higher IPFs and shorter IPIs (Crow, 1952; Falconer, 1971; Haymer and Hartl, 1982; 1983). Thus, a more vigorous and energetic male may vibrate his wings faster giving higher IPFs and shorter IPIs. Thus, the association between lower IPIs and higher IPFs makes mechanical sense.

Given the controversy that had surrounded the existence of song cycles, my initial task was to reanalyse Kyriacou and Hall's original data and attempt to replicate Thackeray's results with $D.$ $yakuba$ (1989), using a new method to analyse the significance of spectral analysis on each individual song. The results were similar to those of Kyriacou and Hall (1989) and Kyriacou et al. (1990), but the new method had the additional advantage that each individual song was acting as its own control. Konopka et al. (1997) have also recently used this new method, to analyse the songs of per^S mosaic flies. The results of my study support Kyriacou and Hall's original (1980) observations on song rhythms, with $D.$ $melanogaster$ and $D.$ $simulans$, and Thackeray's (1989), preliminary observations on song cycles in $D.$ $yakuba$.

The choice of the appropriate upper IPI cut-out points for rhythm analysis (Crossley, 1988; Ewing, 1988; Kyriacou and Hall,1980,1989 and Kyriacou et al., 1990) was further emphasized in this study with $D.$ $melanogaster$. Section 3.3, Figures 3.3.1a and 3.3.2a (for $D.$ $melanogaster$ and $D.$ $simulans$, respectively) aptly demonstrates what happens when 'inappropriate' cut-off points are used; a complete 'masking' of a potential song rhythm is observed. When the different cut-off points are employed, as shown in Figures 3.3.1b and 3.3.2b, an unveiling of a song rhythm occurs. When $D.$ $yakuba$ songs were analysed (see Appendices 3.2a & b), there was not such a striking 'masking' of song cycles with different IPI cut-offs.

In the case of $D.$ $melanogaster$, all songs that satisfied the 50% bins filled criterion, showed significant peaks in their CLEAN and VdB spectrograms, with periods

between ~43-65s. In *D. simulans*, most songs also showed significant peaks with shorter periods than *D. melanogaster*, confirming the results of Kyriacou and Hall (1980; 1986) and Wheeler *et al.* (1991) . In the case of *D. yakuba*, the songs recorded during courtship were treated as three different datasets (Clack + Thud, Thud and Clack). The majority of Clack + Thud (26/37) and Thud (24/35) song types were found to have significant peaks at either the 5% or 1% level of significance. Most of the Clack songs were found to be arrhythmic (17/24); but 7 datasets were found to be rhythmic (see Appendix 3.2a & b). Moreover, ANOVA and χ^2-test revealed that there are species-specific differences in song periods between *D. yakuba*, *D. melanogaster* and *D. simulans*. The mean periods of Clack + Thud and Thud songs of *D. yakuba*, are found to be longer than those of *D. melanogaster*. As far as the Clack song's period is concerned, it is similar to that for *D. melanogaster*. In addition to the determination of the song cycles in *D. yakuba*, the basic analysis of pulse song IPI and IPF confirmed and extended Thackeray's (1989) work.

Since the spectral analyses proved useful in validating Kyriacou and Hall's (1980) data and since the existence of song cycles in *D. melanogaster*, *D. simulans* and *D. yakuba* have been further supported by the results presented in this study; the next logical step was to apply these spectral methods to the other members of the *melanogaster* subgroup (chapter 4) and to their interspecific genotypes (chapter 5) in order to determine whether the song cycles are a ubiquitous feature within the *melanogaster* subgroup and their interspecific hybrids.

Can song rhythms be detected in the other of the members of the *D. melanogaster* subgroup?

CHAPTER **4**

4. Can song rhythms be detected in the other of the members of the
D. melanogaster subgroup?

In this chapter an attempt is made to extend the analysis of song rhythms to the other species of the *melanogaster* subgroup, *D. mauritiana, D. sechellia* (*D. melanogaster* complex) and *D. teissieri, D. erecta* and *D. orena* (*D. yakuba* complex). Previous attempts by Thackeray (1989) to obtain robust song recordings, i.e., recordings with an adequate number of individual IPIs, per unit time, in order to permit IPI rhythm analysis for *D. mauritiana, D. sechellia* and *D. orena*, were unsuccessful. Here, I describe my efforts to obtain robust recordings of all species.

4.1 *D. melanogaster* complex:

D. mauritiana song-(various strains):

D. mauritiana flies produce a similar song to that generated by both *D. melanogaster* and *D. simulans*. The songs contain pulse and sine song components (Cowling and Burnet,1981). Through systematic and empirical alteration of the experimental conditions (the age of both male and female flies, time of the recording, rearing conditions), it has been possible to obtain some songs that had sufficient IPIs, for rhythm analysis.

According to Robertson (1983), the *D. mauritiana* courtship is much more light-dependent than that of *D. melanogaster* and *D. simulans*, so I decided that the males were to be reared in a constant light incubator (25°C) and in solitude, one per vial. Rearing males in solitude increases their sexual vigour (von Schilcher,1976b, C.P. Kyriacou, pers. comm.). Virgin females were maintained at the same temperature, but two females per vial, under light/dark conditions. Evidently, the *D. mauritiana* courtship depends considerably on light stimuli for mating, since ostensibly blind *white* eye *D. mauritiana* mutant males were never observed to court females (Robertson,1983).

D. mauritiana males were observed to perform the 'scissoring' wing motion (Robertson, 1983). One wing vibration style that is also observed, the so-called 'rowing', is a wing motion which involves simultaneous arching and replacing of one wing and extension

of the abdomen during copulation attempts, first described by Robertson (1983). In *D. melanogaster* courtship 'rowing' was never observed; thus superficially, at least, the *D. mauritiana* song is close to that of *D. simulans* (Cowling and Burnet, 1981; Robertson, 1983). The only obvious difference between *D. mauritiana* and *D. simulans* males, during courtship, is that the *D. mauritiana* male flies are swifter in their copulation attempts (Robertson, 1983).

While collecting males and females for recording, using CO_2 anaesthesia under a light microscope (see Chapter 2), certain morphological differences were observed, in both sexes, amongst the different strains. For example, in certain strains such as *D. mauritiana* Isofemale 102, the males have longer and more pointed abdominal ends than the *D. mauritiana* Sweden males.

During recording of *D. mauritiana* songs, I anecdotally observed that sine song was heard when the male was near the female, but at some distance from her, which is in agreement with Robertson's observations (1983). The female fly is seen to brush her wings against her abdomen, perhaps to exude the necessary pheromones in order to excite the male enough to initiate courtship (Jallon *et al.*, 1984; Jallon and David, 1987; Cobb and Jallon, 1990). The song consists of phrases of sine song generated by low amplitude vibration with the wing held horizontally to the body (Cowling and Burnet, 1981; Robertson, 1983) and trains of pulses (involving high amplitude vibration with the posterior margin of the wing lowered) mixed together, in no particular order (Robertson, 1983). Within a train of pulses, the interpulse intervals change, in magnitude, giving rise to three distinct types of phrases (Robertson, 1983):

1) Type A: Irregular and widely spaced IPI's becoming
more regular as courtship progresses;
2) Type B: More regular than the first type with regularly
decreasing IPI's;
3) Type C: Constant short IPI's.

I also observed these three types of IPI's. At the beginning of a burst, the IPIs are long, but become more regular and constant as a burst progresses; towards the end of the burst the IPIs begin to decrease in length, in a regular and constant fashion until the male stops vibrating his wing .

Figure 4.1.1 (below) shows the IPI distribution of a *D. mauritiana* Sweden (red columns), Indiana (blue columns) and France (yellow columns) song. The distribution of IPI for the *D. mauritiana* strains is clearly unimodal, but broader, in shape, than the IPI distribution of the *D. melanogaster* Brighton song and narrower than the *D. simulans* Florida song (see section 3.2- Figure 3.2.1). Modal IPIs in these 3 examples lie between 30-35ms, 30-35ms and 35-40ms, respectively. Note that the IPI distribution of the former two strains is slightly narrower than the latter one.
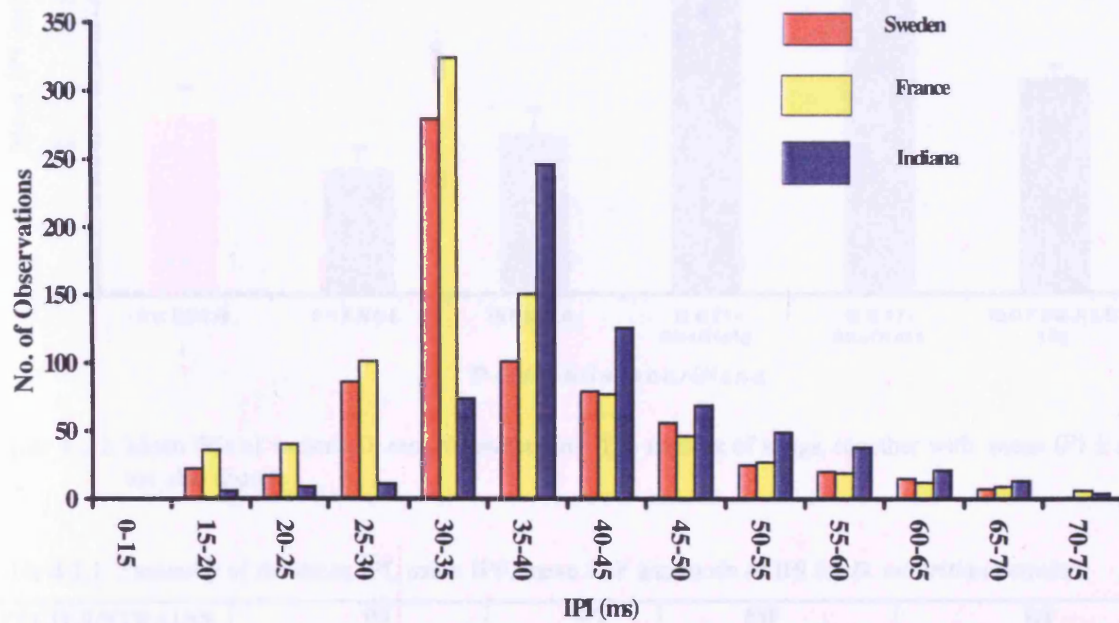
Figure 4.1.1: The IPI distribution of *D. mauritiana* songs - Each strain is represented by one typical song.

Figure 4.1.2 (below) shows the mean IPIs of the various strains that were recorded, but only *D. mauritiana* Sweden, France and Indiana produced robust songs that could be used for rhythm analysis (see section 4.2). The overall mean IPI of the *D. mauritiana* songs of several strains, recorded here, ranged from ~32-61ms. The wide range was due to the longer IPIs of the the three strains with poorer courtship (MG17, MG71 and Isofemale 102). One-way ANOVA of IPI's from all the strains recorded, revealed a significant strain effect (F = 5.50, df = 5, 39, p = 0.001). Unfortunately, it had not been possible to obtain any intraspecific hybrids, as all the intraspecific male hybrids died before reaching the age of 3 days. Since the one-way ANOVA of the mean IPIs between the 'principle' strains of *D. mauritiana*, that were eventually to be used in rhythm analysis, namely *D. mauritiana* Sweden, France and Indiana, did not show any significant differences

67

($p=0.320$), these strains were treated as one overall species. Table 4.1.1 (below) shows a summary of the mean IPI, mean IPF, mean SSF and modal IPI for the three *D. mauritiana* strains, that were used for rhythm analysis (see section 4.2). IPF and SSF measurements were taken from between 2-4 flies for each strain.
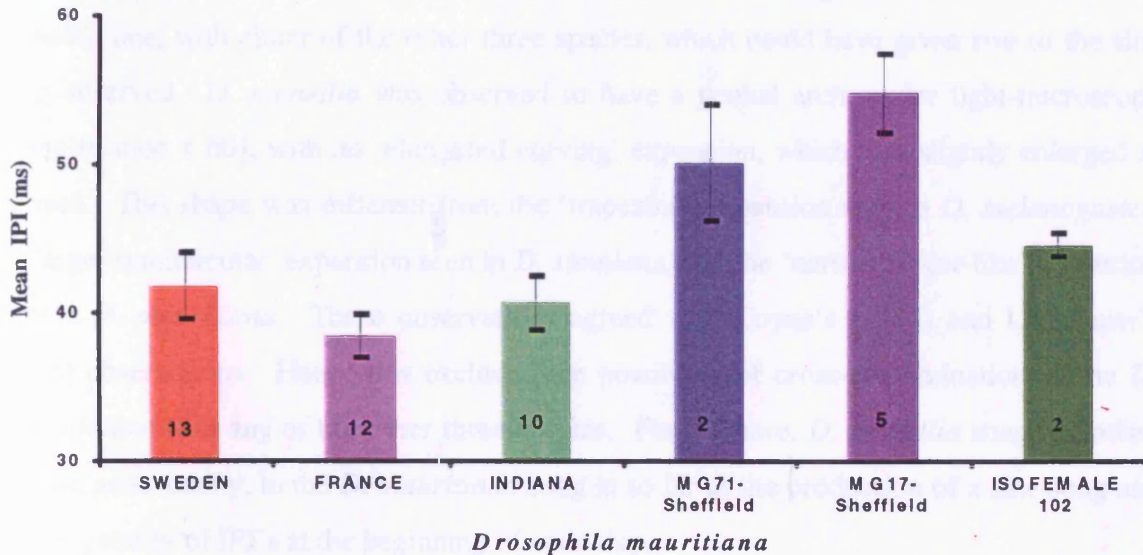
Figure 4.1.2: Mean IPIs of various *D. mauritiana* strains. The number of songs, together with mean IPI ± sem are also shown.

Table 4.1.1: Summary of the mean IPI, mean IPF, mean SSF and mode of IPI for *D. mauritiana* strains

SPECIES/STRAINS	IPI MEAN (ms) ± sem	IPI MODE (ms)	SSF MEAN (Hz) ± sem	IPF MEAN (Hz) ± sem
D. mauritiana Sweden	41.81 ± 2.23	27.31	190.64 ± 5.49	247.75 ± 1.31
D. mauritiana France	38.50 ± 1.53	32.33	175.80 ± 4.44	254.31 ± 1.17
D. mauritiana Indiana	39.74 ± 2.16	30.50	200.20 ± 17.3	269.02 ± 3.64

In summary, the IPI distribution, mean IPI, IPF and SSF results of the *D. mauritiana* strains, presented in this study, are in agreement with Cowling and Burnet's (1981) and Robertson's (1983) observations.

D. sechellia (Cambridge) song:

This song mainly consists of trains of pulses. Contrary to Cobb *et al.* (1989), I found that the *D. sechellia* song does contain some sine song which was not found in every *D. sechellia* song- the sine song was present in 5 out of the 12 *D. sechellia* songs recorded (see Figure 4.1.3-below). Since all the species of the *D. melanogaster* complex are

morphologically similar to one another, the only reliable distinguishing characteristic for their identification are the male genitalia, where the process of the genital arch (epandrium) has a species-distinguishing shape (Coyne, 1983; Lemeunier, 1986). Therefore, an examination of the male genitalia of the four member species of the *D. melanogaster* complex was warranted in order to exclude any possibility of cross-contamination that might had occurred to the *D. sechellia* line, with either of the other three species, which could have given rise to the sine song observed. *D. sechellia* was observed to have a genital arch, under light-microscopy [magnification x 60], with an 'elongated curving' expansion, which was slightly enlarged at its apex. This shape was different from the 'trapezoid' expansion seen in *D. melanogaster*, the large 'semicircular' expansion seen in *D. simulans*, and the 'narrow finger-like' expansion seen in *D. mauritiana*. These observations agreed with Coyne's (1983) and Lemeunier's (1986) observations. Hence this excluded the possibility of cross-contamination of the *D. sechellia* line with any of the other three species. Furthermore, *D. sechellia* song is similar, at least, acoustically, to the *D. mauritiana* song in so far as the production of a sine song and the irregularity of IPI's at the beginning of courtship.

SINE SONG   **PULSES**

~70ms

Figure 4.1.3: The song components of *D. sechellia*.

Figure 4.1.4 (see below) shows the IPI distribution of a *D. sechellia* Cambridge song. Here again, the IPI distribution is unimodal, with a mode IPI varying between 60-65ms. However, there is a much broader IPI distribution than in the rest of the members of the *D. melanogaster* complex:

Figure 4.1.4: The IPI distribution of a *D. sechellia* Cambridge (Se8) song.

The mean IPI of *D. sechellia* songs so far examined range from ~53-89ms, which are in close agreement to the results found by Cobb *et al.* (1989). The wing display repertoire is observed to be limited to 'scissoring' only (Cobb, 1989). The IPI and modal IPI measurements were taken from 12 flies, the SSF measurements were taken from the 5 flies that produce the hum song, whereas the IPF measurements were taken from 4 flies. Table 4.1.2 shows the mean IPI, mean IPF, mean SSF and mode of IPI for *D. sechellia*:

Table 4.1.2: Summary of the mean IPI, mean IPF, mean SSF and mode of IPI for *D. sechellia* Cambridge

SPECIES/STRAIN	IPI MEAN (ms) ± sem	IPI MODE (ms)	SSF MEAN (Hz) ± sem	IPF MEAN (Hz) ± sem
D. sechellia Cambridge	71.93± 2.39	57.33	154.80 ± 21.6	352.89 ± 2.90
	86.30 ± 5.60	76-80	-	252.00 ± 7.20

KEY : Bold - Results from Present Study   Italics - Results from Cobb *et al.* (1989)

4.2 Do song rhythms exist in *D. mauritiana* and *D. sechellia*?

Before applying the spectral analysis methods to the data, the usual preliminary procedures were followed. A song was only considered for rhythm analysis, if more than 50% of its bins were filled. Then the IPI means were analysed with the CLEAN

Figure 4.1.4: The IPI distribution of a *D. sechellia* Cambridge (Se8) song.

The mean IPI of *D. sechellia* songs so far examined range from ~53-89ms, which are in close agreement to the results found by Cobb *et al.* (1989). The wing display repertoire is observed to be limited to 'scissoring' only (Cobb, 1989). The IPI and modal IPI measurements were taken from 12 flies, the SSF measurements were taken from the 5 flies that produce the hum song, whereas the IPF measurements were taken from 4 flies. Table 4.1.2 shows the mean IPI, mean IPF, mean SSF and mode of IPI for *D. sechellia*:

Table 4.1.2: Summary of the mean IPI, mean IPF, mean SSF and mode of IPI for *D. sechellia* Cambridge

SPECIES/STRAIN	IPI MEAN (ms) ± sem	IPI MODE (ms)	SSF MEAN (Hz) ± sem	IPF MEAN (Hz) ± sem
D. sechellia Cambridge	**71.93± 2.39**	**57.33**	**154.80 ± 21.6**	**352.89 ± 2.90**
	86.30 ± 5.60	*76-80*	-	*252.00 ± 7.20*

KEY : Bold - Results from Present Study   **Italics - Results from Cobb *et al.* (1989)**

4.2 Do song rhythms exist in *D. mauritiana* and *D. sechellia*?

Before applying the spectral analysis methods to the data, the usual preliminary procedures were followed. A song was only considered for rhythm analysis, if more than 50% of its bins were filled. Then the IPI means were analysed with the CLEAN

and VdB analyses together with the Monte Carlo simulations, and the results for the various strains of *D. mauritiana* are shown on Table 4.2.1 (below), using different IPI cut-off points. After examining the distributions of the IPIs, for the various strains of *D. mauritiana*, the cut-off points that were eventually employed for the spectral analyses were 75 and 15ms or 80 and 15ms.

Table 4.2.1: Spectral analyses of the songs from the three principle strains of *D. mauritiana*, using lower and higher cut-off points

SPECIES	Clean		Van den Berg		Observations
	1st Peak	**2nd Peak**	**1st Peak**	**2nd Peak**	**Bins Filled**
mauritiana	**CUT Off POINTS : 75 & 15 ms**				
Ma5t10l1	31.20	128.57	125.00	x	29/38
Mas1t13r	173.91	x	181.82	26.85	31/42
Mas1t6l2	30.43	53.91	25.24	x	11/14
Mas1t6r1	30.39	54.03	30.53	x	33/55
Mau2t8l2	20.04	63.55	64.52	444.44	26/42
Mau3t8l2	30.30	x	30.30	27.49	33/38
Mau3t8lr1	39.41	x	26.14	83.33	36/48
Mh1t14l2	54.31	35.71	54.79	x	25/31
Mi3t13l1	30.30	x	67.23	x	25/30
Mi4t10r1	39.52	x	40.00	800.00	51/51
Mi5t10r1	192.31	x	x	x	17/27
Mi6t10r1	47.41	196.43	205.13	47.90	32/46
SPECTRAL ANALYSIS					
SPECIES	Clean		Van den Berg		Observations
	1st Peak	**2nd Peak**	**1st Peak**	**2nd Peak**	**Bins Filled**
mauritiana	**CUT Off POINTS : 80 & 15 ms**				
Ma5t10l1	32.32	24.66	123.08	x	31/38
Mas1t13r	176.99	24.81	24.77	x	31/42
Mas1t6l2	25.42	x	25.40	x	12/14
Mas1t6r1	30.39	54.03	30.53	54.05	33/55
Mau2t8l2	20.04	63.33	64.00	444.44	26/42
Mau3t8l2	30.30	27.32	30.30	27.49	34/38
Mau3t8lr1	x	x	26.14	83.33	39/48
Mh1t14l2	54.31	35.71	54.79	x	25/31
Mi3t13l1	x	x	67.23	x	27/30
Mi4t10r1	39.52	x	40.00	800.00	51/51
Mi5t10r1	x	x	x	x	18/27
Mi6t10r1	47.41	196.43	205.13	47.90	32/47

Any 30s cycle or less is assumed to be arrhythmic, unless a second significant peak in the spectrogram was found. For example, in the 75 & 15ms section of table 4.2.1a for the Mau2t8l2 song, the second significant peak value, in the CLEAN analysis, was taken to be the period because this value is similar to the corresponding primary one in the VdB analysis. Even when the slightly higher cut-off points of 80 & 15ms were employed,

several striking changes occurred to the periods of several songs; for example, songs like Mas1t6l2, Mau3t8r1 and Mi3t13l1 become arrhythmic.

The highlighted values in table 4.2.1 (see above) are the values that have been used to determine the rhythm periods of each song. It can clearly be seen that the CLEAN and the corresponding VdB values, for each individual song, are approximately similar. Song Mi3t13l1 gives a 30.35s period with CLEAN, but a 67.23s period with VdB, and is the only one which is problematic. This song becomes arrhythmic with the 80ms upper cut-offs with CLEAN, so it clearly has a fragile cycle.

Figure 4.2.1 (below) shows the distribution of the significant periods of the *D. mauritiana* songs that were determined with the CLEAN spectral analysis, using the lower cut-off points of 75 & 15ms. The overall species period for *D. mauritiana* is 39.69(s) ± 3.74. If the higher 80 & 15ms cut-off points were used the overall species period becomes 42.51 s ± 4.89. The overall species song period for *D. mauritiana* determined here, is in close agreement with Kyriacou's preliminary investigations into the song periods of this species (C.P. Kyriacou, pers. comm.).

Cut-off Points:- 75 & 15 ms
Mean Period: 39.69(s) ± 3.74 (sem)

N= Number of Observations, Red Line= Expected Normal Distribution
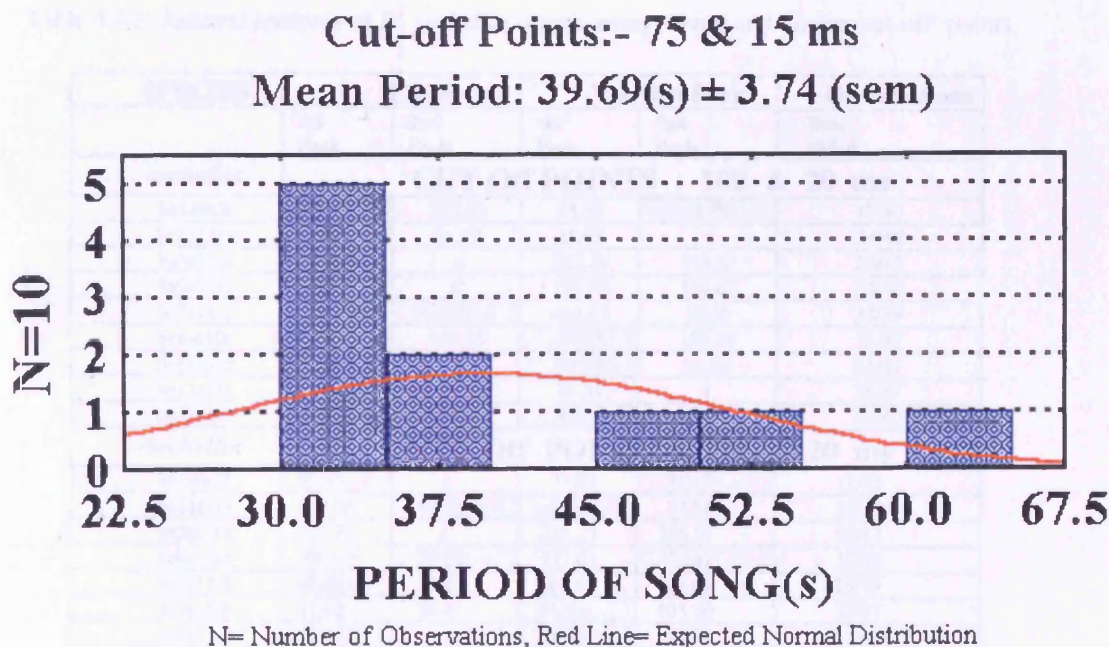
Figure 4.2.1: The distributions of the periods of *D. mauritiana* songs, using the lower cut-off points, after the Clean spectral analysis had been employed. The mean period ± sem is also shown.

Out of the 12 *D. mauritiana* used for rhythm analysis, 2 were found to be arrhythmic. The majority of the rhythmic *D. mauritiana* songs demonstrated periods

between 30-40s. Figures 4.2.2-4.2.4 show the results of representative *D. mauritiana* songs. In all the Figures, graph (a) depicts the regression found initially on the period from the VdB analysis, together with its corresponding F-ratio and degrees of freedom, and (b) and (c) show the spectrograms of the song with the CLEAN and VdB procedures. In Figure 4.2.2a, a ~120s cycle can be seen, on which a period of 32s is superimposed. This 32s cycle is significant with CLEAN, but not quite significant with VdB. Figures 4.2.3 and 4.2.4 show unambiguous 30-40s cycles in both spectral analyses.

D. sechellia is the last member of the *D. melanogaster* complex to be examined for the existence of song rhythms. The same preliminary data manipulations, described in section 3.3 and at the beginning of this present section, were carried out. The results for *D. sechellia* are shown on Table 4.2.2 (below), using different cut-off points. After examining the distributions of the IPIs of *D. sechellia*, the cut-off points that were eventually employed for the spectral analyses were, 100 & 20ms and 120 & 20ms (approximately twice the mean IPI). When 80ms was considered as the upper cut-off point, almost a sixth of the IPIs were discarded, therefore this cut-off point was rejected. Any periods of 30s or less are assumed to be arrhythmic, unless a secondary significant peak in the spectrogram was found.

Table 4.2.2: Spectral analyses of *D. sechellia* songs, using lower and higher cut-off points.

SPECIES	Clean		Van den Berg		Observations
	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
sechellia	CUT Off POINTS : 100 & 20 ms				
Se1e6s2l	52.63	283.02	23.05	36.70	17/43
Se2S1lrl	169.06	24.89	25.08	x	34/49
Se5s1lrl	232.95	x	235.29	106.67	29/43
Se6s1lrl	500.00	x	137.93	106.67	35/50
Se7s1lr2	196.00	31.01	666.67	25.00	24/39
Se8se10r	50.46	103.29	51.61	103.90	33/47
Se8s1lr2	52.11	x	51.95	29.96	34/47
Se11s1lr	26.36	x	21.51	x	35/37
Se12s1lr	43.57	158.73	44.94	22.92	40/42
sechellia	CUT Off POINTS : 120 & 20 ms				
Se1e6s2l	52.63	x	23.05	36.70	17/32
Se2s1lrl	169.06	40.38	41.18	25.08	35/49
Se5s1lrl	232.95	x	235.29	106.67	29/43
Se6s1lrl	500.00	70.29	137.93	68.97	37/50
Se7s1lr2	42.36+	24.85	666.67	25.00	25/39
Se8se10r	51.40	25.17	51.61	103.90	33/47
Se8s1lr2	51.89	x	51.95	29.96	35/47
Se11s1lr	34.31+	26.36	21.51	34.52	36/37
Se12s1lr	43.57	158.73	41.94	22.92	40/42

(A) period = 123.3s F=4.062, df=3,25

(B) 31.20s, 128.57s   CLEAN

(C) 125.00s   VdB
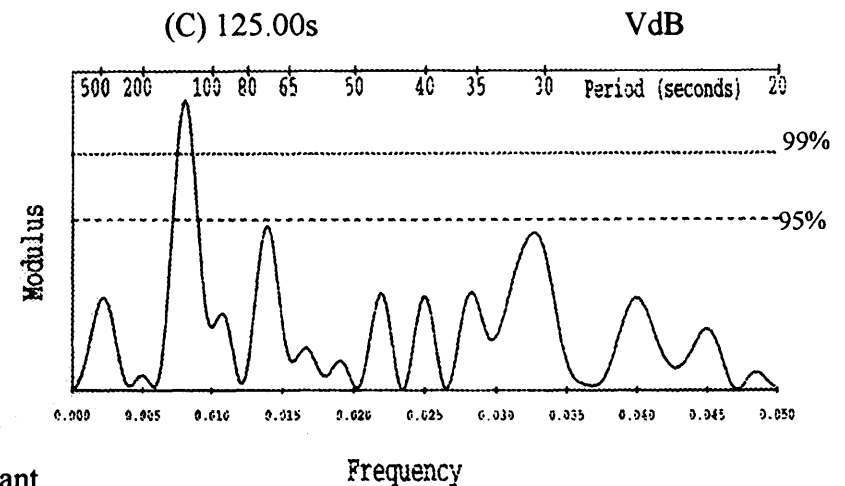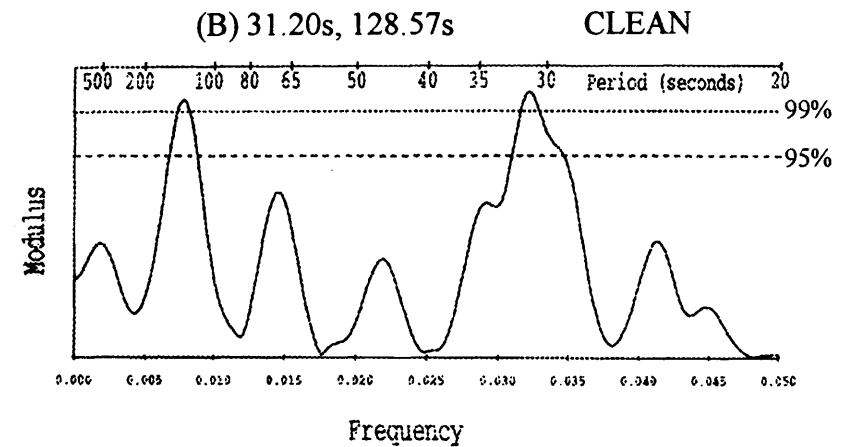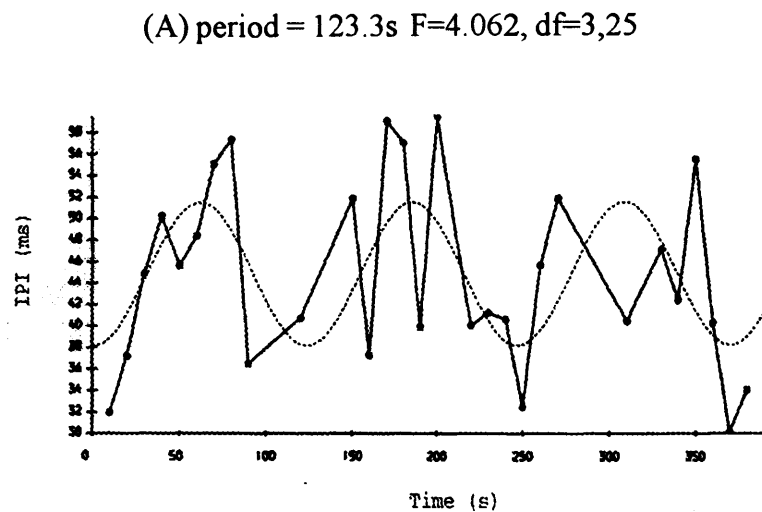
Figure 4.2.2:

D. mauritiana Sweden courtship song (Ma5t10l1)analysed-see table 4.2.1 (upper half), using the lower 75 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure. Note that on a ~120-s cycle, a ~32s period is superimposed.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 31.20s (p<0.01), as well as a weaker secondary peak of 128.57s (p<0.01), with the CLEAN analysis. Also note that there is a significant primary peak of 125s (p<0.01), as well as a weak peak at ~30s that does reach the 5% confidence limit, with the VdB analysis. The 95 and 99% confidence limits are defined by Monte Carlo simulations (see chapter 2).

(A) period = 30.1s F=2.993, df=3,29

(B) 30.30s   CLEAN

(C) 30.30s, 27.49s   VdB
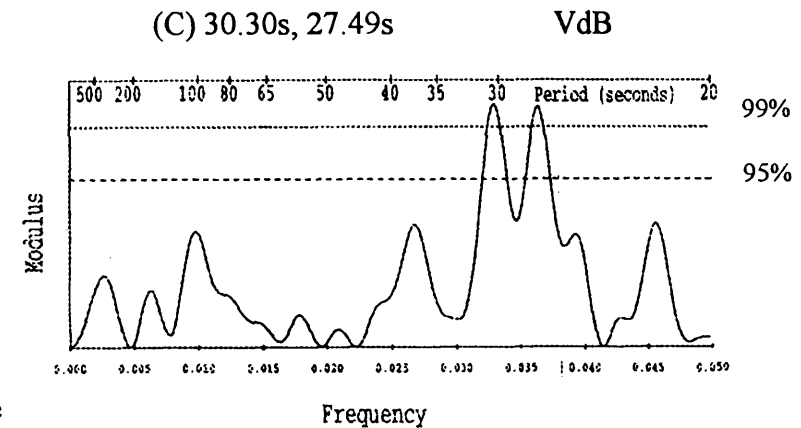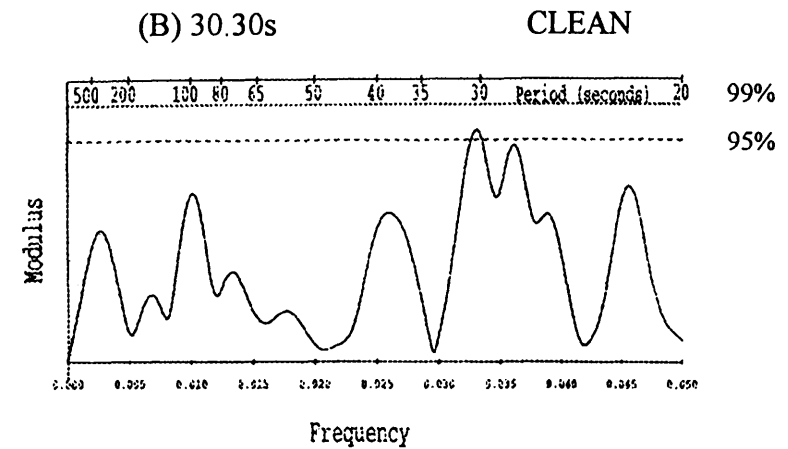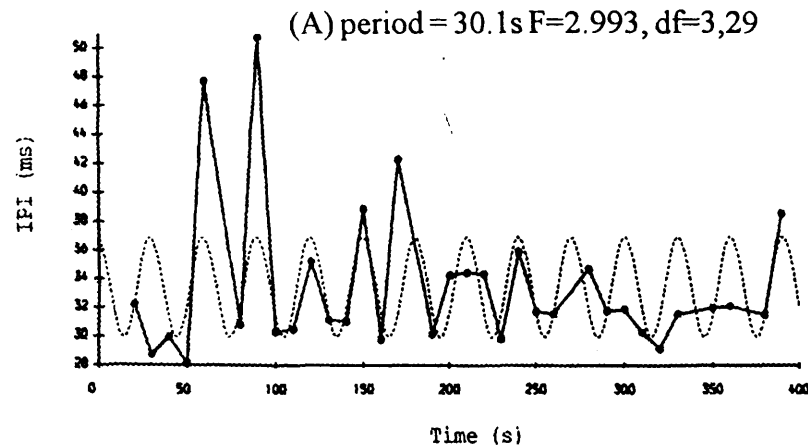
Figure 4.2.3:

D. *mauritiana* France courtship song (Mau3t8l2) analysed-see table 4.2.1 (upper half), using the lower 75 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 30.30s (p<0.05), with the CLEAN analysis. In addition, note there is the highest peak of 30.30s (p<0.01) as well as a high secondary peak of 27.49s (p<0.01), with the VdB analysis. The 95 and 99% confidence limits are defined by Monte Carlo simulations (see chapter 2).

(A) period = 39.8s F=8.182, df=3,47

(B) 39.52s   CLEAN

(C) 40.00s, 800.00s   VdB
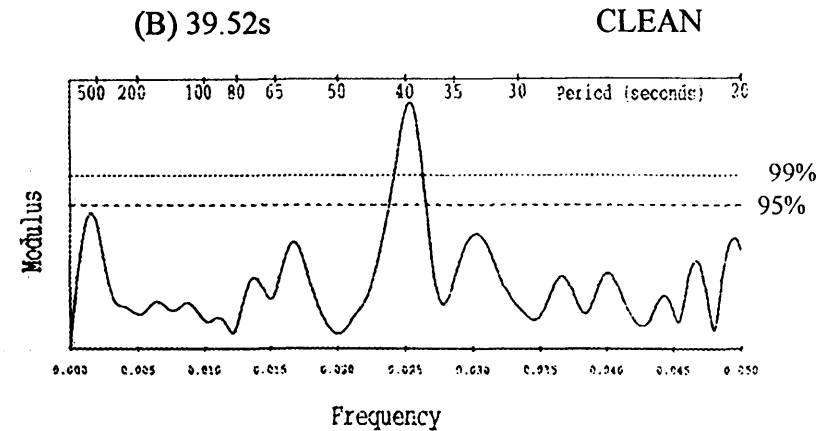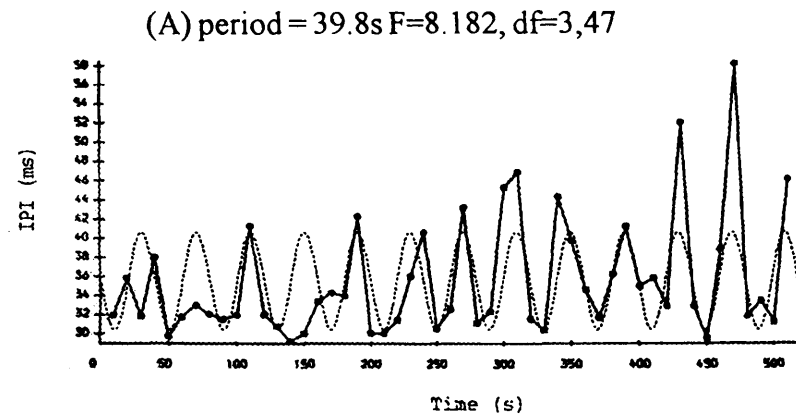
Figure 4.2.4:

D. mauritiana Indiana courtship song (Mi4t10r1) analysed-see table 4.2.1 (upper half), using the lower 75 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 39.52s (p<0.01), with the CLEAN analysis. Also note a significant primary peak of 40s, as well as a weaker secondary peak of 800s ('climber' range) (p<0.01) with the VdB analysis. The 95 and 99% confidence limits are defined by Monte Carlo simulations (see chaper 2).
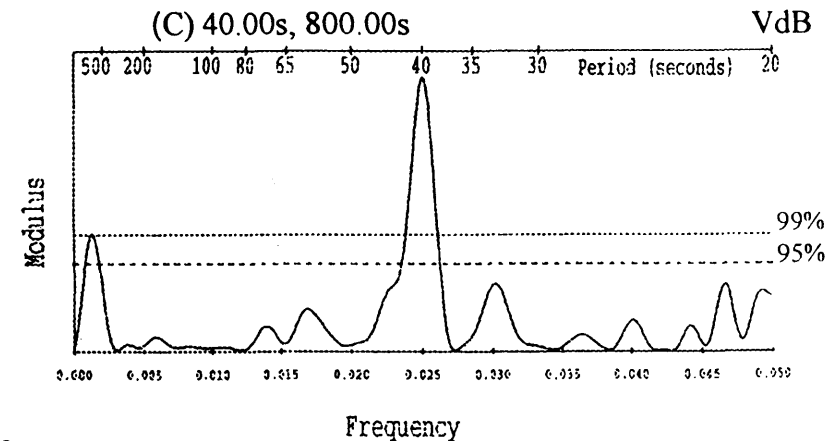
In the 120 & 20ms section of table 4.2.2, the Se2s11r1 song has a primary peak of 169.06s, with the CLEAN analysis, which represents a 'climber', but in addition, it shows a second significant peak of 40.38s and a corresponding primary peak of 41.18s, with the VdB analysis, which is taken to be the period of this song. The Se6s11r1 shows a primary peak, in the 'climber' range, but also has a significant secondary peak of 70.29s, which is also present as a significant secondary peak of ~69s, with the VdB analysis. In the cases of Se8se10r, Se8s11r2, Se11s11r and Se12s11r, there are significant primary or secondary peaks of similar value, with both spectral analyses. With Se1e6s2l and Se7s11r2, there are significant primary peaks of 52.63s and 42.36s, respectively, with CLEAN, but the former song has a 36.7s secondary period with VdB and the latter shows only a climbing frequency with VdB. Finally, Se5s11r1 shows a long-term trend ('climber'), with both of spectral analyses and was therefore considered arrhythmic.

Several *D. sechellia* songs in table 4.2.2 are marked with the (+) sign to indicate changes in period with different cut-offs. Figure 4.2.5 (below) shows the distribution of the periods of the *D. sechellia* songs determined with CLEAN. When using the higher cut-off points (120 & 20ms) the overall species period for *D. sechellia* is 48.35(s) ± 3.87 (n=8). When the lower cut-off points were employed the overall species period changes slightly to 45.96 s ± 4.07 (n=5)-see Figure 4.2.5.

Cut-off Points:- 120 & 20 ms
Mean Period: 48.35(s) ± 3.87 (sem)

Cut-off Points:- 100 & 20 ms
Mean Period: 45.96(s) ± 4.07 (sem)

N = Number of Observations, Red Line = Expected Normal
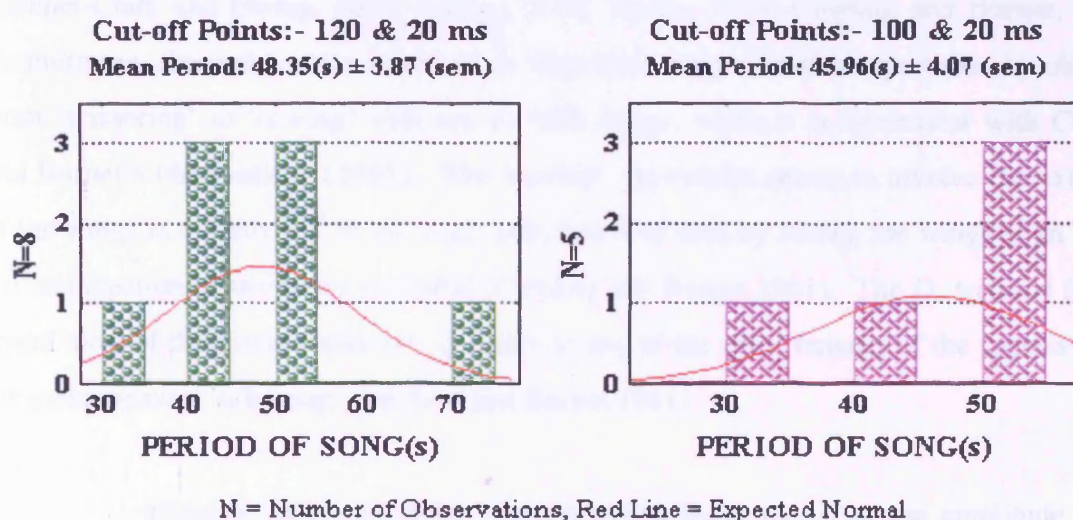
Figure 4.2.5: The distributions of the periods of *D. sechellia* songs, using the different cut-off points, with CLEAN. The mean periods ± sem are also shown.

Out of the 9 *D. sechellia* songs employed for rhythm analysis, one was found to be arrhythmic, using the upper IPI cut-offs of 120ms. From the 8 rhythmic songs, 6 songs had periods between 40-52s, one had a period between 30-40s and one had a period of ~70s. Figure 4.2.6 illustrates the results of a *D. sechellia* song.

4.3 The *D. yakuba* complex: *D. teissieri*, *D. orena* and *D. erecta*.

D. teissieri (Sweden) song:

D. teissieri, unlike *D. yakuba*, produces both sine (see Figure 4.3.1a) and pulse song components. Cowling and Burnet (1981), found that the *D. teissieri* song has a very short mean IPI ~20ms. During recording, I observed that the male seems to come to a complete standstill for an instant, before it resumes running around the recording chamber, while trying to track the female. It is not certain whether this has any behavioural significance, i.e., being some sort of ritual before courtship begins in earnest, but it was observed in almost all the *D. teissieri* songs recorded. Another observation that was almost universal to the songs recorded was that the *D. teissieri* male vibrates both wings in a horizontal orientation and does not track and orientate at the correct position in relation to the female in order to initiate courtship, as observed in the *D. melanogaster* courtship (Bennet-Clark and Ewing, 1968; Ewing, 1970; Tsacas, 1971; Cowling and Burnet, 1981). Furthermore, the males were observed to vary their wing vibration repertoire, by changing from 'scissoring' to 'rowing' with one or both wings, which is in agreement with Cowling and Burnet's observations (1981). The 'rowing' movement seems to involve the extension of the wings at roughly 90^0 to the body axis, followed then by raising the wings at an almost vertical position before being re-folded (Cowling and Burnet,1981). The *D. teissieri* females spend most of their time stationary, contrary to any of the other females of the species within the *melanogaster* subgroup (Cowling and Burnet,1981).

Extreme care was taken when analysing these songs, as the amplitude of the primary and secondary peaks in each "pulse" alter in relation to each other during most song bursts; this means that the "primary" and "secondary" peaks alternate as high and low

(A) period=51.6s F=3.506, df=3,32

Time (s)

(B) 51.89s   CLEAN

Frequency

(C) 51.95s, 29.96s   VdB

Frequency
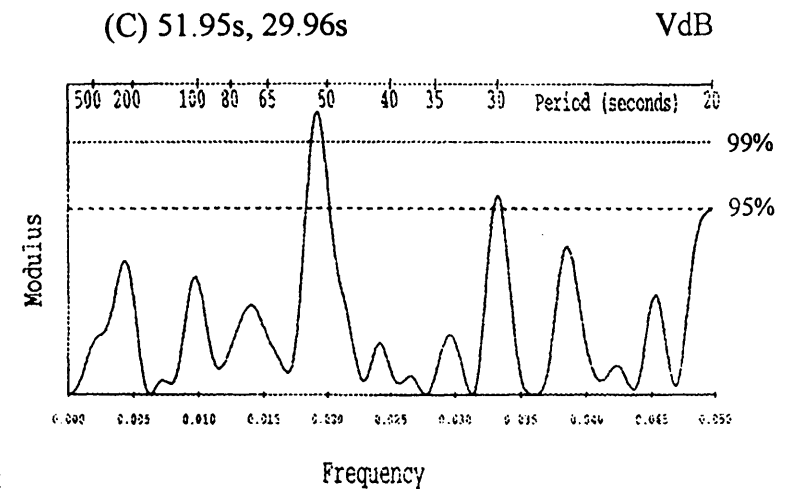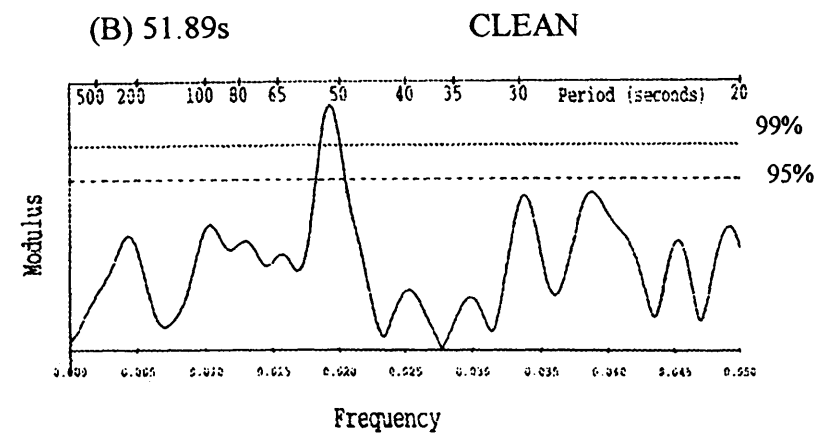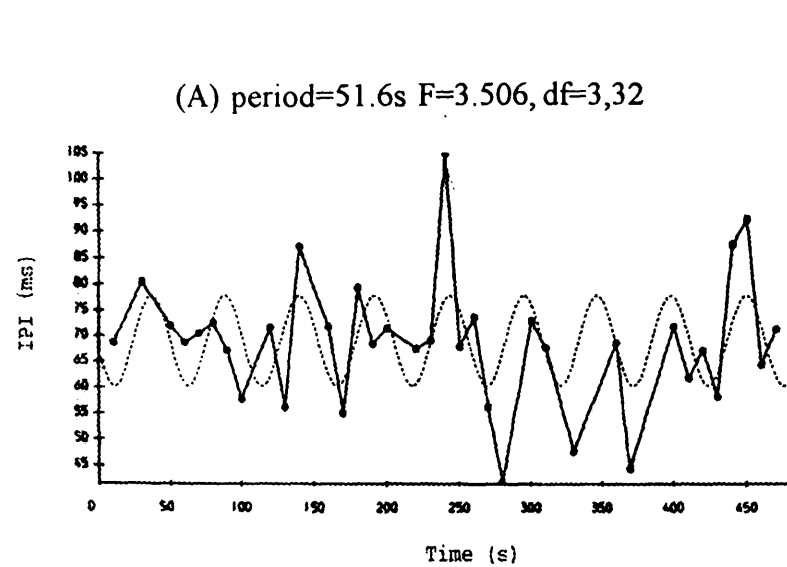
Figure 4.2.6:

D. sechellia courtship song (Se8s11r2) analysed- see table 4.2.2
(lower half), using the higher 120 & 20ms cut-off points, and spectral
analysis.

a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the data, initially defined by VdB spectral analysis,
   and then fitted to the data by non-linear regression, using a least squares
   procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods. Note that there is a significant
   primary peak of 51.89s (p<0.01), with the CLEAN analysis. Also note that there
   is a significant primary peak of 51.95s, as well as a significant secondary peak
   of 29.96s (p<0.05), with the VdB analysis. The 95 and 99% confidence
   limits are defined by Monte Carlo simulations (see chapter 2).

SINE SONG   PULSES

a)

~28ms

D. teissieri   Sweden

b)

~48ms

D. orena   France

c)

~ 47ms
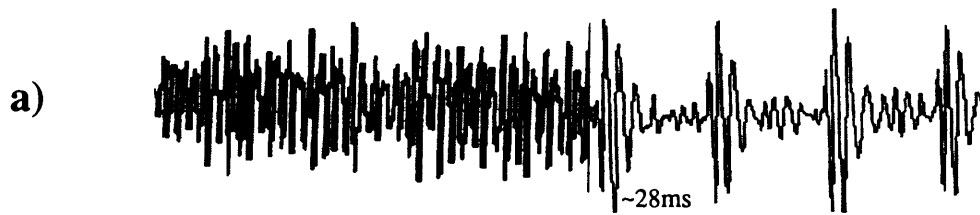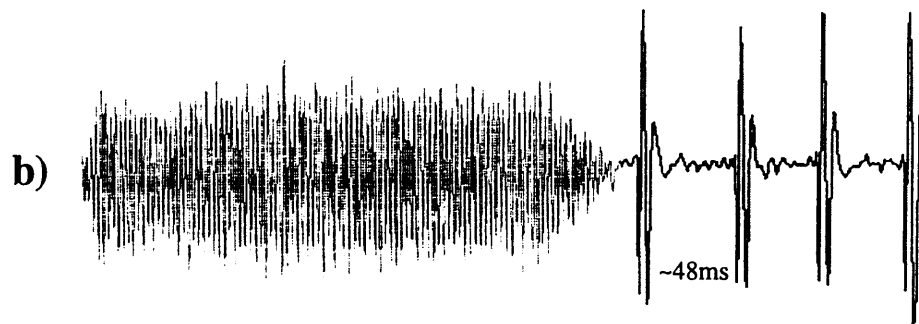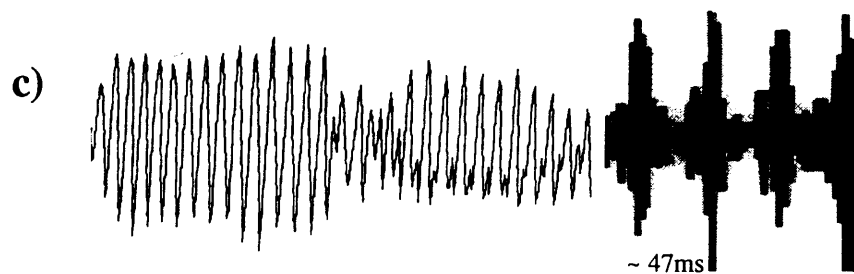
D. erecta   France

Figure 4.3.1 : The principle song elements produced by members
of the *D. yakuba* complex.

amplitude parts of subsequent pulses (see Figure 4.3.2). The IPIs are defined as being the distance between each "primary" peak, even if this 'primary' peak is lower in amplitude than some of the "secondary" pulses later on in the course of a burst.

Figure 4.3.2: The primary and secondary peaks of *teissieri* pulse.
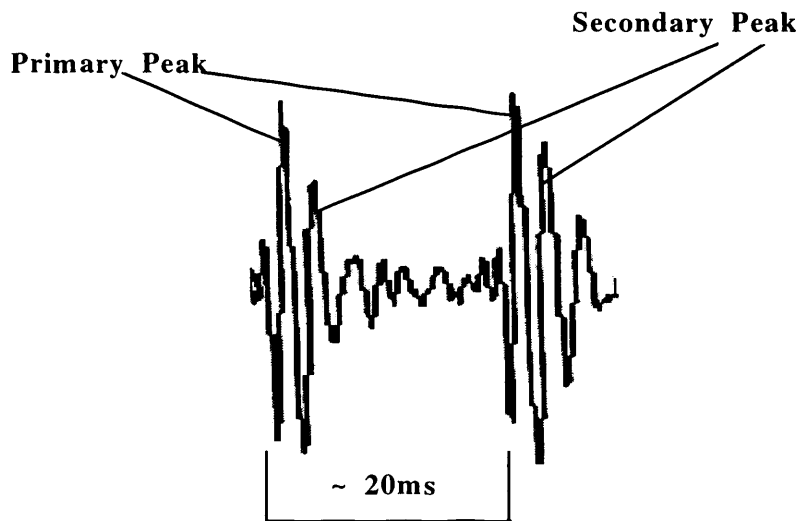
The mean IPI of individual songs ranged from ~20-45ms. The IPI and modal IPI measurement were taken from 20 flies recorded, whereas the IPF and SSF measurents were taken from 5 flies. For the overall species mean IPI, mean IPF, mean SSF and modal IPI, see table 4.3.1 at the end of this section.

D. orena (France) song:

D. orena males produce both pulse and sine song elements (see Figure 4.3.1b). Audibly, the sine song has a high frequency, but not as high as that produced by *D. erecta* (see below) which differs from Cobb *et al.* (1989). The pulse trains, sound like songs produced by certain species of the *D. montana* subgroup species (Hoikkala,1990). The sine song was observed to be produced by a horizontal slow movement of a single wing, which barely rises above the body axis, and in addition constitutes the majority of song; these aforementioned findings are in agreement with Cobb's observations (1989). On the contrary, the pulses are produced by vibrating both wings (Cobb,1989). The individual mean IPIs in this study, range from ~37-60ms. The IPI and modal IPI measurements were taken from

11 flies recorded, whereas the IPF and SSF measurents were taken from 4 flies. The overall species mean IPI, mean IPF and mode of IPI is given in table 4.3.1 (below). Once again, the sine song alternates with trains of pulses during the progression of the courtship without any apparent order. The lack of vigour of the courtship songs meant that certain measures had to be taken in order to obtain songs with an adequate number of individual IPIs, that could be used for subsequent song rhythm analysis. By trial-and-error, it was found that rearing both the females and the males in solitude, seemed to increase courtship intensity and thus generating more vigorous courtship.

D. erecta (France-light-bodied female) song:

D. erecta males also produce the two discrete song types during courtship, a very high frequency sine song (Cowling and Burnet, 1981), the highest in the subgroup, and a pulse song (see Figure. 4.3.1c) with individual songs' mean IPI's ranging from ~40-60ms. The IPI and modal IPI measurements were taken from 21 flies recorded, whereas the IPF and SSF measurents were taken from 5 flies. For the overal mean IPI, mean IPF and mode of IPI see table 4.3.1.

The wing display repertoire observed during courtship is quite different from that seen in the other species. This is comprised of a combination of single wing or simultaneous vibration of both wings (Cowling and Burnet, 1981). The single wing movement looks like a 'scissoring' movement, but is different from that observed in *D. simulans* and *D. mauritiana*. When both wings are used, the vibration is seen to be at 90^0 to the body axis (Cowling and Burnet,1981). The sine song is observed to be generated by vibrating both wings, whereas the pulse song is produced by the single wing (Cowling and Burnet, 1981). Furthermore, Ewing (1977) noted that the song of this species contains a pulse song which is polycyclic. This is confirmed by the results in this study (see Chapter 6). It was extremely difficult to obtain vigorous songs, so once again the males of the species were reared in solitude, in order to increase their courtship vigour.

Table 4.3.1: The mean IPI, mean IPF and the mode of IPI of *D. teissieri*, *D. orena* and *D. erecta*.

SPECIES/STRAINS	IPI (ms) MEAN ± sem	MODE OF IPI(ms)	SSF (Hz) MEAN ± sem	IPF (Hz) MEAN ± sem
D. teissieri (Sweden)	**28.02 ± 1.28**	**21.20**	**231.5 ± 4.35**	**432.66 ± 4.01**
	20.00 ± 0.40	20.00	*105.40 ± 0.90*	*316.50 ± 7.00*
D. orena (France)	**48.44 ± 1.78**	**32.89**	**183.16 ± 1.28**	**285.23 ± 3.42**
	40.00 ± 1.80	30-36	*320.0 ± 2.60*	*322.60 ± 7.30*
D. erecta (France)	**47.01 ± 1.28**	**34.78**	**274.10 ± 11.9**	**291.93 ± 2.56**
	40.90 ± 0.70	35.00	*246.20 ± 4.00*	*327.60 ± 7.30*

KEY : **Bold - Results from Present Study**   **Italics - Results from Cowling & Burnet (1981)**
Shaded-in values - Results from Cobb *et al* (1989)

Table 4.3.1 shows the species/strains mean IPI, mean IPF, mean SSF and mode of IPI for *D. teissieri*, *D. orena* and *D. erecta*, from the present study (Bold), from the Cowling and Burnet study (Italics), and from Cobb *et al.* (Shaded-in). Figure 4.3.3 (below) shows the distribution of IPIs of a *D. teissieri* song (black bars). The distribution is unimodal in shape, as are all the distributions of IPIs of the species/strains examined so far, but here the distribution is much narrower than any other, with a modal IPI between ~24-27ms. The red bars represent the distribution of IPIs for a *D. orena* song which is unimodal (mode 42-45ms) and much broader than the one depicted by the black columns of *D. teissieri*. The yellow bars, in Figure 4.3.3, illustrate the distribution of IPIs of a representative *Drosophila erecta* (light bodied female strain) France song. The distribution of the IPIs is unimodal in shape, broader than the *D. teissieri* IPI distribution, but narrower than the IPI distribution than that seen for the *D. orena* song.
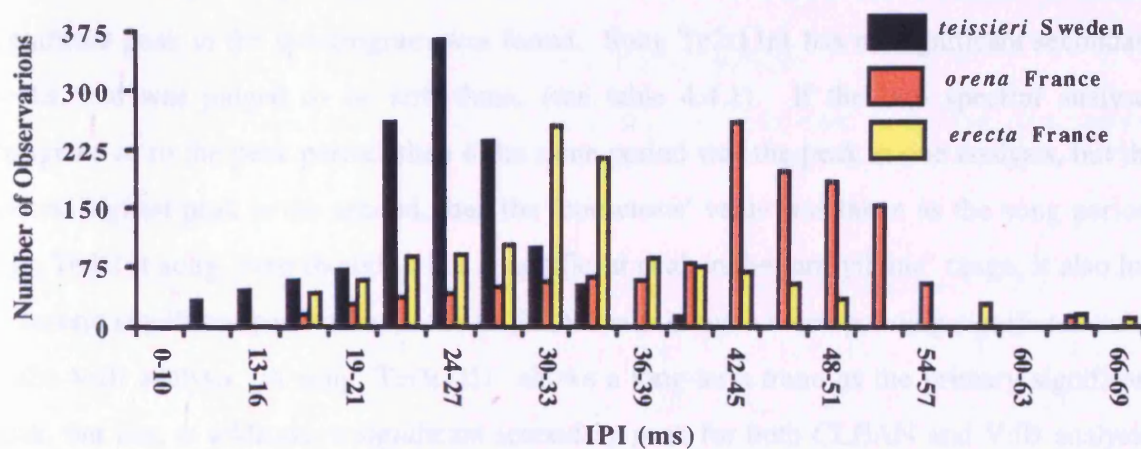
Figure 4.3.3: The IPI distribution of a *D. teissieri* Sweden (Tei1t12l1), *D. orena* France (Or3t11ra) and *D. erecta* France (El1t13l1).

4.4 Do song rhythms exist in *D. teissieri, D. orena* and *D. erecta*?

D. teissieri Sweden:

After examining the histograms of the distributions of IPIs of the *D. teissieri*, the cut-off points of 60 & 10ms were deemed as 'appropriate'-(approximately twice the IPI mean)- see table 4.4.1, upper part-below. When 55 & 10ms were employed (see Appendix 4.1), the two sets of data looked very similar, so I decided to adopt the former cut-off, as in some songs there was a significant number of IPIs in the range of ~50-56ms. Since the distance between the primary and secondary peaks (see Figure 4.3.2) in a pulse lies between ~5-10ms, 10ms were used as the lower cut-off.

Table 4.4.1: Spectral analysis of *D. teissieri* songs using 60 & 10ms cut-off points.

SPECIES	Clean		Van den Berg		Observations
	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
Teissieri	CUT Off POINTS : 60 & 10 ms				
Te1t12l2	31.18	22.50	31.13	36.87	29/57
Te2t1ll1	87.61	40.67	90.91	50.96	27/47
Te2t1lr1	24.46	x	23.05	x	14/20
Te2t6r1	37.14	32.44	148.15	x	30/60
Te3t1lr	21.23	71.22	71.43	26.94	41/79
Tei1t6r2	61.98	23.10	62.50	89.89	35/47
Tei2t6r2	85.33	21.64	86.02	21.68	30/59
Tei2t7r1	125.81	47.10	125.00	46.78	21/41
Tei3t7r1	69.71	92.31	70.80	87.91	62/78
Tei3r1t7	40.97	32.61	40.61	32.92	23/44
Tei3t1ll1	258.62	92.34	320.00	112.68	17/34
Tei6t6r2	88.24	x	97.56	x	14/19

Any 30s periods or less were assumed to be arrhythmic, unless a second significant peak in the spectrogram was found. Song Te2t11r1 has no significant secondary peaks, and was judged to be arrhythmic (see table 4.4.1). If the two spectral analyses disagreed as to the peak period, then if the same period was the peak in one analysis, but the second highest peak in the second, then the 'consensus' value was taken as the song period, e.g., Te3t11r song, even though it has a significant peak in the 'arrhythmic' range, it also has a second significant peak value in the CLEAN analysis and a corresponding significant value in the VdB analysis. A song, Tei3t1ll1 shows a long-term trend as the primary significant peak, but has, in addition, a significant secondary peak for both CLEAN and VdB analysis; therefore that secondary peak was included in the calculations for the overall mean period for the species.

When the lower cut-off points of 55 & 10ms, for *D. teissieri* were employed one song, Tei1t6r2 became arrhythmic (see Appendix 4.1). The highlighted values in Table 4.4.1 (see above) are the values that have been used to determine the rhythm periods of the species, and it can clearly be seen that the CLEAN and the corresponding VdB values, are approximately similar for each individual song.

Out of the 12 *D. teissieri* songs used for rhythm analysis, one was found to be arrhythmic (Te2t11r1-see table 4.4.1). From the 11 rhythmic songs, two had periods between 30-40s, Te1t12l2 and Te2t6r1, the latter having only a CLEAN period, one had a period between 40-50s and one had a period between 120-130s. The majority (7) of the rhythmic *D. teissieri* songs had periods between 60-90s.

Cut-off points: 60 & 10ms
Mean Period : 71.96(s) ± 8.50 (sem)
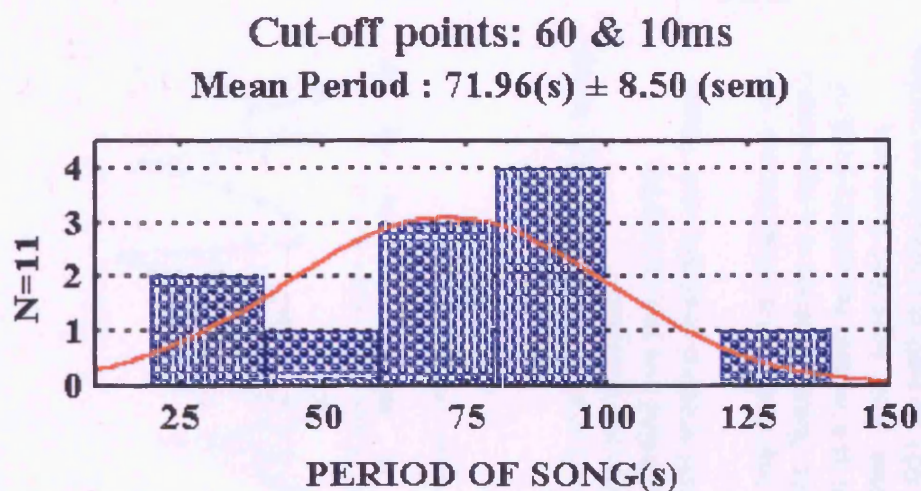
N= Number of Observations, Red Line= Expected Normal Distribution

Figure 4.4.1: The distribution of the significant periods for the *D. teissieri*, using 60 & 10ms cut-off points after CLEAN spectral analysis had been employed. The overall mean period ± sem are also shown.

From Figure 4.4.1, the overall species period for *D. teissieri* is 71.96(s) ± 8.50 (sem)-shown in Figure 4.4.1, which included all significant periods. A similar value of 78.39(s) ± 11.62 (sem) was obtained, using the lower 55ms cut-offs (see Appendix 4.1). Figure 4.4.2 illustrates the results of a representative *Drosophila teissieri* Sweden song.

(A) period = 70.1s F=2.362, df=3,43

(B) 69.71s, 92.31s   CLEAN

(C) 70.80s, 87.91s   VdB
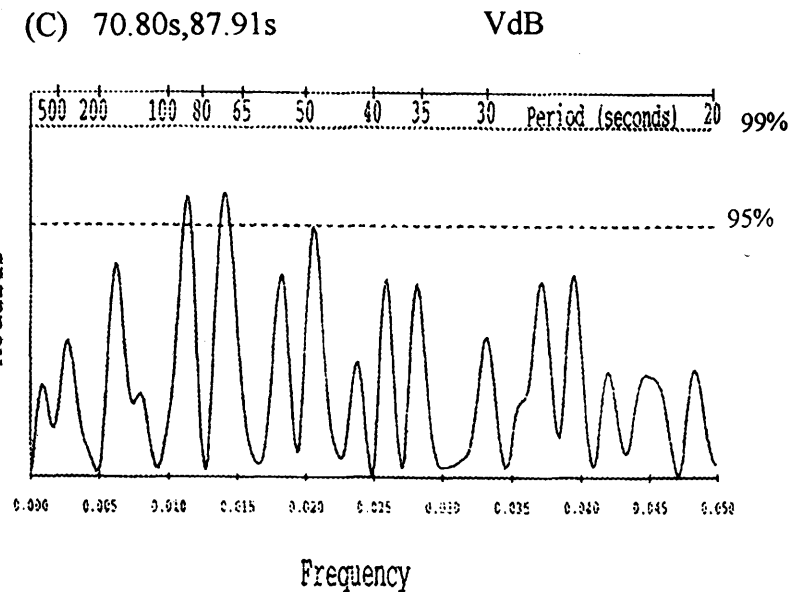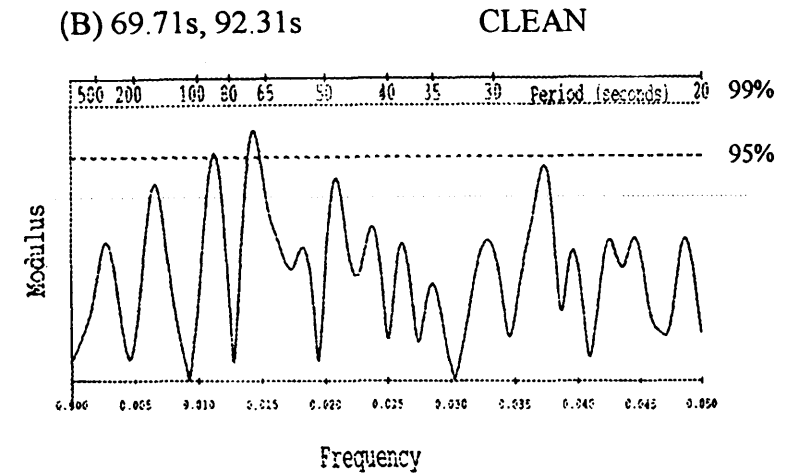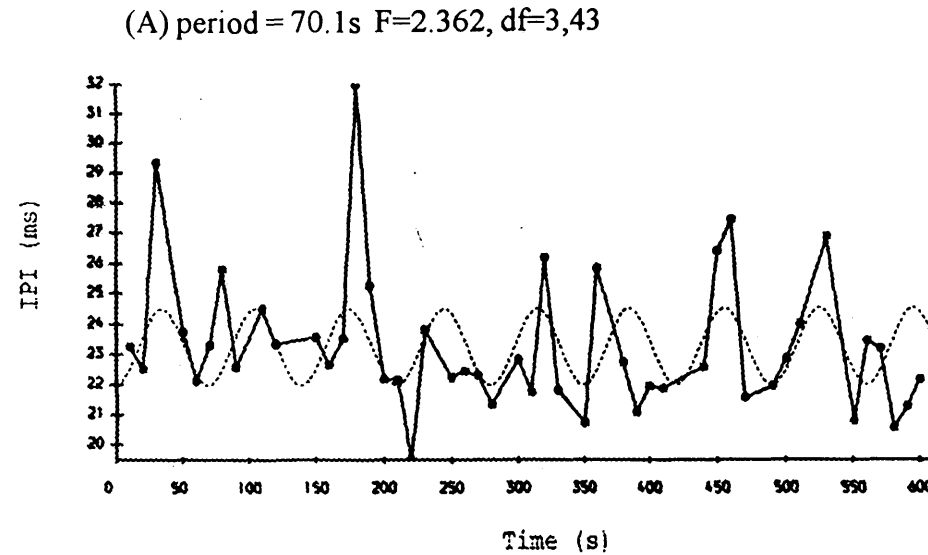
Figure 4.4.2:

D. teissieri Sweden courtship song (Tei3t7r1) analysed-see table 4.4.1, using the higher 60 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, defined by VdB spectral analysis, and fitted to the data by non-linear regression.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 69.71s (p<0.01), as well as a weaker secondary peak of 92.31s (p<0.01), with the CLEAN analysis. Also, note that there is a significant primary peak of 70.80s (p<0.05), as well as a weaker secondary peak of 87.91s (p<0.05), with the VdB analysis. The horizontal lines present the 95 and 99% confidence limits defined by Monte Carlo simulations (see chapter 2).

D. orena France:

After examining the histograms of the distributions of IPIs for *D. orena*, few IPIs were found to be longer than 75ms or less than 15ms. Therefore the cut-offs of 75 & 15ms and 80 & 15ms were employed. The usual rules guided the determination of the periods (see chapters 2 and 3). Table 4.4.2 (below) shows that 6 songs, namely, O1se10r2, O5se10r2, Or1t12l1, Or1t13r2, Or3t11ra and Or5t11r2 have no significant spectrogram peaks between the >30s and <100s range and were therefore judged to be arrhythmic. Song Or4t12l2, even though it has a significant peak of ~137s in the CLEAN analysis, its corresponding VdB value shows a 'long-term trend', so these values were ignored and the second significant peak, for CLEAN analysis was taken as the period, since it has a very similar corresponding VdB secondary value.

Table 4.4.2: Spectral analysis of *D. orena*, 75 & 15ms cut-off points.

SPECTRAL ANALYSIS:					
SPECIES	Clean		Van den Berg		Observations
	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
Orena	CUT Off POINTS : 75 & 15 ms				
O1se10r2	21.48	x	28.78	x	32/34
O2se10r2	46.82	22.29	22.10	x	20/31
O3se10r2	36.75	162.79	73.39	x	26/30
O4se10r2	66.39	326.53	95.24	67.23	33/34
O5se10r2	23.74	x	23.95	x	27/34
O6se10r2	39.07	24.15	39.41	x	29/33
O7se11la	39.27	32.33	38.83	32.79	29/33
O8se10r2	42.24	20.93	42.78	21.11	38/45
Or1t11ra	37.41	74.44	195.12	37.21	31/57
Or1t12l1	416.67	x	47059	x	38/52
Or1t13r2	156.36	x	203.69	x	11/14
Or2t11r2	42.57	343.75	43.48	275.86	33/57
Or2t12l1	34.58	23.57	34.33	23.67	26/45
Or3t11ra	28.21	x	28.37	25.64	30/60
Or3t12l1	52.56	x	52.98	23.39	28/42
Or4t12l1	51.25	21.94	50.96	133.33	58/74
Or4t12l2	137.06	72.97	615.38	71.43	58/74
Or5t11r2	28.69	x	28.88	x	23/30

Out of the 18 *D. orena* songs employed for rhythm analysis, 6 were found to be arrhythmic. From the 12 rhythmic songs, 8 songs had periods between 35-47s, two had a periods between 50-60s, one had a period of ~66s and one had a period of ~72s. Figure 4.4.3 shows the distribution of the CLEAN periods of the *D. orena* songs. The overall species period for *D. orena* is 46.82 s ± 3.50 (see Figure 4.4.3-below).

Figure 4.4.3: The distribution of the significant periods for the *D. orena*, using CLEAN.

Cut-off points: 75 & 15ms
Mean Period: 46.82(s) ± 3.50 (sem)

N= Number of Observations, Red Line= Expected Normal Distribution
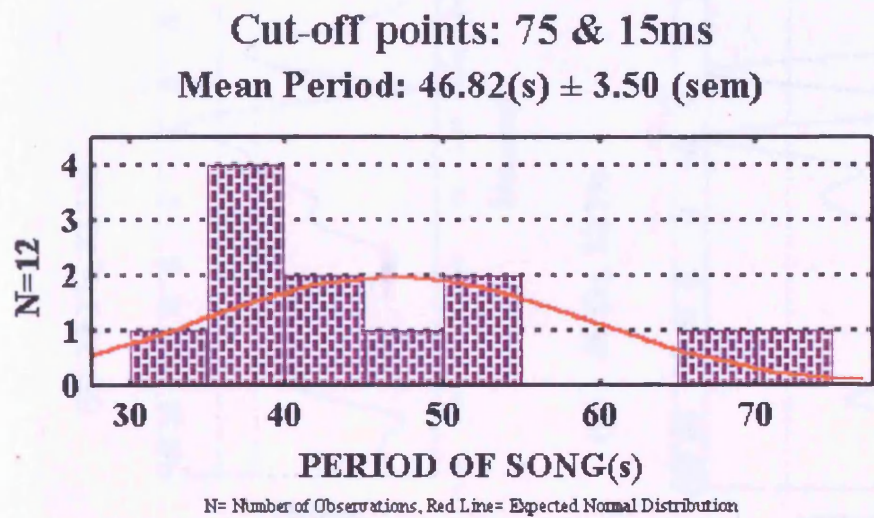
Figure 4.4.4 illustrates the results from a representative *Drosophila orena* France song.

Figure 4.4.4 illustrates the results from a *Drosophila orena* France song.

D. erecta France:

The same preliminary procedures that had been described and mentioned previously, were also applied to the *D. erecta* songs. The results are shown on Table 4.4.3, using different cut-off points, 80 & 15ms and 100 & 15ms (approximately twice the mean). Modifying the IPI cut-off points, gave significant changes. The highlighted values on table 4.4.3 (see below- upper half) are the values that have been used to determine the rhythm periods of the species, which are shown in Figure 4.4.5 (see below). It can, again, clearly be seen that the CLEAN and the corresponding VdB values, for each individual song, are approximately similar.

(A) period=38.8s F=3.622, df=3,25

Time (s)

(B) 39.27s, 32.33s   CLEAN

Frequency

(C)   38.83s, 32.79s   VdB

Frequency
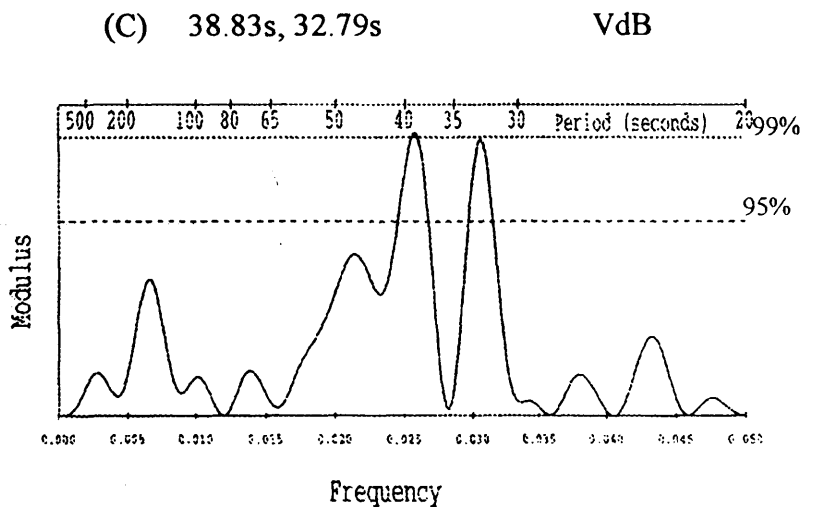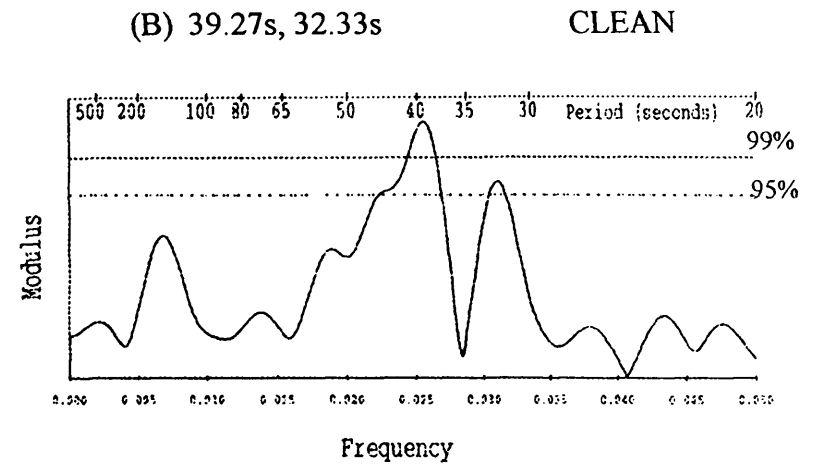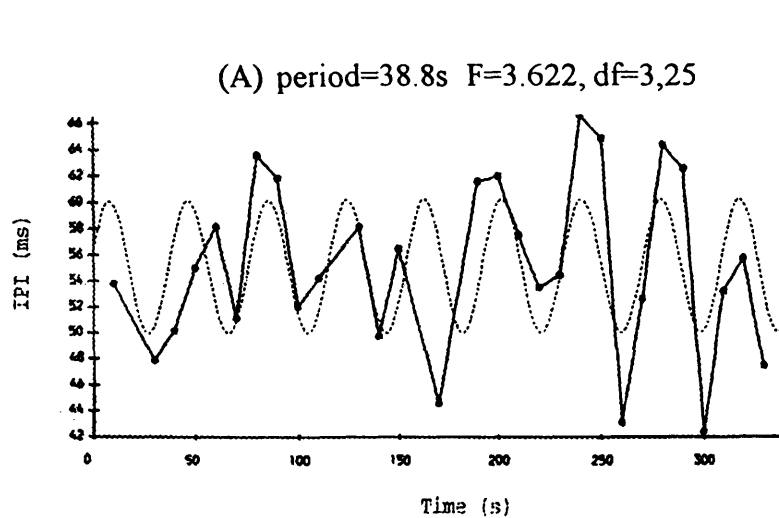
Figure 4.4.4:

D. orena France courtship song (O7se111a) analysed-see table 4.4.2,
using the lower 75 & 15ms cut-off points,and spectral analysis.
a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the data, initially defined by VdB spectral analysis, and
   then fitted to the data by non-linear regression, using a least squares
   procedure.
b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods. Note that there is a significant
   primary peak of 39.27s (p<0.01), as well as a weaker secondary peak of
   32.33s (p<0.05), with the CLEAN analysis. Also note that there is a primary
   peak of 38.83s (p<0.01), as well as a weaker secondary peak of 32.79s
   (p<0.05), with the VdB analysis.The 95 and 99% confidence limits are
   defined by Monte Carlo simulations (see chapter 2).

Table 4.4.3: Spectral analysis of *D. erecta songs*, using different cut-off points.

SPECTRAL ANALYSIS					
SPECIES	Clean		Van den Berg		Observations
	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
D. erecta	CUT Off POINTS : 80 & 15 ms				
Ed1t12l2	x	x	22.10	x	11/17
El1t13l1	47.92	72.43	48.19	75.47	21/33
El1t13r2	x	x	235.29	x	13/26
El2t12l1	45.62	91.15	46.02	93.02	19/37
El2t13l1	43.18	189.02	43.48	200.00	23/34
El3e2s2m	49.56	26.10	49.69	26.32	27/47
El3t12l1	37.45	x	37.56	21.28	52/64
El4e2s2m	49.14	22.83	22.79	49.09	22/44
D. erecta	CUT Off POINTS : 100 & 15 ms				
Ed1t12l2	x	x	21.86	35.71	11/17
El1t13l1	72.43	x	75.47	48.19	22/33
El1t13r2	x	x	285.71	x	13/26
El2t12l1	142.28	x	145.45	33.47	20/37
El2t13l1	43.66	x	45.20	216.22	24/34
El3e2s2m	292.21	60.16	60.61	296.30	27/47
El3t12l1	37.45	x	37.56	21.28	52/64
El4e2s2m	22.83	49.14	22.79	49.09	24/44

The different cut-offs do change the results in a number of songs, with the higher cut-offs resulting in longer periods, particularly with song El2t12l1 which has a period of 142.28s with CLEAN, and 145.45s with VdB ('climber'). Figure 4.4.5 (below) shows the distribution of the periods of the *D. erecta* songs that had been determined from the CLEAN spectral analysis.

Cut-off Points:- 80 & 15 ms
Mean Period: 45.48(s) ± 1.88 (sem)

Cut-off Points: 100 & 15ms
Mean Period: 67.52 (s) ± 15.79 (sem)

N= Number of Observations, Red line= Expected Normal Distribution
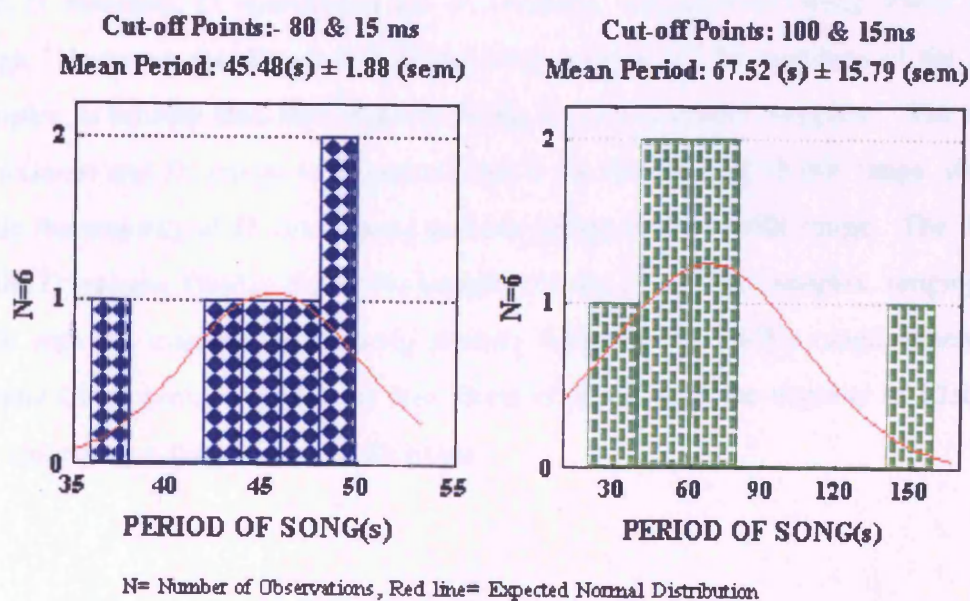
Figure 4.4.5: The distribution of the song periods for *D. erecta*.

When using the lower cut-off points (80 & 15ms) the overall species period for *D. erecta* is 45.47(s) ± 1.88. Moreover, Figure 4.4.5 also shows the distribution of the periods of the *D. erecta* songs, when using the higher cut-off points (100 & 15ms). In this instant, the overall species period for *D. erecta* changes to 67.52 (s) ± 15.8, mainly due to the presence of the 'climber' song (El2t1211). If the period of this 'climber' is excluded, a mean period of 52.57 (s) ± 6.21 is obtained, which is similar to the periods found using the 80 & 15ms cut-offs. Figure 4.4.6 shows the results of a *Drosophila erecta* France song.

One-way ANOVA was employed in order to determine whether there were any differences in the periods between the different species of *D. melanogaster* subgroup. The analysis revealed that there were significant differences (F=3.61, df=8, 78, p=0.001), in the periods, between the different species. Newman-Keuls *a posteriori* test revealed that the *D. yakuba* Thud periods are significantly different, at least at p<0.05, from the periods of *D. simulans*, *D. mauritiana*, *D. sechellia*, *D. orena* and *D. erecta*. The test also revealed that the *D. teissieri* periods are significantly different, at least at p<0.05, from the periods of *D. simulans*, *D. mauritiana*, *D. sechellia*, *D. orena* and *D. erecta*. Figure 4.4.7 shows the histograms of the species periods. As seen from the histograms the members of the *D. melanogaster* complex show a narrower distribution of periods, with *D. melanogaster* having the majority of song rhythms within the 45-60s range, and with *D. simulans*, *D. mauritiana* and *D. sechellia* song periods falling within the 30-45s range. However, the distribution of the song periods, of the members of the *D. yakuba* complex, is broader than that observed in the *D. melanogaster* complex. The majority of *D. teissieri* and *D. erecta* song periods fall in the 60-90s and 45-60s range, respectively, while the majority of *D. orena* song periods falling in the 45-60s range. The distribution of the *D. yakuba* Thud cycles is the broadest of the *D. yakuba* complex, ranging from 30-120s with the majority of the song periods falling in the 75-90s range, whereas the *D. yakuba* Clack periods are shorter than those of Thud, with the majority of Clack rhythms predominantly falling in the 45-60s range.

(A) period=37.4s F=4.097, df=3,45

Time (s)

(B) 37.45s   CLEAN

Frequency

(C) 37.56s, 21.28s   VdB

Frequency
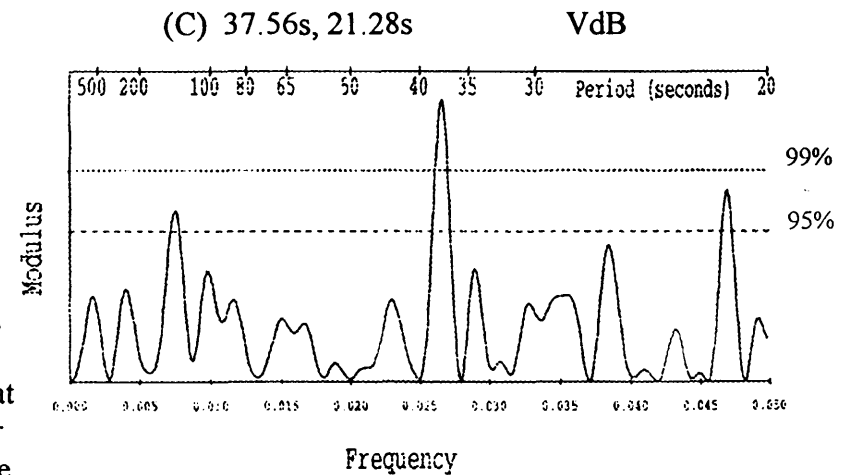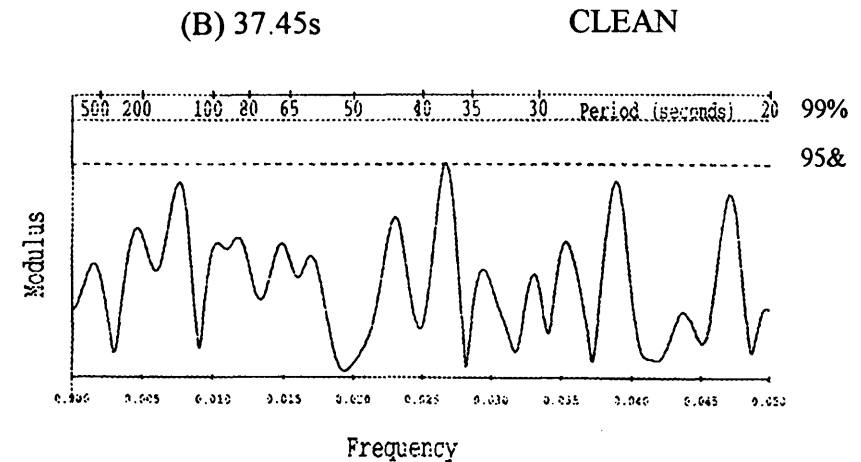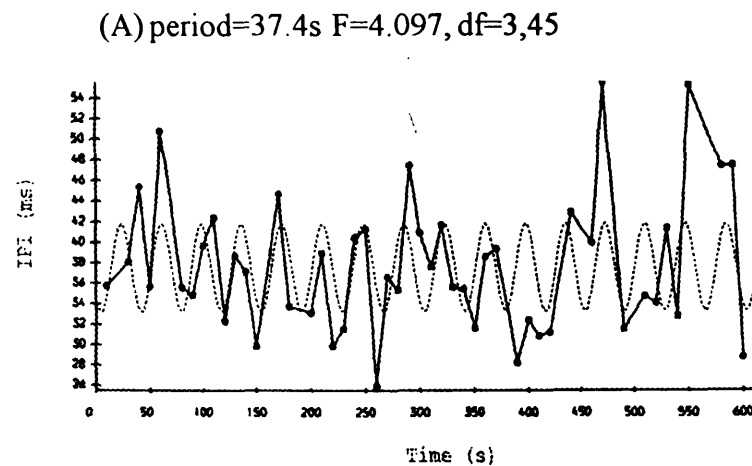
Figure 4.4.6:

D. erecta courtship song (El3t12l1) analysed-see table 4.4.3, using the lower 80 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 37.45s (p<0.05), with the CLEAN analysis. Also note that there is a significant primary peak of 37.56s (p<0.01), as well as a weaker secondary peak of 21.28s (p<0.05- 'arrhythmic' range), with the VdB. The 95 and 99% confidence limits are defined by Monte Carlo simulations (see chapter 2).

D. melanogaster complex

Drosophila melanogaster (Various strains)

Drosophila simulans (Various strains)

Drosophila mauritiana (Various strains)

Drosophila sechellia Cambridge

D. yakuba complex

Drosophila teissieri Sweden

Drosophila orena France

Drosophila erecta France

Drosophila yakuba (Various strains)

GROUP PERIODS OF SONGS (s)
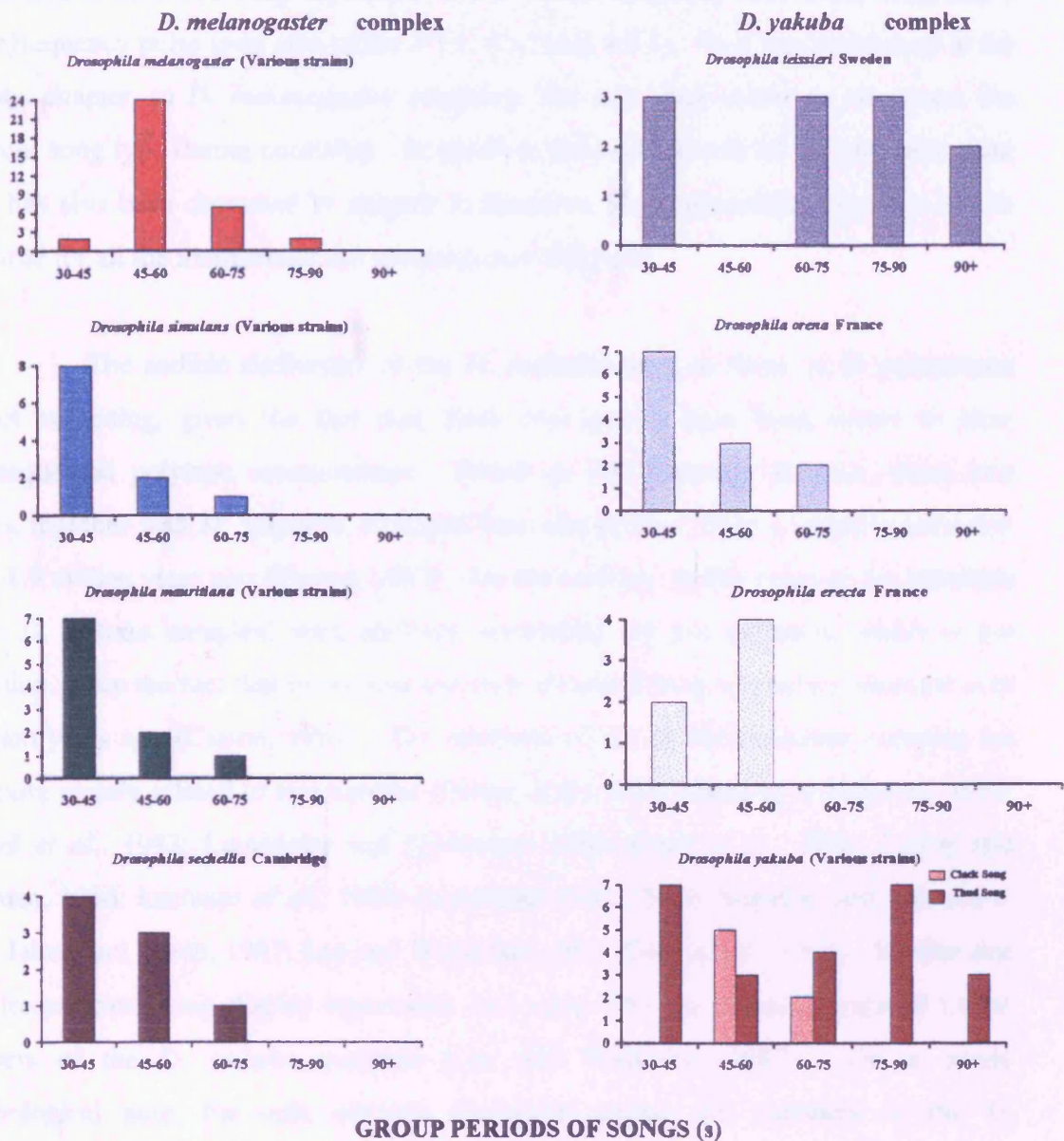
Figure 4.4.7: The distributions of the periods of the various species/song types of the *melanogaster* subgroup

4.5 Discussion:

The songs of all the species examined in this chapter have both song components in their courtship repertoire; a low carrier frequency sine (hum) song and a higher frequency pulse song (see tables 4.1.1, 4.1.2 and 4.3.1). As it was mentioned in the previous chapter, in *D. melanogaster* courtship, the sine song seems to constitute the prevalent song type during courtship. In addition, the possible role of the sine and pulse songs has also been discussed in chapter 3; therefore, it is conceivable that this notion holds true for all the members of the *melanogaster* subgroup.

The audible similarities of the *D. sechellia* song to those of *D. mauritiana* are not surprising, given the fact that these two species have been found to have homosequential polytene chromosomes. Based on Nei's genetic distance, these two species, together with *D. simulans,* diverged from one another, from a common ancestor, about 1.9 million years ago (Cariou, 1987). On the contrary, in the songs of the members of the *D. yakuba* complex, such auditory similarities are not apparent, which is not surprising, given the fact that its various members diverged from a common ancestor over 4 million years ago (Cariou, 1987). The members of the *D. melanogaster* complex are thus more closely related to one another (Eisses *et al.*, 1979; Cowling and Burnet, 1981; Ohnishi *et al.*, 1983; Lemeunier and Ashburner, 1984; Cobb *et al.*, 1986; Coyne and Kreitman, 1986; Lachaise *et al.*, 1986; Lemeunier *et al.*, 1986; Solignac and Monnerot, 1986; Jallon and David, 1987; Lee and Watanabe, 1987; Cobb *et al.*, 1989). Similar sine song frequencies, wing display repertoires and mean IPIs are shared compared to the members of the *D. yakuba* complex (Lee and Watanabe, 1987). On a purely morphological note, the only obvious distinction among the members of the *D. melanogaster* complex is in the shape of the posterior process of the male genital arch (Coyne, 1983).

On the contrary, *D. yakuba* songs have no hum component (Cowling and Burnet, 1981), but have two different song pulse-types (Thackery, 1989), while the other three members of the *D. yakuba* complex, include a sine song with more variable frequencies than those of the *D. melanogaster* complex. Thus, the differences in the mean IPIs between these species are seen to be greater than those seen between the

members of the *D. melanogaster* complex, reflecting perhaps their phylogeny (see Chapter 9).

Further evidence is offered in this chapter towards the existence of song cycles in the other members of the *melanogaster* subgroup. *D. mauritiana* and *D. orena* provide the most convincing evidence of the existence of short song rhythms. *D. erecta* and *D. sechellia* provide strong evidence that song rhythms exist within the same range of *D. melanogaster*, whereas *D. teissieri* provides evidence of the existence of long periods in the same range as *D. yakuba* Thud, which is in agreement with Thackeray's preliminary observations (Thackeray, 1989).
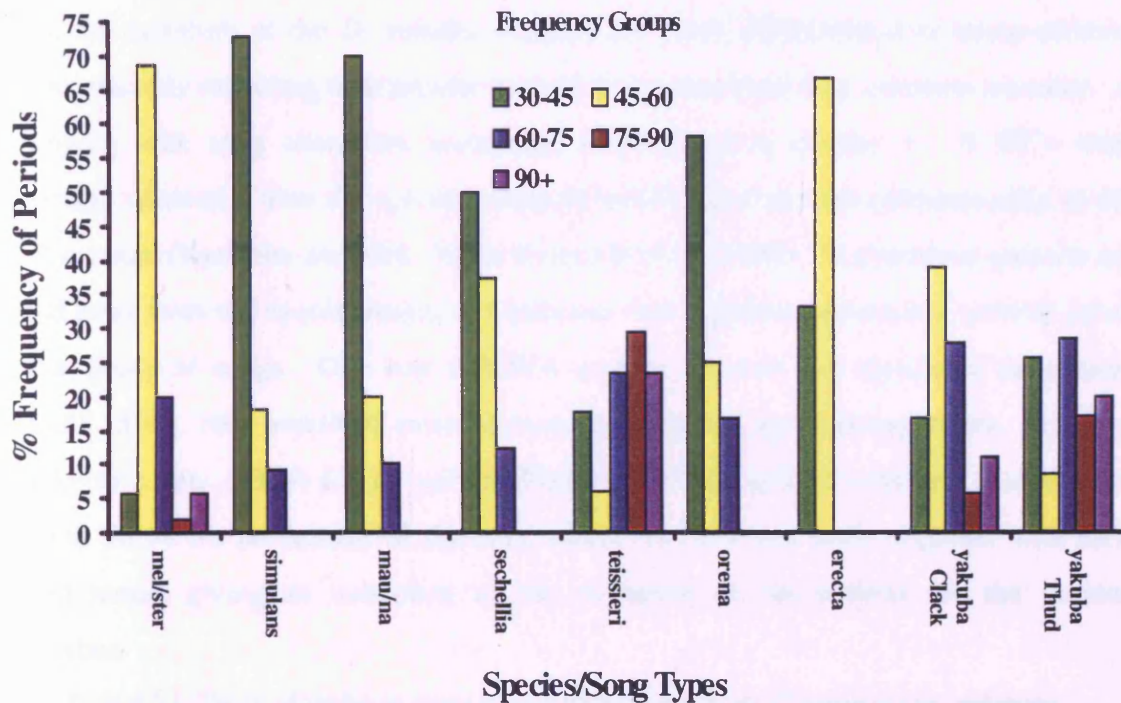
Figure 4.5.1: The frequency distributions of the periods of the members of the *melanogaster* subgroup.

Figure 4.5.1 and Table 4.5.1 illustrate an overall view of all the song periods examined in Chapters 3 and 4. Figure 4.5.1 differs from Figure 4.4.7 in that it includes all the songs reanalysed, songs recorded and analysed by me as well as a few *D. yakuba* and *D. teissieri* analysed by Thackeray (1989). Thackeray (1989) found six rhythmic *D. teissieri* songs, with periods ranging from ~50-130s. Furthermore, Thackeray analysed 12 *D. yakuba* songs, where Clack was the predominant pulse-type;

11 out of the 12 songs were found to be rhythmic with periods ranging from ~40-120s. In addition, Thackeray analysed 12 *D. yakuba* songs, where Thud was the predominant pulse-type; 11 out of the 12 songs were found to be rhythmic with periods ranging from ~32-130s.

The majority of song periods of *D. simulans*, *D. mauritiana* and *D. orena* fall within the 30-45s range, while the majority of song periods of *D. melanogaster*, *D. sechellia*, *D. erecta* and *D. yakuba* Clack fall within the 45-60s range. In addition, *D. teissieri* and *D. yakuba* Thud have song periods falling within the broader range of 60-90s. The clustering of the song periods of the members of the *D. melanogaster* complex is narrower than that observed for the periods of the members of the *D. yakuba* complex. Thus, the members of the *D. yakuba* complex are more differentiated in terms of song period, possibly reflecting their greater time of divergence from their common ancestor. A phylogeny with song characters juxtaposed is presented in chapter 9. If IPI's were generated randomly, then the spectral analyses would show periods predominantly in the 20-30s range (Kyriacou and Hall, 1989; Kyriacou *et al.*, 1990). If consistent periods are found away from the nyquist range, this indicates that a genuine underlying cyclicity exists in this group of songs. One-way ANOVA analysis showed that significant differences (F=4.88, df=8, 106, p=0.000) exist between the different species/song types, including Thackeray's data (1989) for *D. yakuba* Thud and Clack, and *D. teissieri*. Table 4.5.1 (below) shows the percentage of rhythmic songs, found in this study, together with their period range, giving an indication of the clustering of the periods for the various genotypes.

Table 4.5.1: The % of rhythmic songs and the period range in the *D. melanogaster* subgroup.

Species	% of Rhythmic Songs	Majority Period Range
D. melanogaster Complex		
melanogaster	100.00	(24/35)- 45-60s
simulans	91.67	(8/11)- 30-45s
mauritiana	83.33	(7/10)- 30-45s
sechellia	88.89	(4/8)- 30-45s
D. yakuba Complex		
teissieri	94.44	(9/17)- 60-90s
orena	66.67	(7/12)- 30-45s
erecta	75.00	(4/6)- 45-60s
yakuba (thud)	74.47	(16/35)- 60-90s
yakuba (clack)	50.00	(9/18)- 45-75s

What is the nature of a benefit that might be gained by a rhythmically 'singing' male? Kyriacou and Hall (1982; 1989) proposed that a female has a preferred IPI value and so, a successful male would have to generate a variable band of IPI lengths around the overall species mean. Generating this rhythmic variability may just simply be the most efficient way of achieving this short-term goal. Why then, are the periods discovered or confirmed in this study, species-specific? The answer to this may be that the females of the different species need to summate a different number of IPIs at their 'preferred' length, before allowing copulation. According to this proposal, the different periods are produced because females of the various species require different numbers of the 'preferred' IPI(s) to be summated, before allowing copulation. Thus, a slower cycle will allow more IPI's to be generated at each IPI value. A faster cycle would suggest that fewer IPI's of each might be sufficient to trigger female acceptance.

To test this hypothesis of individual IPI preference Greenacre (1990) selected *D. melanogaster* females which either preferred 'high' IPIs (40ms) or 'low' IPIs (30ms) in the presence of courting wingless males. The first females that copulated in either condition were used to produce the next generation, and their daughters were selected in the same way. After only two generations of selection, the females from the 'high' lines were observed to prefer the 40ms IPI over the 30ms IPI song, and the 'low' line females showed a greater response to the 'low' 30ms IPIs song. Unfortunately, this result could not be sustained, and by the sixth generation, females from both sets mated extremely rapidly and indiscriminately. Clearly, it appeared that any possible significant subtle effects of the different IPIs were completely 'overshadowed' by the rapid female mating (a by-product of the selection procedure). Furthermore, Charalambous (1990), selecting for females that mated fastest with males, in the grasshopper *Chorthippus brunneus*, after being stimulated by an artificially-generated male calling with a particular mean length syllable, reported that the apparent response could not be maintained due to the higher responsiveness amongst selected females, after just one generation of selection.

Hence, at present, it can not be stated unequivocally whether the mating effects observed with cycling pulse songs are due to some internal rhythm template within each female or whether the females just simply prefer different IPIs, and

consequently, rhythmically varying IPIs are a more 'powerful' tool in stimulating more females. Therefore with shorter cycles, as in *D. melanogaster* complex, it is conceivable that a female could pick up the relevant IPI's, even after just a few seconds of hearing a song, which in turn implies that females of the different species, within this complex might be more receptive.

Since the spectral analyses proved useful in illustrating the existence of song cycles in *D. mauritiana*, *D. sechellia*, *D. teissieri*, *D. orena* and *D. erecta*, the next logical step is to apply these spectral methods to their interspecific genotypes (chapter 5) in order to initiate a genetic analysis similar to that performed by Kyriacou and Hall (1986).

Characteristics of interspecific hybrid songs between the members of the *D. melanogaster* subgroup

CHAPTER 5

5. Characteristics of interspecific hybrid songs between members of the *D. melanogaster* subgroup

Hybridisation is possible to varying degrees, between species of the *D. melanogaster* subgroup. The first to be achieved was between *D. melanogaster* females and *D. simulans* males, the resulting progeny being females only (Sturtevant, 1920), whereas the reciprocal cross resulted in males only (David, 1974). Other combinations of species hybridisation have been possible under laboratory conditions (von Schilcher, 1976a; Watanabe and Kawanishi,1979; Cowling and Burnet, 1981; Lachaise *et* al., 1986; Lee and Watanabe, 1987; Cobb *et al.*, 1990) which are highly unlikely to occur in the wild, e.g., *D. melanogaster* or *D. simulans* males or females mated with *D. mauritiana* females or males, respectively. *D. melanogaster* and *D. simulans* are cosmopolitan species, whereas *D. mauritiana* is endemic to a few islands of the Seychellian Archipelago, where *D. melanogaster* is absent (Ashburner and Tsacas, 1981).

When the hybrid progeny produced is of both sexes, the hybrid females are usually found to be fertile, whereas the hybrid males are found to be sterile. This phenomenon is called 'Haldane's Rule (Haldane, 1922) and, has stimulated much debate within the speciation community (Muller, 1940; Hennig,1985; Coyne and Orr, 1989; Orr, 1993; Virdee,1993; Wu, 1993; Coyne, 1994; Migeon, 1994; Orr,1995; Turelli and Orr, 1995). As mentioned above, all male hybrids from these species were found to be sterile (David *et al.*, 1976; Lachaise *et al.*,1986; Coyne, 1989). This male sterility condition was found to be reversible, if the hybrid females were backcrossed with males of the parental species (David *et al.*, 1974). This fertility reaches a plateau, where thereafter, there is no further rescue of fertility (David *et al.*,1974). Recently, Davis *et al.* (1996) have isolated a strain of *D. simulans* that produces fertile females in crosses with *D. melanogaster*, which promises to pinpoint some of the genetic components involved in both pre- and post-reproductive isolation in these species.

The first part of each ensuing section of this chapter will be concerned with the setting up of various interspecific hybrid crosses and the analysis of IPI distribution. In this present study some novel interspecific hybrid crosses are reported. In some of these, namely from crosses involving *D. mauritiana* female x *D. yakuba* male, the only way to obtain any hybrid progeny was to 'amputate' the wings of the females. The later

part of each section of this chapter describes my various attempts in trying to detect any possible hybrid song rhythms.

Interspecific Hybrid Genotypes

Table 5.1 illustrates the results of hybridisations giving progeny. It is clear, by examining table 5.1, that obtaining viable hybrids is quite difficult. Figure 5.1a & b depict the mean IPIs of various parental strains and their interspecific hybrids.

5.1 *D. yakuba* (f) *x D. mauritiana* (m) hybrid song:

Several previous attemps to hybridize *D. yakuba* with various other members of the *melanogaster* subgroup have been unsuccessful, except when *D. mauritiana* (Cowling and Burnet, 1981) was used as the paternal parent. Since *D. mauritiana* song differs qualitatively, from the *D. yakuba* one, with respect to the presence of sine song (hum song), the interspecific hybrids could provide at least some preliminary information on the mode of inheritance of this acoustic element.

Preliminary crosses were set up using *D. yakuba* female flies with their wings intact and a second set of female flies with the wings 'amputated'; only vials containing females with their wings intact produced any progeny at all (see Table 5.1). It is, thus, conceivable that the *D. mauritiana* male needs more than just light as a stimulus, to initiate courtship (Robertson, 1983). The female, by brushing her wings against her abdomen, may be exuding the necessary pheromones for courtship initiation (Jallon *et al.*, 1984; Jallon *et al.*, 1987). This 'brushing' behaviour was also observed in all courtships recorded and examined, whether these were of the parental species or of the interspecific hybrids. This is in agreement with the notion that non-visual stimuli also have an important role to play in courtship (Robertson, 1983).

These hybrid males were reared under constant light as their paternal *D. mauritiana* species had been. Externally, the hybrid females of the progeny were observed to be very similar, in body shape, to *D. yakuba* maternal strains, while the hybrid male's genitalia resembled *D. mauritiana* in morphology.

Table 5.1 : Types of interspecific crosses, Total number of crosses set up and percentages of vials giving progeny

Type of Interspecific Cross	1	2	3	4	5	6	7	8	9	10	11	12
Total Number of Crosses set up (vials with females with intact wings)	300	227	176	163	250	80	78	61	52	48	10	10
Number of crosses set up with females with amputated wings	-	-	101	101	-	-	-	-	-	-	-	-
Number of crosses giving rise to progeny	194	156	43	47	143	0	52	0	36	0	0	0
% crosses giving rise to progeny	64.66	69.60	42.57*	46.53*	57.20	0	66.66	0	69.23	0	0	0
Percentage of *successful* crosses giving rise to single sex progeny(%)	63.40	67.09	72.09*	80.85*	61.54	0	65.38	0	75.00	0	0	0
Percentage of *successful* crosses giving rise to two-sex progeny(%)	36.60	32.91	27.91*	19.15*	38.46	0	34.15	0	25.00	0	0	0

Interspecific Crosses:

1. *D. yakuba* France(f) x *D. mauritiana* France(m)
2. *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m)
3. *D.mauritiana* Sweden(f) x *D.yakuba* Ivory Coast(m)
4. *D. mauritiana* Sweden(f) x *D. yakuba* Lamto 3(m)
5. *D. yakuba* France(f) x *D. teissieri* Sweden(m)
6. *D. teissieri* Sweden(f) x *D. yakuba* France(m)

7. *D. simulans* Florida(f) x *D. mauritiana* Sweden(m)
8. *D. mauritiana* Sweden(f) x *D. simulans* Florida(m)
9. *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m)
10. *D. mauritiana* Sweden(f) x *D. teissieri* Sweden(m)
11. *D. erecta* France(f) x *D. orena* France(m)
12. *D. orena* France(f) x *D. erecta* France(m)

*- Females with 'amputated' wings
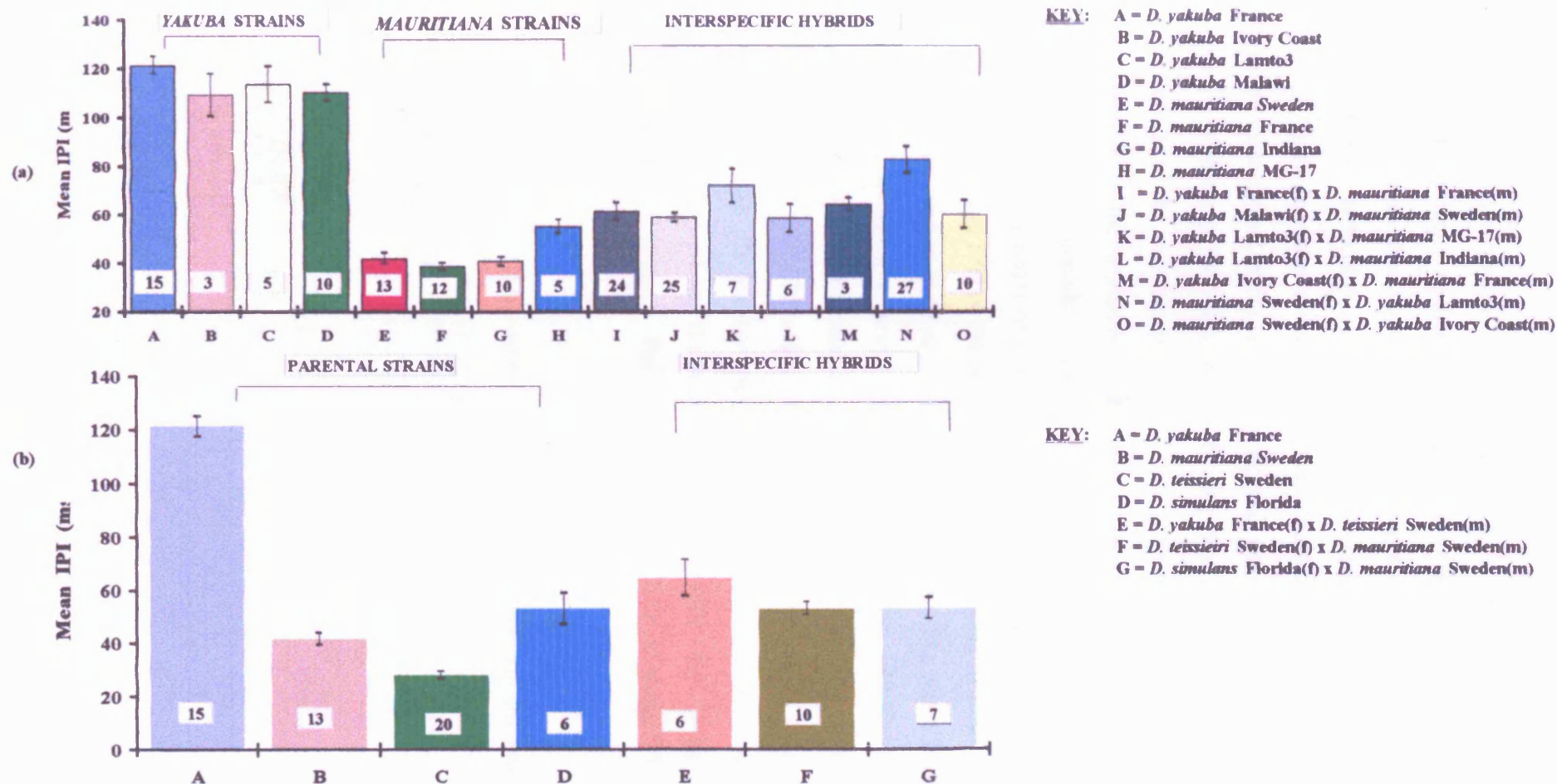
Figure 5.1a: Mean IPIs of various *D. yakuba* and *D. mauritiana* strains and their interspecific hybrids.
Figure 5.1b: Mean IPIs of *D. yakuba*, *D. mauritiana*, *D. teissieri* and *D. simulans* strains and their interspecific hybrids.
NOTE: Number of males recorded and sem's are also shown.

In both crosses 1 & 2 (see Table: 5.1), the percentages of vials set up giving any kind of progeny (either single sex or two-sex progeny) is 64.66% and 69.60% respectively. From these crosses, the percentages of interspecific crosses that produced two-sex progeny is 36.60% (cross 1) and 32.91% (cross 2), respectively. It is apparent that obtaining viable hybrid two-sex progeny is problematic. During recording of the courtship songs of these hybrid males, no sine song was audible (see Table 5.1.1-below). Hybrid males use both wings for 'scissoring'. 'Rowing' was also observed. Both wing displays are characteristic of the *mauritiana* species. In waveform, the IPIs were seen to be short, sharp and irregular at the beginning of the burst (*mauritiana*-like), with a mixture of high frequency, high amplitude Clack-like pulses or lower frequency Thud-like pulses. In between consecutive trains of pulses, there were long periods of silence observed. Figure 5.1a shows the mean IPIs of the parental strains participating in these particular interspecific hybrid crosses, and of their interspecific hybrid males. The mean IPIs, for the individual songs of the various interspecific genotypes, range from ~49-93ms (IPI and modal IPI measurememts were taken from 24 flies recorded, whereas IPF measurements were taken from 3 flies), and ~50-87ms (IPI and modal IPI measurememts were taken from 25 flies recorded, whereas IPF measurements were taken from 3 flies), respectively (see Figure 5.1a:- I & J - K, L & M are the same type of Interspecific cross, using different *yakuba* and *mauritiana* parental strains). The mean IPI, IPF and SSF of these IPI genotypes are given in Table 5.1.1.

Table 5.1.1: Song characteristics of *D. yakuba* and *D. mauritiana*, and their interspecific hybrids.

SPECIES/ STRAINS	OVERALL MEAN IPI ± sem (ms)	MODAL IPI(ms)	MEAN SSF ± sem (Hz)	OVERALL MEAN IPF ± sem (Hz)
yakuba STRAIN:				
FRANCE	124.61±2.71	95.50	X	302.22±3.17
MALAWI	124.80±14.7	96.50	X	309.84±3.34
mauritiana STRAIN:				
SWEDEN	41.81±2.23	27..31	190.64±5.49	247.75±1.31
FRANCE	38.50±1.53	32.33	175.80±8.76	254.31±1.17
STRAINS:	INTERSPECIFIC GENOTYPES			
yakuba France(f) x *mauritiana* France(m)	70.40±9.07	51.30	X	402.46±3.46
yakuba Malawi (f) x *mauritiana* Sweden(m)	68.80±12.7	53.00	X	409.67±3.44

Figure 5.1.2 (see below) shows the IPI distributions of a *D. yakuba* France(f) x *D. mauritiana* France(m)- hybrid male (red columns), and a *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m)-hybrid male (black columns). The distribution is unimodal in shape, in both cases, broader than the one depicted for the *D. mauritiana* (see Figure 4.1.2), but narrower than the one depicted for *D. yakuba* parents (see Figure 3.4.2). The modal values fall between 50-60ms and mean IPIs are generally intermediate between the two parental specis and is consistent with autosomal inheritance of IPI (Cowling and Burnet, 1981; Kyriacou and Hall, 1980).

The fact that no hum song is produced by the hybrid male, indicates X chromosome involvement (Schilcher, 1975; Cowling and Burnet, 1981; Kyriacou and Hall, 1980), as the X chromosome is donated by *D. yakuba* which does not produce sine song (Cowling and Burnet, 1981; Thackeray, 1989). As in Chapter 3 (section 3.4-Table 3.4.1), the mean IPFs of the interspecific hybrids are observed to be higher than their respective parentals ones, suggesting hybrid vigour.
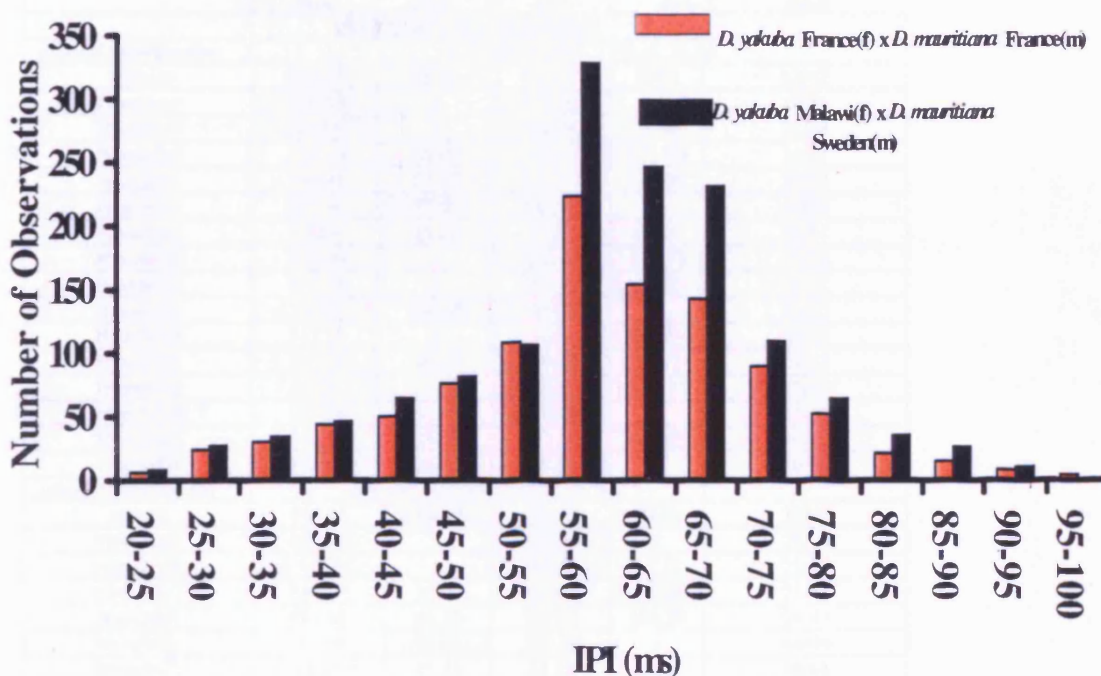
Figure 5.1.2: The distribution of IPI of hybrid males for *D. yakuba* (f) x *D. maurtiana* (m) .

Song rhythms?

The usual preliminary procedures were followed (see Chapter 2). A song was only considered for rhythm analysis, if more than 50% of its bins were filled, and IPI means were analysed with the CLEAN and VdB analyses. The results are shown on Table 5.1.2, using different cut-off points (approximately twice the mean), for each interspecific genotype, which had been decided after individual IPI histograms had been examined.

The highlighted values on Table 5.1.2 (see below) are the values that have been used to determine the rhythm periods of this interspecific genotype. It can clearly be seen that the CLEAN and the corresponding VdB values, for each individual song, are approximately similar. Table 5.1.2 (lower part) shows the results when the lower cut-off points were employed. Any periods of 30s or less were assumed to be arrthyhmic, unless a second significant peak in the spectrogram was found.

Table 5.1.2: Spectral analysis of the *D. yakuba*(f) x *D. mauritiana*(m) interspecific hybrids, using different cut-off points.

SPECIES	SPECTRAL		ANALYSIS		
	Clean		Van den Berg		Observations
	1ST Peak	2ND Peak	1ST Peak	2ND Peak	Bins Filled
INTERSPECIFIC HYBRIDS					
yakuba(f) xmauritiana(m)	CUT Off POINTS : 150 & 20 ms				
Hybrid	454.55	89.69	500.00	29.52	32/42
Hym2smr	120.00	x	119.40	x	25/26
Hym2t10l	34.39	x	34.93	23.12	22/28
Hym2t5r2	256.10	36.21	36.36	275.86	24/47
Hym2t6l1	590.91	24.16	727.27	235.29	29/43
Hym3t5l1	385.96	93.62	380.95	x	46/46
Hym3t6l2	23.67	25.18	23.81	25.24	38/61
Hym4t10l	250.00	x	266.67	133.33	55/58
Hym4t5l2	372.34	x	363.64	67.80	35/37
Hym4t5r1	50.00	136.90	50.00	x	23/25
Hym5t5r2	743.24	x	1000	33.33	33/57
Hym5t6l1	43.73	56.18	43.24	57.97	24/33
Hym6t5r2	84.14	25.62	85.11	133.33	37/61
Hym7t5r2	107.34	31.99	103.90	x	23/41
Hym7t6r2	540.54	23.58	571.43	47.62	30/42
Hym8t5l1	63.06	28.75	28.88	x	30/30
yakuba(f) xmauritiana(m)	CUT Off POINTS : 120 & 20 ms				
Hybrid	454.55	89.69	500.00	29.52	32/42
Hym2smr	120.00	x	119.40	x	25/26
Hym2t10l	34.39	x	34.93	23.12	22/28
Hym2t5r2	260.10	36.02	61.07	36.04	21/47
Hym2t6l1	590.91	24.16	727.27	235.29	29/43
Hym3t5l1	385.96	93.62	380.95	x	46/46
Hym3t6l2	23.67	25.18	23.81	25.24	38/61
Hym4t10l	250.00	x	266.67	133.33	55/58
Hym4t5l2	364.5	x	363.64	67.80	35/37
Hym4t5r1	49.78	136.90	49.38	x	23/25
Hym5t5r2	743.24	x	1000	33.33	33/57
Hym5t6l1	43.73	56.18	43.24	57.97	24/33
Hym6t5r2	98.21	x	135.50	87.91	36/61
Hym7t5r2	20.04	104.40	101.20	x	22/41
Hym7t6r2	540.54	23.58	571.43	47.62	30/42
Hym8t5l1	28.99	x	28.99	x	30/30

Five out of 16 songs showed periods in the 'climber' range and one song (Hym3t6l2) showed periods in the arrhythmic (<30s) range, and therefore were considered arrhythmic (see Table 5.1.2-upper part). Furthermore, it can be seen that the rhythmic songs showed very variable periods. Songs Hym2t5r2, Hym3t5l1 and Hym7t6r2 (see Table 5.1.2-upper part-CLEAN analysis) showed significant primary peaks in the 'climber' range, so the second significant peaks were taken to be the periods of these songs. Song Hym8t5l1 becomes arrhythmic, while songs like Hym4t5r1 and Hym2t5r2 have their periods only slightly changed, when the lower 120 & 20ms cut-off points are employed (see Table 5.1.2 -lower part).

Figure 5.1.3 shows the CLEAN-determined distribution of periods of *D. yakuba* (f) x *D. mauritiana* (m)-interspecific hybrids, whose values were highlighted in Table 5.1.2 (upper part), together with their corresponding mean periods ± sem. Out of the 16 *D. yakuba* (f) x *D. mauritiana* (m) songs examined 6 were found to arrhythmic with CLEAN, and the majority of songs (11/16) were found to be rhythmic with VdB. From the 10 CLEAN-determined rhythmic songs, 6 had periods greater than 60s. If all the rhythmic songs are to be included in the determination of the overall mean period for this genotype, the period is 72.20(s) ± 9.74 (n=10-see Figure 5.1.3). This mean period is more *yakuba*-like than *mauritiana*-like, suggesting the involvement of the X chromosome.

D. yakuba (f) x D. mauritiana (m)
CLEAN-Cut-off Points:- 150 & 20ms
Mean Period: 72.22 (s) ± 9.74
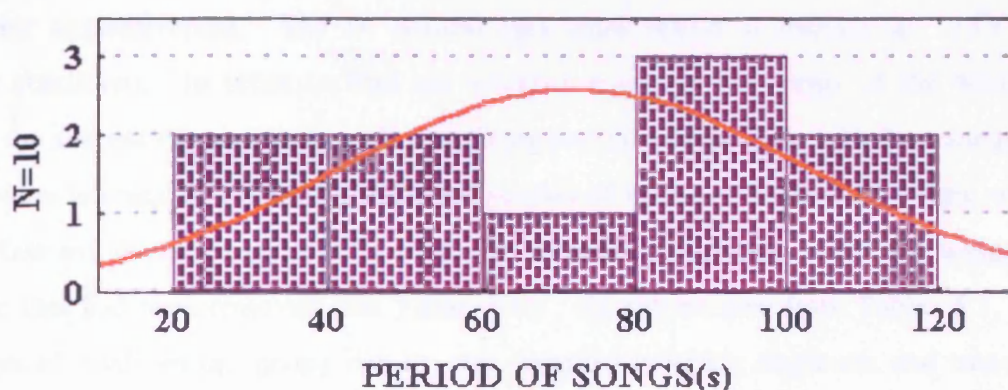
N = Number of Observations, Red Line = Expected Normal Distribution

Figure 5.1.3: The distribution of the periods of songs of the *D. yakuba* (f) x *D. mauritiana* (m) interspecific genotype. The overall mean period ± sem and cut-off points are given.

Figure 5.1.4 shows the results of a *D. yakuba* France(f) x *D. mauritiana* France(m) interspecific hybrid song, after the two different spectral analyses had been employed. It can be seen that there is a ~300s cycle on which a ~90s period is being superimposed. This is significant for CLEAN, giving a period of ~92.63s (see Figure 5.1.4b), but not significant for VdB (see Figure 5.1.4c). Figure 5.1.5 shows the results of a *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) interspecific hybrid song, after the two different spectral analyses had been employed, both of which give significant 50s cycles.

5.2 Hybrids from the reciprocal *D. mauritiana* (f) *x D. yakuba* (m) cross:

Since all previous attempts to obtain any hybrid progeny, from this reciprocal cross were unsuccessful (Lemeunier, 1979; Lee & Watanabe, 1987), a change in the rearing regime of *D. mauritiana* flies was applied, involving the removal of the wings of the females, as described in Chapter 2. This minimized the possibility of the females violently rejecting the males, a behaviour observed during recording of *D. mauritiana* songs. Wingless females in *D. virilis*-group species are mounted less willingly by conspecific males (Vuoristo *et al.*, 1995), primarily because the wings provide a visual acceptance and readiness cue, to the male. Amputating the wings of the *D. mauritiana* females did not seem to impede *D. yakuba* males in attempting to mount the females, clearly showing that this visual cue is not as important in this species. This is also experienced in the cases of *D. ezoana* and *D. novomexicana* (Ewing, 1983; Liimatainen, 1993). Rearing the virgin *D. mauritiana* females in light/dark conditions at 25°C, instead of constant light, also seemed to reduce their aggressiveness. The *D. yakuba* flies were reared in solitude at 25°C in light/dark conditions. In order to find out whether removing the wings of the females improved the success of this cross, vials containing one *D. yakuba* male with four wingless *D. mauritiana* females, or containing the same number of male and intact female flies, were set up. Male and female progeny were produced, but only in the vials where the wings of the female flies had been removed (see Table: 5.1). As can be seen from Table 5.1, the percentages of vials set up giving rise to any progeny (whether single-sex and two-sex progeny) was reduced to 42.57% and 46.53%, respectively. The percentages of the interspecific crosses producing two-sex progeny is also further reduced to 27.91% and 19.15%, respectively.

(A) period =346.9s F=4.113, df=3,42

(B) 385.96s, 93.62s   CLEAN

(C) 380.95s   VdB
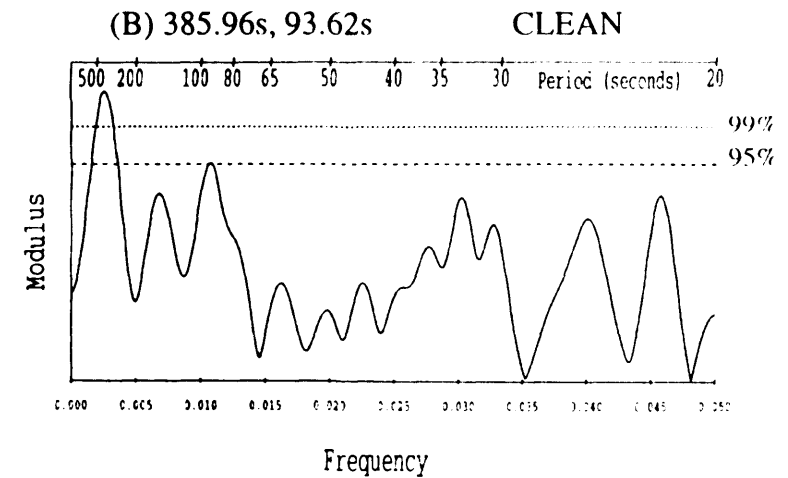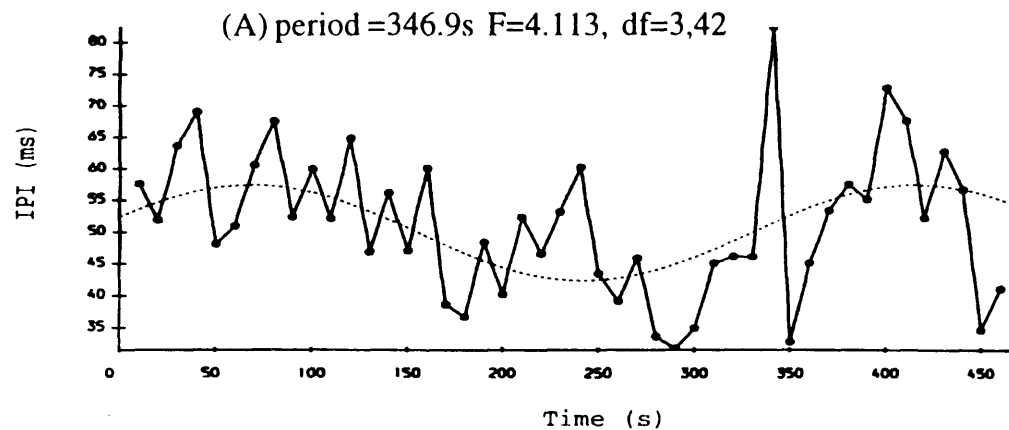
Figure 5.1.4:

D. yakuba France(f) x *D. mauritiana* France(m)-Interspecific hybrid song (Hym3t511) analysed-see table 5.1.2-upper half, using the higher 150 & 20ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure. Note that there is a ~350s sine wave, on which a ~94s period is being super-imposed.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 385.98s (p<0.01-'climber' range), as well as a weaker secondary peak of 93.62s (p<0.05), with the CLEAN analysis. Also, note that there is a significant primary peak of 380.95s (p<0.01- 'climber' range); in addition, note that there is a weak secondary peak at ~90s, which does not reach the 5% confidence limit, with the VdB analysis.The individual lines are the 95 and 99% confidence limits defined by Monte Carlo simulations (see chapter 2).
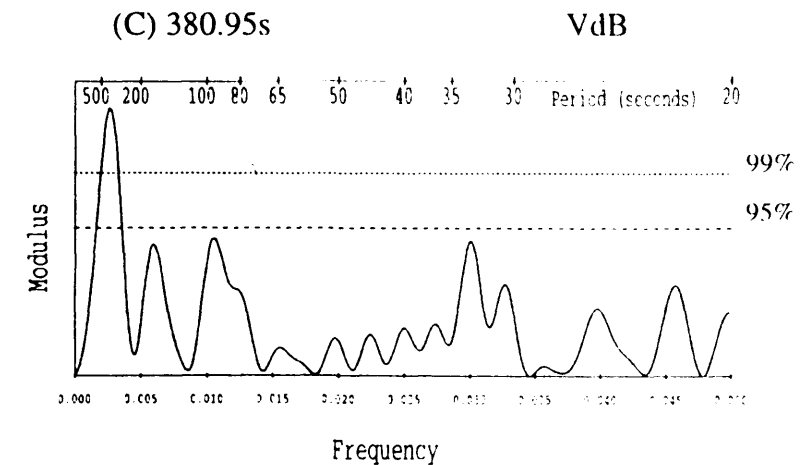
(A) period= 49.9s F=3.335, df=3,19
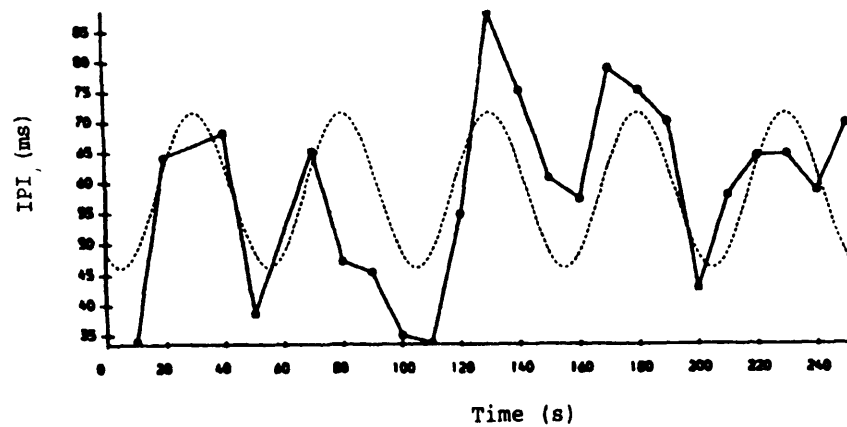
(B) 50.00s, 136.90s   **CLEAN**
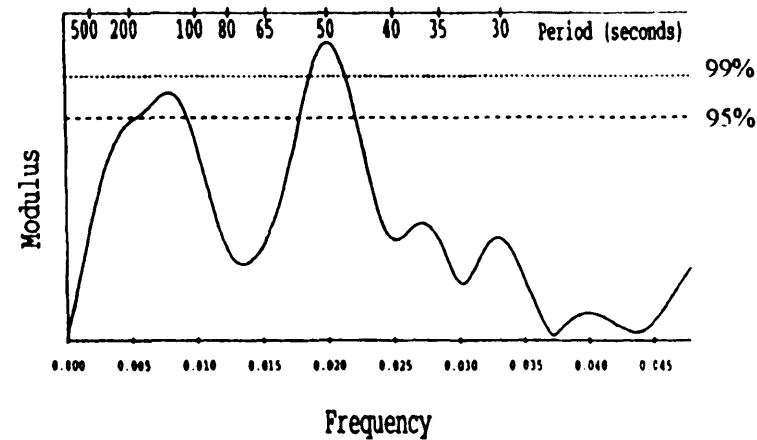
Figure 5.1.5:

D. yakuba Malawi(f) x *D. mauritiana* Sweden(m)-Interspecific hybrid song (Hym4t5r1) analysed- see table 5.1.2-upper half, using the higher 150 & 20ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 50s (p<0.01), as well as a weaker secondary peak of 136.90s (p<0.05), with the CLEAN analysis. Also, note that there is a significant primary peak of 50s (p<0 01), with the VdB analysis. The horizontal lines represent 95 and 99% confidence limits are defined by Monte Carlo simulations (see chapter 2).

(C)   50.00s   VdB
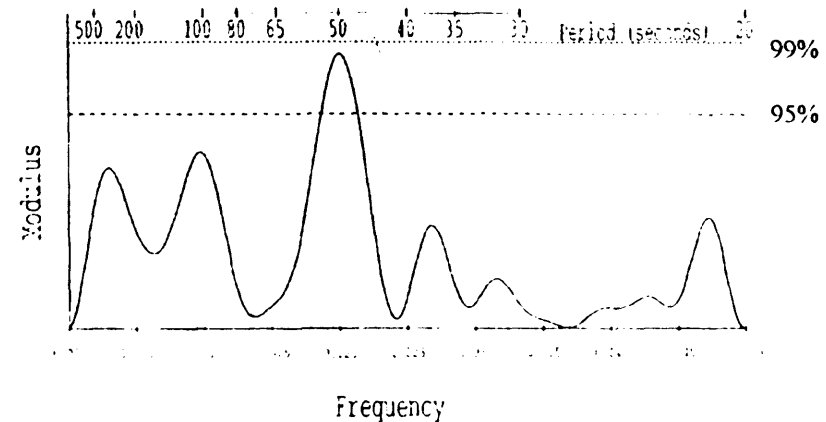
Anecdotal observations of the behaviour of these hybrid males revealed that swift copulations attempts were also encountered, which are characteristic of the *D. mauritiana* species (Robertson, 1983). Wing display repertoire included two types, 'scissoring' (von Schilcher, 1976b) and 'rowing' (Cowling and Burnet, 1981), which were also seen in the two parental species. 'Scissoring' was never observed in D. *yakuba*, only 'rowing' (Cowling and Burnet, 1981). Furthermore, males were seen to continue singing even if they were not in the correct orientation to the female.

The mean IPIs of *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) and *D. mauritiana* Sweden(m) x *D. yakuba* Ivory Coast(m) are shown in Figure 5.1a (Columns:- O & N). The IPI and modal IPI measurements were taken from 27 and 10 flies respectively, whereas the IPF and SSF measurements were taken from 4 and 3 flies respectively. These interspecific males produced both principle acoustic song components. The sine song is clearly audibly (Table 5.2.1 and Figure 5.2.1), which is to be expected, since the presence of sine song is thought to be determined by the X-chromosome (von Schilcher, 1975; Cowling and Burnet, 1981), and which had been donated in this cross by *D. mauritiana*.

Table 5.2.1: Song characters of *D. yakuba, D. mauritiana* and their interspecific hybrids.

SPECIES/ STRAINS	OVERALL MEAN IPI ± sem (ms)	MODAL IPI(ms)	MEAN SSF± sem (Hz)	OVERALL MEAN IPF ± sem(Hz)
yakuba STRAIN:				
IVORY COAST	109.15±8.60	85.50	X	365.99±2.80
LAMTO3	127.60±10.6	88.50	X	311.12±2.58
mauritiana STRAIN:				
SWEDEN	41.81±2.23	27.31	190.64±5.49	247.75±1.31
STRAINS:	INTESPECIFIC GENOTYPES			
mauritiana Sweden(f) x *yakuba* Lamto3(m)	80.70±4.63	74.01	207.00±12.0	394.64±3.44
mauritiana Sweden (f) x *yakuba* Ivory Coast(m)	69.58±6.98	63.48	176.00±4.00	395.00±3.52

Both the modal and mean IPI values are observed to be generally intermediate between the two parental species, which is consistent with autosomal inheritance of IPI (Kyriacou and Hall, 1980; Cowling and Burnet, 1981). Furthermore, the mean IPF of the interspecific hybrids are seeen to be higher than their respective parental ones which suggests hybrid vigour.

Figure 5.2.1: The principle song components of hybrid males from *D. mauritiana*(f) x *D. yakuba*(m) crosses.

SINE SONG   PULSES

(a)

D. *mauritiana* Sweden (f) x
D. *yakuba* Lamto3 (m)

(b)

D. *mauritiana* Sweden (f) x
D. *yakuba* Ivory Coast (m)
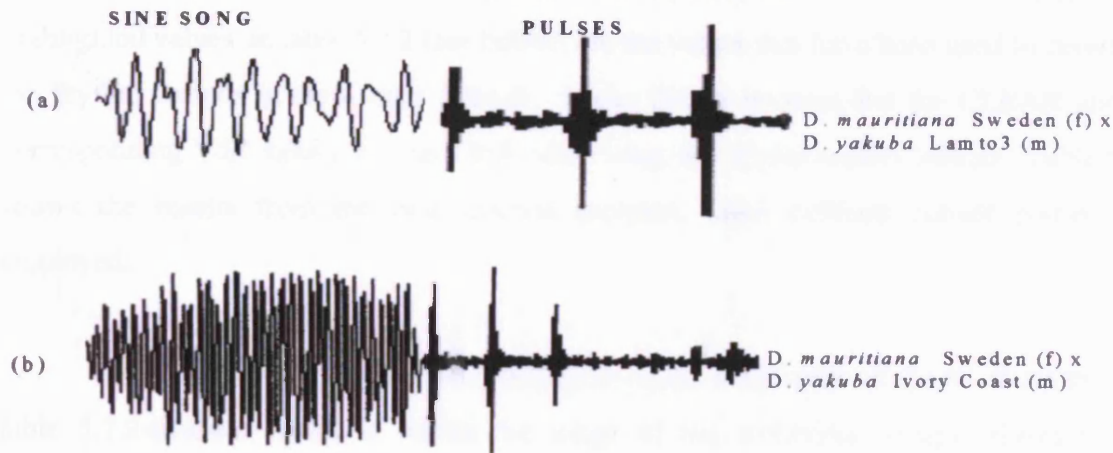
The mean IPIs, for individual songs range from ~49-93ms and from ~46-84ms, respectively (see Figure 5.1a: N & O). At the beginning of the courtship, IPIs are irregular and pulses are short and sharp, reminiscent of *D. mauritiana* males. In the course of courtship low frequency Thud-like pulses (not shown in Figure 5.2.1) and high frequency, high amplitude Clack-like pulses, reminiscent of *D. yakuba* pulses, were audible. Figure 5.2.2 (see below) depicts the distribution of hybrid male IPIs, from the two crosses involving *D. mauritiana* females. The IPI distributions in both cases, have a unimodal shape. Their modal IPIs are between ~60-65 and ~70-75ms, respectively.

Figure 5.2.2: IPI distribution of hybrid males from crosses between *D. mauritiana* Sweden females x *D. yakuba* males.
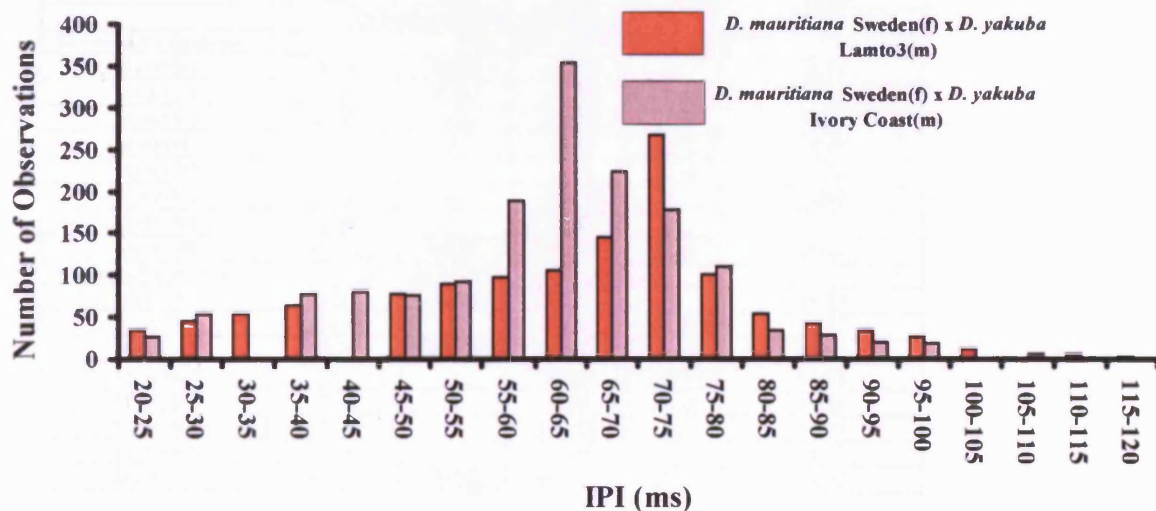
Song rhythms?

All the preliminary procedures and spectral analyses and rules that had been applied in Chapter 3 and 4, and the previous section, have also been followed here. The highlighted values on table 5.2.2 (see below) are the values that have been used to determine the rhythm periods in these male hybrids. It can clearly be seen that the CLEAN and the corresponding VdB values, for each individual song, are approximately similar. Table 5.2.2 shows the results from the two spectral analyses, when different cut-off points were employed.

Song My1t15r1 has a significant Nyquist value with CLEAN, of 20.08s (see table 5.2.2-below), which is within the range of the arrhythmic songs (Kyriacou and Hall,1989; Alt *et al.*, 1997), but in addition it has a second significant CLEAN peak at 30.74s. This was taken to be the period of the song, because it has a corresponding primary 'consensus' value for VdB analysis. My9 t15l1 shows a significcant primary peak in the 'climber' range, for both CLEAN and VdB analyses. In addition, it has a significant secondary peaks of 60.32s and 25.81s (see table 5.2.2-below), with CLEAN and VdB, respectively. Since the CLEAN values have been subsequently used to calculate the mean period of the genotype, 60.32s with CLEAN, is taken to be the period of this particular song.

Table 5.2.2: Spectral analysis of *D. mauritiana*(f) x *D.yakuba*(m) interspecific hybrids, using different cut-off points.

SPECIES	SPECTRAL		ANALYSIS		
	Clean		Van den Berg		Observations
	1ST Peak	2ND Peak	1ST Peak	2ND Peak	Bins Filled
INTERSPECIFIC HYBRIDS					
Mauritiana(f) x *Yakuba*(m)	CUT Off POINTS : 120 & 20 ms				
My1t15r1	20.08	30.74	31.37	x	14/23
My2t15l1	23.13	47.89	444.44	47.90	33/56
My4t15r2	46.02	26.29	45.98	26.23	39/56
My4t16l2	x	x	25.48	31.37	15/18
My5t15r2	25.88	x	21.05	26.14	19/24
My6t15r1	291.67	x	307.69	x	24/29
My8t15r1	77.84	x	77.67	x	18/27
My9t15l1	562.50	60.32	560.31	25.81	43/47
Mauritiana(f) xYakuba(m)	CUT Off POINTS : 150 & 20 ms				
My1t15r1	20.08	30.74	31.37	x	14/23
My2t15l1	354.55	x	380.95	47.90	34/57
My4t15r2	26.26	**45.86**	45.98	26.23	40/57
My4t16l2	x	x	25.48	31.37	15/18
My5t15r2	25.88	x	21.05	26.14	19/24
My6t15r1	294.12	x	250.00	x	24/29
My8t15r1	79.86	x	78.43	x	18/27
My9t15l1	562.50	59.32	560.31	25.81	43/47

Figure 5.2.3 shows the distribution of periods of hybrid songs from the *D. mauritiana*(f) x *D. yakuba* (m) crosses, whose values were highlighted in Table 5.2.2, together with their corresponding mean period ± sem. Of the 8 *D. mauritiana* (f) x *D. yakuba* (m) songs examined, 3 songs were found to be arrhythmic, one had a period between ~30-40s, two had a period between ~40-50s, one had a period between ~60-70s and one had a period between ~70-80s. If all the rhythmic songs were to be included in the determination of the overall mean period for this genotype, the period would be 52.53(s) ± 7.88, n=5 (Figure 5.2.3), which is slightly nearer the *D. mauritiana* value of ≈ 40s than the *D. yakuba* Thud value. Also, the distribution of periods in the hybrids is similar to *D. mauritiana*, in that the clustering of the period in Figure 5.2.3 is reminiscent of the *D. mauritiana* clustering of periods seen in Figure 4.2.1 (see chapter 4), where the majority of periods lay between 30-40s, and several between 50-70s.

D. mauritiana Sweden (f) x D. yakuba (f)
CLEAN-Cut-off Points:- 120 & 20ms
Mean Period: 52.53 (s) ± 7.88
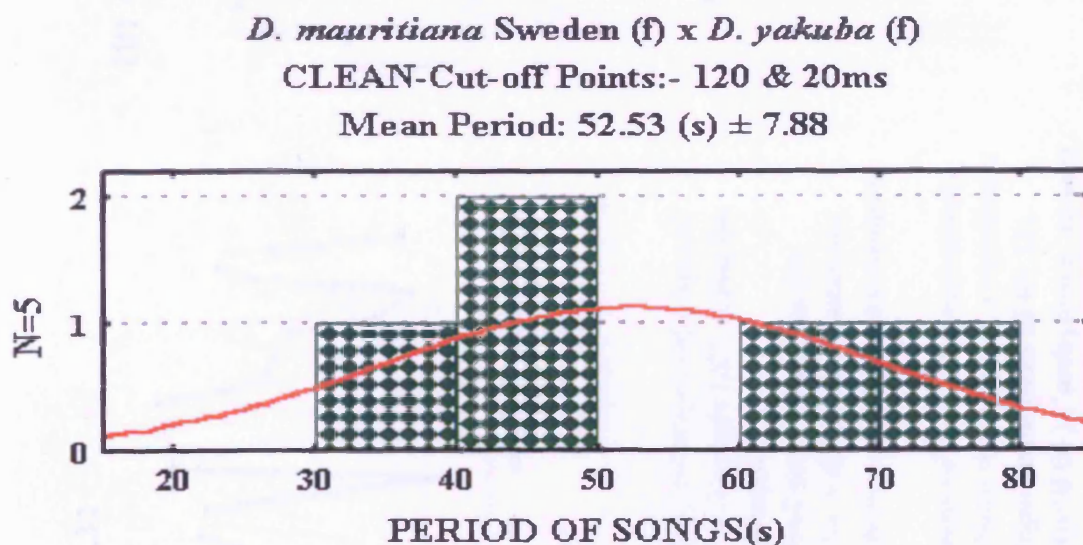
N = Number of Observations, Red Line = Expected Normal Distribution

Figure 5.2.3: The distribution of the periods of hybrid songs from the *D. mauritiana*(f) x *D. yakuba(m)* crosses.

Figure 5.2.4 shows the results of a *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) hybrid song, after the two different spectral analyses had been employed, both of which give significant ≈ 46s cycles. Figure 5.2.5 shows the results of another hybrid song. Significant 'climbing' frequencies are seen with a significant 60s period in CLEAN, and 25.281 and 59.70s in VdB.

(A) period = 45.8s F= 4.164, df= 3,32

Time (s)
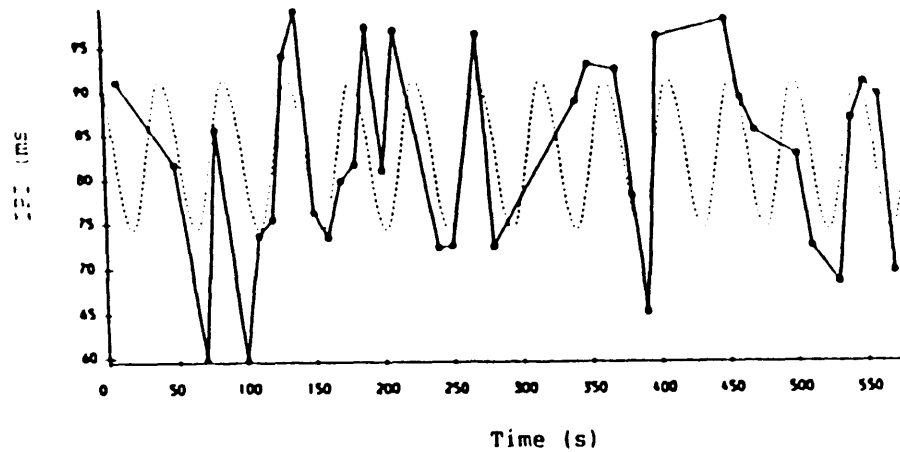
(B) 46.02s, 69.52s, 26.29s, 216.67s   CLEAN

Frequency
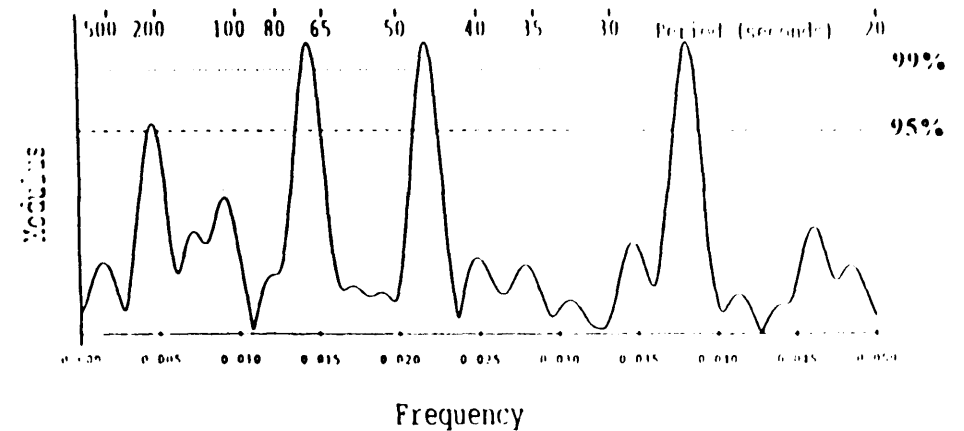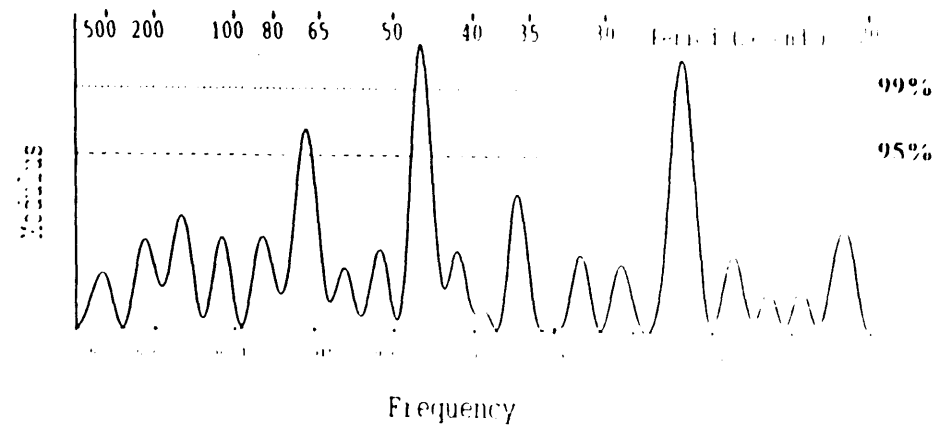
Figure 5.2.4:

D. mauritiana Sweden(f) x *D. yakuba* Lamto3(m)-Interspeciefic hybrid courtship song (My4t15r1) analysed-see table 5.2.2-uuper half, using the lower 120 & 20ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 46.02s (p<0.01), a second significant peak of 69.52s (p<0.01), a third significant peak of 26.29s (p<0.01) ('arrhythmic' range), as well as a fourth significant peak of 216.67s ('climber' range) (p<0.05), with the CLEAN analysis. Also note that there is a significant primary peak of 45.98s (p<0.01), as well as a second significant peak of 26.23s (p<0.01), with the VdB analysis. The individual lines represent the 95 and 99% confidence limits defined by Monte Carlo simulations (see Chapter 2).

(C) 45.98s, 26.23s, 68.97s   VdB

Frequency

(A) period = 25.7s F=3.154, df=3,39

(B) 562:50s, 60.32s   CLEAN

(C) 560.31s, 25.81s, 59.70s   VdB
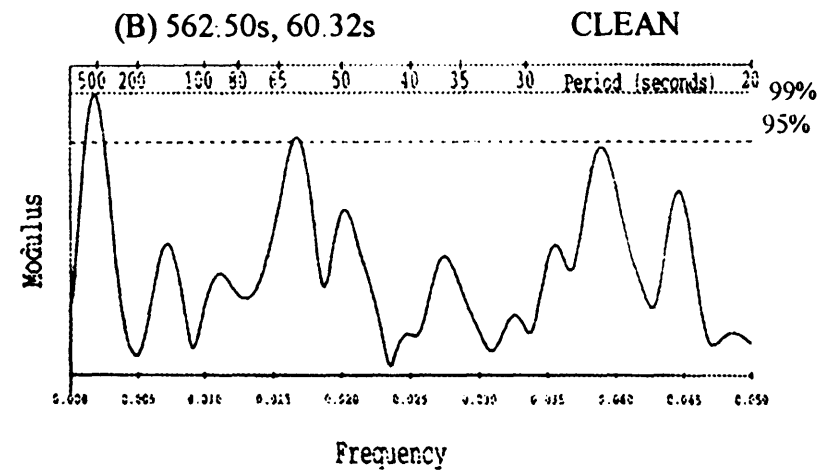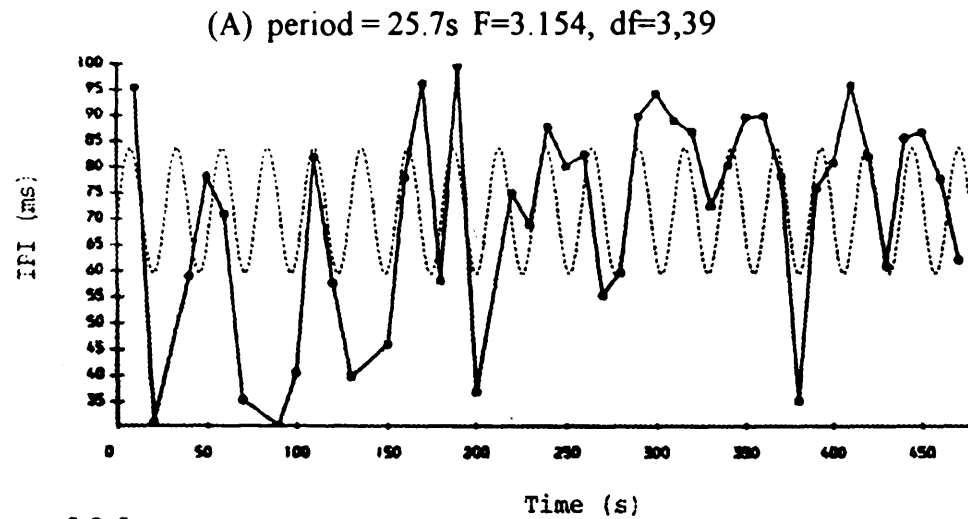
Figure 5.2.5:

D. mauritiana Sweden(f) x *D. yakuba* Ivory Coast(m)-Interspecific hybrid song (My9t1511) analysed-see table 5.2.2-upper half, using the lower 120 & 20ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 562.50s ('climber' range) (p<0.01), as well as a weaker secondary peak of 60.32s (p<0.05), with the CLEAN analysis. Also note that there are two significant peaks of 560.31s and 25.81s (p<0.01)('climber' and arrhythmic range) respectively, as well as a weaker tertiary peak of 59.70s (p<0.05), with the VdB analysis. This is taken to be the correct period, because it has a similar value to its CLEAN-derived counterpart. The individual lines are the 95 and 99% confidence limits defined by Monte Carlo simulations (see Chapter 2).
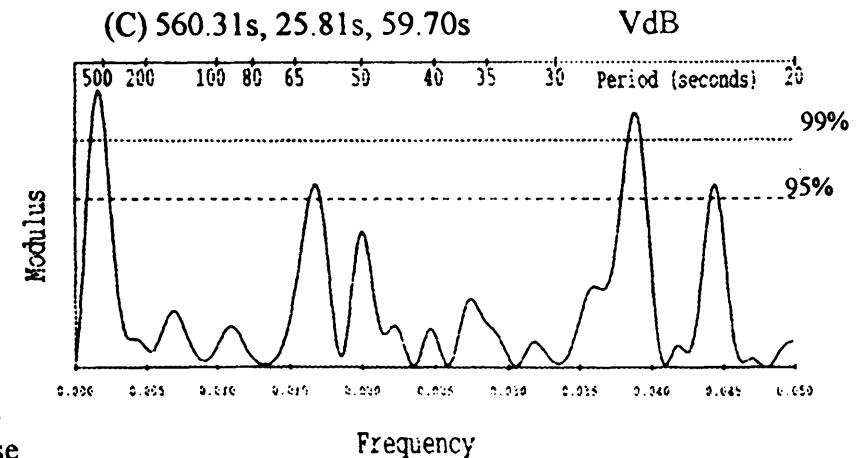
One-way ANOVA between the periods of the parental strains and the reciprocal hybrids showed significant differences (F=3.63, df=3, 47, p=0.020). Newman-Keuls *a posteriori* test showed significant differences, at least at p<0.05, between the periods of *D. mauritiana* and the *D. yakuba*(f) x *D. mauritiana*(m) hybrids, but no significant differences were observed between these hybrids and *D. yakuba*. The test also revealed that there were no significant differences between the periods of the *D. mauritiana*(f) x *D. yakuba*(m) hybrids and *D. mauritiana* (for ANOVA and Newman-Keuls *a posteriori* test results see Appendix 5.2.1). Therefore, the mean periods for the hybrid crosses suggest X chromosome involvement, which is in agreement with Kyriacou and Hall (1980, 1986).

5.3 *D. yakuba, D. teissieri* and their hybrids:

Both reciprocal crosses have been attempted. After persistent attempts, it was possible to obtain two-sex progeny by using *D. yakuba* females as the maternal participant in the cross. The integral role of non-visual stimuli in the courtship ritual (Bennet-Clark and Ewing, 1969; Schilcher, 1976a; 1976b) was further demonstrated, since when 'mute' *D. yakuba* females (females with their wings removed) were courted by *D. teissieri* males, no progeny was produced (Table 5.1). The percentage of vials set up, giving rise to any progeny is down to 57.20%, while the percentage of the interspecific cross producing both male and female offspring is 38.46%. The reciprocal cross, involving *teissieri* females, was unsuccessful in producing any progeny. Since *D. teissieri* song differs qualitatively from *D. yakuba* with respect to the presence of sine song (hum song), the interspecific hybrids could provide further information on the mode of inheritance of this acoustic element. The IPI and modal IPI measurements were taken from 6 flies, whereas the IPF measurements were taken from 3 flies.

Table 5.3.1: Song characters of *D. yakuba, D. teissieri* and their interspecific hybrids.

SPECIES/ STRAINS	OVERALL MEAN IPI ± sem (ms)	MODAL IPI(ms)	MEAN SSF ± sem (Hz)	OVERALL MEAN IPF ± sem(Hz)
yakuba STRAIN:				
FRANCE	124.61±2.71	95.50	X	302.22±3.17
teissieri STRAIN:				
SWEDEN	28.02±1.28	21.20	231.5±4.35	432.66±4.01
STRAINS:	INTERSPECIFIC GENOTYPE			
yakuba France (f) x *teissieri* Sweden(m)	64.76±6.00	60.07	X	411.77±3.58

The mean IPI for the individual songs in this hybrid genotype ranges from ~44-95ms, intermediate between the parental values (see Figure 5.1b:-E), and suggesting autosomal inheritance (Cowling and Burnet, 1981; Kyriacou and Hall, 1980). This is confirmed with the overall mean IPI and modal IPI's of the hybrids, which are also intermediate between the parental values. The mean IPF of the hybrids is more *teissieri*-like, suggesting dominance of *D. teissieri* autosomal genes.
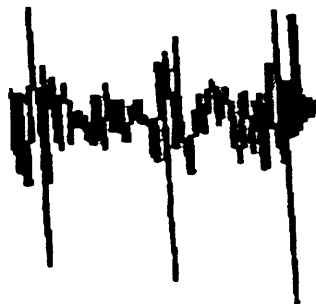
Figure 5.3.1: The principle song component of a D. *yakuba* France(f) x D. *teissieri* Sweden(m) song.

Figure 5.3.1 illustrates some song pulses. At certain instances in the course of the courtship song, pulses were sharp, with high amplitudes, reminiscent of the Clack-type pulses, and at other instances, the pulses were dense, with lower amplitude, resembling the *D. teissieri* pulses. Figure 5.3.2 (see below) shows the distribution of IPI of a *D. yakuba* France(f) x *D. teissieri* Sweden(m) interspecific hybrid. The distribution is unimodal in shape, as all the distributions examined so far with a modal IPI of ~60-65ms.
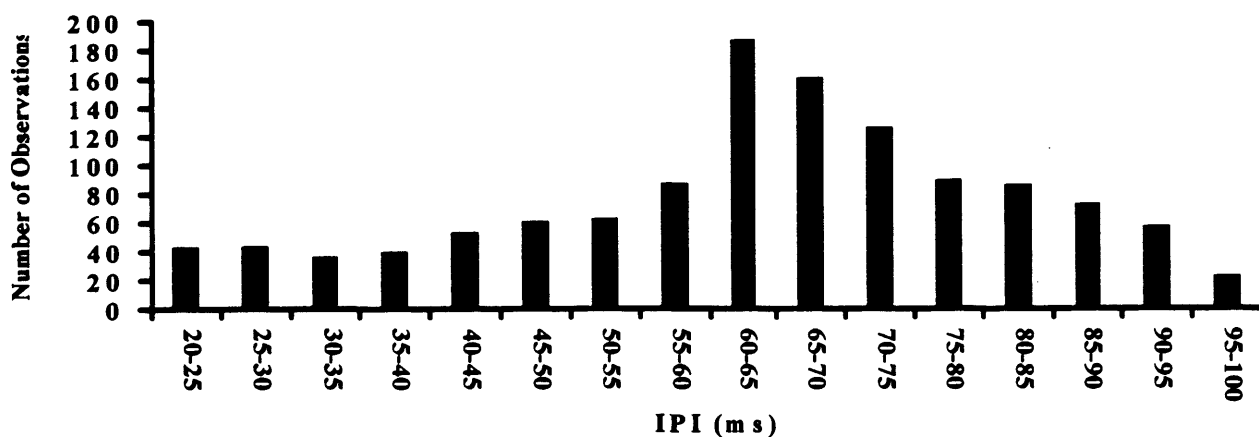
Figure 5.3.2: The distribution of IPI of a D. *yakuba* France(f) x D. *teissieri* Sweden(m) interspecific male (Hyb6t7l1).

102

Song rhythms?

The highlighted values on table 5.3.2 (see below) are the values that have been used to determine the rhythm periods of this interspecific genotype, using different IPI cut-off points. It can clearly be seen that the CLEAN and the corresponding VdB values, for each individual song, are quite similar. Song Hyt9t6l2 has a significant primary peak in the 'climber' range, but also shows a second significant peak of 34.68s (CLEAN), with a very similar significant primary peak VdB value of 34.63s. Songs, Hyt6t6l2 and Hyt9t6l2, become arrhythmic with CLEAN, when the lower 90 & 15ms cut-offs are used (see Table 5.3.2-lower part). The reason for choosing 90ms instead 120ms, as the 'alternative' upper cut-off was that in the majority of this type of interspecific genotype, no IPIs were found to be over 100ms.

Table 5.3.2: Spectral analysis of *D. yakuba* France(f) x *D. teissieri* Sweden(m) interspecific hybrid, using different cut-off points.

SPECIES	SPECTRAL	ANALYSIS		
SPECIES	Clean		Van den Berg	Observations
	1ST Peak	2ND Peak	1ST Peak	2ND Peak	Bins Filled
INTERSPECIFIC HYBRIDS				
yakuba(f) x teissieri(m)	CUT Off POINTS : 100 & 15 ms			
Hyt4t7ll	24.12	199.28	24.17	205.13	36/57
Hyt6t6l2	39.55	x	26.49	40.00	24/30
Hyt6t7ll	87.46	x	86.96	50.63	31/61
Hyt8t6l2	65.98	25.18	66.67	25.81	23/30
Hyt9t6l2	283.51	34.68	34.63	250.00	39/58
yakuba(f)x teissieri(m)	CUT Off POINTS : 90 & 15 ms			
Hyt4t7ll	24.12	199.28	24.17	205.13	36/57
Hyt6t6l2	29.41	x	45.71	142.86	22/30
Hyt6t7ll	88.04	x	87.91	275.86	31/61
Hyt8t6l2	25.71	66.18	66.67	25.72	22/30
Hyt9t6l2	20.45	x	20.67	x	36/58

Of the five *D. yakuba* France(f) x *D. teissieri* Sweden(m) songs examined, one was found to be arrhythmic. The periods are quite variable, with two longer than 60s and two shorter (see Table 5.3.2). Both parental species have long rhythms (see chapter 3, section 3.5- ~63s for *D. yakuba* Thud+Clack, ~ 67s for *D. yakuba* Thud and ~ 57s for *D. yakuba* Clack, and chapter 4, section 4.4- ~72s for *D. teissieri*), and so, the overall mean of ~57s is slightly shorter than the parental values. One-way ANOVA between the parental species (*D. teissieri* and *D. yakuba* Thud + Clack) and the hybrids showed no significant differences (F=0.67, p=0.52, df=2, 38). The small sample size and low vigour of the courtships (the % of filled bins is quite low) have not helped the analysis. It is,

however, encouraging that the hybrid rhythms are not significantly different from those of the parents. Figure 5.3.3 shows one of these hybrid songs with a period of ≈ 87s.

5.4 *D. simulans*, *D. mauritiana* and their interspecific hybrids:

The cross, involving *D. simulans* females, was achieved with comparative ease (David *et al.*, 1974), although, it has not been possible to obtain any progeny from the reciprocal cross, involving *D. mauritiana* females, in this present study. The percentage of vials set up producing any kind of offsprings is 66.66% (either single-sex or two-sex progeny), while the percentage of interspecific crosses, with *D. simulans* females (see table 5.1:-Column 7) giving rise to two-sex progeny is 34.15%. The reciprocal cross, with *D. mauritiana* females (see table 5.1:-Column 8) was completely unsuccessful, even when *D. mauritiana* females with 'amputated' wings were used.

During the recording of these songs, both basic components of a courtship song, sine and pulse songs (see Figure 5.4.1-below) were present. It was often obesrved that at the beginning of a burst the pulses had irregularly long IPIs, which became more uniform in shape as the burst progressed, which was reminiscent of the *mauritiana* pulses.

SINE SONG   PULSES
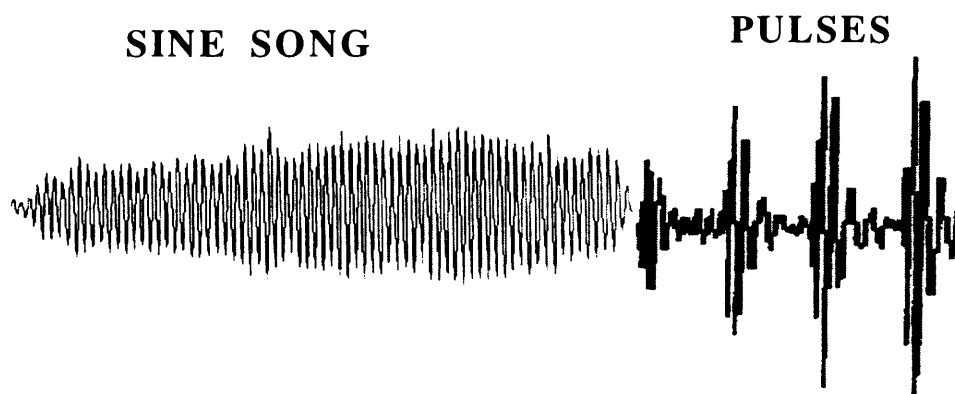
Figure 5.4.1: The principle song components of a *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) song.

The mean IPI of individual songs for this genotype ranges from ~36-61ms (see Figure 5.1b:- G). The IPI and modal IPI measurements were taken from 7 flies, whereas the IPF and SSF measurements were taken from 3 flies. Table 5.4.1 gives the values for the major song characters.

(A) period = 85.9s F=4.216, df=3,26

(B) 87.46s   **CLEAN**

(C) 86.96s   **VdB**
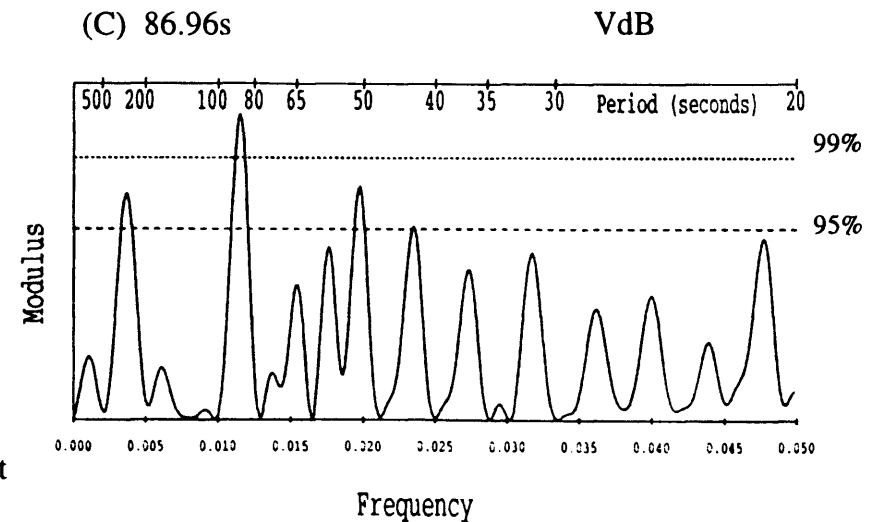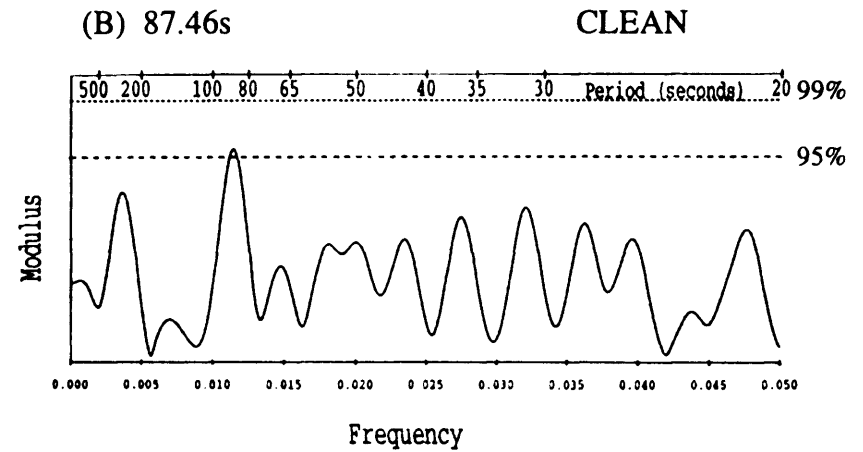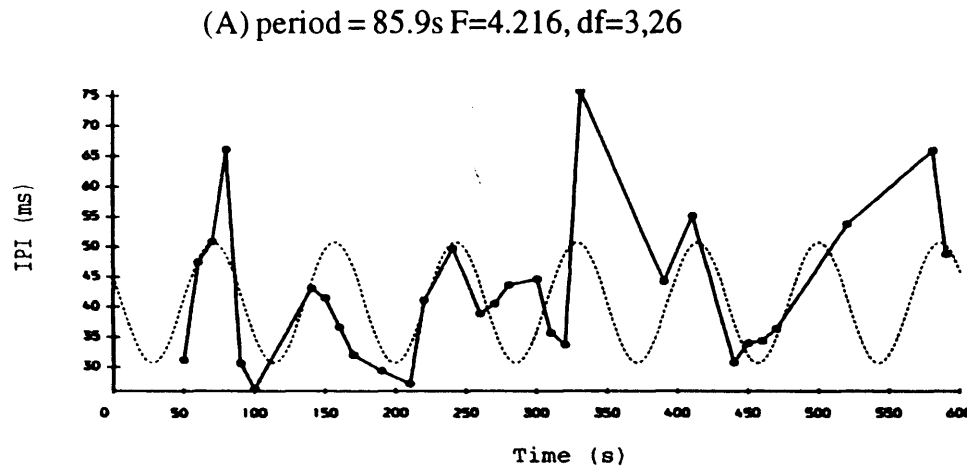
Figure 5.3.3:

D. yakuba France(f) x *D. teissieri* Sweden(m)-Interspecific hybrid courtship song (Hyt6t711)-see table 5.3.2-upper half, using the higher 100 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 87.46s (p<0.05), with the CLEAN analysis. Also note that there is a significant primary peak of 86.96s (p<0.01), as well as a weaker secondary peak of 50.63s (p<0.05), with the VdB analysis. The individual lines are the 95 and 99% confidence limits defined by Monte Carlo simulations (see chapter 2).

Table 5.4.1: Song characters of *D. simulans*, *D. mauritiana* and their hybrids.

SPECIES/ STRAINS	OVERALL MEAN IPI ± sem (ms)	MODAL IPI(ms)	MEAN SSF± sem (Hz)	OVERALL MEAN IPF ± sem(Hz)
simulans STRAIN:				
FLORIDA	53.34±5.74	36.17	205.75±6.21	408.01±3.55
mauritiana STRAIN:				
SWEDEN	41.81±2.23	27.31	190.64±5.49	247.75±1.31
STRAINS:	INTERSPECIFIC GENOTYPE			
simulans Florida (f) x *mauritiana* Sweden(m)	46.15±1.04	30.14	204.19±3.00	500.29±8.69

The mean IPI of these hybrids are intermediate to the parental values, suggesting autosomal control. One-way ANOVA showed significant differences between the parental species and these hybrids (F=3.48, p=0.048 and df=2, 23-see Appendix 5.4.1). Newman-Keuls *a posteriori* test revealed significant differences, at least at p<0.05, between *D. mauritiana* and *D. simulans*. The test also revealed no significant differences between *D. simulans* and *D. mauritiana*, and these hybrids (see Appendix 5.4.1). The modal IPI of the hybrids are slightly more *mauritiana*-like, suggesting the presence of dominant *D. mauritiana* autosomal factors. One-way ANOVA of the modal IPI values between the parental species and these hybrids showed no significant differences (F=1.69, df=2, 23, p=0.21). The mean SSF is more *simulans*-like, suggesting either X chromosome involvement, or autosomal dominant factors for *D. simulans*. One-way ANOVA between the parental species and these hybrids revealed significant differences (F=5.42, df=2, 8, p=0.038-see Appendix 5.4.2). Newman-Keuls *a posteriori* test revealed significant differences, at least at p<0.05, between *D. mauritiana*, and *D. simulans* and these hybrids. The test also revealed no significant differences between *D. simulans* and these hybrids (see Appendix 5.4.2). The IPF of these hybrids is higher than both the parental values, suggesting hybrid vigour. Figure 5.4.2 (below) shows the distribution of IPI of a *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) interspecific hybrid song. The distribution is unimodal in shape, broader than the distribution of *D. mauritiana* Sweden (see Figure 4.1.2- red columns) and of *D. simulans* Florida (see Figure 3.2.1), with a modal IPI between ~27-30ms.
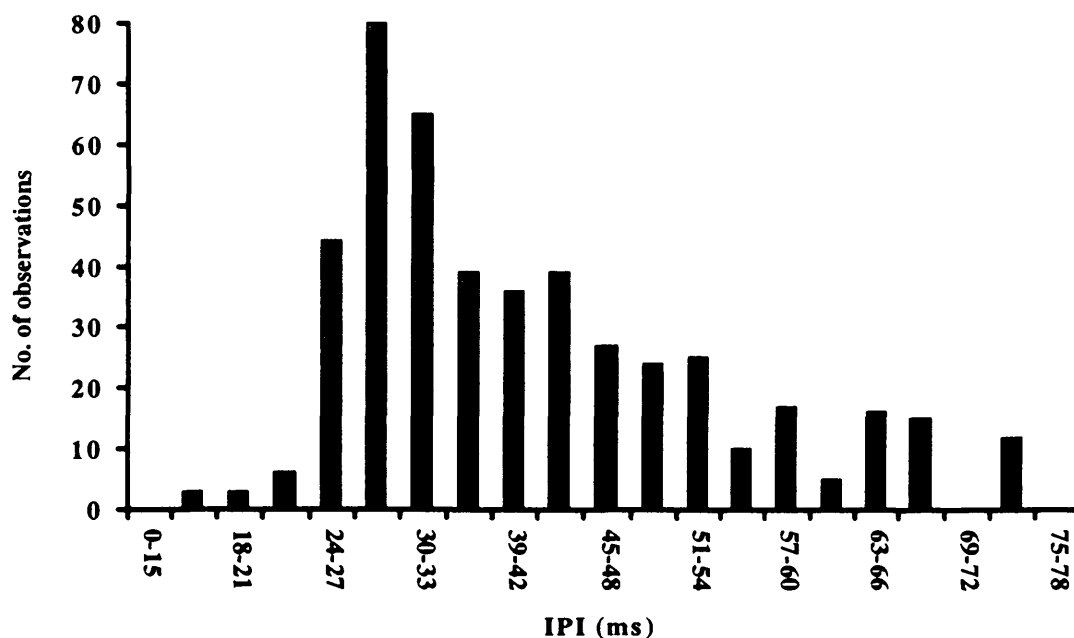
Figure 5.4.2: The distribution of IPI of hybrid male from the *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) cross(SM1t1711).

Song rhythms?

The results are shown on Table 5.4.2, using different cut-off points (approximately twice the mean), which had been decided after the individual IPI histograms had been examined. The highlighted values in table 5.4.2 (see below) are the values that have been used to determine the rhythm periods of this interspecific genotype. As seen, the CLEAN and the corresponding VdB values, for each individual song, are approximately similar. However, the songs are not very robust, in two cases only just passing the 50% bins filled criterion. The most vigorous song (Sm3e2s2m, 29/37 bins filled) has a 45s cycle.

Table 5.4.2: Spectral analysis of the *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) hybrid genotype, using different cut-off points.

	SPECTRAL	ANALYSIS			
SPECIES	Clean		Van den Berg		Observations
	1ST Peak	2ND Peak	1ST Peak	2ND Peak	Bins Filled
INTERSPECIFIC HYBRIDS					
simulans(f) x mauritiana(m)	CUT Off POINTS : 80 & 15 ms				
Sm1t1711	63.60	22.47	65.04	x	19/38
Sm3e2s2m	45.34	24.07	24.24	x	29/37
Sm4e2s2m	68.44	23.44	23.46	68.97	28/53
simulans(f)xmauritiana(m)	CUT Off POINTS : 75 & 15 ms				
Sm1t1711	63.60	22.47	65.04	x	19/37
Sm3e2s2m	26.04	44.32	20.57	222.22	28/37
Sm4e2s2m	68.44	23.44	23.46	68.97	28/53

106

Out of the three *D. simulans* (f) x *D. mauritiana* (m) songs examined, all of them were found to be rhythmic with periods between ~45-70s. The overall mean period for this genotype is 59.13(s) ± 7.03, n=3 (see Figure 5.4.3-below), which is longer than both parental values. One-way ANOVA between the periods of the parental species and the hybrids showed no significant differences (F=3.24, p=0.07, df=2, 14). However, the small sample size and poor vigour of these hybrids means that little confidence can be placed in the data, even though the statistical analysis gives no significant differences between the parents and the hybrids, which is what it would be expected from the parental values.

Figure 5.4.3 shows a hybrid song which just reach the 50% bins filled criterion. The missing data points make this song presentation ambiguous, as can be observed in Figure 5.4.3a. In Figure 5.4.3b, a secondary peak of ~34s is also significant, a reflection of the gaps in the data series, because gaps will also allow shorter cycles to be compressed within the data.

5.5 *D. teissieri*, *D. mauritiana* and their interspecific hybrids:

The percentage of vials set up, involving *teissieri* females, giving rise to either single-sex or two-sex progeny is 69.23%; only 25% of these vials set up produced viable two-sex progeny (see Table 5.1). The reciprocal cross, involving *D. mauritiana* females, was unsuccessful; even when 'mute' *D. mauritiana* females (females with their wings removed), were set up with *D. teissieri* males, no viable progeny were produced.

Both principal acoustic components were observed to be present in the songs of this genotype (see Figure 5.5.1- below). When the hybrid courtship songs from the *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m) cross were printed on dot matrix computer paper, it was observed that in some instances, the pulses (irregular in shape and value) resembled the *D. mauritiana* pulses and at other instances, in the course of the song, the pulses became dense, reminiscent *D. teissieri*.

(A) period =63.9s F=2.503, df=3,14

Time (s)

(B) 63.60s, 22.47, 34.62s   CLEAN

Frequency
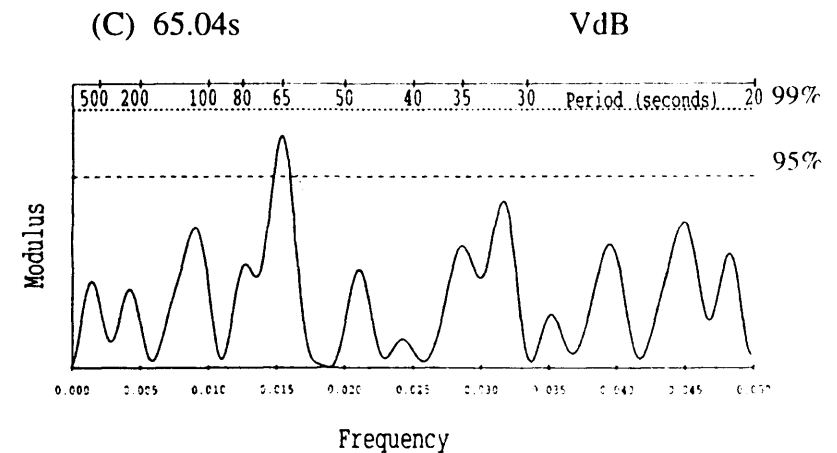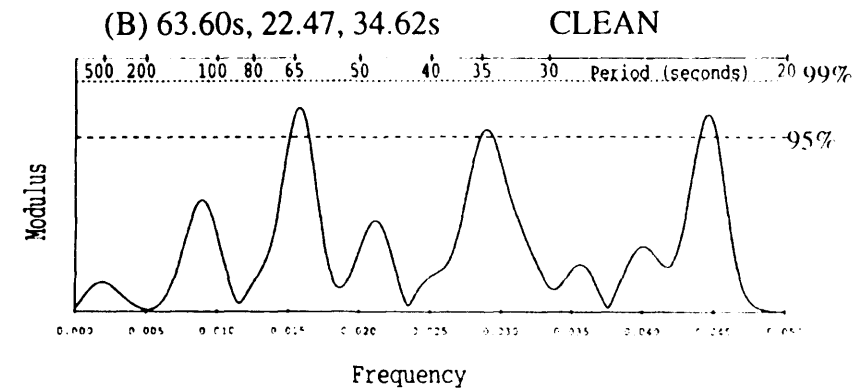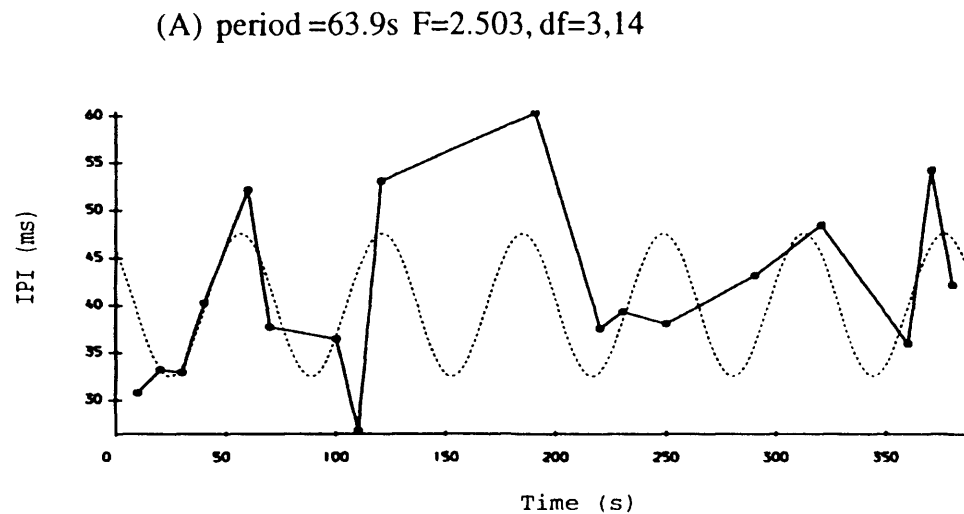
Figure 5.4.3:

D. simulans Florida(f) x *D. mauritiana* Sweden(m)-Interspecific hybrid courtship song (Sm1t1711) analysed-see table 5.4.2-upper half, using the higher 80 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the strongest cycle in the data, initially defined by VdB spectral analysis, and then fitted to the data by non-linear regression, using a least squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively, each indicating the corresponding periods. Note that there is a significant primary peak of 63.60s (p<0.05), as well as two weaker secondary and tertiary peaks of 22.47s (arrhythmic range) and of 34.62s (p<0.05), respectively, with the CLEAN analysis. Also note that there is a significant primary peak of 65.04s (p<0.05), with VdB.The individual lines are the 95 and 99% confidence limits defined by Monte Carlo simulations (see Chapter 2).

(C) 65.04s   VdB

Frequency

Figure 5.5.1: The principle song components of a *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m) song.

SINE SONG　　　　　　**PULSES**
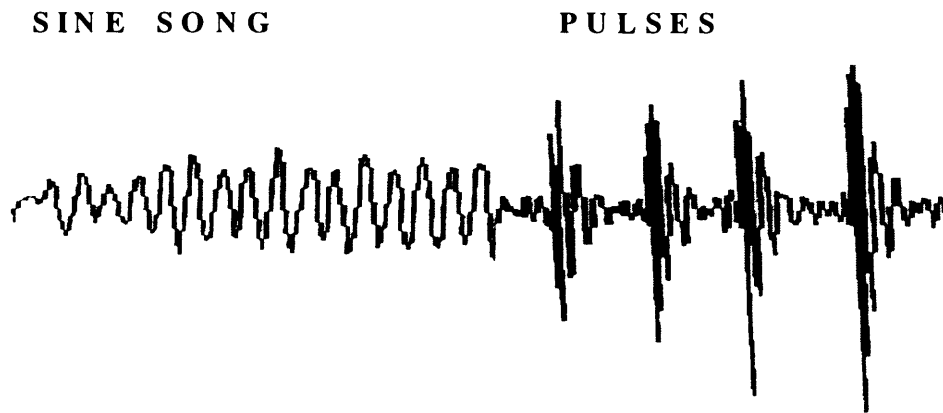
The mean IPI, of the individual songs, for this genotype ranges from ~36-59ms (see Figure 5.1b:- F, and table 5.5.1). The IPI and modal IPI measurements were taken from 10 flies, whereas the IPF and SSF measurements were taken from 3 flies.

Table 5.5.1: Song characteristics for *D. teissieri*, *D. mauritiana* and their interspecific hybrids.

SPECIES/ STRAINS	OVERALL MEAN IPI ± sem (ms)	MODAL IPI(ms)	MEAN SSF ± sem (Hz)	OVERALL MEAN IPF ± sem (Hz)
teissieri STRAIN:				
SWEDEN	28.02±1.28	21.20	231.50±4.35	432.66±4.01
mauritiana STRAIN:				
SWEDEN	41.81±2.23	27.31	194.64±5.49	247.75±1.31
STRAINS:	INTERSPECIFIC GENOTYPE			
teissieri Sweden(f) x *mauritiana* Sweden(m)	34.70±1.59	31.00	219.76±2.89	537.42±7.53

The table 5.5.1 shows that the mean IPI of these hybrids are intermediate between the parental values, which is suggestive of autosomal control for this character. One-way ANOVA revealed significant differences between the parental species and these hybrids ($F=18.58$, $df=2$, 40, $p=0.000$-see Appendix 5.5.1). Newman-Keuls *a posteriori* test revealed significant differences, at least at $p<0.05$, between the parental species and the hybrids, as well as differences between the IPIs of the parental species (see Appendix 5.5.1). The modal IPI (for ANOVA and Newman-Keuls *a posteriori* test results see Appendix 5.5.2), and mean IPF are seen to be higher than both the parental values, which suggests hybrid vigour. The SSF is observed to be more *teissieri*-like which suggests X chromosome involvement. One-way ANOVA between the parental species and these

hybrids revealed significant differences (F=42.75, df=2, 8, p=0.000-see Appendix 5.5.3). Newman-Keuls *a posteriori* test revealed significant differences, at least at p<0.05, between *D. mauritiana*, and *D. teissieri* and these hybrids. The test also showed that there were no significant differences between *D. teissieri* and these hybrids (see Appendix 5.5.3).

Figure 5.5.2 (see below) shows the distribution of IPIs of a representative hybrid male which is unimodal, but broader than the distributions of both the parental strains (see Figure 4.3.3-black columns and Figure 4.1.2).
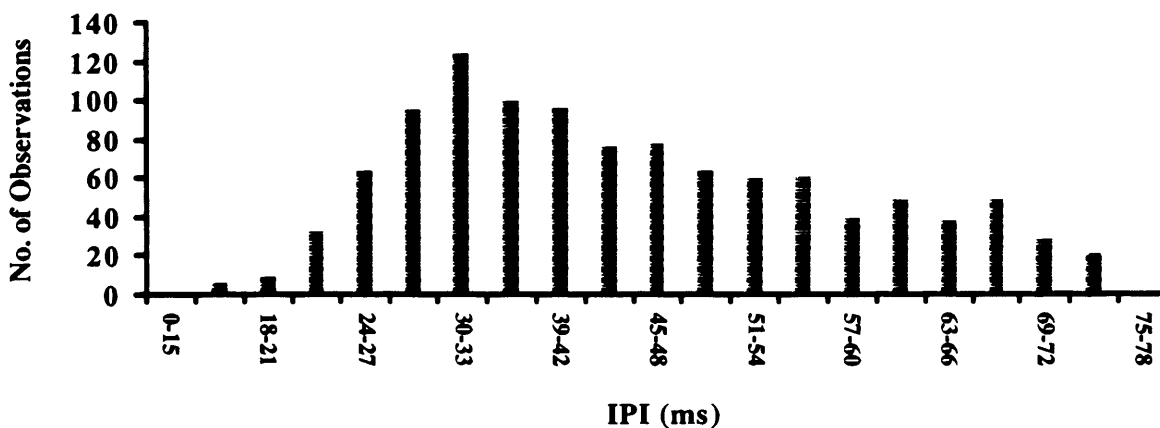
Figure 5.5.2.: The distribution of IPI of a hybrid male from the *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m) cross-(Tm3se2s1m2).

Song rhythms?

The results are shown on Table 5.5.2, using different cut-off points (approximately twice the mean), which had been decided after the individual song histograms had been examined. The highlighted values in table 5.5.2 (see below) are the values that have been used to determine the rhythm periods of interspecific genotype. It can clearly be seen that the CLEAN and the corresponding VdB values, for each individual song, are approximately similar.

Table 5.5.2: Spectral analysis of hybrid songs from the *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m) cross.

SPECIES	SPECTRAL	ANALYSIS		
	Clean		Van den Berg	Observations
	1ST Peak	2ND Peak	1ST Peak	2ND Peak	Bins Filled
INTERSPECIFIC HYBRIDS					
Teissieri(f) x Mauritiana(m)	CUT Off POINTS : 80 & 15 ms				
Tm1e2s1m	50.62	22.18	44.94	24.96	56/86
Tm1e2sm1	45.15	40.00	90.91	75.47	33/46
Tm3e2s1m	283.33	62.35	307.69	1000	48/54
Tm4e2s1m	73.72	x	26.58	x	19/37
Tm5e2sm2	92.39	65.22	94.12	65.04	39/54
Tm6e2s2m	66.67	54.05	24.39	65.57	40/49
Tm7e2s1m	27.31	x	22.28	26.94	28/31
Tm8e2s2m	52.25	40.73	25.56	51.95	41/82
Tm9e2s2m	107.42	36.47	35.89	800.00	43/66
Teissieri(f) x Mauritiana(m)	CUT Off POINTS : 75 & 15 ms				
Tm1e2s1m	22.18	50.18	50.31	33.06	56/86
Tm1e2sm1	43.12	24.63	45.71	x	32/46
Tm3e2s1m	289.77	62.35	307.69	1000	46/54
Tm4e2s1m	x	x	26.49	22.99	17/37
Tm5e2sm2	64.79	x	94.12	75.47	38/54
Tm6e2s2m	x	x	65.57	20.62	38/49
Tm7e2s1m	29.77	x	26.76	22.16	26/31
Tm8e2s2m	41.20	x	25.56	51.95	40/82
Tm9e2s2m	878.79	23.60	30.30	800.00	42/66

Songs Tm6e2s2m and Tm9e2s2m become arrhythmic, while songs Tm3e2s1m, Tm5e2sm2 and Tm8e2s2m lengthen their periods when the lower 75 & 15ms cut-offs are used (table 5.5.2). Figure 5.5.3 shows the distribution of periods of *D. teissieri* Sweden (f) x *D. mauritiana* Sweden (m)-interspecific hybrids, whose values were highlighted in Table 5.5.2, together with their corresponding mean period of $\approx$ 60s. Out of the 9 hybrid songs examined, one song was found to arrhythmic. 6 of the remaining 8 songs had periods >50s. These periods fall much closer to the *D. teissieri* value of $\approx$ 70s, than those of *D. mauritiana* ones, suggesting X chromosome determination of the song period. One-way ANOVA between the periods of the parental species and the hybrids revealed significant differences (F=6.29, df=2, 26, p=0.006). Newman-Keuls *a posteriori* test showed significant differences between *D. mauritiana* and, *D. teissieri* and the hybrids, whilst no significant differences were observed between *D. teissieri* and the hybrids (see Appendix 5.5.4). This suggests X chromosome involvement, which is in agreement with Kyriacou and Hall (1980; 1986). Of the various hybrids examined in this chapter, these by far generate the most vigorous courtships, and therefore more confidence can be placed in the results.

D. teissieri(f) x **D. mauritiana**(m)
CLEAN-Cut-off Points: 80 & 15ms
Mean Period: 59.95 (s) ± 6.28
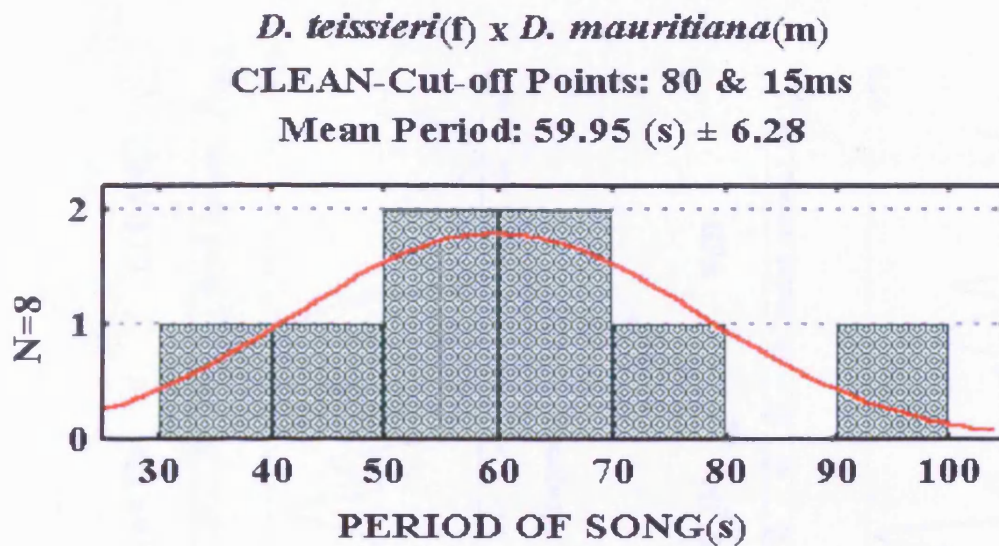
Figure 5.5.3: The distribution of the periods of hybrid songs from the *D. teissieri* Sweden (f) x *D. mauritiana* Sweden *(m)* cross.

Figure 5.5.4 shows a hybrid song with an approximate 90s cycle.

5.6 *D. erecta*, *D. orena* and their interspecific hybrids:

Both types of hybrid crosses failed to produce any progeny.

(A) period =92.8s F=3.562, df=3,36

(B) 92.39s, 64.72s   CLEAN
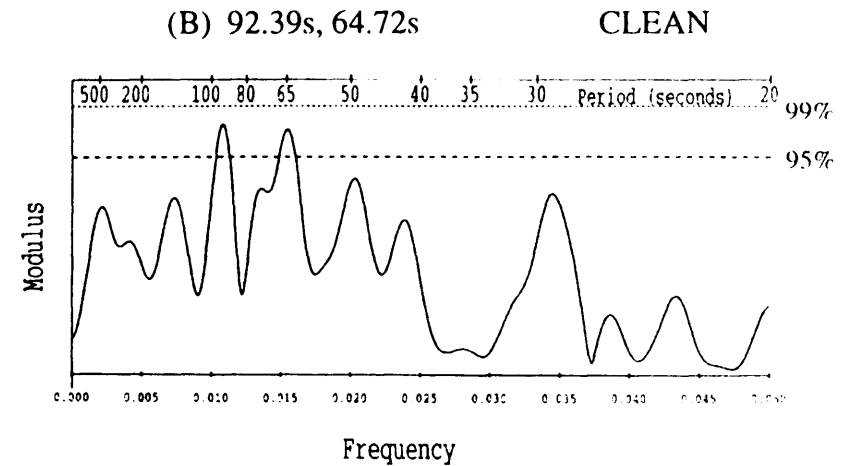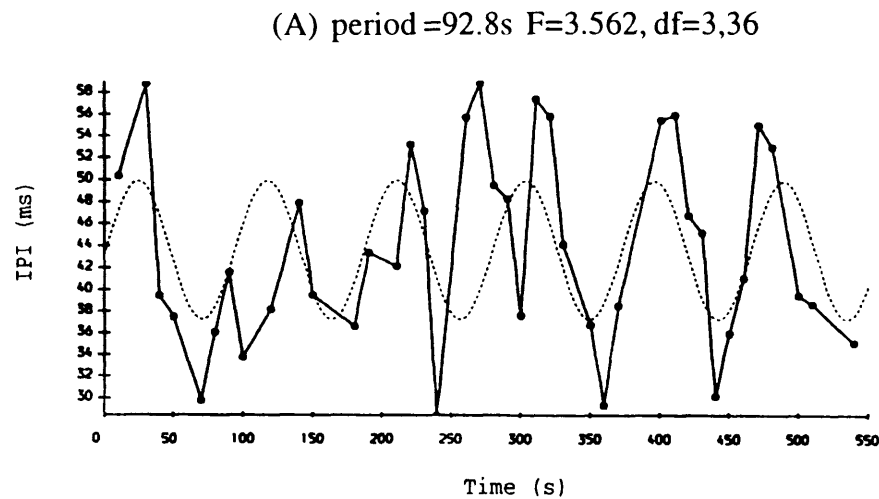
Figure 5.5.4:

D. teissieri Sweden(f) x *D. mauritiana* Sweden(m)-Interspecific hybrid
courtship song (Tm5e2sm2) analysed-see table 5.5.2-upper half, using the
higher 80 & 15ms cut-off points, and spectral analysis.

a) A plot of the mean IPI against time. The dotted line represents the
   strongest cycle in the data, initially defined by VdB spectral analysis,
   and then fitted to the data by non-linear regression, using a least
   squares procedure.

b) and c) show the CLEAN and VdB spectrograms of this song respectively,
   each indicating the corresponding periods. Note that there is a significant
   primary peak of 92.39s ($p<0.05$), as well as a weaker secondary peak of
   64.72s ($p<0.05$), with the CLEAN analysis. Also note that there is a significant
   primary peak of 94.12s ($p<0.05$), as well as a weaker secondary peak of 65.04s
   ($p<0.05$), with the VdB analysis. The 95 and 99% confidence limits are defined
   by Monte Carlo simulations (see chapter 2).

(C) 94.12s, 65.04s   VdB
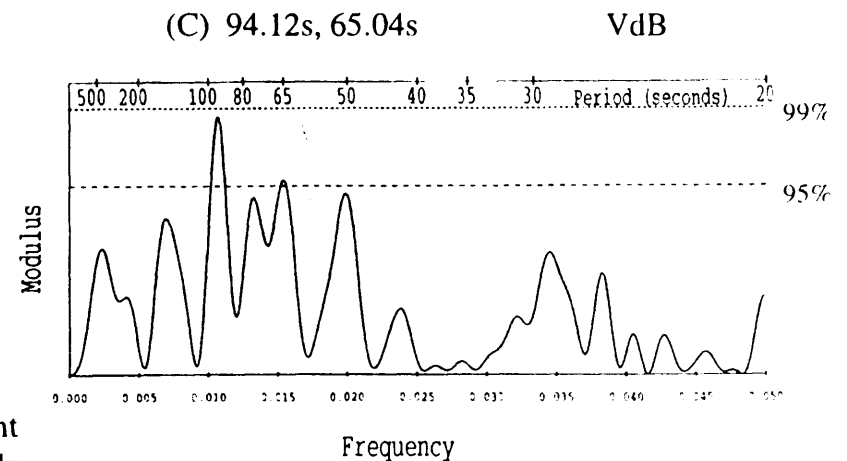
5.7 Discussion:

A number of different song characteristics have been studied in hybrids. Several conclusions can be drawn:

1) The mean and modal IPI seem to be autosomally determined, since the mean IPI value of the interspecific hybrids is approximately intermediate between the two parental strains' values. This observation reconfirms the original von Schilcher (1976a), Cowling and Burnet (1981) and Kyriacou and Hall (1986) observations;

2) As in the case of the *D. yakuba* intraspecific hybrids (see chapter 3), the mean IPF is seen to be usually higher than the parental values, which is suggestive of hybrid vigour implying that the parents have undergone inbreeding depression, which may suggest that superior fitness may be associated with higher IPFs. However, the mean IPF in the hybrid males involving *D. teissieri* (*D. yakuba* France(f) x *D. teissieri* Sweden(m)), tend to be *teissieri*-like, suggesting dominant autosomal *teissieri* factors (table 5.3.1). Clearly, the genetic control of IPF is more complex than originally perceived by Cowling and Burnet (1981);

3) The presence or absence of sine song was observed to depend on which species was the maternal participant in the cross. In interspecific hybrid crosses where the maternal participant was *D. yakuba* (known not to produce a sine song- Cowling and Burnet (1981) and Thackeray (1989)), no sine song was produced by those interspecific hybrid males. When *D. yakuba* is the paternal participant, as in the case of *D. mauritiana* Sweden(f) x *D. yakuba* Ivory Coast(m) and *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) (Table 5.2.1), sine song is present, suggesting X chromosome inheritance, and reconfirming the observations by Cowling and Burnet (1981). Moreover, the SSF in hybrid males involving *D. simulans* and *D. teissieri*, as the maternal parent, tend to be closer to the maternal values, suggesting again X chromosome involvement (tables 5.4.1 and 5.5.1) for this acoustic component;

4) As far as song rhythms are concerned, some hybrid males generated very little song. Consequently, the results can not be considered to be reliable (*D. yakuba* France(f) x *D. teissieri* Sweden(m) and *D. simulans* (f) x *D. mauritiana* (m)). However, the *D.*

yakuba France(f) x *D. mauritiana* France(m) and *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) interspecific males were quite vigorous (see section 5.1), and the majority of song rhythm periods were >60s, reflecting the maternal species value of *D. yakuba*. In the reciprocal crosses to the above (*D. mauritiana* Sweden(f) x *D. yakuba* Ivory Coast(m) and *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m)), song periods (see Figure 5.2.3) appeared to lie closer to the mean period of *D. mauritiana* than *D. yakuba*, suggesting that there is X chromosome involvement in the song rhythm, which is in agreement with Kyriacou and Hall's (1986) original observations with *D. melanogaster* and *D. simulans*. In *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m) cross, the majority of the periods are >50s. These periods fall much closer to the *D. teissieri* mean period value of ≈ 70s than that of *D. mauritiana*, which again suggests X chromosome determination of the song period. However, additional autosomal factor association cannot be ruled out. Indeed, some autosomal gene involvement has been implicated in Kyriacou and Hall's (1986) study in *D. melanogaster/D. simulans* hybrids.

The contribution of the X chromosome, in the species-specificity of the male song cycles has been carried to the molecular level by Wheeler *et al.* (1991). *D. melanogaster per^{01}* males, transformed with the *per* gene of *D. simulans*, sang with a characteristic *D. simulans* 40s cycle. Moreover, transformed *D. melanogaster* males, carrying a *D. simulans*-Thr-Gly encoding fragment in an otherwise *D. melanogaster per* background, were also found to sing with a typical 40s *D. simulans* song cycle. In addition, male flies carrying the reciprocal hybrid constructs, were found to behave in a characteristic *D. melanogaster* fashion. This small fragment (≈700 bp) of the *per* gene, that was interchanged between the two species *per* genes, has been found to be particularly variable in the *D. melanogaster* subgroup (Peixoto *et al.*, 1992). Moreover, the amino acids substitutions encoded within this fragment, which were assigned by Wheeler *et al.* (1991) as being critical in the song rhythmicity of *D. melanogaster* and *D. simulans* are observed to be unique among the different members of the *D. melanogaster* subgroup (Peixoto *et al.*, 1992). We can therefore imagine that the 'key' gene for the male song cycle in all these species is *per*. Consequently, since the orthologues of *D. melanogaster per*, in one other member of the *D. melanogaster* subgroup species, *D. yakuba*, has been cloned (e.g., Thackeray and Kyriacou, 1990), it is possible to transform *per* genes from the different species of the *D. melanogaster* subgroup, similar to those

constructed by Wheeler *et al.* (1991), into *per*[01] *D. melanogaster* hosts. This should determine whether *per* is the species-specific cycle gene within the *D. melanogaster* subgroup.

Since the existence of X-linked species-specific song rhythms was further supported, my attention now turned to short-term temporal changes in song characters, which are dealt in the ensuing Chapter 6.

Short-term temporal changes in song characters

CHAPTER 6

6. Short-term temporal changes in song characters

I have shown that song rhythms exist in the courtship songs of members of the *melanogaster* subgroup, over the 30-100s time scale. However, I have not examined any changes within the various courtship song components, namely IPI, IPF and CPP over much shorter time scales, such as song bursts. Ewing (1983) provided some anecdotal evidence that IPI's get longer over the course of a burst in *D. melanogaster*. I therefore chose at least ten of the longest bursts within a song, and using criteria discussed below, examined temporal changes.

The determination of all three song components, IPI, IPF and CPP, was carried out manually and details of how this was done, are described in Chapter 2. Briefly, once a song was traced onto dot-matrix paper, I inspected the entire song, choosing the ten longest bursts. Changes in a character over a burst were plotted, against the pulse sequence order within the burst. Regression was used to examine the relationship, by taking a mean value at each pulse number. For each individual song, the regression lines were determined for each acoustic component under examination, and a graph was constructed. For the overall species trend in a particular characteristic, all the bursts of all the songs of a species were stacked together, and the various genotype/species regression equations and lines were determined from mean column values. Figures 6.1-6.17 show the results for all the species/strain/intraspecific genotypes examined in this study. Table 6.1 gives an overall synopsis of the results. (For all species/intra- and interspecific hybrid results and individual song regressions see Appendices 6.1, 6.2 and 6.3.).

6.1 *D. melanogaster* complex:

Figure 6.1 A illustrates the *D. melanogaster* IPF values, which appear to oscillate every 7-8 pulses, before becoming irregular towards the end of the burst, as fewer and fewer values contribute. The middle section of the figure gives the regression based on 12 pulses, taken from a minimum of ten bursts which had at least 12 pulses per burst. An asterisk (*) represents a significant ($p<0.05$) regression. The oscillation in IPF can be seen in the first 7 pulses. The top right-hand panel shows the individual song
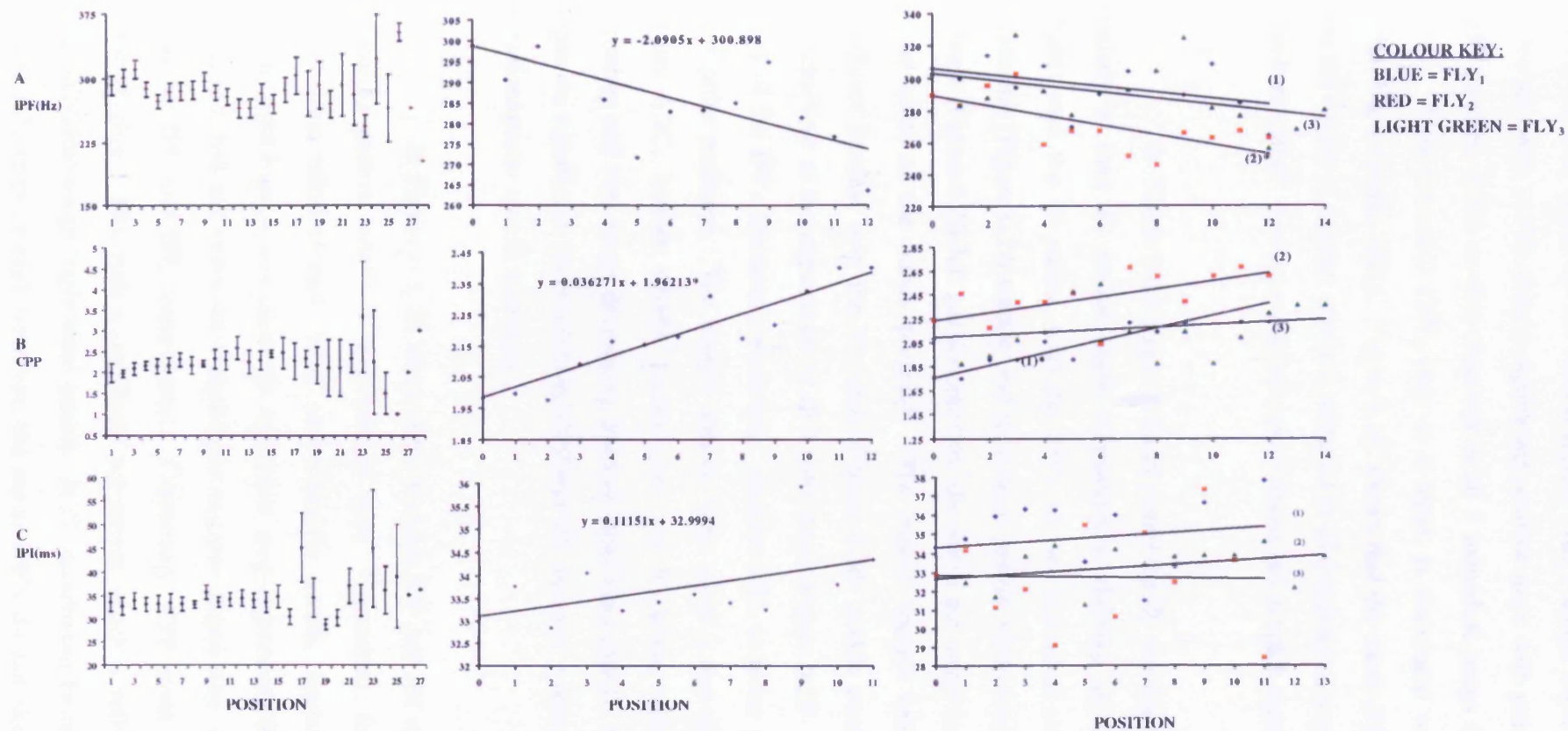
Figure 6.1 : *Drosophila melanogaster* Brighton : Song elements versus pulse position in burst.

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

regressions that make up the central figure. All 3 songs show a negative slope. The cycles per pulse (CPP) show a significant positive slope with pulse number (Figure 6.1B: middle panel), which is also observed in all 3 individual songs (Figure 6.1B: right-hand panel). Thus increased CPP, later in a burst, is associated with lower IPF, possibly suggesting a fatigue effect. Figure 6.1C shows that the mean IPI shows a slight increase over the course of a burst, which is reflected in all 3 individual songs (Figure 6.1C: bottom right-hand panel). However, all individual slopes fail to reach significance.

In Figure 6.2A (top left-hand panel) the *D. simulans* IPF values behave very similarly to their *D. melanogaster* counterparts, showing an overall species decrease (slight) over the 10 pulses, with the IPF's of two individual songs increasing and two decreasing (Figure 6.2A-middle and right-hand panels, respectively). The CPPs show an increase (Figure 6.2B-left panel)-note how the sem's get larger as fewer pulses contribute to the score as the bursts progress. The overall species value shows a slight non-significant increase over the 10 pulses (Figure 6.2B- middle panel), which is reflected in the behaviour of the regressions of all 4 individuals (Figure 6.2B- right-hand panel). The sem's of the IPI's fluctuate enormously, (bottom left), as fewer pulses contribute to the latter pulse positions. The overall species value gives a non-significant increase in IPI (Figure 6.2C, bottom middle panel), over the 9 pulses, with two individual songs increasing and two songs decreasing (bottom right-hand panel). However, none of these slopes are significant. In conclusion, CPP and IPI increase, whereas IPF decreases in both *D. melanogaster* and *D. simulans*.

In Figure 6.3, *D. mauritiana* Sweden IPF and IPI values show a significant decreasing pattern, which is supported in both regressions, for individual songs and overall strain values (Figure 6.3A and C-middle panels, respectively). The top- and bottom- right-hand panels show the individual song regressions that make up the central figure, and five out of six show significant negative slopes (top and bottom right-hand panels for IPF and IPI, respectively). Conversely, CPP gives a consistent increasing profile (Figure 6.3B), with a significant regression, which is reflected in two out of the three individual songs (right-hand panel). In *D. mauritiana* France and Indiana (Figures 6.4 and 6.5, respectively), however, the mean IPF's do not show a consistent pattern

COLOUR KEY:
BLUE = FLY₁
RED= FLY₂
LIGHT GREEN = FLY₃
DARK RED = FLY₄

POSITION
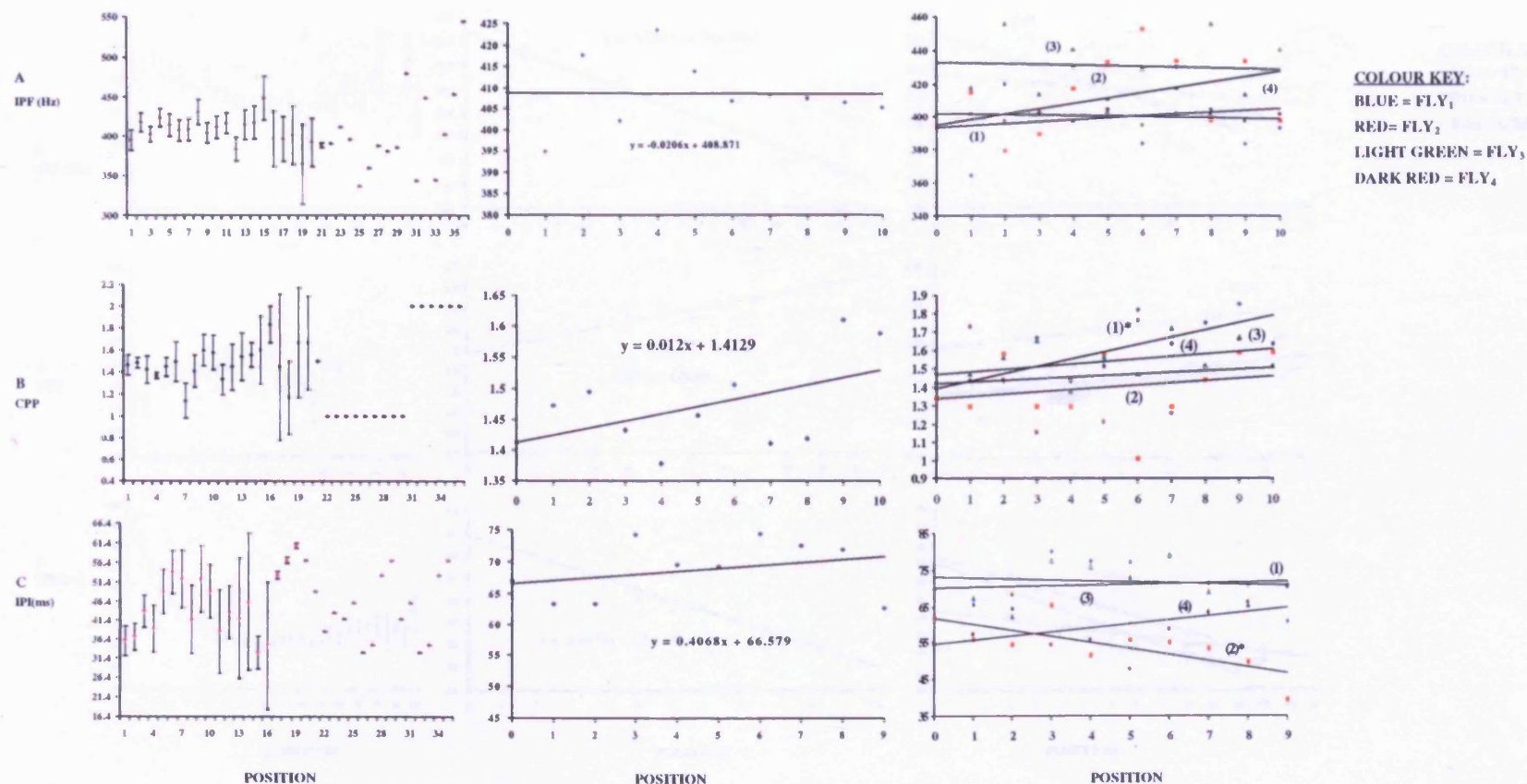
POSITION

POSITION

Figure 6.2 : *Drosophila simulans* Florida : Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSION (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃

$y = -2.4324x + 269.698*$

$y = 0.0552x + 1.6666*$

$y = -2.4674x + 57.235*$

POSITION
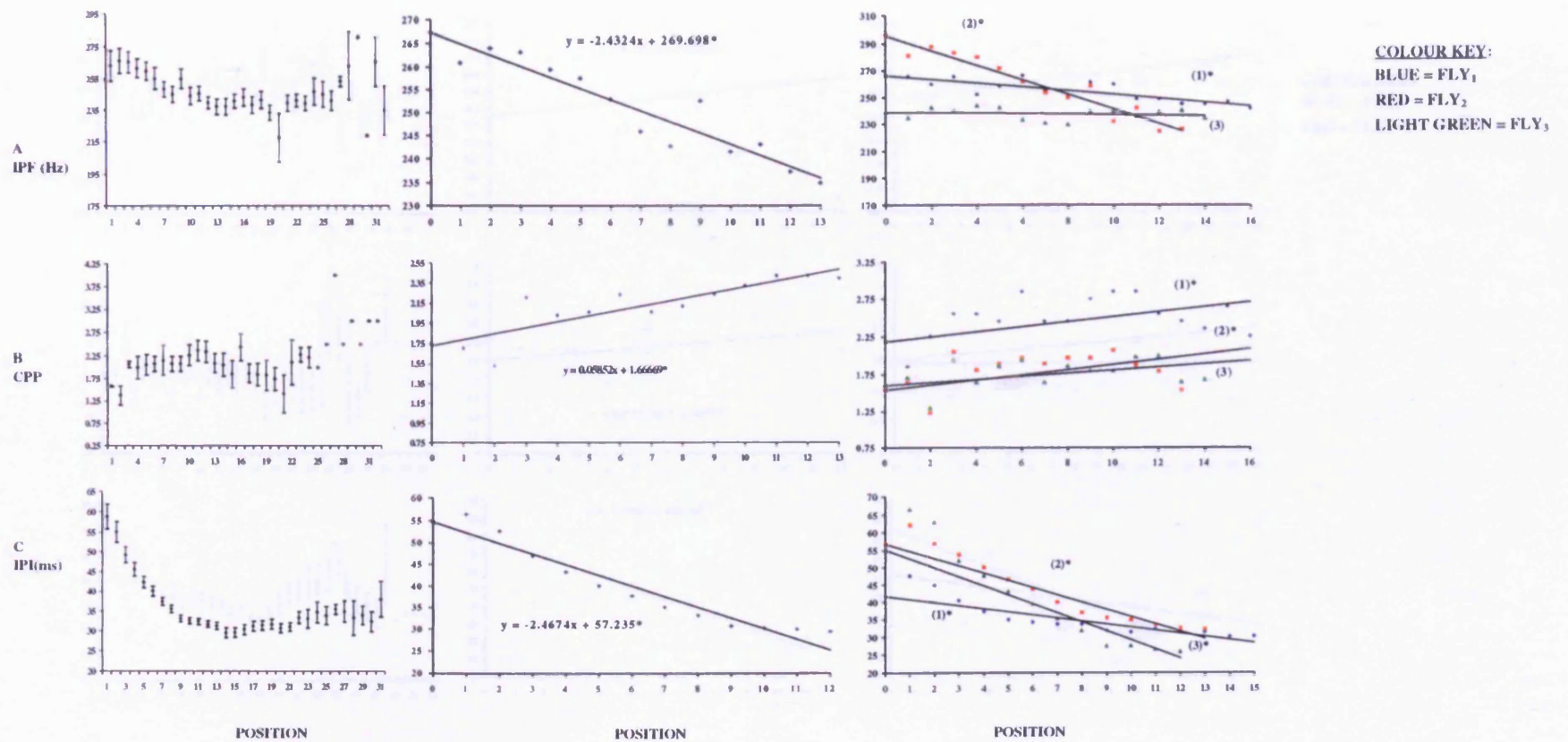
POSITION

POSITION

Figure 6.3 : *Drosophila mauritiana* Sweden: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT -   MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT -   REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY$_1$
RED = FLY$_2$

A
IPF (Hz)

$y = 0.3906x + 250.648$

(2)
(1)

B
CPP

$y = 0.01424x + 2.48974$

(2)
(1)

C
IPI(ms)

$y = -1.4305x + 444.361*$

(1)
(2)*

POSITION
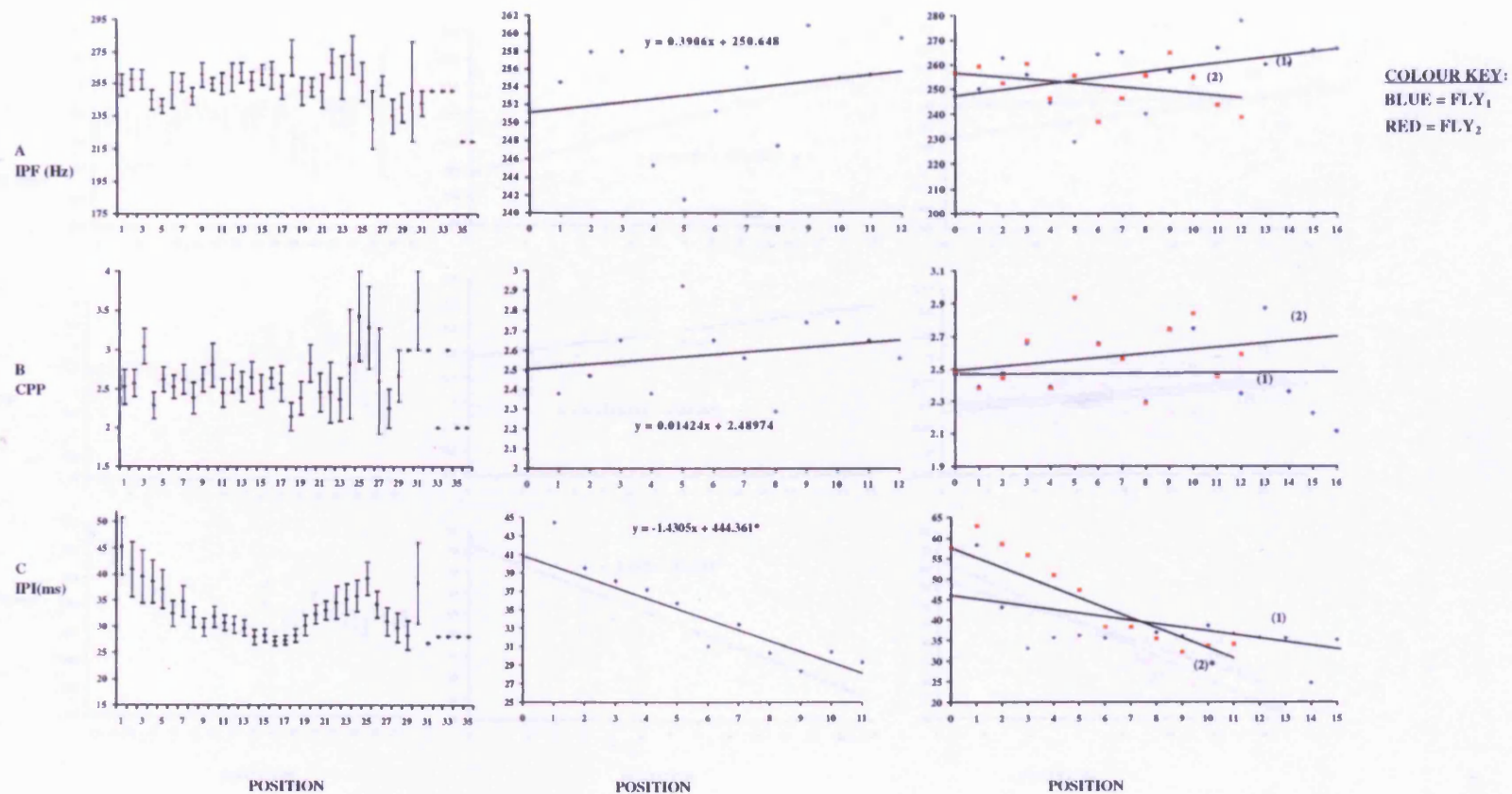
POSITION

POSITION

Figure 6.4 : *Drosophila mauritiana* France : Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE aPPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂

A
IPF (Hz)

$y = 2.0289x + 376.322*$

(1)

(2)

B
CPP

$y = 0.016827x + 2.15241$

(1)

(2)

C
IPI(ms)

$y = -2.619x + 64.149*$

(1)*

(2)*

POSITION
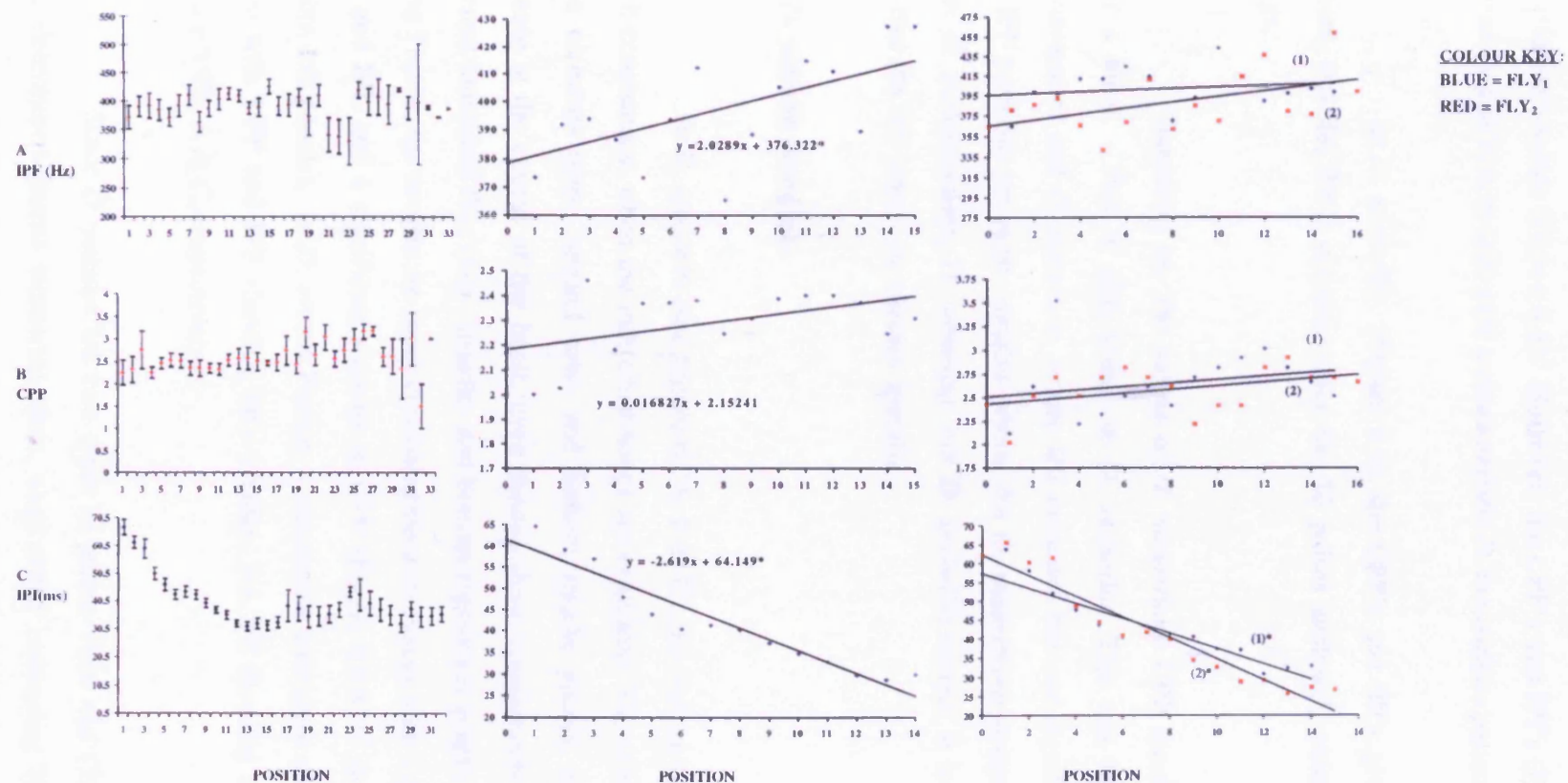
POSITION

POSITION

Figure 6.5 : *Drosophila mauritiana* Indiana : Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL(IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

either at the level of the individual songs, or the overall strain pattern compared to *D. mauritiana* Sweden (Figure 6.3). However, the CPP's and IPI's (Figures 6.4 & 6.5-B & C: middle and bottom left) give a characteristic *D. mauritiana* pattern over a burst.

In *D. sechellia* (Figure 6.6), the CPP's and IPI's give the *mauritiana*-like pattern, but the IPF's decrease over the 10 pulses analysed, which is reflected in all 4 songs.

Summing up, the values of *D. mauritiana* CPP increase and IPI decrease over a burst. This is also found in *D. sechellia*. This can be contrasted with *D. melanogaster* and *D. simulans*, where IPI increases but non-significantly. Nonetheless, the IPF patterns are more variable within the *D. mauritiana* strains. However, without other *D. melanogaster*, *D. simulans* and *D. sechellia* strains, it is difficult to speculate whether the IPF trends are species-specific.

6.2 *D. yakuba* complex:

In *D. teissieiri* (see Figure 6.7A, B & C), the various characters do not show much consistency, when the individual songs are examined. The overall regressions for all three elements (top-, second row- and bottom middle panels, respectively) show an increase in the course of the burst, even though these components behave differently in different individual flies (top-, middle- and bottom right-hand graphs, respectively). In *D. orena* France, the results are more consistent between songs with significant decreases in IPF and IPI, and a significant increase in CPP (Figure 6.8A, C & B-top-, middle- and bottom-left panels). In *D. erecta* France, a reasonably consistent picture also emerges in songs with CPP and IPF showing little change, but IPI showing a significant decrease (Figure 6.9B, A & C, respectively).

Since *D. yakuba* has two types of pulses-Thud and Clack, two sets of data were determined-(bursts examined, here, were either containing Thud or Clack pulses exclusively). In *D. yakuba* France, the Thud and Clack IPF decrease consistently over the course of a burst (Figure 6.10: top row). The Thud and Clack CPP give consistent increasing patterns in the two songs examined, whereas the Thud songs change only

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃
DARK RED = FLY₄
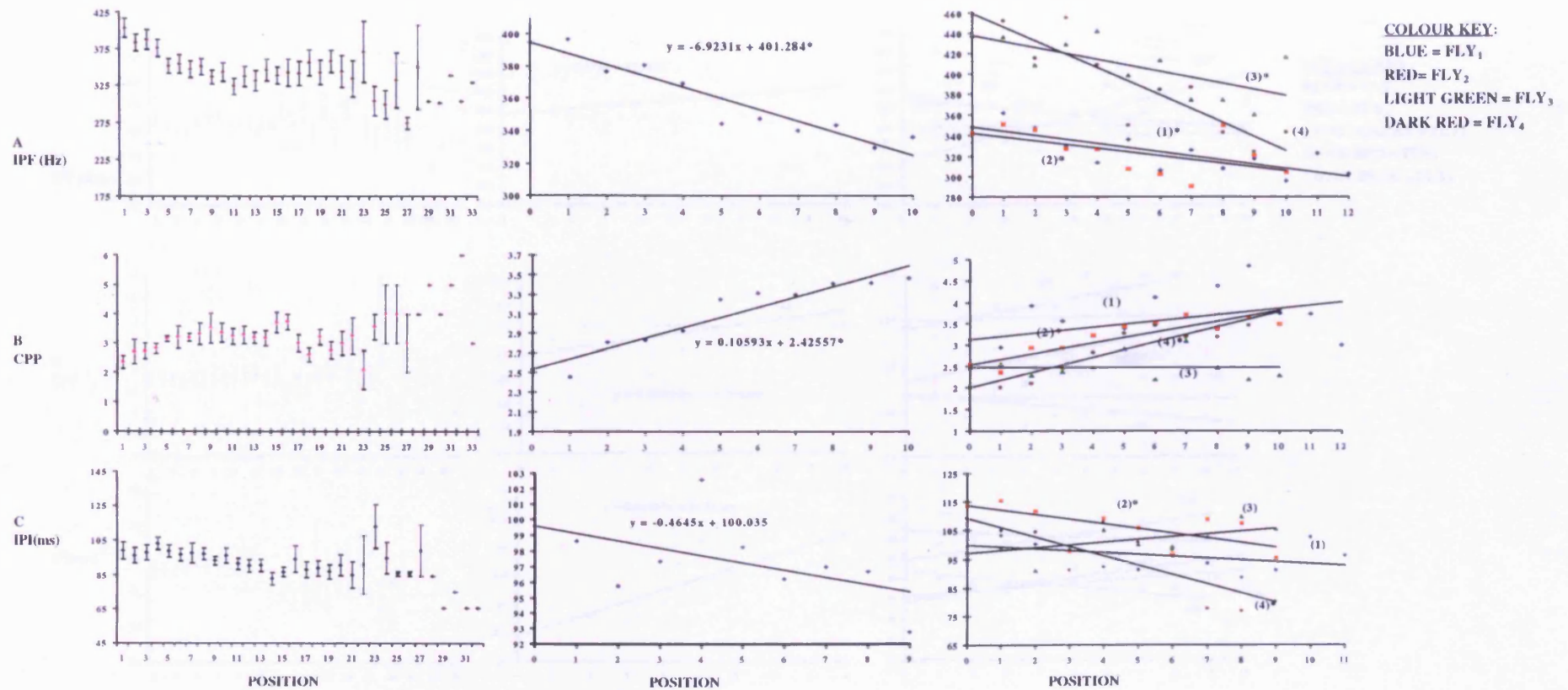
Figure 6.6 : *Drosophila sechellia* Cambridge: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃
DARK RED = FLY₄
LIGHT BLUE = FLY₅
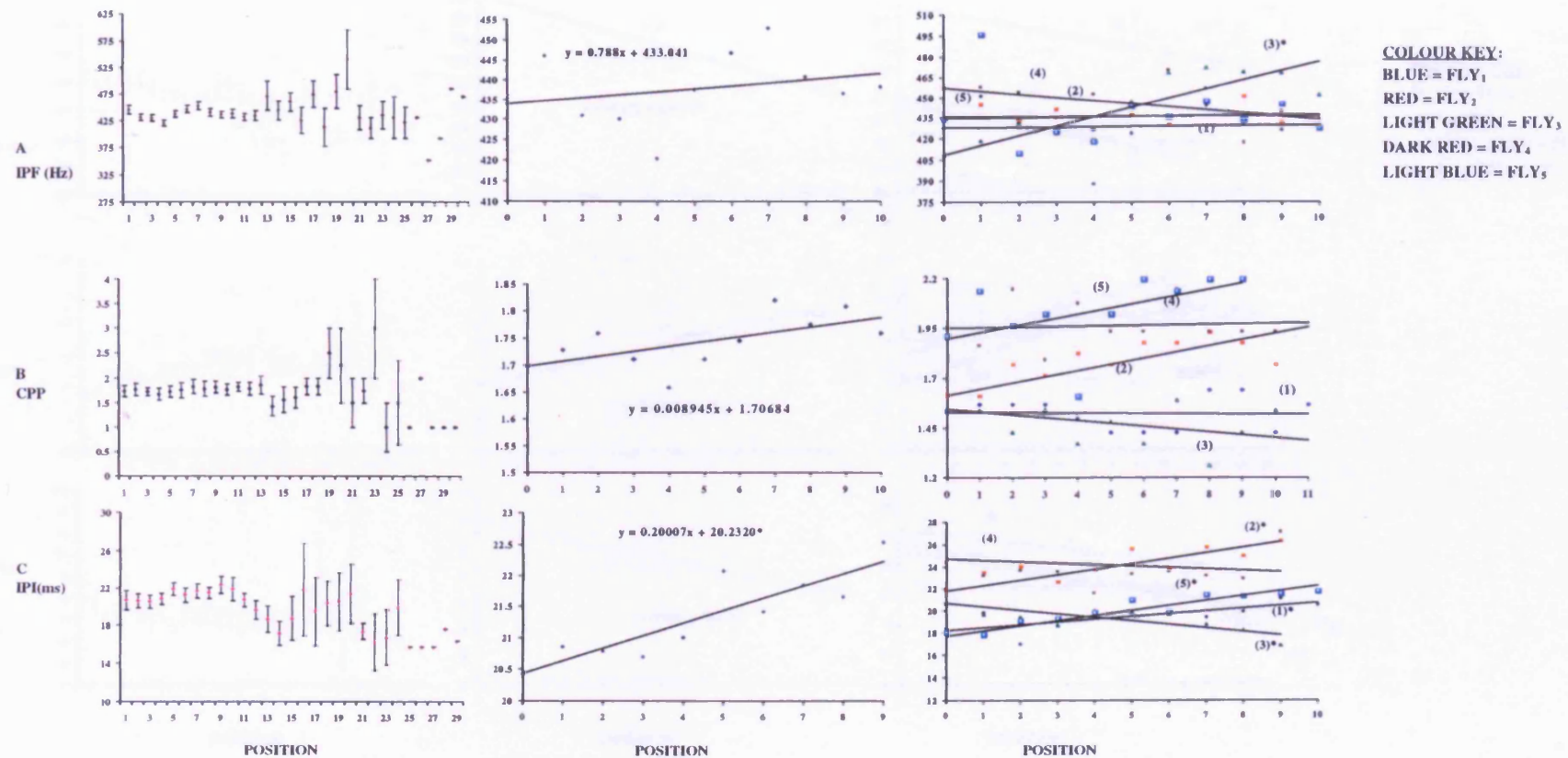
Figure 6.7 : *Drosophila teissieri* **Sweden : Song elements versus pulse position in burst**

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED= FLY₂
LIGHT GREEN = FLY₃
DARK RED = FLY₄

$y = -9.17x + 639.09*$

$y = 0.04982x + 1.36593*$

$y = -1.526x + 66.801*$

POSITION   POSITION   POSITION
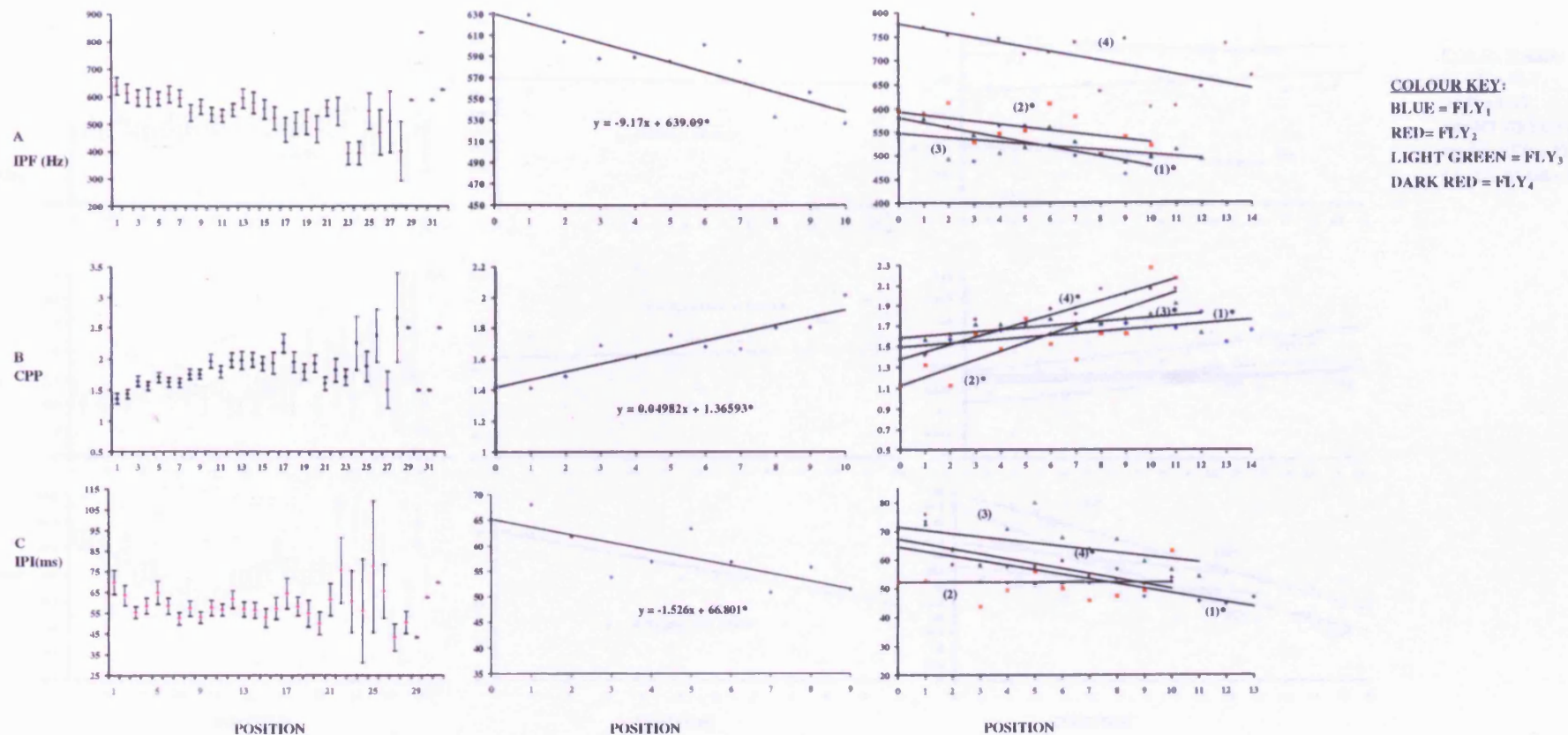
Figure 6.8 : *Drosophila orena* France: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY$_1$
RED = FLY$_2$
LIGHT GREEN = FLY$_3$
DARK RED = FLY$_4$
LIGHT BLUE = FLY$_5$

A
IPF (Hz)

$y = -0.1738x + 389.688$

B
CPP

$y = 0.00256x + 2.7388$

C
IPI(ms)

$y = -0.9126x + 51.0904*$

POSITION   POSITION   POSITION
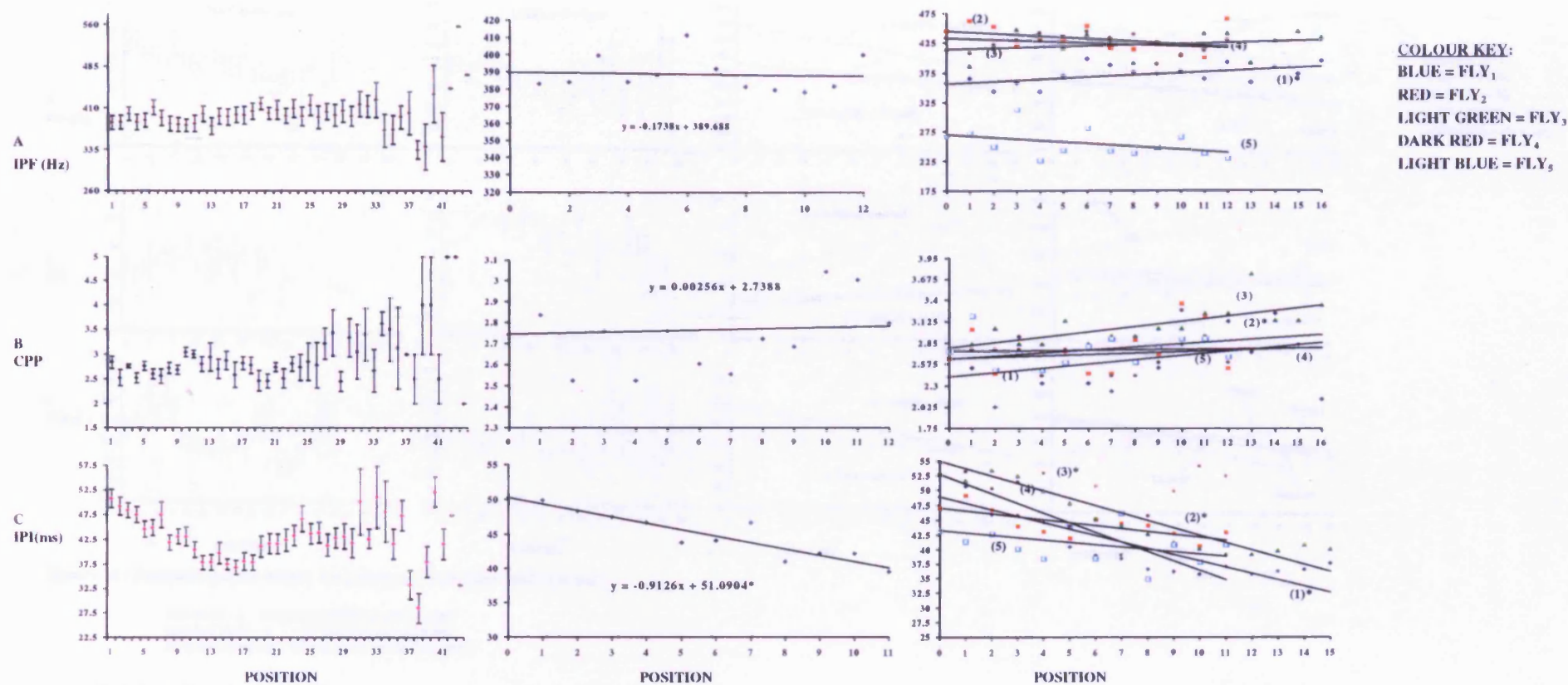
Figure 6.9 : *Drosophila erecta* France: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
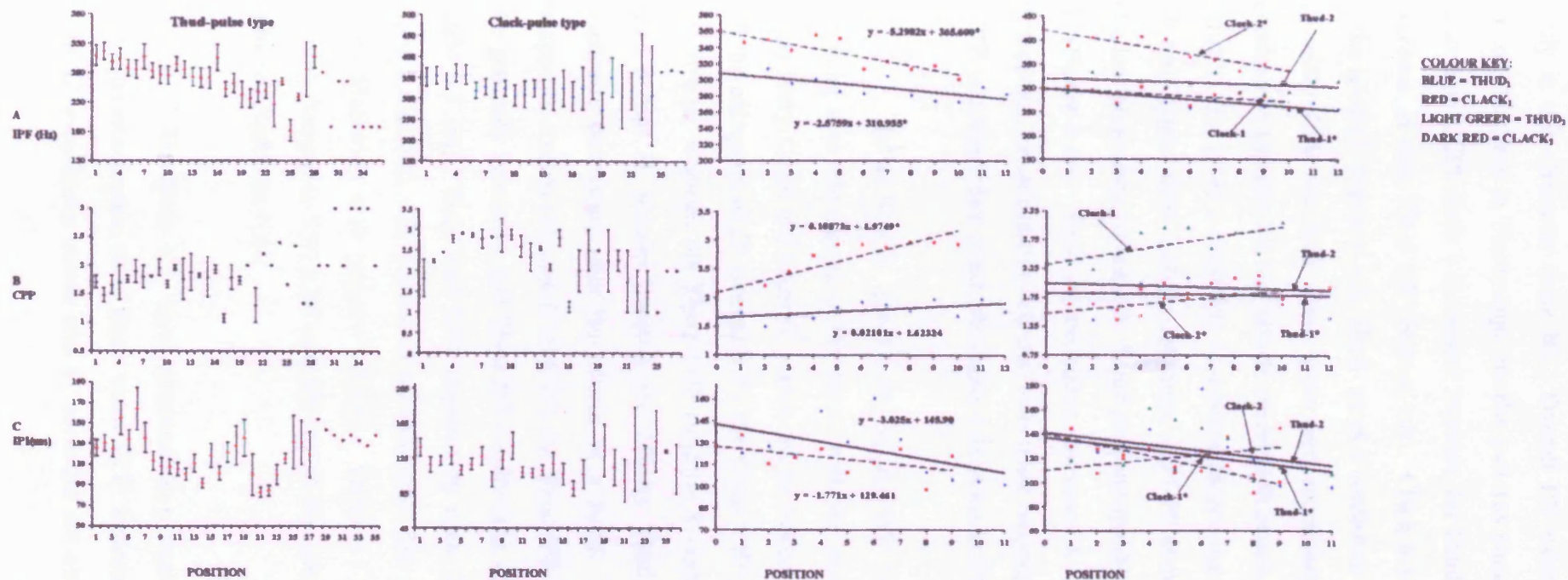
Figure 6.10 : *Drosophila yakuba* France: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
SECOND LEFT - CLACK PULSE-TYPE
THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
DOTTED LINE= CLACK REGRESSION LINE
SOLID LINE- THUD REGRESSION

slightly in this character (row B). Overall IPI values (row C), for Thud and Clack decrease, but only in Thud songs are the patterns consistent. In *D. yakuba* Ivory Coast (Figure 6.11), CPP show a consistent increase, for Thud and Clack. Again Thud IPI gives a decrease, as does Thud IPF (bottom-left). Clack is inconsistent for IPF and IPI. In *D. yakuba* Lamto3 (Figure 6.12), Thud gives a consistent decrease in IPF and an increase in IPI, while CPP for both song types are inconsistent, and Clack IPIs show some inconsistency (middle left and middle second left panels, respectively). *D. yakuba* Malawi (Figure 6.13), gives a strikingly consistent decreasing pattern in IPI, while the IPF, for both song types, show a slight decrease, and CPP show a significant increase, for Clack, and a less pronouced increase for Thud (top and middle rows, respectively). In *D. yakuba* S.T. (Figure 6.14), Thud IPI decreases, increases in CPP, while Thud IPF is stable. In Clack songs, for all three characters, a decrease ranging from slight in IPF, to a noticeable in CPP, to a significant more pronounced decrease in IPI is observed.

In all five *D. yakuba* strains, the IPF for Thud and Clack appears to be decreasing over the course of the burst, with the exception of S.T. Thud IPF, and *D. yakuba* Ivory Coast and Lamto3, which are increasing during the progression of a burst. With the exception of *D. yakuba* S.T., the Clack CPP are observed to increase in all the other strains, whereas the Thud CPP increase in most strains, except Lamto3. In all strains except *D. yakuba* Lamto3, the IPI for Thud decreases, and the Clack IPI is consistently decreasing over the course of a burst. Upon inspection of the data for intraspecific crosses (Figures 6.15-6.17), the Thud IPF falls slightly, the Thud CPP is seen to be generally increasing and Thud IPI usually falls, except in *D. yakuba* Malawi(f) x *D. yakuba* S.T.(m). The Clack CPP consistently rises, Clack IPF decreases, except in *D. yakuba* Lamto3(f) x *D. yakuba* Ivory Coast(m), and Clack IPI decreases, except in *D. yakuba* Malawi(f) x *D. yakuba* S.T.(m). Table 6.1 (below) shows a synopsis of the temporal changes in IPF, CPP and IPI for all the subgroup species-(for individual song results see Appendix 6.4).

The table 6.1, upon examination, reveals that of the members of the *D. melanogaster* complex, all of them show CPP increases as the burst develops. The IPI, in the *D. mauritiana* strains and *D. sechellia* are consistently decreasing, while in the
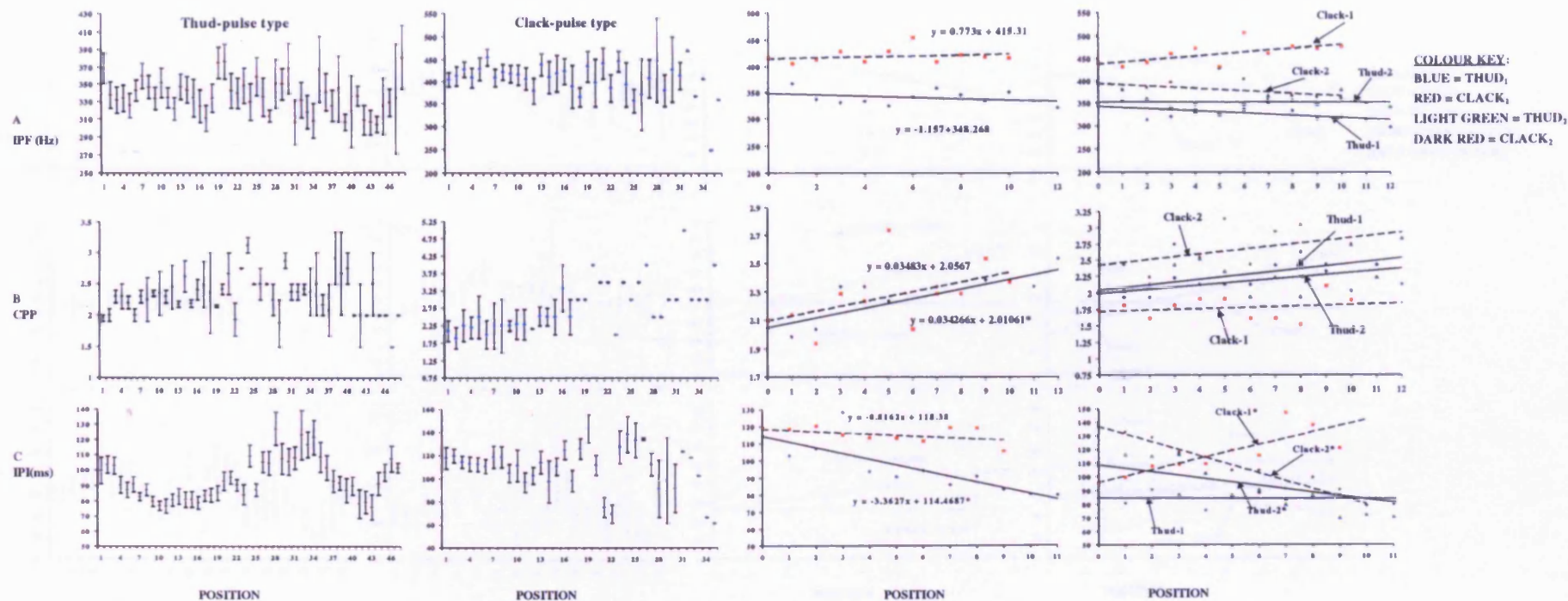
Figure 6.11 : *Drosophila yakuba* Ivory Coast: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
SECOND LEFT - CLACK PULSE-TYPE
THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
DOTTED LINE= CLACK REGRESSION LINE
SOLID LINE- THUD REGRESSION
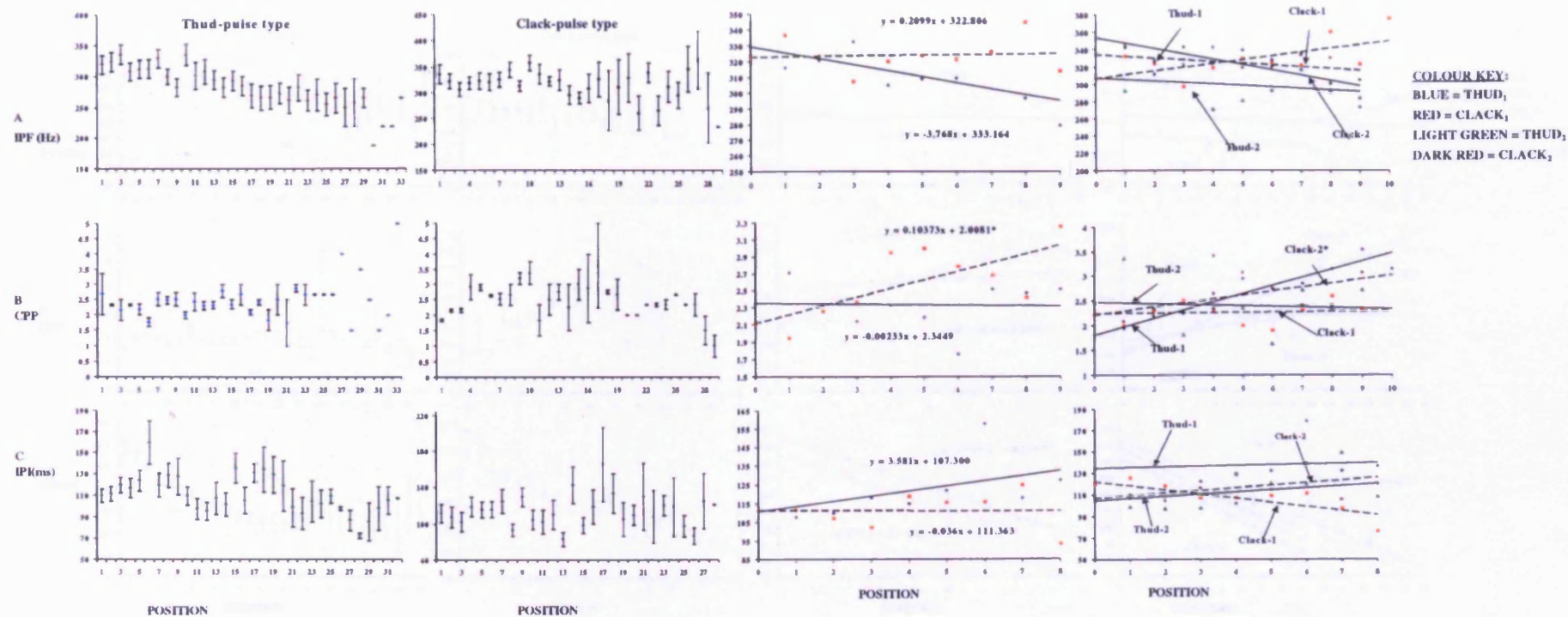
Figure 6.12 : *Drosophila yakuba* Lamto3: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
SECOND LEFT - CLACK PULSE-TYPE
THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
DOTTED LINE= CLACK REGRESSION LINE
SOLID LINE- THUD REGRESSION
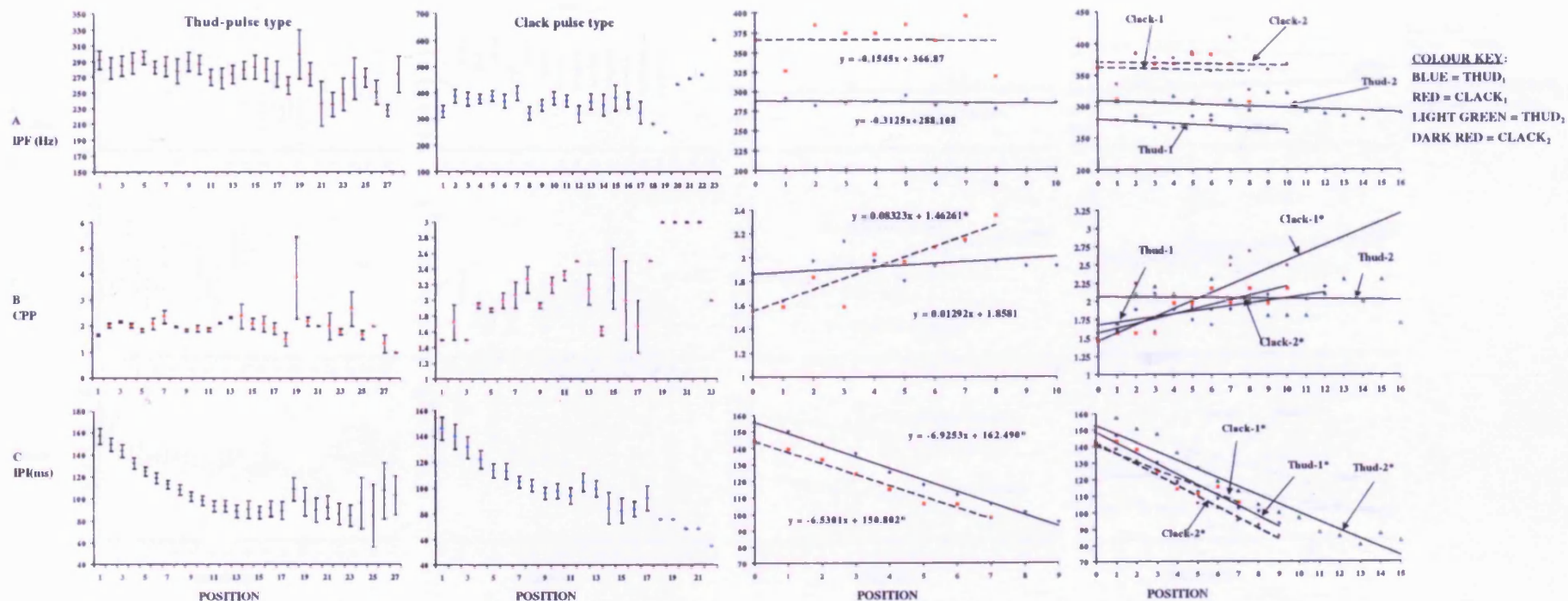
Figure 6.13 : *Drosophila yakuba* Malawi: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
SECOND LEFT - CLACK PULSE-TYPE
THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
DOTTED LINE= CLACK REGRESSION LINE
SOLID LINE- THUD REGRESSION
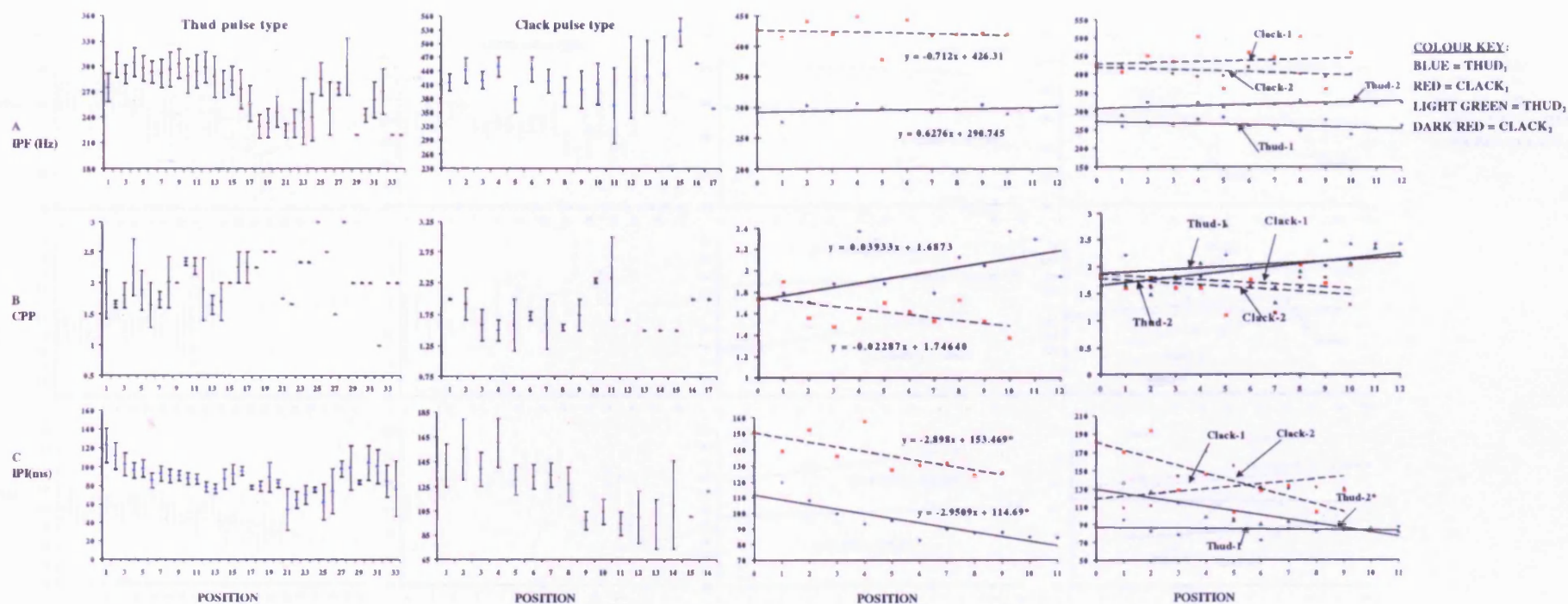
Figure 6.14 : *Drosophila yakuba* S.T.: Song elements versus pulse in position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
SECOND LEFT - CLACK PULSE-TYPE
THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
DOTTED LINE= CLACK REGRESSION LINE
SOLID LINE- THUD REGRESSION
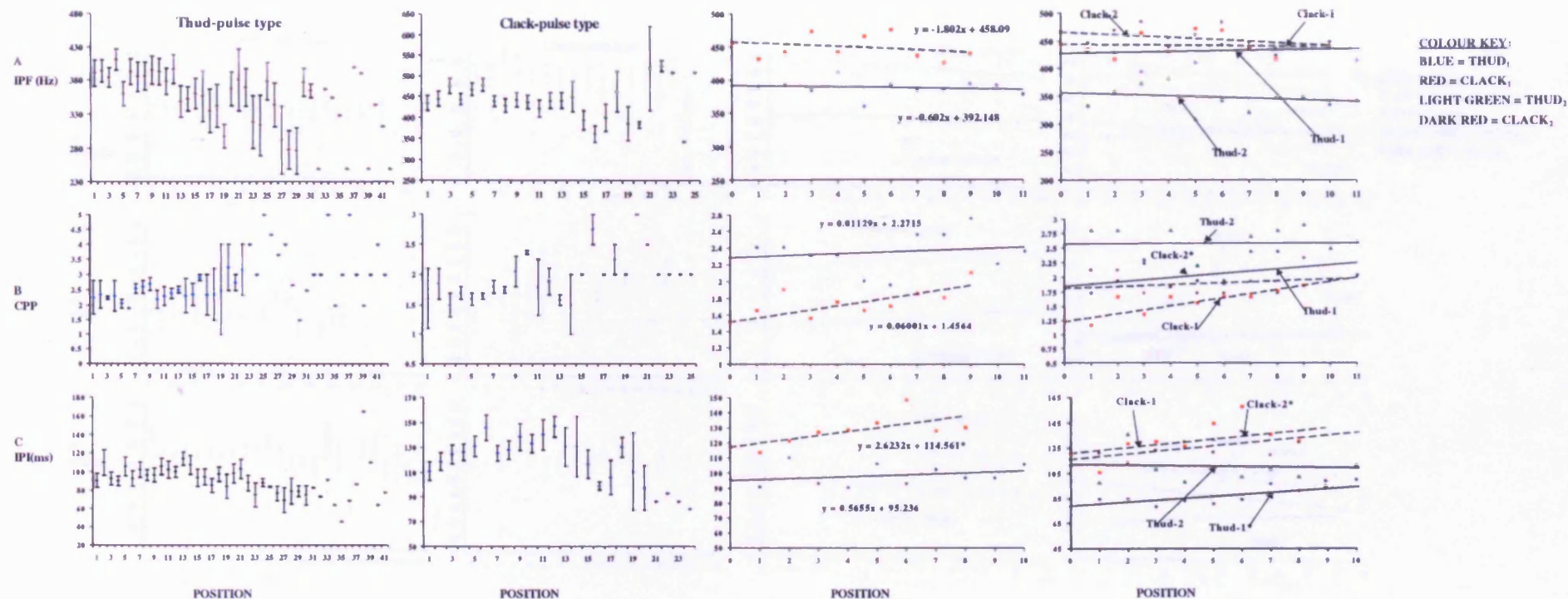
Figure 6.15 : *D. yakuba* Malawi(f) x *D. yakuba* S.T.(m)-Hybrid: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
SECOND LEFT - CLACK PULSE-TYPE
THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
DOTTED LINE= CLACK REGRESSION LINE
SOLID LINE- THUD REGRESSION
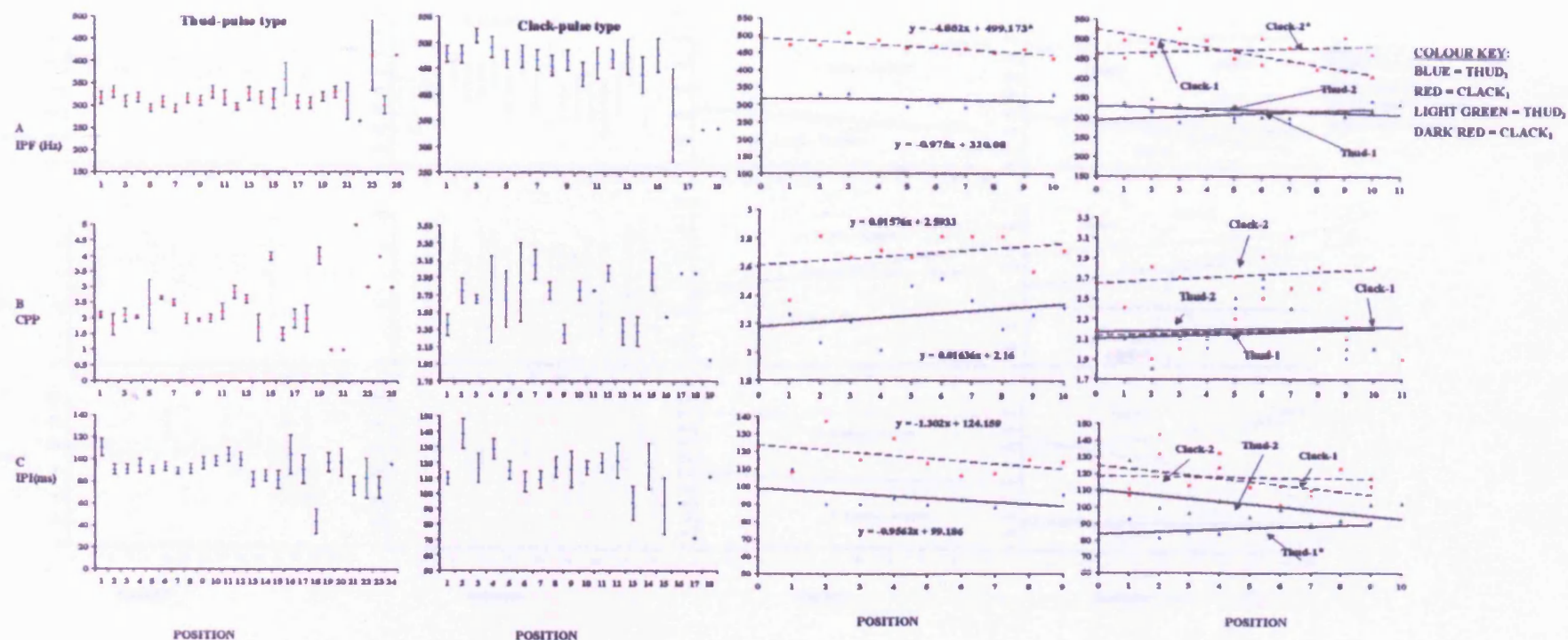
Figure 6.16 : *D. yakuba* S.T.(f) x *D. yakuba* Lamto3(m)-Hybrid: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
             SECOND LEFT - CLACK PULSE-TYPE
             THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
             RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
             ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
             DOTTED LINE= CLACK REGRESSION LINE
             SOLID LINE- THUD REGRESSION
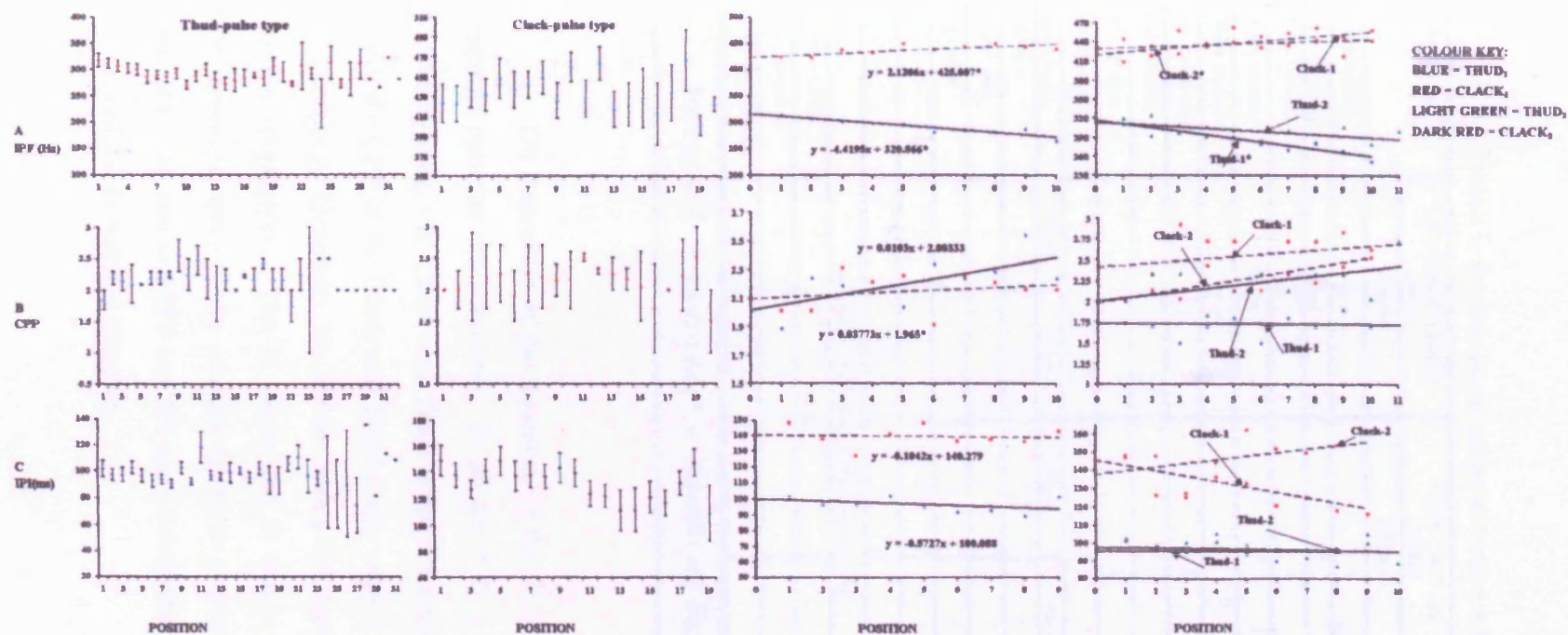
Figure 6.17 : *D. yakuba* Lamto3(f) x *D. yakuba* Ivory Coast(m)-Hybrid: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - THUD PULSE-TYPE
         SECOND LEFT - CLACK PULSE-TYPE
         THIRD LEFT - SPECIES REGRESSION OF CLACK AND THUD FOR ELEMENT VERSUS POSITION OF PULSE IN BURST
         RIGHT - CLACK AND THUD REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
         ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
         DOTTED LINE- CLACK REGRESSION LINE
         SOLID LINE- THUD REGRESSION

cases of *D. melanogaster* and *D. simulans* are constantly increasing, in the course of a burst. The IPF shows a more variable pattern.

Table 6.1: Synopsis of the temporal changes in IPF,CPP and IPI.

Species/Strain	Intrapulse Frequency (IPF)		Cycles per Pulse (CPP)		Interpulse Interval (IPI)	
	Overall		Overall		Overall	
melanogaster(Brighton)	↓ *		↑ *		↑	
simulans (Florida)	Slight ↓		↑		↑	
mauritiana (Sweden)	↓ *		↑ *		↓ *	
mauritiana (France)	Slight ↑		↑		↓ *	
mauritiana (Indiana)	↑ *		↑		↓ *	
sechellia(Cambridge)	↓ *		↑ *		↓	
teissieri (Sweden)	↑		↑		↑ *	
orena (France)	↓ *		↑ *		↓ *	
erecta (France)	↓		↑		↓ *	
yakuba Strains	Thud	Clack	Thud	Clack	Thud	Clack
France	↓ *	↓ *	↑	↑ *	↓	↓
Ivory Coast	↓	↑	↑ *	↑	↓ *	↓
Lamto3	↓	↑	↓	↑ *	↑	↓
Malawi	↓	↓	↑	↑ *	↓ *	↓ *
S.T.	↑	↓	↑	↓	↓ *	↓ *
yakuba Intraspecific Hybrids						
Malawi(f) x S.T.(m)	↓	↓	↑	↑ *	↑	↑
S.T.(f) x Lamto3(m)	↓	↓ *	↑	↑	↓	↓
Lamto3(f) x Ivory Coast(m)	↓ *	↑ *	↑ *	↑	↓	↓

KEY: ↑ = Increase, ↓ = Decrease, * = Significant Regressions

On inspection of the members of the *D. yakuba* complex, from Table 6.1, more variable patterns are observed. In all the 5 *D. yakuba* strains, as well as the 3 intraspecific hybrids, the Clack and Thud song IPI consistently decrease over a burst, except one; the CPP of the Clack and Thud songs show a consistent increase over a burst with one exception. Generally, the IPF for both Clack and Thud songs decrease, except in two and one, respectively. The *D. orena* and *D. erecta* IPF and IPI generally decrease and CPP increases, while in *D. teissieri* all three characters consistently increase. The behaviour of the *D. teissieri* IPF and IPI song components is unique and opposite to the other species of the *D. yakuba* complex.

6.3 Interspecific Hybrid Crosses:

The graphs of the individual types of interspecific genotypes, examined in this study, are shown in Appendix 6.5. Table 6.1.2 gives a synopsis of the temporal changes in IPF, CPP and IPI over the course of burst-(for all individual song trends see Appendix 6.6).

Table 6.2: Synopsis of the temporal changes in IPF, CPP and IPI of the interspecific genotypes

Interspecific genotypes	Intrapulse Frequency (IPF)	Cycles per Pulse (CPP)	Interpulse Interval (IPI)
	Overall	Overall	Overall
yakuba France(f) x *mauritiana* France(m)	↓	↑	↑ *
yakuba Malawi(f) x *mauritiana* Sweden(m)	↓	↑ *	↓
yakuba France(f) x *teissieri* Sweden(m)	↑	↓	↓
mauritiana Sweden(f) x *yakuba* Ivory Coast(m)	↓	↓	↑
mauritiana Sweden(f) x *yakuba* Lamto3(m)	↓	↑	↑
simulans Florida(f) x *mauritiana* Sweden(m)	↓ *	↑	↑ *
teissieri Sweden(f) x *mauritiana* Sweden(m)	↓	↑	↓

KEY: ↑ = Increase, ↓ = Decrease, * = Significant Regressions

IPF:

In all interspecific crosses except one, the IPF shows a consistent decrease over the course of a burst. The IPF's in *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m), *D. mauritiana* Sweden(f) x *D. yakuba* Ivory Coast(m), *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m), and *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) show the same patterns as the parental strains (not informative). In *D. yakuba* France(f) x *D. mauritiana* France(m), IPF exhibits a *yakuba*-like pattern, suggesting either X chromosome influence or autosomal dominant *yakuba* factors, whereas in *D. yakuba* France(f) x *D. teissieri* Sweden(m), and *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m), the IPF's exhibit a *teissieri*-like and *mauritiana*-like patterns, respectively, implying the involvement of dominant *D. teissieri* and *D. mauritiana* autosomal factors, respectively.

CPP :

In the majority of the interspecific crosses the CPP consistently increases over the course of a burst, except in *D. yakuba* France(f) x *D. teissieri* Sweden(m) and *D. mauritiana* Sweden(f) x *D. yakuba* Ivory Coast(m). The CPP, except in the above mentioned interspecific crosses, exhibit the same patterns as their corresponding parental strains (not informative). Perhaps the decrease in the CPP, observed in *D. yakuba* France(f) x *D. teissieri* Sweden(m) and *D. mauritiana* Sweden(f) x *D. yakuba* Ivory Coast(m) may be a reflection of these hybrids being unfit (Haldane, 1922).

IPI :

In the majority of interspecific crosses, the IPI pattern consistently increase as a burst progresses, except in *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m), *D. yakuba*(f) x *D. teissieri*(m), and *D. teissieri*(f) x *D. mauritiana*(m). In *D. yakuba* France(f) x *D. mauritiana*(m) and *D. mauritiana* Sweden(f) x *D. yakuba* Ivory Coast(m), the IPI patterns show a consistent increase over the course of a burst, which differ from their corresponding parental ones. Perhaps, the observed decrease in IPI in the parental strains may be a result of inbreeding depression. In *D. yakuba* France(f) x *D. teissieri* Sweden(m) and *D. simulans* Florida(f) x *D. mauritiana* Sweden(m), the IPI exhibits a *yakuba*-like and *simulans*-like patterns, respectively, suggesting either X chromosome involvement or dominant maternal autosomes. In *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) and *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m), the IPI exhibits a *yakuba*-like and a *mauritiana*-like patterns, respectively, suggesting the influence of *D. yakuba* and *D.mauritiana* dominant autosomal factors respectively. In *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m), the IPI behaves in the same way as both parental strains (not informative).

In conclusion, it is clear that the mode of inheritance for the overall trends for all three song components, over the course of a burst, is complex. There is evidence for autosomal dominance and, perhaps X chromosome involvement, depending on the type of cross. When *D. yakuba* or *D. simulans* females are used, the IPI might either be under the influence of the X chromosome or corresponding dominant autosomal factors, whereas when *D. yakuba* or *D. mauritiana* males are used, the IPI's behave in a way which is consistent with dominant autosomal factors. When *D. teissieri* or *D.*

mauritiana males are used, the CPP patterns imply dominant autosomal influence, whereas when *D. mauritiana* females are used, the CPP's show a pattern which is consistent with either X chromosome or *D. mauritiana* dominant autosomal involvement. When *D. teissieri* or *D. mauritiana* males are used, the IPF's exhibit a pattern which is consistent with dominant autosomal factors, whilst when *D. yakuba* females are used, the IPF behaviour is observed to be either under X chromosome influence or show *D. yakuba* dominant autosomal effects.

6.4 Burst Duration and Interburst Interval over time

Byrne and Kyriacou (pers. comm.) have observed, in simulator experiments, that the burst length difference between *D. mojavensis* and *D. arizonae* males is a cue which females use as a species-specific cue for enhanced conspecific mating. This observation stimulated the ensuing investigation of the grosser aspects of temporal changes in the songs, in order to uncover any possible species-specific patterns that could be used by females for selecting conspecific mating. The procedures that had been followed in order to determine the Mean Burst Duration (**mbd**) and Mean Interburst Interval (**mibi**) for an entire song, as well as for the first and second halves of the songs are described in detail in Chapter 2. Briefly, the procedure involved was as follows:- Each song was divided into two equal halves and the length of each burst (burst duration, bd) measured in ms, and the distance between consecutive bursts (interburst interval, ibi) was measured in secs (see chapter 2). The rationale behind this exercise was simply to see whether there were any time-dependent changes in courtship intensity.

Kolmogorov-Smirnov and Shapiro-Wilks' tests are well-known tests which are suitable for small and large samples, respectively, and were employed, in order to test the normality of the data. Raw data and $Log_{[x]}$-transformation were employed, but the two categories of data gave approximately similar results with the two tests. Only a few samples were non-normally distributed. Thus, it was decided that the Raw datasets should be used for any further statistical analysis. In order to obtain the overall picture for any individual species, the first and second half values, and the overall mean ± sem for each component were determined. The different strains within a species were treated as one entity.

On examination of the different species, most species were shown to have a shorter overall mbd and mibi for the first half of the song (mbd_1 and $mibi_1$, respectively), while having longer mbd and mibi in the course of the second half of the song (mbd_2 and $mibi_2$, respectively), with the exception of *D. simulans* songs, where the converse was true. Figure 6.4.1 depicts the mbd and mibi ($\pm$ sem) results for the first and second halves of song. Appendices 6.7, 6.8 and 6.9 give the results and a brief discussion of the individual songs for all the species and interspecific hybrid songs examined.

Two-way ANOVA, for mbd between the different species and their interspecific hybrids, revealed that there are significant differences between the species (F=8.87, df=12, 129, p=0.000), but no significant differences between the first and second halves of song (F=1.04, df=1, 129, p=0.309), and no significant interaction between the species and the two halves of song (F=0.339, df=12, 129, p=0.980, see Figure 6.4.2, below). Newman-Keuls *a posteriori* test revealed significant differences, at least at the p<0.05, between *D. yakuba* and the rest of the species. The test also revealed significant differences, at least at p<0.05, between *D. yakuba* and, *D. yakuba*(f) x *D. mauritiana*(m) and *D. yakuba*(f) x *D. teissieri*(m) hybrids, and between *D. mauritiana* and *D. mauritiana*(f) x *D. yakuba*(m), but no significant differences between the hybrids and the paternal parents, implying that the mbd may be influenced by Y chromosome or dominant autosomal factors, since the hybrid mean mbd's have values similar to their corresponding paternal parents (see Appendix 6.10). In addition, the test revealed no significant differences between *D. yakuba*(f) x *D. mauritiana*(m), and its reciprocal cross (see Appendix 6.10). Furthermore, the test revealed no significant differences between the *D. simulans*(f) x *D. mauritiana*(m) and *D. teissieri*(f) x *D. mauritiana*(m) hybrids and their corresponding parents. The hybrid mean values are slightly higher than their corresponding parental ones, suggesting hybrid vigour.

(A)

(B)
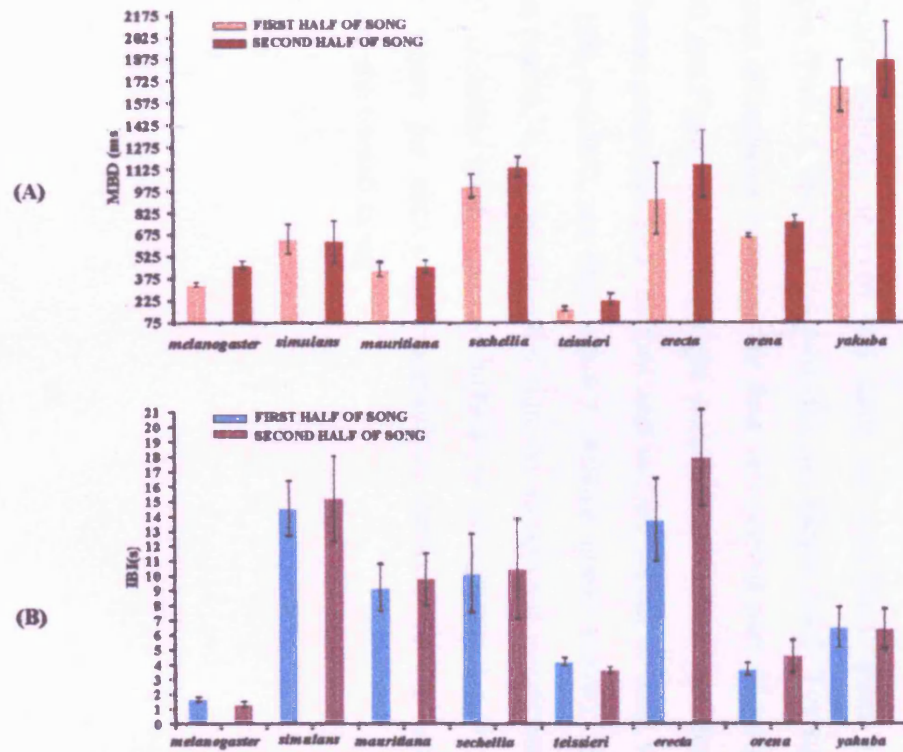
D. melanogaster Subgroup
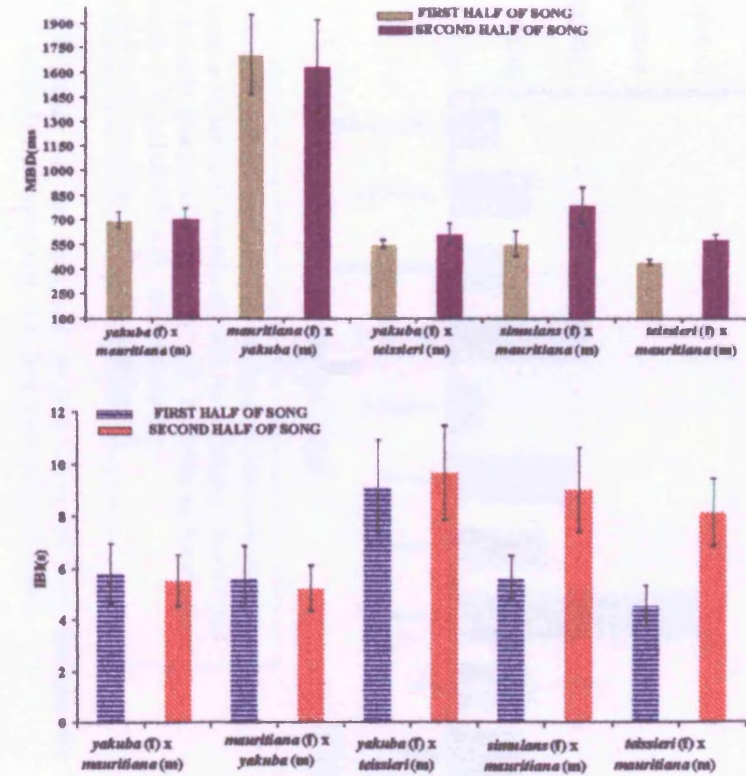
Interspecific Hybrids

Figure 6.4.1: (a) Mean Burst Duration(ms)
(b) Interburst Interval(s)

Genotype Effect

F(12,129)=8.87; p<.0000

KEY : yfts = *D. yakuba* France(f) x *D. teissieri* Sweden(m)
tsms = *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m)
sfms = *D. simulans* Florida(f) x *D. mauritiana* Sweden(m)
ymhyb = *D. yakuba*(f) x *D. mauritiana*(m)
myhyb = *D. mauritiana*(f) x *D. yakuba*(m)
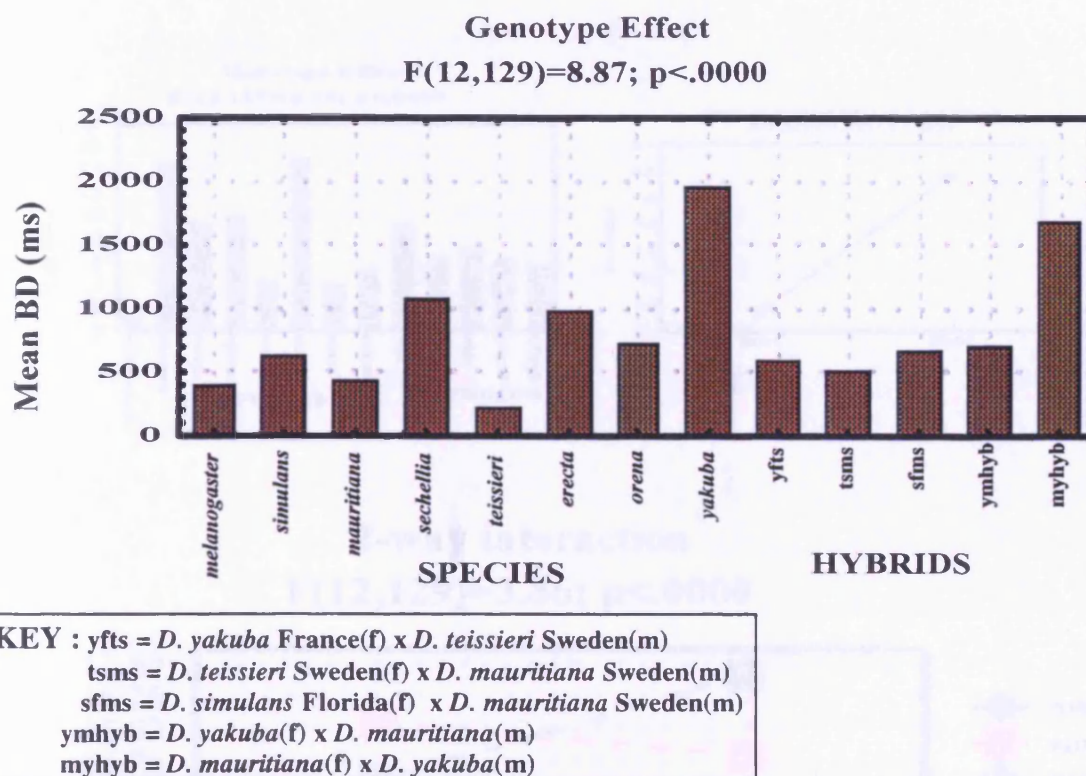
Figure 6.4.2: MBD for the first and second halves of song between the different species of the *melanogaster* subgroup and their interspecific hybrids.

Two-way ANOVA for mibi between the different species and their interspecific hybrids, showed that there are significant main effects for the different genotypes (F=6.16, df=12, 129, p=0.000, see Figure 6.4.3: Top right panel). In addition, significant differences between the first and second part of song (F=15.81, df=1, 129, p=0.000, see Figure 6.4.3: Top right panel), as well as a significant interaction between the different genotypes and the first and second halves of song were revealed (F=3.86, df=12, 129, p=0.000, see Figure 6.4.3: Bottom panel, below). Examining Figure 6.4.3 (Bottom panel), it can be observed that the significant interaction involves *D. simulans* (red), *D. sechellia* (pink), and my-hybrid (*D. mauritiana*(f) x *D. yakuba*(m) (yellow and black)), where the mibi's are observed to decrease in the second half of the song, contrary to the overall trend.

124

Genotype Effect
F(12,129)=6.16; p<.0000

First or Second Part of Song Effect
F(1,129)=15.81; p<.0001

2-way interaction
F(12,129)=3.86; p<.0000

KEY : yfts = *D. yakuba* France(f) x *D. teissieri* Sweden(m)
tsms = *D. teissieri* Sweden(f) x *D. mauritiana* Sweden(m)
sfms = *D. simulans* Florida(f) x *D. mauritiana* Sweden(m)
ymhyb = *D. yakuba*(f) x *D. mauritiana*(m)
myhyb = *D. mauritiana*(f) x *D. yakuba*(m)
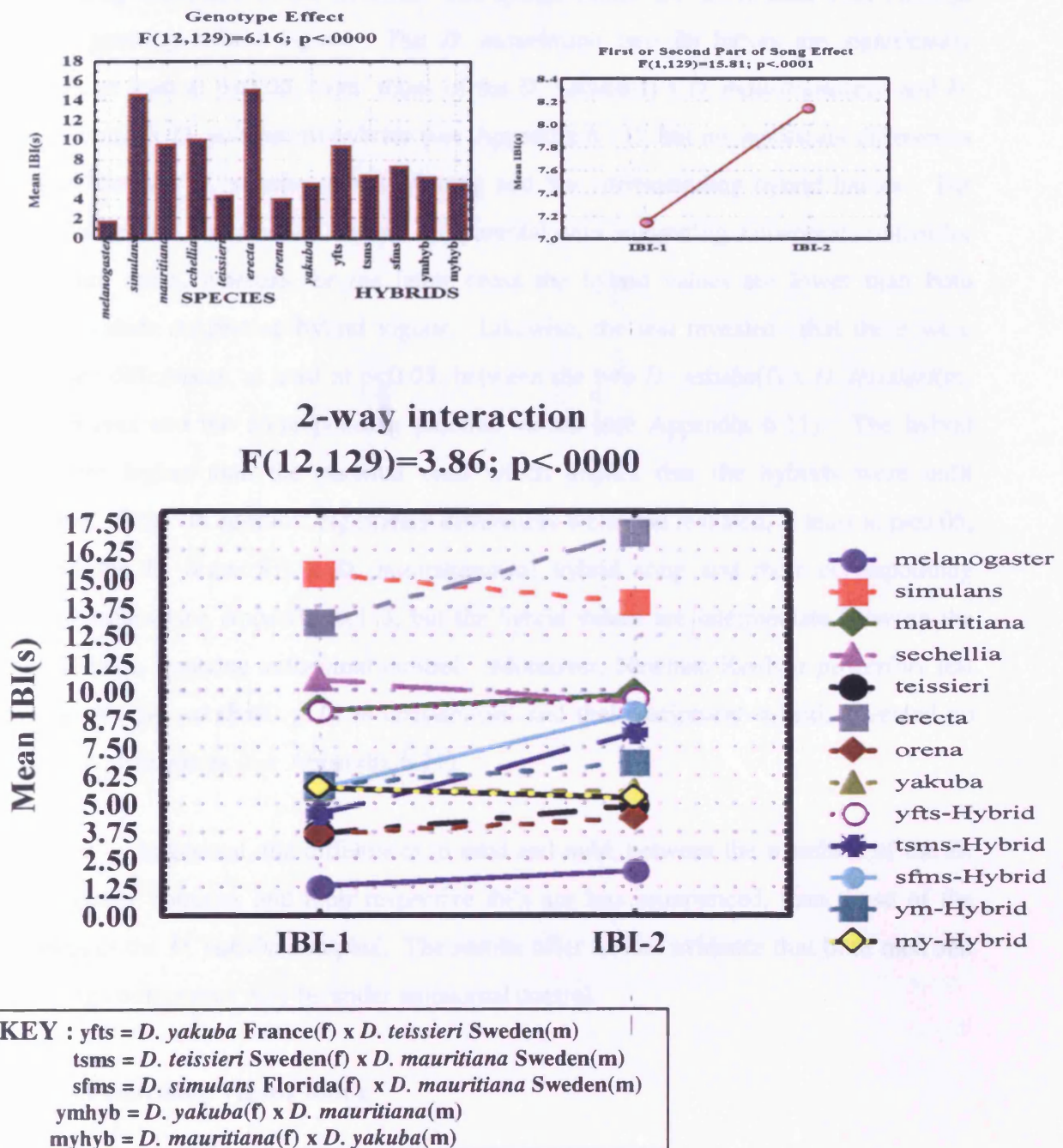
Figure 6.4.3: MIBI for the first and second halves of song between the different species and their interspecific hybrids.

Newman-Keuls *a posteriori* test for the two ibi halves of song between the members of the *melanogaster* subgroup are given in Appendix 6.11. Several significant differences were revealed. The test revealed significant differences between the two halves of *D. simulans* song and the respective halves of the *D. simulans*(f) x *D. mauritiana*(m) hybrids, and no significant differences between the *D. mauritiana* second

125

half of song and those of the hybrids. The hybrid values are lower than both parental ones, suggesting hybrid vigour. The *D. mauritiana* two ibi halves are significantly different, at least at p<0.05, from those of the *D. yakuba*(f) x *D. mauritiana*(m), and *D. mauritiana*(f) x *D. yakuba*(m) hybrids (see Appendix 6.11), but no significant differences between the two *D. yakuba* halves of song and the corresponding hybrid halves. The hybrid values are intermediate betwen the parental ones suggesting autosomal control for the former cross, whereas for the latter cross the hybrid values are lower than both parental values suggesting hybrid vigour. Likewise, the test revealed that there were significant differences, at least at p<0.05, between the two *D. yakuba*(f) x *D. teissieri*(m) hybrid halves and the corresponding parental values (see Appendix 6.11). The hybrid values are higher than the parental ones which implies that the hybrids were unfit (Haldane, 1922). In addition, significant differences were also revealed, at least at p<0.05, between the *D. tessieri*(f) x *D. mauritiana*(m) hybrid song and their corresponding parental values (see Appendix 6.11), but the hybrid values are intermediate between the parental ones, implying autosomal control. Moreover, Newman-Keuls *a posteriori* test between the *D. yakuba*(f) x *D. mauritiana*(m) and their reciprocal hybrids revealed no significant differences (see Appendix 6.11).

In general, the differences in mbd and mibi, between the members of the *D. melanogaster* complex and their respective ibi's are less pronounced, than those of the members of the *D. yakuba* complex. The results offer further evidence that both mbd and mibi song components may be under autosomal control.

6.5 % of courtship vigour index

In order to validate the results for the above exercise in terms of what they might mean in courtship, a % courtship vigour index was calculated. Briefly, the mbd for the entire song (mbd_e) of song was divided by mbd_e + the mean ibi for the entire song ($mibi_e$). This determined the courtship vigour index for the song (vig_e). The resultant values were converted into a percentage and arcsin-transformation was employed to transform the data. One-way ANOVA of the arcsin-corrected % vigour indices of song between the different species and their hybrids revealed significant

differences (F=9.12, df=12,129, p=0.000-see Figure 6.5.1, below). Figure 6.5.1 shows that both *D. melanogaster* and *D. yakuba* have the highest % vigour.
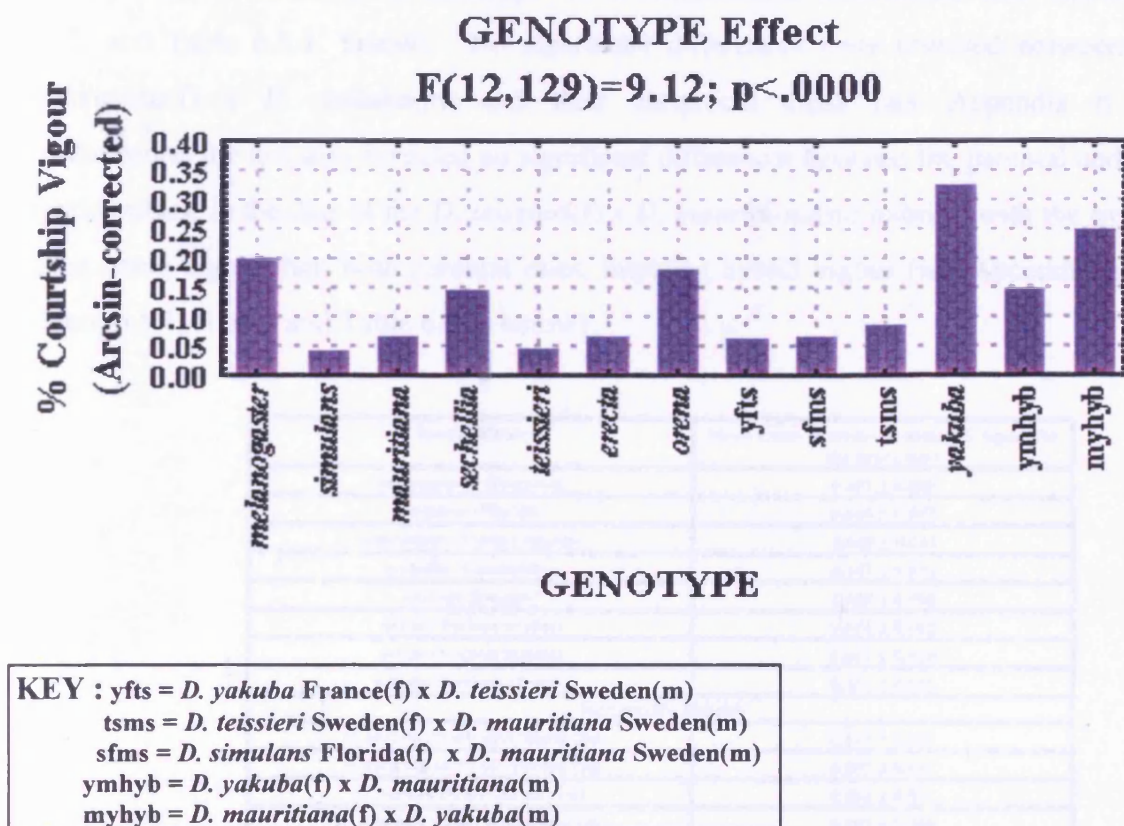
Figure 6.5.1: Arcsin-corrected % courtship vigour between the various members of the *D. melanogaster* subgroup and their interspecific hybrids.

Newman-Keuls *a posteriori* test revealed significant differences, at least at p<0.05, between *D. yakuba* and the rest of the member species of the *melanogaster* subgroup, except *D. orena* and *D. melanogaster*. The test also revealed significant differences, at least at p<0.05, between *D. yakuba*, and the *D. yakuba*(f) x *D. teissieri*(m) and *D. yakuba*(f) x *D. mauritiana*(m) hybrids (see Appendix 6.12). In the case of the former cross, the results imply *D. teissieri* dominant autosomal effects (the hybrid value is closer to that of *D. teissieri*, see Figure 6.5.1, above, and Table 6.5.1, below), whereas in the case of the latter cross, it is observed that the hybrid value is intermediate between the two parental ones, suggesting autosomal control (see Figure 6.5.1, above and Table 6.5.1, below). In the case of the *D. simulans*(f) x *D. mauritiana*(m), Newman-Keuls *a posteriori* test showed no significant differences between the hybrids and the parental species, with the hybrid value being closer to that of *D. mauritiana*, implying *D. mauritiana* dominant autosomal effects (see Appendix 6.12, Figure 6.5.1, above and Table 6.5.1, below). In the case of the *D. mauritiana*(f) x *D. yakuba*(m) hybrids, the test

revealed no significant differences between the parental values and those of the hybrids, with the hybrid value being closer to that of *D. yakuba* suggesting either *D. yakuba* dominant autosomal factor involvement or Y chromosome involvement (see Appendix 6.12, and Table 6.5.1, below). No significant differences were revealed between *D. mauritiana*(f) x *D. yakuba*(m) and their reciprocal cross (see Appendix 6.12). Furthermore, the test also revealed no significant differences between the parental and the hybrid values, in the case of the *D. teissieri*(f) x *D. mauritiana*(m) hybrids, with the hybrid value being higher than both parental ones, implying hybrid vigour (see Appendix 6.12, Figure 6.5.1, above, and Table 6.5.1, below).

Species/Strain	Mean ± sem of arcsin-corrected % vigour for the entire song
melanogaster (Brighton)	0.201 ± 0.020
simulans (Florida)	0.044 ± 0.007
mauritiana (Various strains)	0.069 ± 0.012
sechellia (Cambridge)	0.147 ± 0.022
teissieri (Sweden)	0.045 ± 0.010
orena (Various strains)	0.181 ± 0.021
erecta (Various strains)	0.067 ± 0.010
yakuba (Various Strains)	0.324 ± 0.031
Intraspecific Hybrids	
D. yakuba (f) x*D. mauritiana* (m)	0.145 ± 0.032
D. mauritiana (f) x*D. yakuba* (m)	0.250 ± 0.030
D. yakuba (f) x*D. teissieri* (m)	0.063 ± 0.007
D. simulans (f) x*D. mauritiana* (m)	0.067 ± 0.030
D. teissieri (f) x*D. mauritiana* (m)	0.085 ± 0.011

Table 6.5.1: The mean ± sem for the arcsin-corrected % vigour for the entire song.

Upon examination of Table 6.5.1, the overall picture that emerges is that *D. melanogaster* and *D. sechellia* have the highest % courtship in the *melanogaster* complex, whereas *D. yakuba* and *D. orena* have the highest % courtship in the *yakuba* complex. Summing up, it is clear that the mode of inheritance for this song element may involve polygenic autosomal partially dominant factors.

The question of how many IPIs are presented to a female, in an IPI cycle, can now be tackled. The mean IPI cycle for each species (see chapters 3, 4 and 5), was divided by the [mbd + mibi] (see Appendices 6.7, 6.8 and 6.9 for individual song mbd and ibi). This gives the number of bursts (NBC) per cycle. This was then multiplied by the mbd of each particular genotype to give the amount of pure pulse song present in an entire cycle (PSC). The PSC was then divided by the species-specific mean IPI for each

species, to give the number of IPI's per cycle (NIC-see chapters 3, 4 and 5). Table 6.5.2 shows (below) the results of this empirical analysis.

SPECIES	MBD(ms)	MIBI(s)	MEAN PERIOD (s)	MEAN IPI (ms)	Number of bursts per cycle (NBC)	Total amount of Pulse Song in Cycle (PSC)	IPI in a Cycle (NIC)
melanogaster	397.504	1.64726	54.37	35.60	26.6	10.57	297
simulans	632.898	14.57126	47.99	53.34	3.16	1.99	37.5
mauritiana	432.456	9.476	39.69	38.05	4.00	1.73	45.5
sechellia	1065.361	10.1714	48.35	71.93	4.00	4.26	59.2
yakuba	1768.646	5.5973	63.18	117.76	7.34	12.98	110.2
teissieri	215.394	4.2208	71.96	28.02	16.22	3.49	124.7
orena	730.361	3.9828	46.82	48.44	9.93	7.25	149.7
erecta	968.569	15.018	45.48	47.01	2.84	2.75	58.5
INTERSPECIFIC HYBRIDS							
yakuba(f) x mauritiana(m)	700.713	6.3938	72.22	69.60	10.18	7.13	102.5
mauritiana(f) x yakuba(m)	1673.747	5.4638	52.53	75.14	7.36	12.32	163.9
yakuba(f) x teissieri(m)	582.894	9.4086	56.92	64.76	5.70	3.32	51.3
simulans(f) x mauritiana(m)	674.862	7.2891	59.13	46.15	7.42	5.01	108.5
teissieiri(f) x mauritiana(m)	514.262	6.3499	59.95	34.70	9.44	4.85	139.9

Table 6.5.2: The mbd, mibi, mean period, mean IPI, NBC and IPIs in a Cycle of the different species of the *melanogaster* subgroup and their interspecific hybrids.

As it can be seen from Table 6.5.2 (above), *D. melanogaster* males present the highest number of IPIs in one entire cycle, from any of the other member species of the *melanogaster* subgroup. Within the *yakuba* complex, *D. orena* is observed to produce the most IPI's in an entire song cycle. On inspection of the pulse song production of the different hybrids, it can be seen that in the case of the *D. mauritiana*(f) x *D. yakuba*(m), and *D. simulans*(f) x *D. mauritiana*(m) hybrids, the hybrid pulse song production (number of IPIs in an entire cycle) is seen to be greater than both parental values. The *D. yakuba*(f) x *D. teissieri*(m) hybrids produced far fewer IPIs than both parents, which suggests hybrid unfitness (Haldane, 1922). Taking into consideration that the mode of inheritance for the IPI's is autosomal, and that the song rhythm cycle is under X chromosome influence and, mbd and mibi is autosomal, we cannot draw too many conclusions from the *D. yakuba*(f) x *D. mauritiana*(m), and *D. teissieri*(f) x *D. mauritiana*(m) hybrids. My initial idea, however, that the number of IPI's in a cycle might be a constant between species however, is clearly incorrect.

6.6 Discussion:

Differences in the courtship song elements in *Drosophila* are considered to play an important role in species sexual isolation, for two reasons: a) the absence of significant variation within species (Ewing and Bennet-Clark, 1968; Hoikkala, 1985; Cobb *et al*, 1990, Ritchie *et al.*, 1994), and b) the occasional large differences observed even between closely related species (Kyriacou and Hall, 1980; Cowling and Burnet, 1981; Hoikkala and Lumme, 1987; Lee and Watanabe, 1987; Thackeray *et al.*, 1990). Different species show different short-term fluctuation of behaviour for the three acoustic characters, under investigation, i.e., IPF, CPP and IPI, respectively, within a burst. Trends in acoustic elements between individual songs within the species, may sometimes vary, i.e., within a species, some trends (regression lines), when examining a particular song component, may either show a positive or negative tendency. For example, three songs may give two regression lines with a slight decrease, whereas the third one may show a strong positive trend, and the resultant overall species trend may conform to a strong positive regression line, 'masking' the behaviour of the former two. Therefore, only the most consistent patterns within a species are given any attention in this discussion.

The IPI of *D. melanogaster* and *D. simulans* increases over a burst. This concurs with Ewing's (1983) observations. On the contrary, the IPI of *D. mauritiana* and *D. sechellia* decreases in the course of a burst, and therefore, the IPI trend seems to be species-specific. Simulator experiments, with decreasing or increasing IPI's over a burst, could be carried out in order to test if indeed IPI trend behaviour is species-specific among the different members of the subgroup. On inspection of the members of the *D. yakuba* complex more variable patterns are observed. These differences could be a reflection on the phylogenetic relationships between the members of the *melanogaster* subgroup, which concurs with Lee and Watanabe (1987) and Cobb *et al.*'s (1990) findings. The *D. teissieri* IPF and IPI characters increase over the course of a burst, and these differ from the other three species in the *yakuba* complex. Since, *D. teissieri* lives in sympatry with *D. yakuba*, in the Afrotropical region (Tsacas, 1971; Burla, 1954), it is possible the differences obseved in both IPI and IPF may represent a case of character displacement (Grant, 1984; Schluter and McPhail, 1992; Schluter, 1994), which is the

exaggeration of species markers such as courtship rituals or song characters, in sympatric populations. In this way, the species barriers are reinforced and maintained. In the intraspecific *D. yakuba* Malawi(f) x *D. yakuba* S.T.(m) hybrids, the IPI for Clack and Thud songs consistently increases, which differ from both parental trends. Perhaps, a decrease in IPI over a burst, for the parental *D. yakuba* strains may be a result of inbreeding depression.

Examination of the overall temporal trends, for all three song characters, in interspecific hybrids (see Table 6.2), gave a complex pattern of results which is difficult to interpret. In the case of IPF, it is observed that generally this character may be under the influence of the X chromosome, but dominant autosomal factors can not be excluded in some interspecific crosses. The same can be stated for both CPP and IPI trends. Thus, the mode of inheritance of these three characters may be complex interaction between the X chromosome and autosomal factors.

Results for mbd and mibi for the member species within the *melanogaster* subgroup and their interspecific hybrids, revealed significant differences between the species, for these two characters. *D. sechellia* was observed to have the longest mbd among the members of the *D. melanogaster* complex, and *D. yakuba* among the member species of the *D. yakuba* complex, which could be a reflection on their long mean IPI's, compared to the other member species of the subgroup. Byrne and Kyriacou (pers. comm.) reported, on the basis of simulator experiments, that the burst length differences between *D. arizonae* and *D. mojavensis* may be a species-specific cue, which the females might use for enhanced conspecific mating. Whether or not mbd is a species-specific cue for the females of the member species of the *melanogaster* subgroup remains to be tested. A more variable picture emerges from examining the members of the *D. yakuba* complex. *D. erecta* has the longest mibi of the complex, which suggests, bearing in mind its short IPI (see Chapter 4-section 4.3), that *D. erecta* males are the least vigorous courters, which is further supported by the fact that they produce the least number of IPIs, in an entire cycle (see section 6.5.). The differences in both mbd and mibi are far more obvious among the members of the *D. yakuba* complex, which could reflect upon their comparatively distant evolutionary relationship. Examining the interspecific hybrids produced among the members of the *melanogaster* subgroup gave further evidence as to

the mode of inheritance for mbd and mibi song elements. The results suggest that these two song characters are under autosomal control, but Y chromosome involvement could not be excluded.

The empirical analysis for the number of IPIs produced in an entire IPI cycle, revealed large differences among the members of the *melanogaster* subgroup. Within the *melanogaster* complex, *D. melanogaster* males produce the largest number of IPI's in an entire cycle, than any other of its sibling species. In general, the members of the *yakuba* complex produce more IPIs in an entire cycle than the members of the *melanogaster* complex, except for *D. melanogaster*.

Within the *D. yakuba* complex, *D. orena* males are observed to produce the largest number of IPIs in an entire cycle. The courtship vigour analysis showed that within the *melanogaster* subgroup *D. melanogaster* produce most IPI's in a complete cycle of song, which could be attributed to their longer adaptation to the laboratory environment (stock over 10 years), while the rest of the member species of the *D. melanogaster* complex seem to be poorly adapted to laboratory conditions, especially *D. sechellia* which is known to breed exclusively on fallen fruits of the maritime rubiaceous shrub *Morinda citrifolia* (Tsacas and Bächli, 1981). Within the *yakuba* complex, *D. yakuba* seems to be most well-adapted, and *D. erecta* the least-adapted to laboratory conditions, whose major breeding site is the fallen fruit of the tree *Pandanus candelabrum* in equitorial West Africa (Tsacas and Lachaise, 1974). Examing the interspecific hybrids it is apparent that the mode of inheritance of courtship vigour is under autosomal control. The *D. teissieri*(f) x *D. mauritiana*(m) hybrids show higher % courtship vigour than both their respective parental ones, suggesting hybrid vigour.

In conclusion, examination of all the song components from the interspecific hybrids, in this study, revealed complex modes of inheritance for all the song elements. Another avenue that could be explored is to carry out a detailed investigation into the contributions of the various individual song elements and mating success. This could be achieved, by producing hybrid progeny between the different member species of the *D. melanogaster* subgroup, and examining the patterns of these song elements in the backcross hybrids that are viable. This sort of study would investigate further the genetic

bases for the phenotypic cues that may form a foundation for species discrimination, and could possibly give a further insight into speciation.

Locomotor activity rhythms in the *melanogaster* subgroup in constant darkness (DD).

CHAPTER 7

7. Locomotor activity rhythms in the *melanogaster* subgroup in constant darkness (DD).

The chapters 3 and 4, and 5 described attempts to investigate ultradian period rhythms in the courtship songs of the member species of the *melanogaster* subgroup, and some of their interspecific hybrids, respectively, with varying success. Another common behavioural character to be examined for the presence of rhythms is the locomotor activity of individual flies. This locomotor activity character has been studied in a great variety of species, one of earliest examples being the house cricket, *Acheta domestica* (Lutz,1932).

The two most commonly studied aspects of locomotor activity are either under constant darkness (DD or "free-run") or artificial light/dark regimes (LD); while the former conditions will uncover the period of the endogenous circadian oscillator which controls activity, the latter can allow the detailed pattern of activity throughout the day to be investigated under light entrainment. Aside from the free-running period of the circadian locomotor activity, the daily pattern of activity, including the absolute level of activity, may be another aspect of behaviour under the influence of *per* (Petersen et al.,1988). This latter aspect is being dealt with in detail in Chapter 8. Although the locomotor activity rhythms of several *Drosophila* species have been examined and documented (Saunders, 1982), most of the species dealt with in this chapter were not included. Here I report on 'free-run' experiments in DD for all the member species of the *D. melanogaster* subgroup, several strains, and their interspecific hybrids, and an attempt is made to investigate any species-specific differences.

Males from each species/strain and interspecific hybrid genotype were monitored as described in Chapter 2 for 5-7 days, in constant darkness (DD) at 25^{0}C. The flies had been previously entrained in light/dark cycles (LD12:12) for 3 days (see Chapter 2). Initially, the activity data from each fly was analysed for circardian rhythmicity by an autocorrelation procedure (Chatfield, 1984). Spectral analysis was employed in order to obtain a more precise measure of the endogenous period (see Chapter 2). A fly was

considered to be rhythmic, if both analyses gave a consensus of rhythmicity (see Chapter 2). If no consensus rhythmicity was observed, the fly was considered arrhythmic.

Table 7.1 shows the total number of flies examined that survived the duration of the experiments (Column A). From these, the number of flies that had revealed significant autocorrelation and spectral analyses (Column B), and the number of 'arrhythmic' flies (Column C) are shown. Thereafter, a percentage of rhythmicity was determined (Column D). A mean period ± sem for all the species/strains and interspecific hybrids was then determined (Column E). It can be observed that with the exception of *D. sechellia*, all other mean periods range from 24 to 24.5 h. However, it was observed that individual fly's free-running periods differ quite markedly within each species, for example, a range of 21.10-26.78 h in *D. orena*, and a range of 22.57-25.00 h in *D. teissieri* was observed. This prevents an overall picture of the daily pattern of activity from being assembled from the activity data of all the animals of each species. One way to solve this problem is to examine the activity of all the flies under light/dark cycles entrainment. Under these artificial conditions (dealt in Chapter 8), with instantaneous light to dark transitions and *vice versa* rather than the more gradual transitions that would occur in nature, all participating flies would be entrained to a 24 h cycle, which would allow the construction of a single plot by superimposition, giving the pattern of daily activity within that circadian cycle (see Chapter 8). Another way is to superimpose that data for each species for only a subset that exhibits identical periods. This is discussed in section 7.2.

7.1 Locomotor activity of the members of the *D. melanogaster* subgroup and their interspecific hybrids.

One-way ANOVA for the spectrally-determined periods of the various members species of the *melanogaster* subgroup and their hybrids revealed significant differences (see Figure 7.1.1). Newman-Keuls *a posteriori* test for the spectrally-determined periods of the various species/strains and hybrids revealed significant differences, at least at $p<0.05$, between *D. sechellia* (shorter mean period), and the rest of the species (see

TABLE 7.1 : Locomotor Activity:- % of rhythmic flies in DD and their mean periods.

SPECIES/STRAINS	No. of flies examined (A)	Rhythmic flies (B)	Arrhythmic flies (C)	% of rhythmic flies (D)	Mean Period(h) ± sem (E)
melanogaster (Brighton)	31	30	1	85.71	24.45± 0.112
simulans (Florida)	22	21	1	95.45	24.03 ± 0.092
mauritiana (France)	54	42	12	77.78	24.15 ± 0.097
mauritiana (Sweden)	19	14	5	73.68	23.96 ± 0.111
mauritiana (MG17)	8	8	-	100.00	24.53 ± 0.405
mauritiana (Isofemale 72)	7	6	1	85.71	24.48 ± 0.344
mauritiana (Isofemale 105)	3	3	-	100.00	23.34 ± 0.491
mauritiana (Isofemale 152)	4	4	-	100.00	24.31 ± 0.297
mauritiana (Isofemale 197)	3	3	-	100.00	24.19 ± 0.302
sechellia (Cambridge)	57	52	5	91.23	23.16 ± 0.077
teissieri (Sweden)	34	18	16	52.94	23.94 ± 0.170
orena (France)	35	23	12	65.71	24.21 ± 0.242
erecta (France)	35	19	16	54.29	23.97 ± 0.189
yakuba (France)	54	35	19	64.81	24.06 ± 0.104
yakuba (Malawi)	12	11	1	91.67	23.90 ± 0.126
yakuba (Ivory Coast)	7	6	1	85.71	24.57 ± 0.280
yakuba (Lamto3)	15	11	4	73.33	24.39 ± 0.179
yakuba (Subtaome)	11	11	-	100.00	24.40 ± 0.232
yakuba (Japan)	6	5	1	83.33	23.85 ± 0.485
INTERSPECIFIC HYBRIDS					
yakuba France(f) x *mauritiana* France(m)	106	90	16	84.91	24.09 ± 0.054
yakuba Malawi(f) x *mauritiana* Sweden(m)	10	5	5	50.00	24.91 ± 0.711
yakuba Lamto3(f) x *mauritiana* Isofemale72(m)	9	8	1	88.89	23.79 ± 0.131
yakuba France(f) x *teissieri* Sweden(m)	5	4	1	80.00	23.25 ± 0.469
simulans Florida(f) x *mauritiana* Sweden(m)	8	7	1	87.50	24.35 ± 0.156
mauritiana Sweden(f) x *yakuba* Lamto3(m)	17	13	4	76.47	24.16 ± 0.208

Appendix 7.1). The test also revealed significant differences (p<0.05), between the *D. yakuba*(f) x *D. teissieri*(m) hybrid, and their corresponding parental periods, with the hybrid periods being shorter than those of the parents (see Appendix 7.1). Furthermore, the test revealed no significant differences between the *D. yakuba*(f) x *D. mauritiana*(m), *D. mauritiana*(f) x *D. yakuba*(m), and *D. simulans*(f) x *D. mauritiana*(m) hybrids, and their corresponding parental species (see Appendix 7.1), with hybrid mean period values being slightly shorter, intermediate and longer than their corresponding parents, respectively (see Table 7.1).

Figure 7.1.1: Results for the spectrally-determined mean periods of the different species of the *melanogaster* subgroup and their interspecific hybrids.

SPECIES Effect
F(11,440)=10.18; p<.0000
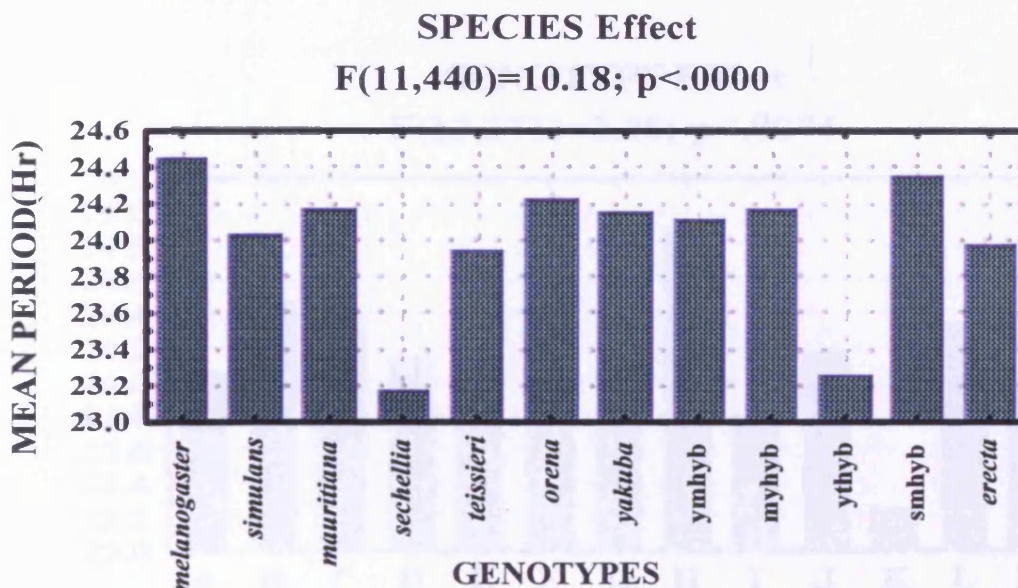
KEY :

ymhyb = *D. yakuba*(f) x *D. mauritiana*(m)
myhyb = *D. mauritiana*(f) x *D. yakuba*(f)
ythyb = *D. yakuba*(f) x *D. teissieri*(m)
smhyb = *D. simulans*(f) x *D. mauritiana*(m)

The analysis above pools all the strains within a species, and hybrid types together. One-way ANOVA of the spectrally-determined periods of the various strains used in the crosses, and their respective hybrids only, also revealed significant differences (see Figure 7.1.2). Newman-Keuls *a posteriori* test revealed significant differences (p<0.05),

between the *D. yakuba* France(f) x *D. teissieri* Sweden(m) (Column K), and *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) (Column H) hybrids, and their corresponding parental strains (Columns A & E, and M & N, respectively), with the hybrid mean periods being shorter, and longer respectively (see Appendix 7.2). No significant differences were revealed between the *D. yakuba* France x *D. mauritiana* France(m) (Column G), *D. yakuba* Lamto3(f) x *D. mauritiana* Isofemale 72(m) (Column I), *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) (Column J), and *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) hybrids (Column L), and their corresponding parental strains (Columns A & D, C & B, N & C, and F & M, respectively, see Appendix 7.2). It is observed that none of the other hybrids revealed significant differences from their respective parental strains.

GENOTYPE Effect
$F_{(13,271)}=2.28$; $p<.0074$
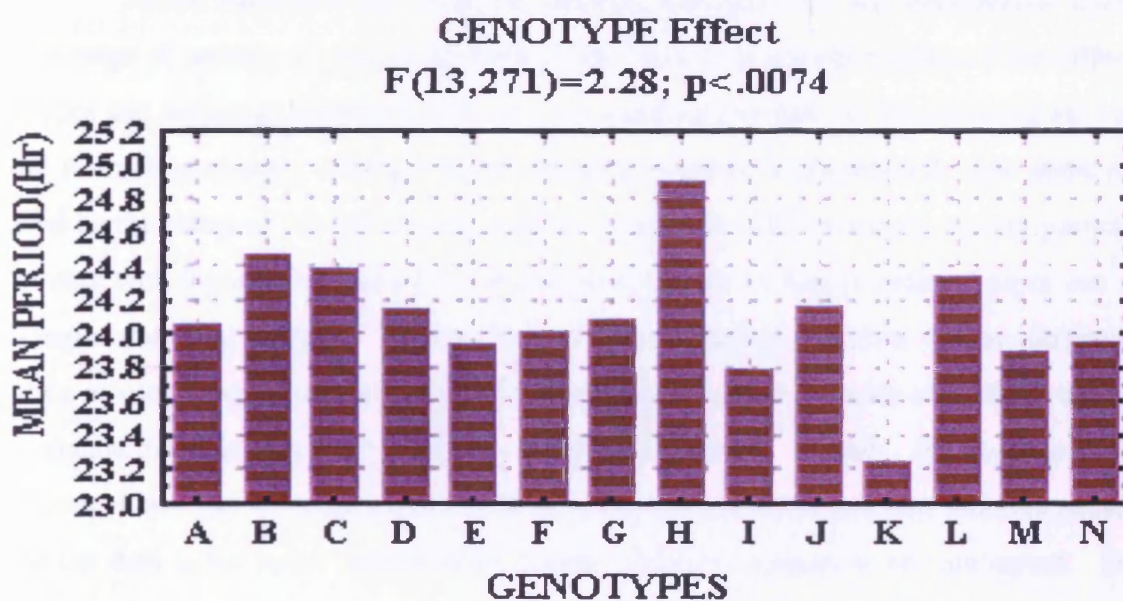
KEY:

A = *D. yakuba* France	G = *D. yakuba* France(f) x *D.mauritiana* France(m)
B = *D. mauritiana* Isofemale 72	H = *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m)
C= *D. yakuba* Lamto3	I = *D. yakuba* Lamto3(f) x *D. mauritiama* Isofemale 72(m)
D= *D. mauritiana* France	J = *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m)
E = *D. teissieri* Sweden	K = *D. yakuba* France(f) x *D. teissieri* Sweden(m)
F = *D. simulans* Florida	L = *D. simulans* Florida(f) x *D. mauritiana* Sweden(m)
M = *D. yakuba* Malawi	
N = *D. mauritiana* Sweden	

Figure 7.1.2: Results for the spectrally-determined mean periods of the different parental strains and their corresponding interspecific hybrids.

One-way ANOVA for the spectrally-determined periods within the different *D.* *yakuba* and *D. mauritiana* strains also revealed no significant differences (F=1.777, df=5, 73, p=0.128, and F=1.452, df=6, 68, p=0.207, respectively). Summing up, *D. sechellia* gives a shorter period, but the hybrids do not shed any light as to the paternal or maternal contributions to the determination of the period, because the maternal and paternal values are so similar.

7.2 Locomotor activity profiles of the different members of the *melanogaster* subgroup, various *D. mauritiana* and *D. yakuba* strains, and their interspecific hybrids.

Since individual flies from the different species/strains and interspecific hybrids show a range of periods, a general overview of the locomotor activity profiles of the different genotypes and statistical analysis is difficult. If a significant number of flies showing the same period from all genotypes could be found, the data could be superimposed, thus allowing a detailed investigation of the DD activity profiles to proceed. Unfortunately, no one particular set of data with the same period provided adequate number of flies in order to carry out any meaningful statistical analysis. The only way to gain a general overview was to standardise the data in such a way, so that some sort of comparison could be achieved. How the data were standardised is described in more detail in Chapter 2. Briefly, the standardisation procedure allows the comparison of DD data which has been wrapped into different periods, but as the data is no longer independent, formal statistical analysis is not attempted. Data recording was initiated at CT21, 9h after the last lights-off which was at CT12.

D. melanogaster complex:

D. melanogaster is characterised by a unimodal pattern activity, with the 'subjective morning'peak being maintained into the evening at CT24, with a slight fall in

activity during the day and night (see Figure 7.2.1: Top left panel). *D. simulans* shows a similar profile, with the evening slightly later at CT35, which is followed by a fall in activity during the night which gradually increases as daytime approaches (see Figure 7.2.1: Top second left panel). Furthermore, it shows an unusual short-lived burst of increased activity at CT30. *D. sechellia* is characterised by a strong 'subjective evening' peak (see Figure 7.2.1.: Top third left panel) at CT35, followed by a rapid fall in activity, which is sustained throughout the 'subjective night'; the 'subjective morning' peak is not pronounced at all, but a rather gradual increase in activity is seen, as seen in *D. melanogaster*. *D. mauritiana* is characterised by a more bimodal pattern of activity (see Figure 7.2.1: Top right panel), with slight morning (CT25) and evening (CT34-35) peaks, but with generally high activity levels during the day and low activity during the night. In addition, *D. mauritiana* is observed to be less active compared to the othe members of the *melanogaster* complex.

D. yakuba complex:

D. teissieri (see Figure 7.2.1: Bottom left panel) shows both strong 'subjective' morning (CT25-26) and evening (CT34) peaks, with reduced activity during the night. *D. erecta* (see Figure 7.2.1: Bottom second panel) reveals a more pronounced evening than morning peak at CT33-34 and CT22 respectively, which is followed by a fall in activity during the night, and a gradual increase of activity as daytime approaches. *D. orena* (see Figure 7.2.1: Bottom third left panel) also shows a bimodal profile, with a pronounced evening peak at CT33, followed by a sudden fall in activity which is sustained at low levels during the night. This is followed by a small increase of activity at CT24, which signifies the morning peak. *D. yakuba* (see Figure 7.2.1: Bottom right panel) shows less pronounced morning and evening peaks, high activity levels during the day and a fall in activity during the night. *D. yakuba* is the most active species within the *D. yakuba* complex.

In summing up, the *D. melanogaster* complex shows a unimodal pattern of locomotor activity, whereas the members of the *D. yakuba* complex exhibit bimodal activity profiles, except *D. yakuba*. *D. yakuba* has a broader evening peak, whereas the other sibling
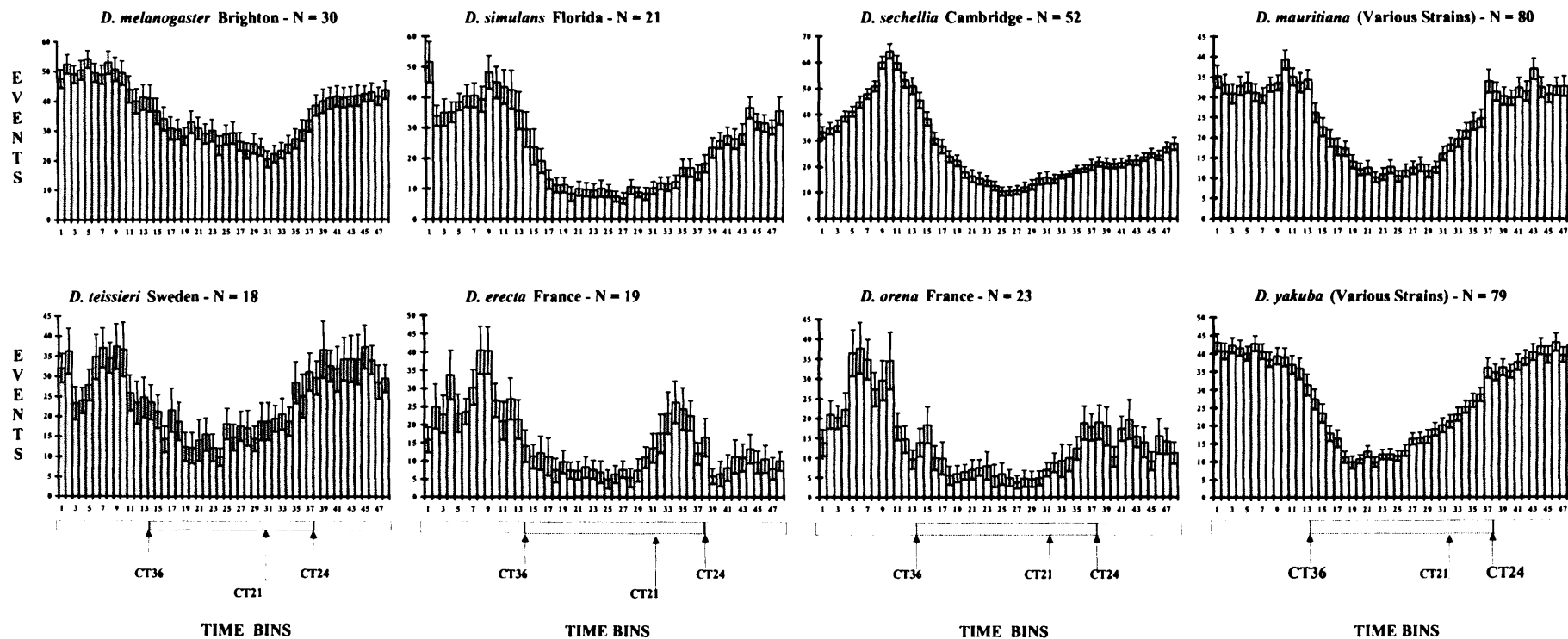
Figure 7.2.1: Locomotor Activity Profiles of the members of the *D. melanogaster* subgroup, in DD, after standardisation of the data.

KEY:
The CT regime is shown below the graphs.
CT24 = Lights-On
CT36 = Lights-Off
CT21 = Data collection commencement

species in the *yakuba* complex exhibit sharper and more transitory evening peaks. In addition, *D. melanogaster* is seen to be the most active, whereas *D. erecta* and *D. orena* are the least active species.

D. mauritiana strains:

I also examined the different *D. mauritiana* strains in search of intraspecies heterogeneity. All the *D. mauritiana* strains show a slight bimodal pattern of locomotor activity (see Figure 7.2.2), with reduced activity during the night, and a small burst of activity around the subjective light transitions. As it can be seen from Figure 7.2.2, there is some heterogeneity in the activity levels. All the strains which had reasonable n's ($\geq$ 8) showed levels of activity around the evening peak (CT36) that are higher than around the morning peak. Considerable variation in the free-running periods between different individuals within the Isofemale 152, 197 and David 105 strains, in conjunction with their small sample sizes may have contributed to the large sem's observed in these strains. However, *D. mauritiana* France, Sweden and Isofemale 72 exhibit almost identical patterns, with similar activity levels.

D. yakuba strains:

In general, all the *D. yakuba* strains (see Figure 7.2.3) are characterised by a broad peak of activity, with no strongly defined morning and evening peaks. The Malawi, Lamto3 and Ivory Coast strains (Figure 7.2.3: Top centre, Top right and Bottom left panels, respectively) show no pronounced morning and evening peaks, and varying levels of activity during the night and daytime. The Japan and Subtaome strains (Figure 7.2.3: Bottom centre and right panels, respectively) show some phase-delay of their morning activity levels at CT28-29 and CT28 respectively, and little activity during the night. It is clear that variation, apart from the slightly different levels of activity observed between the different strains, there not much heterogeneity in the activity profiles of the *D. yakuba* strains.

Figure 7.2.2: Locomotor Activity Profiles of the different *D. mauritiana* strains, in DD, after standardisation of the data.

        KEY :

        The CT regime is shown below the graphs.

            CT24 = Lights-On

            CT36 = Lights-Off

            CT21 = Data collection commencement
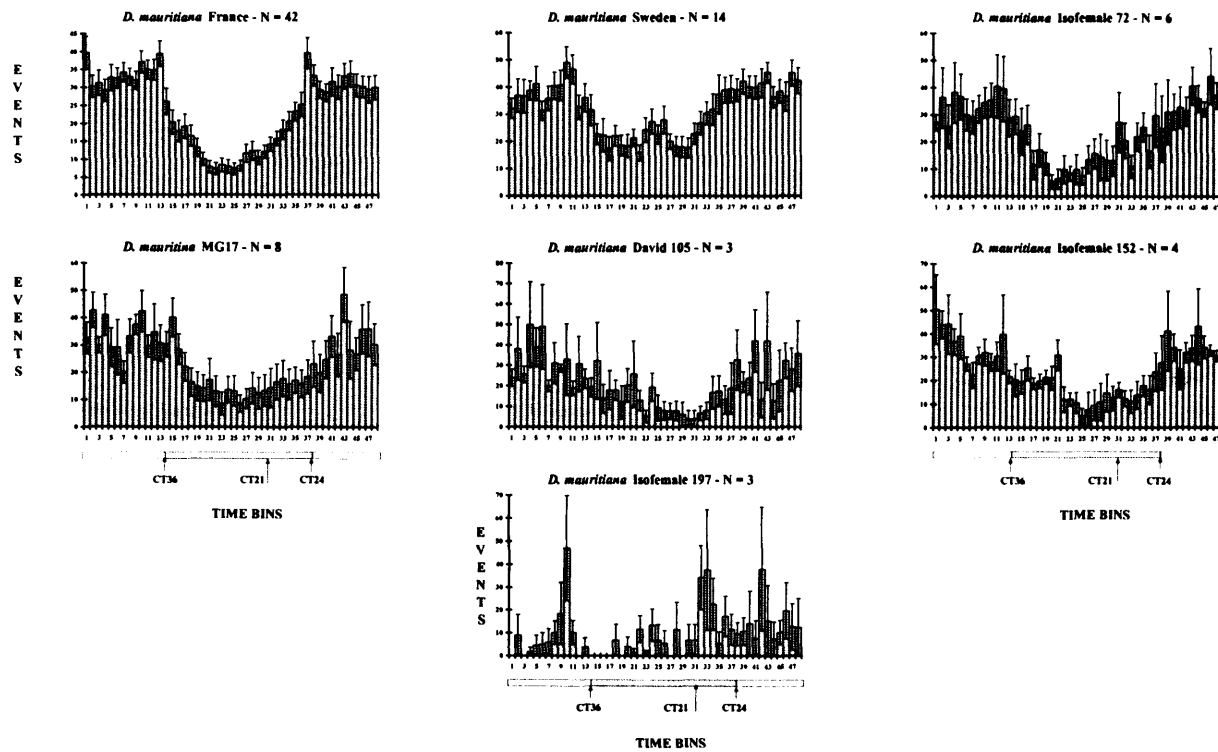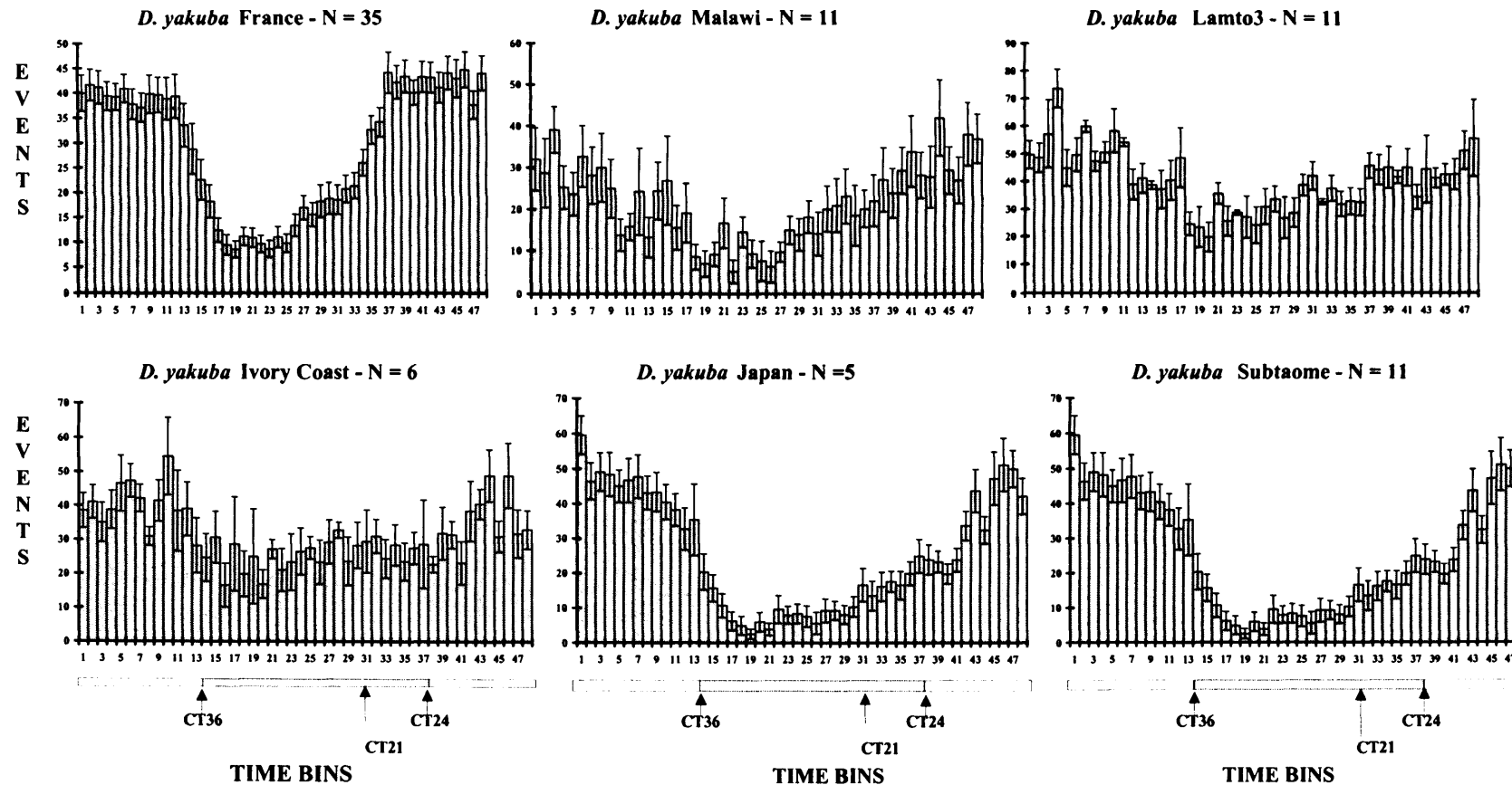
Figure 7.2.3: Locomotor Activity Profiles of the different *D. yakuba* strains, in DD, after standardisation of the data.

KEY:

The CT reigime is shown below the graphs.
CT24 = Lights-On
CT36 = Lights-Off
CT21 = Data collection commencement

Consequently in both *D. mauritiana* and *D. yakuba*, the general lack of intraspecific variation suggests that species-specific patterns of locomotor behaviour are reasonably robust.

Different species and their interspecific hybrids:

Figure 7.2.4 depicts the various parental strains (Top graph for the female and Bottom graph for the male parents) and their respective interspecific hybrids (Middle graphs).

The *D. yakuba* France(f) x *D. mauritiana* France(m) (Figure 7.2.4: Column A, Middle left panel) hybrids show an overall activity profile that is similar to both parents. The rise in activity at ≈ CT30 resembles the paternal parent, as does the burst of morning activity around the time of the subjective transition to lights-on.

The *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) hybrids (Figure 7.2.4: Column B, Middle second left panel) shows slightly higher levels of activity than both parental strains which may suggest hybrid vigour. The overall pattern of activity resembles the paternal pattern, with a definite evening peak. However, because the sample size of these hybrids is small, these results should be considered with some caution.

The *D. yakuba* Lamto3(f) x *D. mauritiana* Isofemale 72(m) hybrids (Figure 7.2.4: Column C, Mddle third left panel) shows an overall pattern of activity that resembles that of the paternal parent, as well as similar levels of activity, and having a pronounced evening peak at CT32-33 resembling that of the paternal participant, and a morning peak at CT25 also resembling the paternal parent.

The *D. yakuba* France(f) x *D. teissieri* Sweden(m) hybrids (Figure 7.2.4: Column D, Middle fourth left panel) show an overall pattern of activity that is different from those exhibited by both parents. There is a burst of an evening surge of activity which also
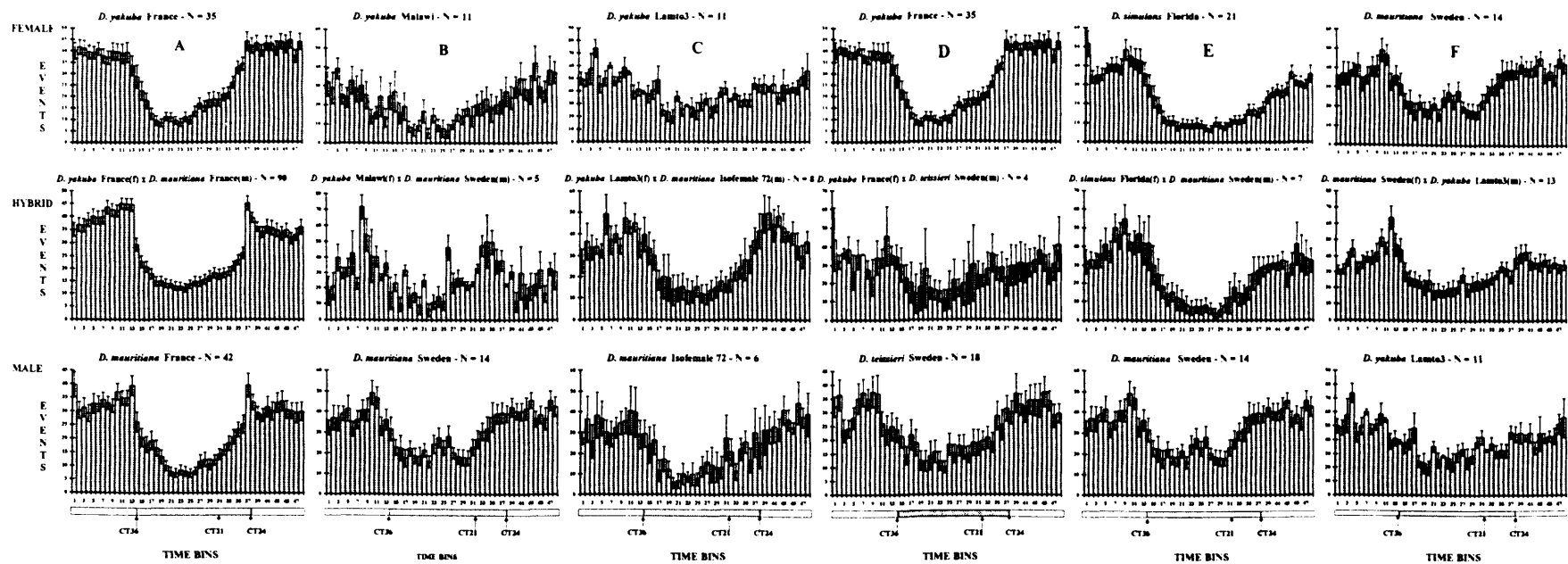
Figure 7.2.4: Locomotor Activity Profiles of the different strains and their interspecific hybrids, in DD, after standardisation of the data.

KEY:
    The CT reigime is shown below the graphs.
        CT24 = Lights-On
        CT36 = Lights-Off
        CT21 = Data collection commencement

resembles the paternal parent. Because the hybrid sample size is small, these results must be treated with caution.

The *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) hybrids (Figure 7.2.4: Column E, Middle fifth panel) show a typical *simulans*-like activity profile overall, with their evening peaks in phase with the maternal parent at CT33-34, and also exhibit a more pronounced fall in the activity during the night and a less pronounced morning peak resembling the maternal participant.

The *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) hybrids (Figure 7.2.4: Column F, Middle right) exhibit an overall activity profile, relative activity levels, relative fall in activity during the night, and pronounced evening peak at CT 35 (albeit slightly phase-delayed) similar to that of the maternal parent. However, the less pronounced morning peak resembles the paternal parent.

In short, the hybrid activity patterns, in their majority, tend to follow the paternal parent, particularly when *D. mauritiana* contributes the father. The *D. mauritiana* pattern appears to dominate that of *D. yakuba* (Columns A, B & C). When *D. mauritiana* is the maternal participant, the hybrid pattern is similar to that of *D. mauritiana* (Column F). Consequently, the *D. yakuba* x *D. mauritiana* reciprocals (Columns A, B, C & F) clearly reveal an autosomal control, with *D. mauritiana* carrying the dominant factors. Similarly, the *D. teissieri* pattern seems to be dominant to *D. yakuba* (Column D), whereas *D. simulans* is dominant to *D. mauritiana* (Column E). However, in other aspects of activity, such as the relative amounts of activities around the morning and evening peaks, maternal or paternal dominance is-, suggesting again probably autosomal involvement.

7.4 Discussion:

Not surprisingly, all eight species, various strains and interspecific hybrids revealed circadian rhythmicity during the free-run experiments. The maintenance of circadian behaviour, despite the lack of environmental cues, is a demonstration of the existence of an endogenous oscillator. With the exception of *D. sechellia* which has a period of ~23h, the periods of the rest of the species of the *melanogaster* subgroup are close to 24 h. With the exception of the *D. yakuba* France(f) x *D. teissieri* Sweden(m) hybrids, which exhibit short periods of ~ 23h, which are outside the range of both parents, all the other interspecific hybrids exhibited circadian periods of 24 h. The mean period value of the hybrid, was usually intermediate, and not significantly different from the parental values, suggesting autosomal control. This is surprising given the influence of the sex-linked *per* on the circadian period (Konopka and Benzer, 1971; Petersen *et al.*, 1988). However, several autosomal genes are known to affect the circadian period (see below).

Another feature often observed in circadian rhythm studies, is that a proportion of individuals are arrhythmic. Species like *D. teissieri, D. orena*, and *D. erecta* are observed to have as many arrhythmic flies compared to rhythmic ones (see Table 7.1). A possible explanation for this could be that these individuals are the least active animals, with only a few events scored per bin, rendering the statistical analysis susceptible to small n's. However, this simplistic explanation is incorrect due to the fact the most of the arrhythmic animals are highly active. A more plausible explanation might involve the multioscillatory nature of rhythmicity (e.g., Saunders, 1982), whereby the oscillator spontaneously 'splits-up' into two or more independent components, with different periods during lengthy DD or LL (constant illumination) experiments. This was first described by Pittendrigh (1960) studying the activity patterns of the arctic rodent *Spermophilus undulatus*, during extended LL conditions. Therefore these hypothetical multioscillators could have become uncoupled in the arrhythmic flies generating the apparent arrhythmicity.

Although rigorous statistical analysis could not be carried out due to the standardising of the raw data, some useful information on the overall activity profiles of the different species and interspecific hybrids could be drawn. With the possible exception of *D. mauritiana*, all the other species in the *melanogaster* subgroup showed broad levels of activity during the daytime with no morning or evening peaks, as shown in LD (see Chapter 8, Startle responses). *D. sechellia* gives the most pronounced evening peak, amongst the subgroup. The patterns of activity of the *D. yakuba* complex are quite different with *D. teissieri* and *D. erecta* showing prominent morning and evening peaks, whereas *D. yakuba* has a more *melanogaster*-complex profile. Two species that live in sympatry in the Afrotropical region, *D. teissieri* and *D. yakuba* (Tsacas, 1971; Burla, 1954), show distinctly different patterns of locomotor activity behaviour. This may represent an example of character displacement caused by reinforcement, in order to prevent sterile interspecific hybridisation (Grant, 1984; Schluter and McPhail, 1992; Schluter, 1994, see Chapter 9). Examination of the various *D. mauritiana* and *D. yakuba* strains revealed little heterogeneity in the overall activity profiles of the different strains. Consequently, there is some justification in assuming that the patterns observed are species-specific.

A more detailed inspection of the interspecific hybrids and the corresponding parental strains gave some evidence as to the mode of inheritance of the locomotor activity patterns. When *D. mauritiana* was used as the paternal or maternal participant in crosses with *D. yakuba* (see Figure 7.2.4: Column F), the hybrid pattern of activity was *mauritiana*-like, which implies autosomal dominant genes in *D. mauritiana*. However, *D. simulans* was dominant to *D. mauritiana*, and although the *D. simulans* contributed the mother, the results from the *D. yakuba* x *D. mauritiana* reciprocal crosses could suggest that this may not be due to sex-linkage, but to dominant autosomal *D. simulans* factors.

Given the general lack of maternal influence in the hybrids, these results are not consistent with the sex-linked *per* locus having a significant influence over the activity patterns. This directly contradicts Petersen's (1988) and Hennessey's (pers. comm.) work on *D. melanogaster* and *D. pseudoobscura* patterns. In these experiments, the *per* gene of *D.*

pseudoobscura was transformed into *D. melanogaster per*^{0} hosts, with the transformed flies showing typical *pseudoobsura* activity profiles. So, what could have caused this discrepancy? A possibility could be that sex-linked genes may have evolved further than the autososomal ones, because the effective population size of the X is smaller than the autosomes, and as the X chromosome is hemizygous and lacks recombination in one sex accumulates some evolutionary changes faster than do the autosomes (Charlesworth *et al.*, 1987; Coyne and Orr, 1989; Charlesworth, 1991). Thus, between distantly related species such as *D. pseudoobscura* and *D. melanogaster* sex-linked *per* locus variation may have gone to fixation earlier (in phylogenetic terms) than autosomal variation. Consequently, between more closely related species such as the species in the *melanogaster* subgroup, autosomal variation may reflect more recent divergence. Several autosomal loci are known to affect circadian length, such as *psi-2*, *psi-3* and *gate* (Jackson, 1983), and *timeless* (Seghal *et al.*, 1994). Any species-specific variation at these loci could conceivably generate the results observed here. An interesting line of investigation might be to examine the activity profiles of *D. melanogaster* transformants that carry the cloned *tim* gene (Gekakis *et al.*, 1995; Myers *et al.*, 1995) of the other species, as in the experiments performed by Petersen *et al.* (1988) and Wheeler *et al.* (1991). In this way the candidate loci could be identified.

A more detailed examination of the circadian locomotor activity patterns of the different species/strains and interspecific hybrids is provided in the ensuing Chapter 8, where the activity profiles are examined in light/dark (12:12 LD) conditions.

145

Locomotor activity patterns of the different members of the *melanogaster* subgroup and their interspecific hybrids.

CHAPTER 8

8. Locomotor activity patterns of the different members of the *melanogaster* subgroup and their interspecific hybrids.

Circadian rhythmicity exists in nature to anticipate daily environmental fluctuations. One of the most intensively investigated circadian systems is that of eclosion of *D. pseudoobscura* (Pittendrigh, 1960; 1974), and *D. melanogaster* (Konopka and Benzer,1971). While the free-running conditions reveal the endogenous periods of the oscillators which control locomotor activity, artificial light/dark cycles (LD) provide an opportunity to identify any finer details in the patterns of activity throughout the day. Wild type *D. melanogaster* flies, in light:dark (LD) conditions demonstrate a bimodal pattern of activity, being most active at dawn and dusk, with morning and evening bursts of activity commencing a few hours before lights-on and lights-off respectively (Petersen *et al.*, 1988; Hamblen-Coyle *et al.*, 1992). Konopka and Benzer (1971) identified the *period* (*per*) locus in *Drosophila*, as being responsible for controlling the period of locomotor activity cycles in *D. melanogaster*. The effects of the *per* mutations on the locomotor activity patterns of *D. melanogaster* in LD conditions have been thoroughly studied. The *per*^{L} mutation is observed to delay the onset of the evening peak, while the *per*^{S} mutation is seen to advance the evening peak in LD (Dushay *et al.*, 1990; Hamblen-Coyle *et al.*, 1992; Konopka *et al.*, 1995). On the contrary, the morning peak seems relatively inert in these mutations. In addition, the original *per*^{01} arrhythmic allele tends to be more active during lights-on than lights-off, with activity changing in response to, rather than in anticipation of the light transitions (Petersen *et al.*, 1988; Hamblen-Coyle *et al.*, 1989).

Here, I report on the patterns of locomotor activity demonstrated by the various member species of the *D. melanogaster* subgroup, as well as by their interspecific hybrids, in LD conditions (see Chapter 2). Briefly, adult males from each of the eight species, various strains and interspecific hybrids were monitored for 5-7 days in a regime of 12 h light/12 h dark (LD12:12), at a constant 25°C (see chapter 2), and a synopsis of these is given in Appendix 8.0.

8.1 Overall patterns of LD activity

Figure 8.1.1 shows that although broadly similar in shape, with reduced activity during lights-off ('subjective night') compared to lights-on ('subjective day'), and the majority of activity around the two light/dark transitions ('subjective dusk and dawn'), some interesting differences are evident between the 8 panels, which depict the results from the different species. The various *D. yakuba* and *D. mauritiana* strains have been pooled. The 'evening' peaks are higher than the corresponding 'morning' peaks in *D. melanogaster, D. simulans, D. erecta* and *D. orena*, whereas in *D. sechellia, D. mauritiana, D. yakuba* and *D. teissieri* the converse is true . *D. melanogaster* is observed to be the most active of the species, while *D. erecta* is the least active species as shown by the mean number of recorded events per time bin.

D. melanogaster complex:

D. melanogaster is characterised by a bimodal pattern, with the 'morning' peak of activity present within 2 h, after lights-on, followed by a fall in activity through the middle part of the subjective day. Several hours before lights-off, the activity increases, in clear anticipation of the subsequent LD transition. Maximum activity includes the 'startle' response to the lights-off signal, and thereafter activity falls steadily throughout the night (Figure 8.1: Top left panel). *D. simulans* (Figure 8.1: Top second left panel) demonstrates the same bimodal pattern seen in *D. melanogaster*. Increased activity in anticipation of lights-on begins roughly ~2 h before the lights-on signal, with activity reaching its maximum ~30 mins after lights-on. At ZT2, there is an abrupt drop in activity, which is maintained at a low level, before a sudden burst of increased activity at ZT10 anticipating lights-off, before activity falls to a low night level. *D. sechellia* (Figure 8.1: Top third left panel) shows more pronounced diurnal activity, with virtually no night activity. There is a sudden burst of activity ≈ 2 h before lights-off (ZT10), which is maintained for 1-2 h in the night phase (ZT13-14), before falling to a low level during the day. Activity increases ~ 2 h before the lights-off signal. *D. mauritiana* (Figure 8.1: Top right panel), like the rest of the species of the *melanogaster* subgroup, exhibits a bimodal pattern of activity. The activity begins to increase at ZT22,
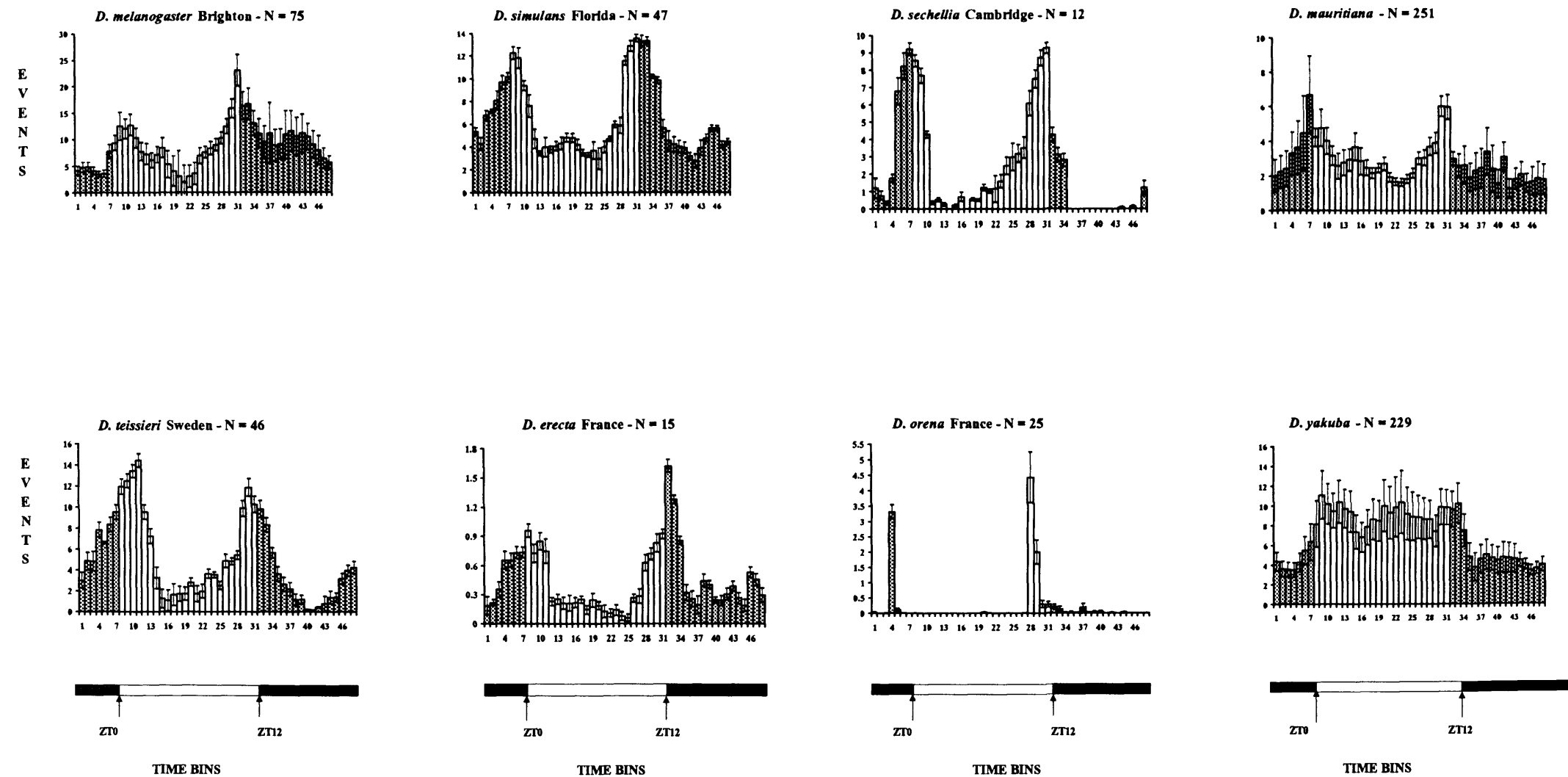
Figure 8.1 : Locomotor activity of members of the *melanogaster* subgroup in LD 12:12.

KEY : The ZT regime is given below the graphs
        Night Activity is shaded

reaching its maximum ~30 mins before lights-on, in clear anticipation of the oncoming lights-on transition, after which there is an abrupt decrease of activity. This low activity pattern is maintained throughout the daytime, until ZT12 when there is a burst of activity in clear anticipation of the oncoming lights-off signal, after which the activity drops to a relative low level which is sustained throughout the night. The *D. mauritiana* activity (described in greater detail in section 8.2) is an amalgamation of activities from 11 different strains, which could explain the observed large sem's.

D. yakuba complex:

D. teissieri (Figure 8.1: Bottom left panel) shows a gradual increase of activity ~3 h before lights-on, which reaches its maximum ~ 2 h into lights-on (ZT2). Thereafter, the activity falls and is sustained at a low level. Anticipation of lights-off begins a few hours before the ZT12. The activity continues to fall gradually after the lights-off transition and is maintained at low levels during the night. In *D. erecta* (Figure 8.1: Bottom second left panel) activity (smallest amount) increases sharply 2 h before the lights-on transition. Activity falls at ZT2, and this pattern is sustained until ~2 h before lights-off. The 'evening' peak is delayed slightly into the dark phase, followed by a gradual decay of activity until ZT14, then the activity drops and is sustained at a low night-time level. *D. orena* (Figure 8.1: Bottom third left panel) shows very little activity overall. There are two bursts of activity, one comes just before lights-on and the other ~2 h before lights-off (4 bins). The 'evening' peak is higher than the 'morning' peak. Thereafter activity ceases. *D. yakuba* (Figure 8.1: Bottom right panel) also shows a bimodal pattern of activity. There is a gradual increase of activity during the lights-on transition reaching a maximum ~ 1 h (ZT1) into the 'day'. The high level of activity is sustained throughout the 'day', contrasting with the rest of the member species of the subgroup, and which is maintained for ~ 1 h (ZT13) into the dark phase. Thereafter, the activity falls to a lower 'night' level which is maintained throughout the night. The large sem's observed could be due to the fact that the *D. yakuba* activity presented here is an amalgamation of the activities from 10 different strains (described in greater detail in section 8.3).

Other Indices: Total Activity, Day Activity, Night Activity, Night/Day Activity Ratio, Startle-On and -Off, and Anticipation-Off and -On Responses.

Subgroup: Various other indices were calculated and their mean values appear in Appendix 8.0. The indices are described in Chapter 2.
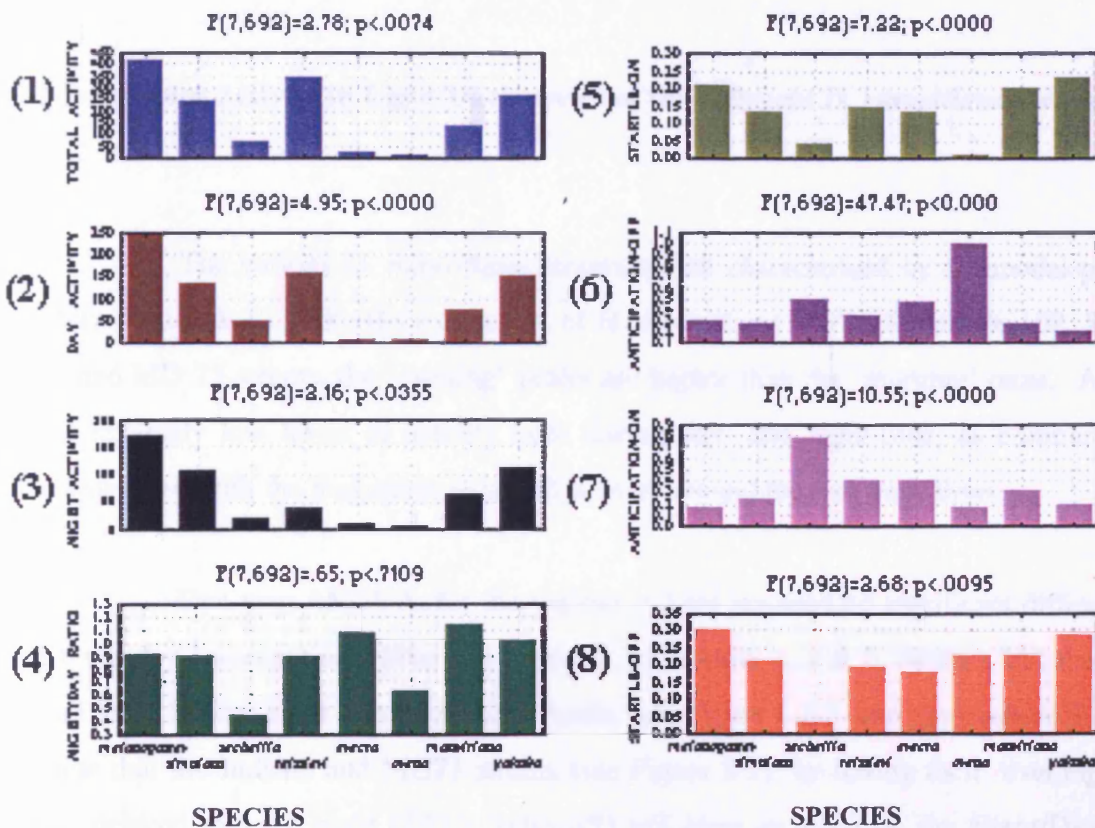
Figure 8.1.2: Results for Total, Day, Night Activity, Night/Day, Startle-On, Anticipation-Off, Anticipation-On and Startle-Off responses of the members of the *melanogaster* subgroup (see Table 8.1 for data).

One-way ANOVA between the above mentioned species for total amount of activity (TA, Column 1 in Table 8.1) showed significant differences ($F=2.78$, $df=7$, 692, $p=0.007$, see Figure 8.1.2: Panel 1, above). Furthermore, all the other indices gave significant F-ratios (see Figure 8.1.2, and Appendices 8.1a-f), except the Night/Day ratio. The most striking observations are that *D. erecta* and *D. orena* exhibit the least activity throughout the day (see Figure 8.1.2: Panel 2), although the night/day ratios of these species

do not differ significantly from those of the other species. In addition, *D. orena* shows no startle-on response, but exhibits the largest anticipation-off response. *D. sechellia* shows the greatest anticipation-on and the smallest startle-off responses (ANOVAs and Newman-Keuls *a posteriori* test results can be found in Appendices 8.1a-f). Within the *melanogaster* complex, *D. sechellia* is the one that shows different behaviour, whereas *D. erecta* and *D. orena* exhibit the more unusual activity patterns in the *yakuba* complex.

8.2 Locomotor activity in Light/Dark cycles of the different *D. mauritiana* strains.

The various *D. mauritiana* strains are all characterised by a bimodal pattern of activity (Figure 8.2). With the exceptions of *D. mauritiana* MG71, Isofemale 102, Isofemale 197, and MD 75 strains, the 'evening' peaks are higher than the 'morning' ones. All strains show relatively low levels of activity both during day- and night-time, as compared *to D. melanogaster*, with the maximum recorded activity around the two transitions.

One-way ANOVA, for the various indices revealed no significant differences for the total, day and night activities (see Figure 8.2.2: Panels 1, 2 & 3, below), but the F-ratios for all other indices were significant (see Panels 4-8, Figure 8.2.2, and Appendices 8.2a-e). It is clear that the Indiana and MG71 strains (see Figure 8.2), by having their 'evening activity peaks delayed into the night (ZT13, lights-off) will have an effect on the Night/Day activity ratio (see Figure 8.2.2: Panel 4). For the startle and anticipation indices, there are various significant differences (see Appendices 8.2a-e) between the strains, and so some heterogeneity in behaviour within the species can be observed. However, the significant differences are usually caused by either one or two strains showing higher deviation from the rest of the strains, e.g. in the anticipation-off (see Figure 8.2.2: Panel 6), the Isofemale 152 strain exhibits an atypically high anticipation response to the light-off transtion.
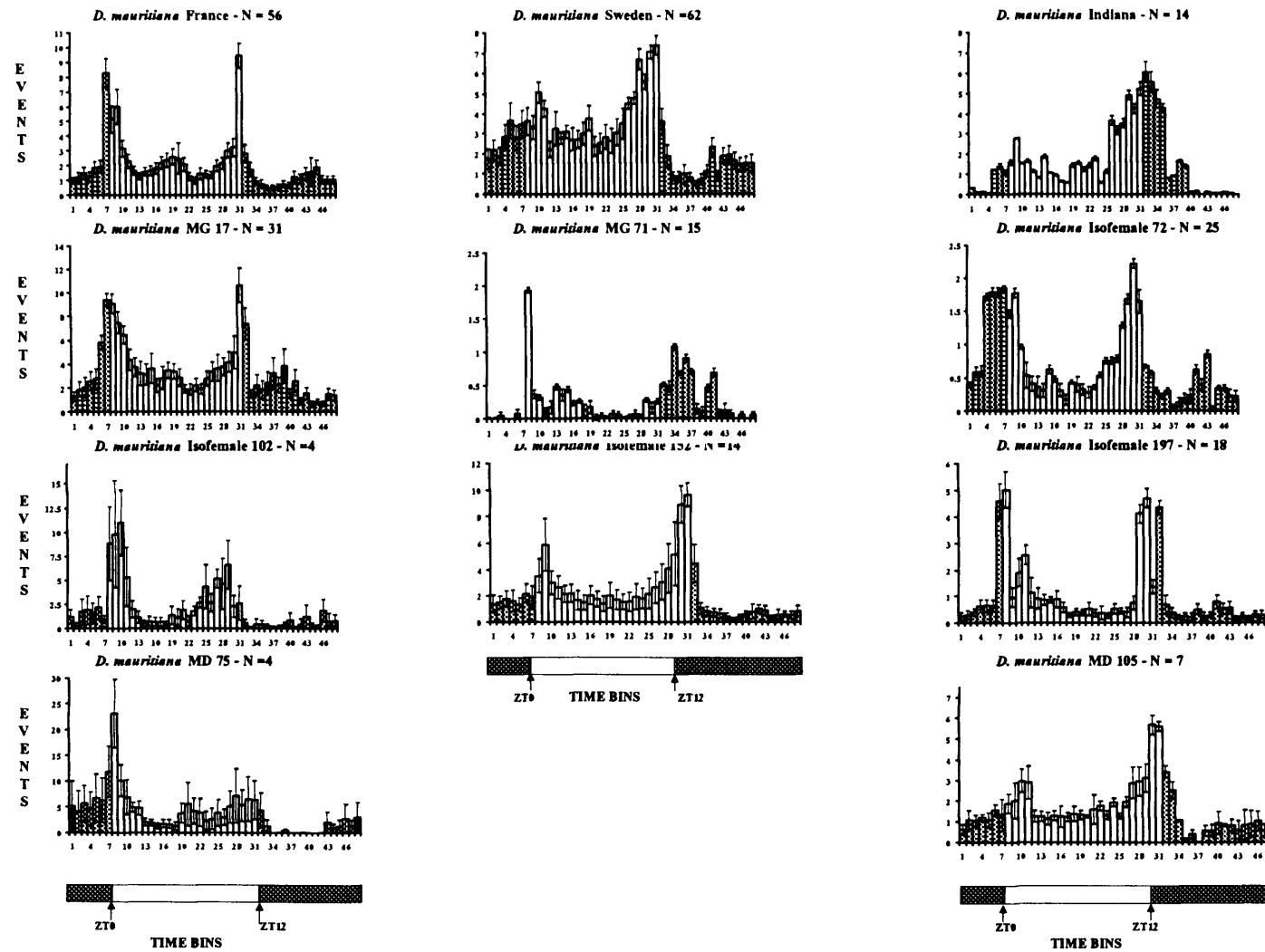
Figure 8.2 : Locomotor activity of the different *D. mauritiana* strains in LD 12:12.

KEY :   The ZT regime is given below the figures
        Night Activity is shaded
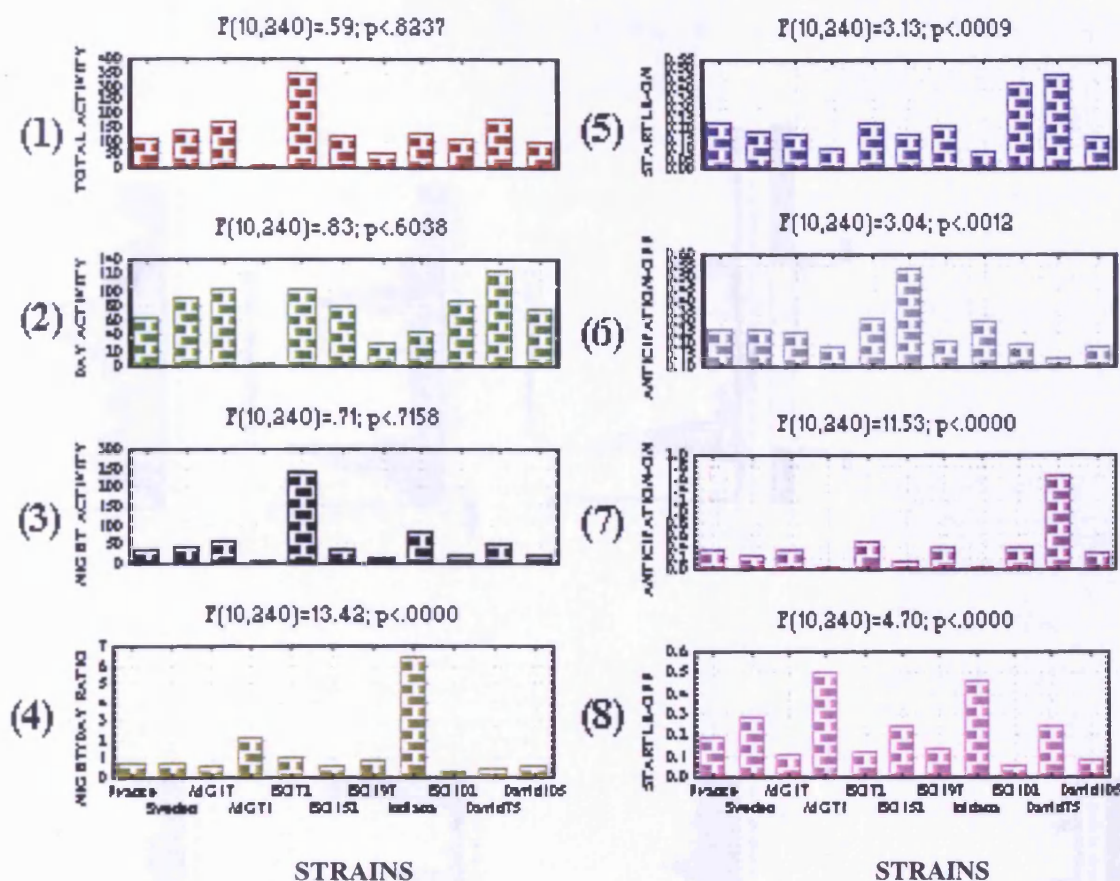
Figure 8.2.2: Results of ANOVA for all the indices between the different *D. mauritiana* strains, under examination.

8.3 Locomotor activity in Light/Dark cycles of the different *D. yakuba* strains.

The various *D. yakuba* strains are all characterised by a bimodal pattern of activity (Figure 8.3). With the exceptions of *D. yakuba* France, Ivory Coast, Indiana, and EL8 strains, the 'morning' peaks are higher than the 'evening' ones. All strains show low levels of activity during daytime, while the night-time activity is sustained at even lower levels than daytime activity, with the maximum recorded activity around the two transitions, e.g., lights-off-to-on and lights-on-to-off transitions. The most unusual profile is that of *D. yakuba* S.T., with its trimodal pattern which indicates a burst of activity in the middle of the night phase.

151

Figure 8.3 : Locomotor activity profiles of the different *D. yakuba* strains in LD 12:12

KEY : The ZT regime is shown below the graphs.
Night Activity is shaded
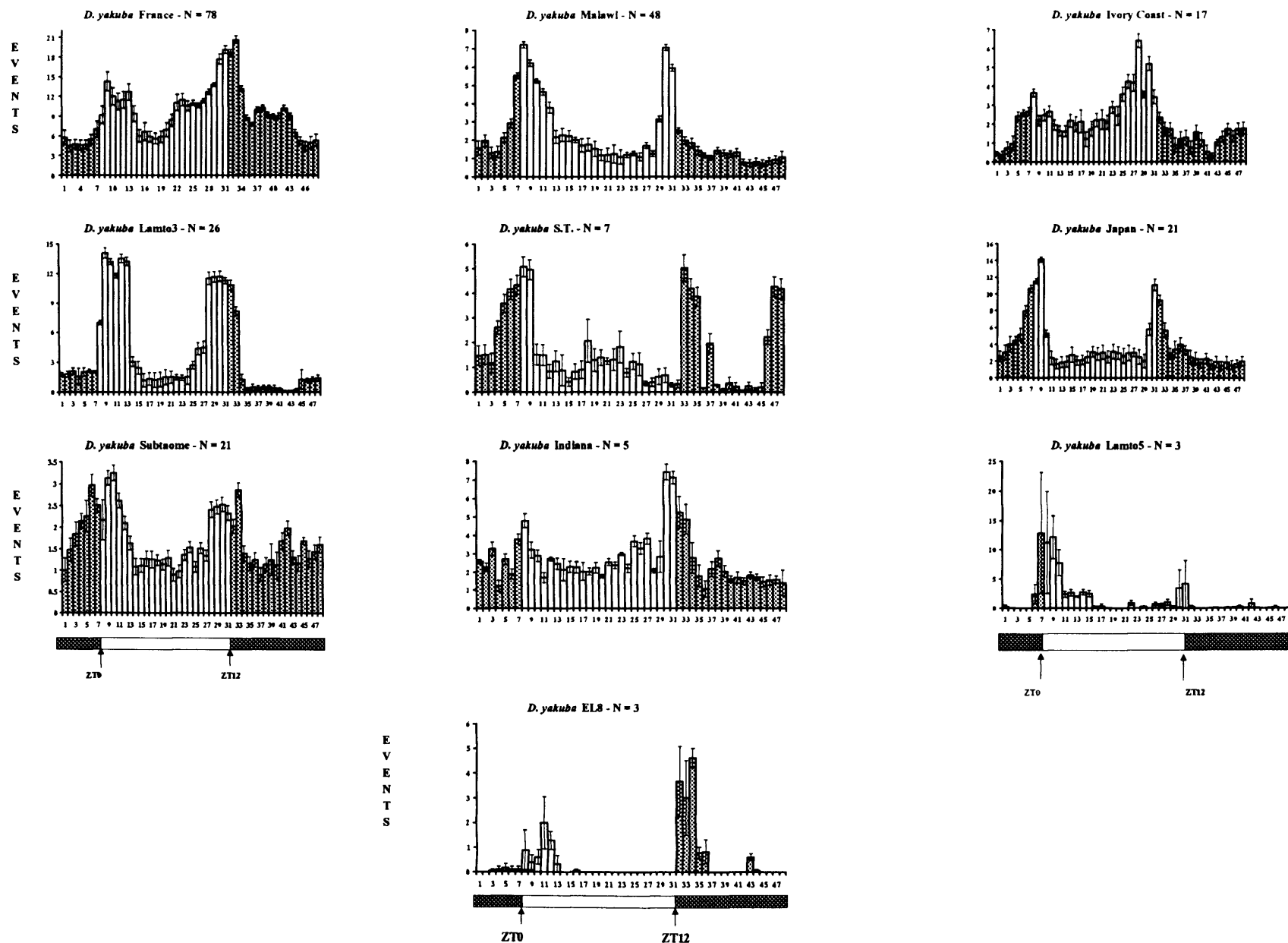
One-way ANOVA for total and night activities, between the various *D. yakuba* strains showed no significant differences (see Figure 8.3.2: Panels 1 & 3 respectively, below), whereas all the other indices gave significant effects (see Figure 8.3.2: Panels 2 & 4-8). Newman-Keuls *a posteriori* test results are shown in Appendices 8.3a-e, but a simple visual inspection of the Figure 8.3.2 reveals major differences in the data between the various strains. Again, as in *D. mauritiana*, it is clear that there is heterogeneity between strains in their behaviour.
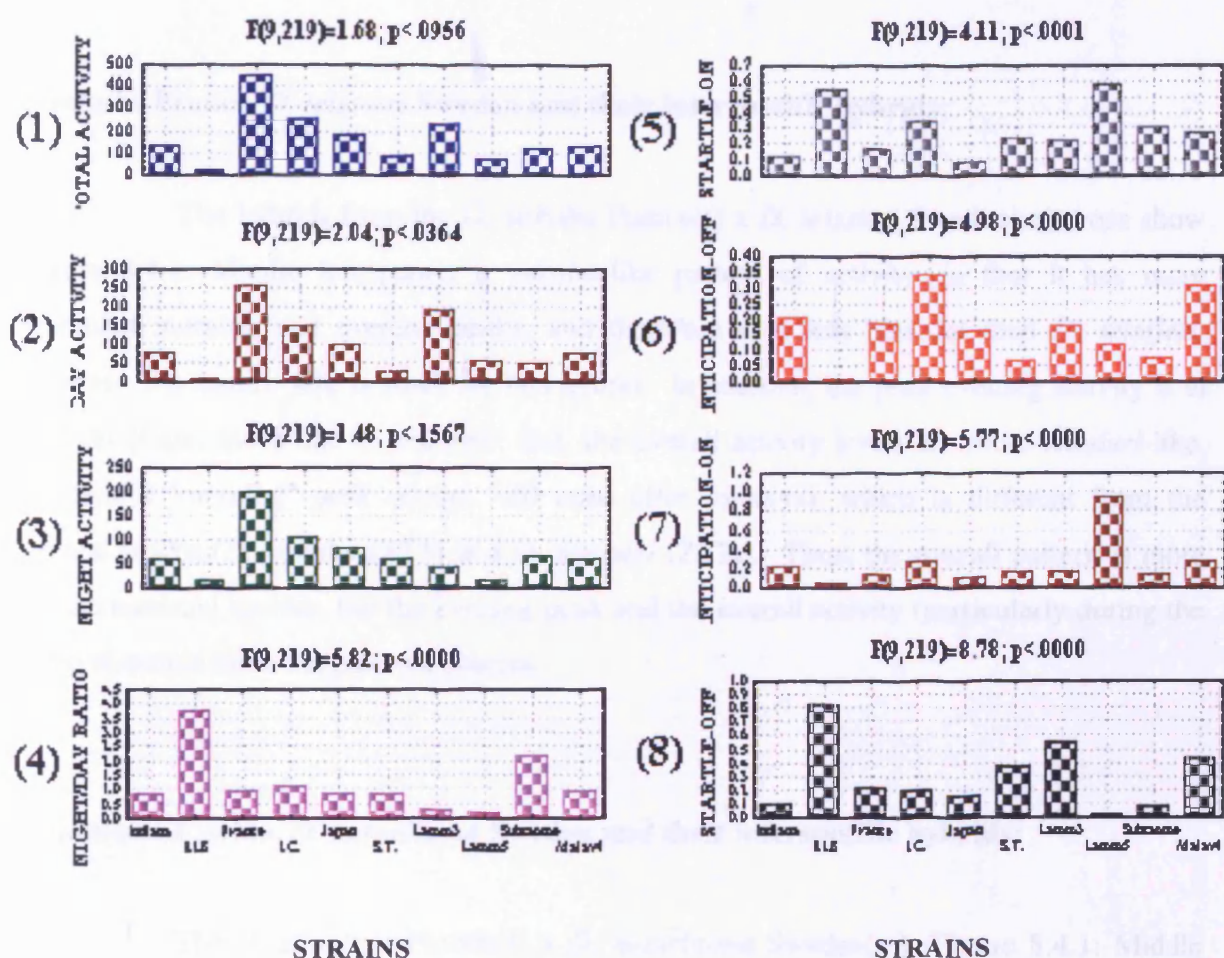
Figure 8.3.2: Results for ANOVA for all the indices between the different strains of *D. yakuba*.

Summing up, for the latter two sections, it is clear that there is some intra-species heterogeneity within both the *D. mauritiana* and *D. yakuba* strains, which must temper any conclusions about the species-specific aspects of the LD activity patterns.

8.4 Locomotor activity in Light/Dark cycles of the different Interspecific Hybrids.

The different interspecific hybrids are characterised by a bimodal pattern of activity, as all their respective parental strains. Figures 8.4.1, 8.4.2 and 8.4.3 depict the patterns of locomotor activity of the parental strains (Top row: maternal participant, and Bottom row: paternal participant), together with their corresponding interspecific hybrid patterns (Middle row).

D. yakuba France, *D. teissieri* Sweden and their interspecific hybrids:

The hybrids from the *D. yakuba* France(f) x *D. teissieri* Sweden(m) cross show (Figure 8.4.1: Middle left panel) a *yakuba*-like pattern of activity, in that it has more prominent morning and evening peaks, and therefore it is less bimodal than *D. teissieri*. However, the sample size is small for this hybrid. In addition, the peak evening activity is in the light phase, more like *D. teissieri*, and, the overall activity levels are more *teissieri*-like. The hybrid 'morning' peak occurs ~30 mins after lights-on, which is different from the parental strains (*D. yakuba* (ZT1) and *D. teissieri* (ZT2)). Thus, the overall pattern is more like the maternal species, but the evening peak and the overall activity (particularly during the night) resemble more the paternal species.

D. simulans Florida, *D. mauritiana* Sweden and their interspecific hybrids:

The *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) (Figure 8.4.1: Middle right panel) hybrid shows a typical 'clean' *simulans*-like 'morning' peak of activity (~ 30 mins into lights-on), and the prominent morning and evening peaks give an overall *simulans*-like appearance. The immediate fall in activity after lights-off follows that of the paternal species. The overall levels of activity of the hybrids are greater than those of the parents.
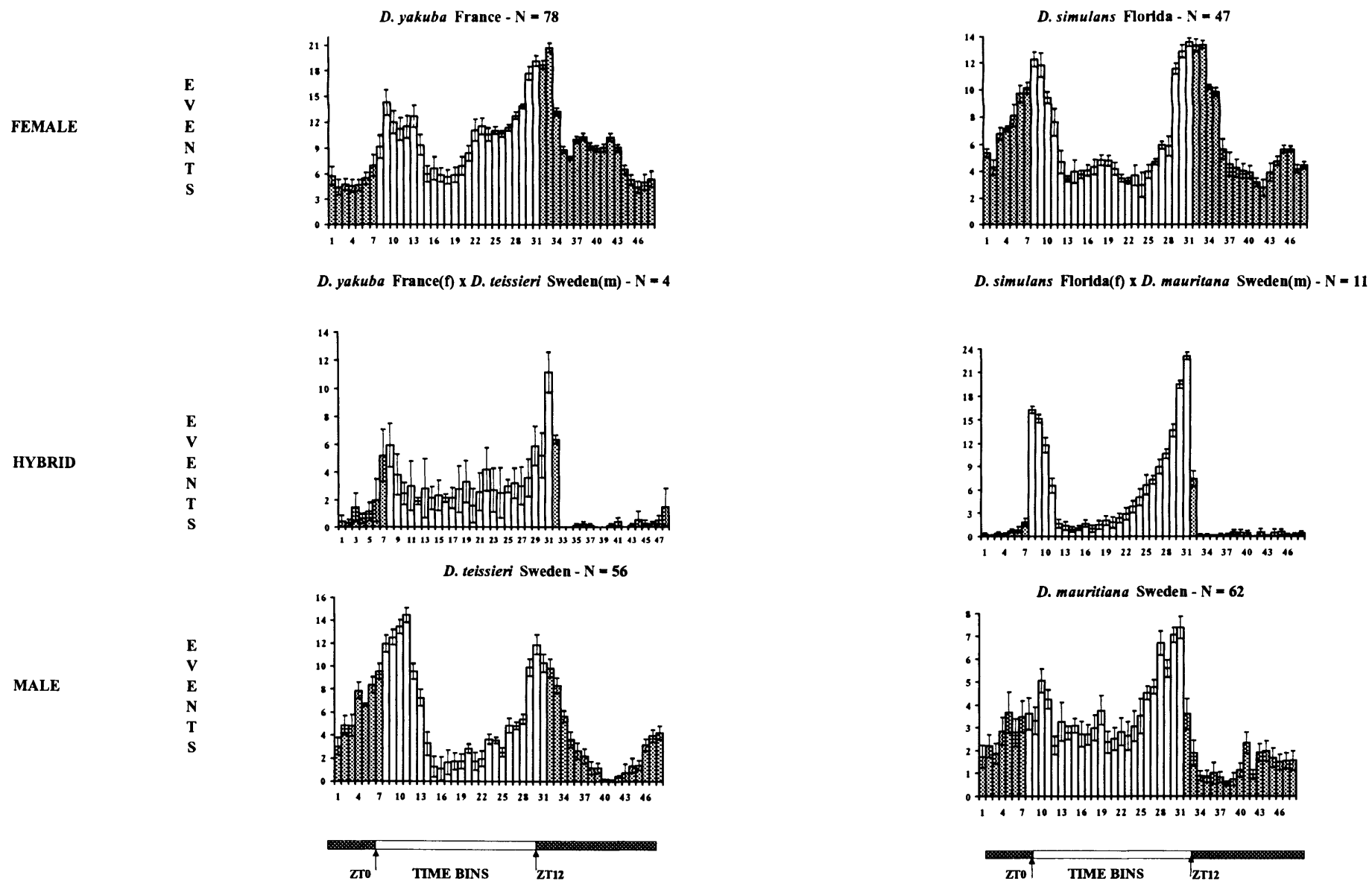
Figure 8.4.1 : LD Locomotor activity profiles of *D. yakuba* France, *D. teissieri* Sweden, *D. simulans* Florida, *D. mauritiana* Sweden and their interspecific hybrids.

KEY : The ZT regime below the figures
         Night Activity is shaded

Thus, in both crosses illustrated in Figure 8.4.1, the overall pattern of behaviour in these hybrids has the maternal 'signature', but the evening peak takes a paternal character, suggesting autosomal dominant or even Y chromosomal influence.

D. yakuba (females), *D. mauritiana* (males) strains and their interspecific hybrids:

The *D. yakuba* France(f) x *D. mauritiana* France(m) (Figure 8.4.2: Middle row left panel) hybrid shows an activity pattern reminiscent of *D. yakuba* France, in that the morning and evening peaks are less prominant, and the overall activity levels are more *yakuba*-like. The hybrid and paternal species show a startle response to lights-on. As the light comes on either at the end of bin 7 or the beginning of bin 8 the startle response becomes more *mauritiana*-like. The daytime activity decays gradually until midday when thereafter it begins to increase, until a sudden burst of activity ~ 30 mins into lights-off ('evening' peak) which is similar to that seen for *D. yakuba* France.

The *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) hybrid (Figure 8.4.2: Middle row centre panel) also shows an overall activity pattern resembling *D. yakuba* Malawi in the prominance of the morning and evening peaks, but with higher levels of activity than both parents. Furthermore, the startle response to light-on, the trough of midday inactivity, the gradual increase of activity occurring preceeding the lights-on-to-off transition and the sudden burst of activity (Startle response to lights-off, reaching its maximum at ZT13) are all reminiscent of *D. yakuba* Malawi.

The *D. yakuba* Lamto3(f) x *D. mauritiana* Isofemale 72(m) hybrid (Figure 8.4.2: Middle row right panel) shows little anticipation and startle responses to lights-on, while showing clear anticipation to the lights-off, similar to *D. yakuba* Lamto3 also. The evening peak is also more similar to *D. yakuba*, with high levels of activity in the hour after lights-off. In addition, the overall levels of activity of the hybrid resembles that of *D. yakuba* Lamto3.
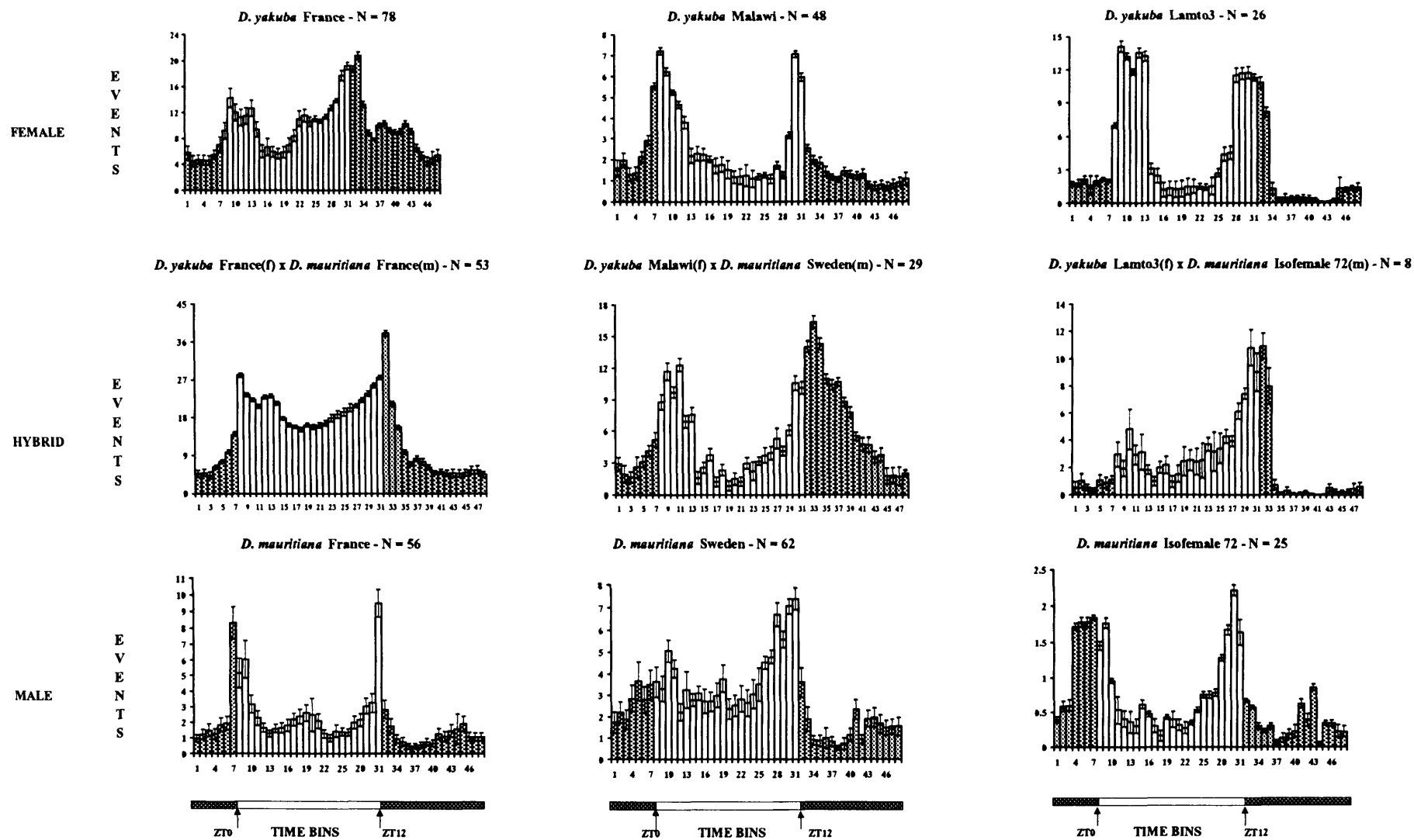
Figure 8.4.2 : Locomotor activity profiles of *D. yakuba* France, *D. yakuba* Malawi, *D. yakuba* Lamto3, *D. mauritiana* Isofemale 72, *D. mauritiana* Sweden, *D. mauritiana* France and their interspecific hybrids in LD 12:12.

KEY : The ZT regime is shown below the figures
      Night Activity is shaded

D. mauritiana (females) and D. yakuba (males) strains and their interspecific hybrids:

The *D. mauritiana* Isofemale 72(f) x *D. yakuba* Lamto3(m) hybrid (Figure 8.4.3: Middle row right panel) reciprocal cross produces a hybrid whose 'evening' peak is higher than its 'morning' peak, which is similar to *D. mauritiana* Isofemale 72 (Figure 8.4.3: Top right panel). The less prominent morning and evening peaks also resemble the maternal parent. Furthermore, the levels of activity of the hybrid resembles that of the paternal parent, which may imply paternal dominant effects on the levels of activity.

The *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) hybrid (Figure 8.4.3: Middle row left panel) shows a bimodal pattern of activity which resembles the *D. mauritiana* Sweden, with less prominent morning and evening peaks. The overall activity levels resemble the paternal species, but the reduced anticipation of lights-on, and the higher evening than morning activity levels are more in keeping with the maternal species.

Thus, the cursory examination of these hybrids' behaviour reveals that the overall patterns of activity are more similar to the maternal than the paternal contributors. A more detailed examination of the parental and hybrid behaviour, using the indices described earlier (see Appendix 8.0), together with ANOVA results are shown in Appendix 8.4a-f. For brevity, a single one-way ANOVA was performed for the parental and hybrid strains. Figure 8.4.4 (sse below) gives the results.

If the 7 different crosses were to be presented separately, it would have entailed 56 separate figures. Hence I present the parental strains and their interspecific hybrids together in the various panels, on the same figure. The sequence of the parental and hybrid crosses, from A→O, in the figure is a feature of the Statistica computer programme, over which I have no control. This makes the discussion of the data quite difficult. However, I will attempt to make this as painless to the reader as possible!

All one-way ANOVAs, except for the night activity are significant (see Figure 8.4.4: Panels 1, 2 & 4-8 for significant effects, above). Newman-Keuls *a posteriori* tests are presented in Appendices 8.4a-f. The *D. mauritiana* Isofemale 72(f) x *D. yakuba* Lamto3(m)
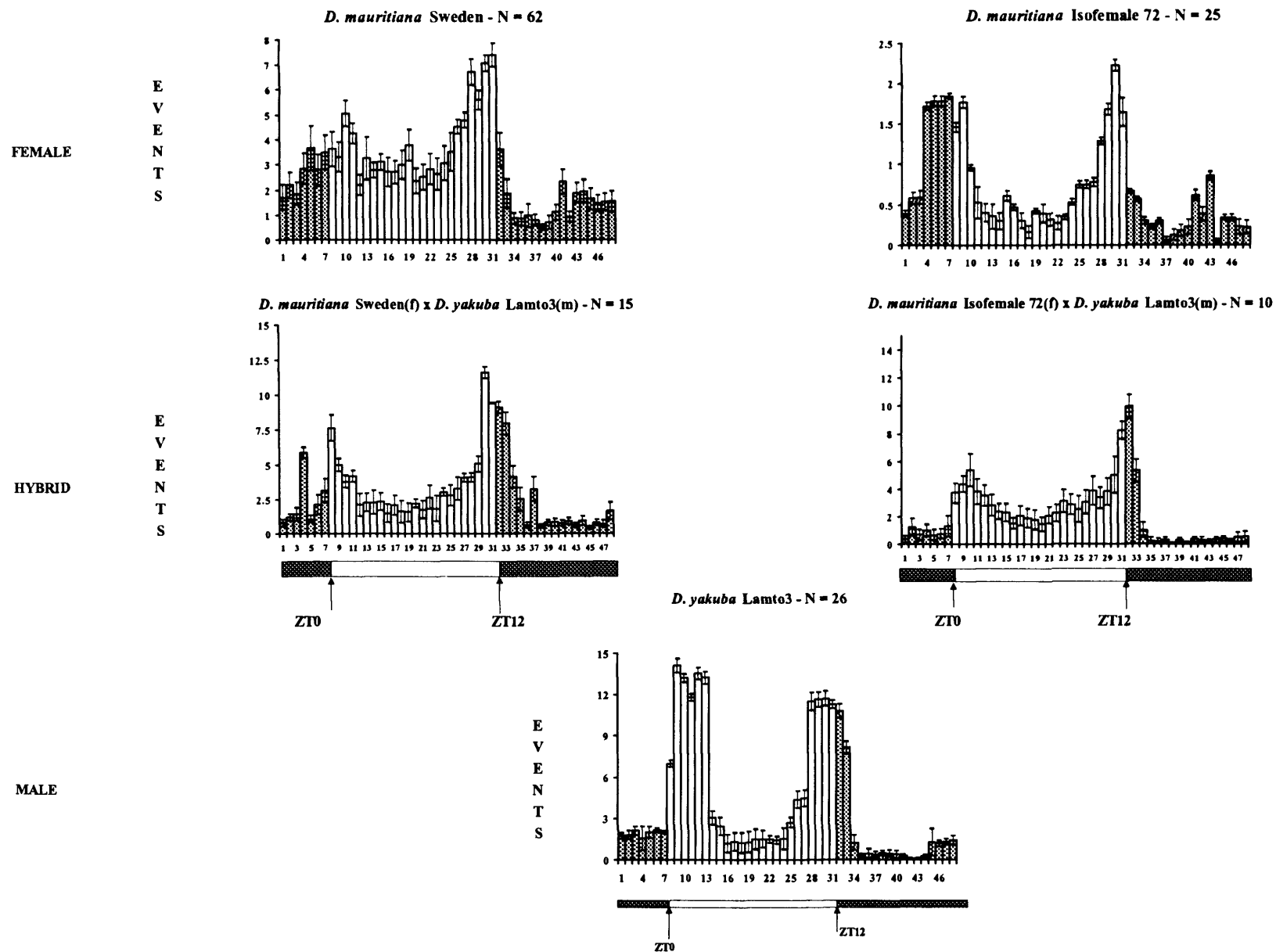
Figure 8.4.3: Locomotor activity of *D. yakuba* Lamto3, *D. mauritiana* Isofemale 72, *D. mauritiana* Sweden and their interspecific hybrids LD 12:12.

KEY :   The ZT regime is depicted below the graphs
                     Night Activity is shaded

(Column G), the reciprocal (Column K), and *D. yakuba* France(f) x *D. teissieri* Sweden(m) (Column L) hybrids show lower amounts of activity compared to the parental strains (Columns C, E, N, and O), implying hybrid unfitness (Haldane, 1922). The *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) (Column J), and *D. yakuba* France(f) x *D. mauritiana* France(m) (Column I) exhibit higher amounts of activity as compared to their corresponding parental strains (Columns D, B, N, and A, respectively), suggesting that the parental strains may have undergone inbreeding depression, while the *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m) (Column H) and *D. simulans* Florida(f) x *D. mauritiana* Sweden(m) (Column M) hybrids show intermediate amounts of activity to the parental ones, implying autosomal control (Colomns B, E, and F).

Figure 8.4.4: ANOVA results between the various parental strains and their interspecific hybrids for all the indices- Arrows show parental strains (C & E) and the reciprocal crosses (G & K) for *D. yakuba* Lamto3 and *D. mauritiana* Isofemale 72.
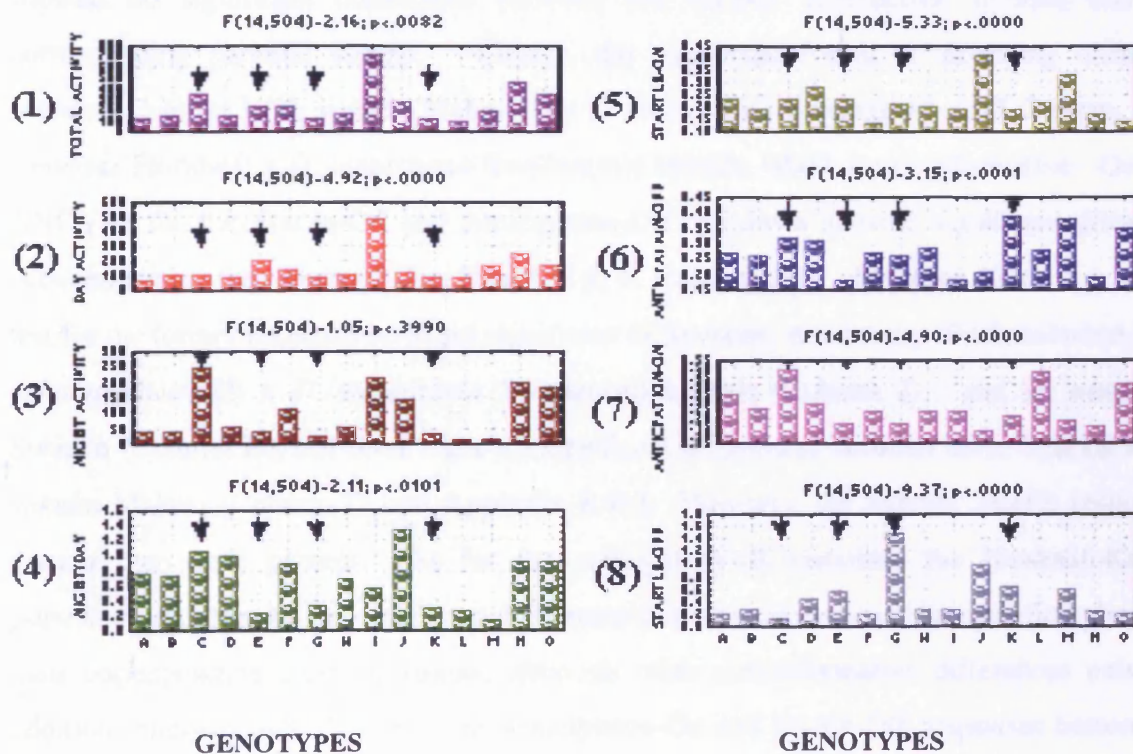
KEY:

A = *D. mauritiana* France	G = *D. mauritiana* Isofemale 72(f) x *D. yakuba* Lamto3(m)
B = *D. mauritiana* Sweden	H = *D. mauritiana* Sweden(f) x *D. yakuba* Lamto3(m)
C = *D. mauritiana* Isofemale 72	I = *D. yakuba* France(f) x *D. mauritiana* France(m)
D = *D. yakuba* Malawi	J = *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m)
E = *D. yakuba* Lamto3	K = *D. yakuba* Lamto3(f) x *D. mauritiana* Isofemale 72(m)
F = *D. simulans* Florida	L = *D. yakuba* France(f) x *D. teissieri* Sweden(m)
N = *D. yakuba* France	M = *D. simulans* Florida(f) x *D. mauritiana* Sweden(m)
O = *D. teissieri* Sweden	

One-way ANOVA for the day activity between the various parental strains and hybrids revealed significant differences (see Figure 8.4.4: Panel 2). Newman-Keuls *a posteriori* test for the day activity revealed significant differences, at least at p<0.05, between the *D. yakuba* France(f) x *D. mauritiana* France(m) (Column I) hybrids and the parental strains (Column N & A respectively, see Appendix 8.4a). Apart from the *D. yakuba* France(f) x *D. mauritiana* France(m) hybrids, which show higher amounts of day activity from their corresponding parents suggesting hybrid vigour, all the rest of the hybrids show intermediate amounts of day activity to their parental strains, implying autosomal control.

One-way ANOVA for the Night/Day activity ratio showed significant differences between the various strains(see Figure 8.4.4: Panel 4), but Newman-Keuls *a posteriori* test showed no significant differences between the various interspecific hybrids and their corresponding parental strains. Clearly, the significant F-ratio is detecting differences between Column J (*D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m)), and Column M (*D. simulans* Florida(f) x *D. mauritiana* Sweden(m)) hybrids, which is not informative. One-way ANOVAs for the Startle-On and Anticipation-Off responses showed significant differences between strains (see Figure 8.4.4: Panels 5 & 6, respectively). Newman-Keuls *a posteriori* test for the former response revealed significant differences, at least at p<0.05, between the *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) hybrids (Column J), and *D. mauritiana* Sweden (Column B), but there were no significant differences between these hybrids and *D. yakuba* Malawi (Column D, see Appendix 8.4b). However, the hybrids' startle response is greater than both parents. As for the anticipation-off response, the Newman-Keuls *a posteriori* test revealed no significant differences between the various interspecific hybrids and their corresponding parental strains, although other non-informative differences exist. In addition, one-way ANOVAs for the Anticipation-On and Startle-Off responses between the different hybrids and their corresponding parental strains showed significant differences (see Figure 8.4.4: Panels 7 & 8, respectively). Newman-Keuls *a posteriori* test for the former response revealed significant differences, at least at p<0.05, between *D. mauritiana* Isofemale72 (Column C), and *D. yakuba* Lamto3(f) x *D. mauritiana* Isofemale72(m) (Column K) with the hybrid value falling between those of the parental strains, but closer to the *D. yakuba* Lamto3 maternal parent and *D. mauritiana* Isofemale72(f) x *D. yakuba* Lamto3(m)

(Column H) hybrids, but no significant differences between *D. yakuba* Lamto3 (Column E) and these hybrids (see Appendix8.4c). Since, this is the only true reciprocal cross between *D. mauritiana* and *D. yakuba* strains (labelled with an ↓ for instant recognition), this imples that *D. yakuba* is dominant. The test also revealed significant differences, at least at $p<0.05$, between *D. yakuba* France (Column N) and the *D. yakuba* France(f) x *D. teissieri* Sweden(m) (Column L) hybrids(see Appendix 8.4c), with the hybrid value higher than both parental ones but closer to the paternal value, which may suggest *D. teissieri* dominant autosomal factors may be involved. Newman-Keuls *a poesteriori* test for the latter response revealed significant differences, at least at $p<0.05$, between the *D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m) (Column J) hybrids and their corresponding parental strains (Columns D & B respectively, see Appendix 8.4d). The test also revealed significant differences, at least at $p<0.05$, between *D. mauritiana* Isofemale72 (Column C) and *D. yakuba* Lamto3 (Column E), and the *D. mauritiana* Isofemale72(f) x *D. yakuba* Lamto3(m) (Column G) hybrids (see Appendix 8.4d). In both cases the hybrid values are higher than both parental ones, which suggest hybrid vigour.

Summing up, it can be stated that the overall activity profile is significantly influenced by the maternal origin, which could imply X-linked factors, whereas the activity levels and the phasing of the evening peak may either be under dominant autosomal or even Y-chromosomal factor control. The only reciprocal crosses between *D. yakuba* Lamto3 and *D. mauritiana* Isofemale 72 strains, support the evidence that the overall pattern of activity profile may be under X chromosome control. Furthermore, certain features of the locomotor activity patterns, such as the morning and evening peaks may be either under X chromosomal or cytoplasmic factors. However, the overall activity levels are seen to be under autosomal dominant control.

8.6 Discussion:

The diurnal distribution of activity observed in light/dark cycles, which is universally observed among the member species of the *melanogaster* subgroup, is in line with a previous comprehensive study of *Drosophila* species (Hardeland and Strange, 1973). The peaks of activity were close to dawn and dusk, with a relative 'suppression' of activity during the remainder of lights-on. Previous studies have described an anticipatory increase in activity before lights-on (Petersen *et al.*, 1988), yet a less pronounced increase of activity is observed here, which is more gradual than previously reported. A superficial explanation of this pattern of activity might be that, in the Afrotropical regions where most of the species examined are found, a bimodal pattern with peaks at the beginning and end of the day would confine activity to the cooler time of day, thus avoiding the midday sun, and thereby reflecting a behavioural strategy which minimises the chances of dessication (Kalmus, 1940; 1945). However, the same pattern of activity has been observed in species from non-arid clines (Hardeland and Strange, 1973).

It is interesting to note, however, that the one species which exhibited a relatively reduced 'suppression' of its activity during the 'midday' period, *D. teissieri*, tends to inhabit evergreen rainforest, with a high canopy of trees which would maintain a relatively moist environment (Tsacas *et al.*, 1981). The *D. melanogaster* strain from Brighton (U.K.) is observed to have midday activity, which may reflect on the cooler climate of its area of collection than the Afrotropical region. The remaining species inhabit more exposed areas of forested savannah and mountainous evergreen forest (Tsacas et al., 1981). *D. simulans* which runs the greatest risk of encountering the cosmopolitan *D. melanogaster*, exhibits prominent morning and evening peaks which are quite different from those of *D. melanogaster*. *D. sechellia* (Tsacas and Bächli, 1981), and *D. mauritiana* (Tsacas and David, 1974) exhibit *simulans*-like and *melanogaster*-like activity patterns respectively, which is not surprising, given the fact that are endemic to Seychelles and Mauritius isles. In addition, this cursory resemblance is also in line with the phylogenetic relationship described by Nei (1983). *D. yakuba* is active throughout the daytime and the midday sun does not seem to affect it as much as the other species living in this region, found in shadowy places in the Afrotropical, and may be found in sympatry with *D. melanogaster, D. simulans, D. teissieri*, and *D. erecta*

(Tsacas *et al.*, 1981; Lachaise *et al.*, 1981; Tsacas and Lachaise, 1974). As it can be observed the overall patterns of activity exhibited by members of the *D. yakuba* complex, is more varied than those exhibited by the members of the *D. melanogaster* complex, which could probably reflect on their phylogenetic relationships (Nei, 1983; Cariou, 1987).

Another possibility that may explain the differences in the patterns of locomotor activity observed among the various species of the *melanogaster* subgroup is that different locomotor activity profiles have evolved so as to minimise the chances of disadvantageous interspecific hybridisations between species that live in sympatry in the Afrotropical regions. This is called 'character displacement' and can be caused by reinforcement (Butlin and Ritchie, 1989), and contributes further to the isolation of the species. For example, take a species such as *D. yakuba*, which is active throughout the daytime (see Figure 8.1: Bottom right panel) and living in sympatry with *D. teissieri*, which shows reduced daytime activity (see Figure 8.1: Bottom left panel). Bearing in mind that courtship is greatly aided by light and other visual cues (Burnet and Connolly, 1974; Robertson, 1983; Tompkins, 1984), their vastly different activity profiles could prevent interspecific hybridisation by minimizing the opportunities for hybridisation, since the latter species is almost idle during the day. Therefore, the evolution of such activity profiles may play an important part in speciation, for it develops further prezygotic reproductive isolation mechanisms, that may rival the 'mate recognition systems' in such organisms such tree frogs (Doherty and Gerhardt, 1984), crickets (Hoy *et al.*, 1977), and the courtship song in the *D. melanogaster* (Kyriacou and Hall, 1986, reviewed in Butlin and Ritchie, 1989).

Hennessey (pers. comm.) has observed the activity patterns of various *D. melanogaster* strains at different temperatures. At 29^0C, the patterns of activity are more bimodal, resembling that of *D. sechellia* (see Figure 8.1: Top third left panel), and at 18^0C the patterns become more unimodal. This was taken to mean that by changing the pattern of activity, these flies could accommodate these temperature changes. This flexibility in the locomotor activity of *D. melanogaster* might be the reason why this species can be found from temperate to hot climates (Ashburner, 1989). Similar experiments could be carried out using different species in the *D. melanogaster* subgroup in order to see whether this flexibility of the locomotor patterns is a common characteristic of the *D. melanogaster* subgroup or

whether is only a property of the cosmopolitan *D. melanogaster*. If *D. melanogaster*, at 29^0C, exhibits the same pattern of activity as that of *D. sechellia* at 25^0C, this could suggest that perhaps *D. sechellia* is more sensitive to higher temperatures.

Saunders (1982) observed that the 'morning' peak of activity seems to be a 'Startle' response to lights-on in *D. pseudoobscura*, in part because there appears to be no anticipation of that environmental transition. In contrast, the results presented here, indicate that all the species, and the majority of strains and, interspecific hybrids begin to increase their activity 1-2h before lights-on (Figure 8.1.1-8.6.1), in addition to showing an even more pronounced anticipation of lights-off (which incidentally also occurs in *D. pseudoobscura*, Petersen *et al.*, 1988). These results are in agreement with the Hamblen-Coyle *et al.* (1992) observations on *D. melanogaster* locomotor activity patterns. In addition, Hamblen-Coyle *et al.* (1992) monitored the activity patterns of the various *per*-mutants, which were observed to cause the free-running period (τ) to be lengthened or shortened (reviewed by Kyriacou and Hall, 1990; Vosshall and Young, 1992). Hence, the evening peak could be phase-shifted by endogenous τ-altering mutations in a way that is generally consistent with the effects of the genetic variations on the 'evening' peak (Hamblen-Coyle *et al.*, 1992). Therefore a species such as *D. sechellia* (Figure 8.1: Top third left panel), which has an endogenous free-running period of ~23h (see chapter 7), is observed to have advanced both its 'morning' and 'evening' peaks, as compared to the rest of the species examined.

In addition, the locomotor activity patterns for the different species have been divided into various components, such the Startle-On and Anticipation-Off, and Anticipation-On and Startle-Off components in order examine any possible different responses that the different species may exhibit during the lights-on-to-off transitions and *vice versa*. The different indices showed significant differences between the members of the *melanogaster* subgroup (see Figure 8.1.2). *D. melanogaster* is the most active, while *D. sechellia*, *D. orena* and *D. erecta* are the least active of the species. The same holds true for the amounts of day and night activities. *D. mauritiana* and *D. erecta* show the highest Night/Day activity ratios, indicating that they are more active during the night. *D. yakuba* is observed to react profoundly to the lights-on, while *D. orena* shows the highest anticipation for the oncoming lights-off transition. *D. sechellia* shows the highest anticipation of the oncoming lights-on

transition from the rest of the species, while *D. melanogaster* and *D. yakuba* show the highest 'Startle' response to the lights-off , and *D. sechellia* the least.

It is difficult to make any strong statements on the species-specificity of locomotor activity patterns, if only one strain of a species is examined. Inspecting the patterns of activity of the different *D. mauritiana* and *D. yakuba* strains, it is observed that they show similar relative amounts of activity. The differences appear in the bursts of activity around the 'dawn' and 'dusk' peaks, as well as their Night/Day activity ratios (see section 8.2 & 8.3). The intraspecific heterogeneity that is observed between the various *D. mauritiana* and *D. yakuba* strains, is usually caused by either one or two strains which show higher deviation, in a particular variable (index) from the rest of the strains. Therefore, any conclusion that might be given about any species-specific aspects of LD activity profile, must be treated with some caution.

This diurnal locomotor pattern may in fact be an exogenous effect of the abrupt changes between light and dark phases in these laboratory experiments (Hamblen-Coyle *et al.*, 1992; Petersen, 1988). In *D. mauritiana* MG71 and David75, for example, a clear burst of activity occurred in the bin immediately following lights-on, with little or no activity either directly before or directly after this bin. This seems to be an example of a "startle" reaction to the lights coming on, since the free-running records did not show this burst in both *melanogaster* subgroup complexes (see chapter 7). Indeed, there is a more gradual increase of activity around the two 'subjective' light transitions, and more gradual decrease in activity after the two light transitions. This kind of effect has been described in flight activity of the mosquito *Anopheles gambiae*, in which a dawn burst of activity disappeared if the sudden dark to light transition was replaced by a steady increase in light intensity (Jones *et al.*, 1972). An entrainment and run schedule of this graduated type could be used to examine which of the peaks observed in this study were exogenously created, and which are clock-controlled. The question of anticipation of either or both of the light transitions, which must clearly involve some form of clock input, could not accurately be addressed in this study, due to the activity being collated into 30 minute bins. In several cases anticipation clearly occurred, whereas in others the changes in activity were too close to the light signal to allow a definite statement

about whether anticipation had occurred. A minute-by-minute basis might enable us to observe the exact phasing of activity changes during each day.

Petersen *et al.* (1988) reported that species-specific locomotor activity information was transferred along with the *per⁺-pseudoobscura* DNA to *D. melanogaster per⁺* hosts. Thus *per* carries species-specific activity information. Examining the interspecific hybrids of the *melanogaster* subgroup and/or strains may help in discovering if the locomotor activity components were under X chromosome influence or whether some other autosomal factor may also play a role in the patterns of activities displayed. The mode of inheritance is more complex than originally thought. In general, the overall activity patterns of the various hybrids are broadly similar in shape to the corresponding maternal parent, implying that the overall pattern may be under the influence of the X chromosome, although maternal or paternal dominant factors can not be ruled out. Specifically, in *D. simulans* Florida(f) x *D. mauritiana* Sweden(m), the overall activity pattern is *simulans*-like, but the fall in activity after the two light transitions is more similar to the paternal participant. In *D. yakuba* France(f) x *D. teissieri* Sweden(m) hybrids the overall activity profiles are resembling the maternal parent, but the advancement of the evening peak in the light phase, as well as the overall levels of activity are more similar to the paternal parent. In *D. yakuba*(f) x *D. mauritiana*(m) crosses, hybrids exhibit a typical *yakuba*-like activity profile, and in *D. mauritiana*(f) x *D. yakuba*(m) crosses, the hybrids exhibited high morning and evening peaks, but less prominent than those of the paternal parent (*D. yakuba*), thus resembling the maternal activity profile. This has been further supported by the results from the only genuine reciprocal cross between *D. mauritiana* Isofemale72 and *D. yakuba* Lamto3, and their hybrids. This reciprocal cross also revealed that the overall locomotor activity profile of the hybrids resemble that of the maternal parent. In addition, their phasing of the morning and evening peaks resemble the maternal participant. Furthermore the levels of activity, in this reciprocal cross showed that they may be influenced by *D. yakuba* Lamto3 autosomal dominant factors. Yet, the relative amounts of activity, any possible advancement or delay in the evening peaks, as well as the magnitude of the responses to the light transitions of the hybrids were observed to be intermediate between the corresponding parental strains, in the other interspecific crosses. If these aspects of the locomotor activity profile are under autosomal control, then perhaps other autosomal clock genes such as *psi-2* and *psi-3*

163

(Jackson, 1983), *gate* (Jackson, 1983), *timeless* (Seghal *et al.*, 1994), and *ebony* (Newby and Jackson, 1991) may play an important part in the observed locomotor activity patterns. If such genes are involved, then variation in any one or more such genes could be responsible for the observed sensitivity to lights-on-to-off and *vice versa* transitions.

In short, the overall locomotor activity patterns may have the maternal 'signature', but the amount and pattern of activity around the light transitions, especially the evening peak, demonstrates paternal characteristics, suggesting either dominant autosomal factors or even Y chromosomal involvement.

GENERAL DISCUSSION

CHAPTER **9**

9. GENERAL DISCUSSION :

The experimental work was divided into two discrete sections. Specifically, the first section (Chapters 3-6) involved a search for, and subsequent characterisation of, species-specific differences, within the *melanogaster* subgroup in the courtship song rhythms. The second section involved an exploration into the circadian locomotor activity patterns between the different member species of the *D. melanogaster* subgroup and their interspecific hybrids under DD and LD conditions. The unifying feature in these two phenotypes is the influence of the *period* gene (Hall, 1995; Kyriacou *et al.*, 1996).

9.1 Courtship song:

The ultradian oscillation in the courtship song of *Drosophila* (Kyriacou and Hall, 1980), was the subject of the first three experimental chapters. This is a controversial topic, in the wake of two reports denying the existence of these ultradian cycles (Crossley, 1988; Ewing, 1988). The controversy has finally been put to rest by Alt *et* al. (1997), who by using new advanced spectral methods, confirmed all the early results of Kyriacou and Hall (1980; 1989), including the effects of the *per*-mutants on the 60s song cycle. Furthermore, Noor and Aquadro (1997) found evidence of rhythmicity in the *D. persimilis* song. Therefore, courtship song rhythmicity may be more widespread among the *Drosophila* genus than was originally thought. My work was initiated during the 'controversial' period and my results further extend the existence of song cycles in the *D. melanogaster* subgroup of species.

The courtship song of all the member species of the *D. melanogaster* subgroup, as well as different interspecific hybrids were recorded and analysed. A problem that was encountered to a varying degree was the low courtship vigour of the various species under study. Rearing the males in solitude, and in the case of *D. mauritiana* rearing the males in solitude and under constant illumination conditions, seemed to overcome the problem of low courtship vigour. As it had been stressed on several occasions by Crossley (1988) and Ewing (1988), and subsequently by Crossley

(1989), Ewing (1989), Logan and Rosenberg (1989) and Kyriacou and Hall (1989), *Drosophila* courtship has a drawback as far as time series analysis is concerned, in that it is a rather transitory event. It would be an exception for a male to court a female consistently, even for five or six minutes. However, this probably reflects the situation imposed upon courtship in laboratory conditions, rather than being representative of the situation in the wild, whereby Ewing (1983) reported that the *Drosophila* courtship durations are even shorter.

Reanalysis of the Kyriacou and Hall's (1980) original data, with new and more advanced spectral analyses, reconfirmed their findings. Furthermore, recording and analysing *D. yakuba* courtship songs supports the existence of long ~65-70s song cycles, first detected by Thackeray (1990). In addition, recordings and analysis of courtship songs from the rest of the member species of the *melanogaster* subgroup revealed that song rhythms exist which could be broadly categorised into 4 separate classes, 30-45s found in *D. mauritiana* and *D. simulans*, 45-50s found in *D. sechellia*, *D. orena*, and *D. erecta*, 50-60s found in *D. melanogaster*, and 60-75s found in *D. yakuba* and *D. teissieri*. With respect to *D. simulans*, my small sample size of 4 songs included an outlier (see Chapter 3). Therefore, I used the value of ≈ 40s from Kyriacou and Hall (1980; 1986) (see below). The mode of inheritance of IPI periodicity was further studied by analysing interspecific hybrids. That the X chromosome is contributing to the species-specificity of the male song cycles has first been reported by Kyriacou and Hall (1986), and confirmed with the transformation experiments carried out by Wheeler *et al* (1991). The findings here, reinforce this notion, in that hybrids usually expressed the song period of the maternal species. However, additional autosomal factors could not be ruled out. Indeed, some autosomal gene involvement has been implicated by Kyriacou and Hall's (1986) study in *D. melanogaster/D. simulans* hybrids.

Wheeler *et al.* (1991) reported that apart from the varying lengths of the Thr-Gly repeat, 4 amino acid substitutions encoded downstream of the Thr-Gly repeat in *D. melanogaster* and *D. simulans* were species-specific, and therefore may be responsible for the differences in the observed courtship song rhythms. Two of these (A/T and K/R) are

highlighted in bold letters in Figure 9.1.1, and fall within a common phosphorylation site (wheeler *et al.*, 1991). The other two are encoded further downstream. These two amino acid sustitutions seen in *D. simulans* are also found in both *D. mauritiana* and *D. sechellia* (Peixoto *et al.*, 1992). These are Threonine at position 102 and Arginine at position 107. In addition, *D. mauritiana* has an Alanine residue at position 122, which is substituted by an Isoleucine in *D. sechellia*. *D. yakuba* and *D. teissieri* both show an Alanine and Arginine residues at positions 102 and 107 (Peixoto *et al.*, 1992), as well as an Asparagine at position 105 which is not found in the other species. Finally, *D. erecta* and *D. orena* share the same amino acid substitutions. Furthermore, *D. erecta* has Theonine residue at position 120, which is unique from the other species. Thus, the amino acid changes encoded immediately downstream of the repeat are species-specific, and given the fact that this region is heavily involved in the species-specificity of the courtship song (Yu *et al.*, 1987a; Wheeler *et al.*, 1991), perhaps these differences correlate with the observed song cycles in the different species.

In order to study potential evolutionary changes in song rhythms a phylogenetic tree was used, where an attempt is made to determine the song cycle of the common ancestor of the *melanogaster* subgroup. Figure 9.2.2 shows a phylogenetic tree which is based on Nei's map on the phylogenetic relationship between the members of the subgroup (Nei, 1983; Cariou, 1987). This has also recently been used to study the evolution of the male-specific abdominal muscle of Lawrence (Lawrence and Johnston, 1984; 1986) by Gailey *et al.* (1997). The courtship song periods of the species were categorised into 4 different states I (35-45s), II (45-50s), III (50-60s) and IV (60-75s) (see Figure 9.2.2). It was assumed that song cycles can mutate between the different states in single steps only. By superimposing the various song cycle states, the most parsimonious solution (smallest number of steps) to the identity of the common ancestral song cycle can be empirically determined, by examining the number of single steps that are required to generate the extant species song cycles.

If the common ancestral species had a state I song cycle, then to reach state III there had to occur two changes at the *erecta-orena* branch, one at the *melanogaster*

N-TERMINAL CONSERVED REGION	VARIABLE REGION	C-TERMINAL CONSERVED REGION

melanogaster ...............AGTG....GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG..........................NGTNSG..........TGTGTASSS...KGGS.....................AAIPPVTLTESLLNK } **II**

simulans (Aus) ..............AGTG.....GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG..........NGTNSG..........TGTGTTSSS...RGGS....................TAIPPVTLTESLLNK } **IV**

mauritiana ...............AGTG.....GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG.NGTNSC..........TGTGTTSSS...RGGS....................AAIPPVTLTESLLNK } **IV**

sechellia ...............AGTG.....GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG......................NGTNSG....................TTSSS...RGGS....................AAAPPVTLESLLNK } **III**

yakuba .............AGTG.....GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG............................NGTNSG.......TGTGTASSNYRGGG...................VAIQPVTLESLLNK } **I**

teissieri .............AGTG.....GTGTGTGTGTGTGTGTGTGTGTGTGTGTG......................................NG............................SASSNYRGGG....................VAIQPVTLESLLNK } **I**

erecta ............AGTGGTGGTGTGTGTGTGTGTGTGTG.........TDTGTGTG.............................TR.................NGTNSGTNSGTRTGTASS-YRGGGGGAGGGGGVTIQHLTLTESLLNK } **III**

orena .........AGTGGTGGTGTGTGTGTGTGTGTGTG TGTDTGTGTGTG TETGTGTGTGTR.............. NGGTNSG........TKTGTASS-YRGGG....................VAIQPVTLTESLLNK } **III**

↑　　　　　↑　　　　　↑
100　　**110**　　**120**

Fiure 9.2.1: **Part of the amino acid seuence of the Thr-Gly region in the PER protein of the eight species in the *melanogaster* subgroup. implicated by Wheeler *et al.* as contributing to the species-specificity of the song cycle (Wheeler *et al.*, 1991). The bold letters designate the species-specific amino acid substitutions (Figure taken from Peixoto *et al.*, 1992). Roman Numerals designate the different song cycle states. Arrows designate the relative positions in the amino acid sequence.**

branch (state II), two at the *mauritiana* branch (state IV), and one at the *simulans* branch (state IV), resulting in a total of 6 single step changes (see Figure 9.2.2: triangle), in order to obtain the observed song cycle states. If the ancestral species had a state II song cycle, then there had to occur one change at the *erecta-orena* branch (state III), one at the *teissieri-yakuba* branch (state I), two changes at the *mauritiana* branch (state IV), and one at the *simulans* branch (state IV), giving a total of 5 single step changes (see Figure 9.2.2: rectangle), in order to reach the different song states. If the common ancestor had a state III song cycle, then to reach state I there had to occur two changes on the *teissieri-yakuba* brach, one change on the *melanogaster* branch (state II), one change on the *mauritiana* brach (state IV), and one change on the *simulans* brach (state IV), giving again a total of 5 single step changes (see Figure 9.2.2: circle), in order to achieve the different observed song states. If the ancestral species had a state IV song cycle, then to reach state III there had to occur one change at the *erecta-orena* branch, three changes to reach state I at the *teissieri-yakuba* branch, two changes at the *melanogaster* branch, and one change at the *sechellia* branch (see Figure 9.2.2: rhombus), resulting in a total of 6 single step changes in order to reach the observed song states. So, it can be speculated that the common ancestral song cycle could have been either 50-60 or 45-50s. Given the number of amino acid changes between the species in this region, it is very difficult to correlate song changes with specific substitutions.

The species-specific song differences could potentially be used for species recognition (Kyriacou and Hall, 1982; 1986). If this is the case then we might expect that species which live in sympatry, e.g, *D. simulans* and *D. melanogaster* to have different rhythms, thereby reducing the chances of hybridisation. This exaggeration of species markers such as visual clues, scents or courtship rituals, or adaptations in their anatomy, physiology and behaviour, in sympatric populations is called 'Character displacement' (Grant, 1984; Schluter and McPhail, 1992; Schluter, 1994). In this way, the species barriers are reinforced and maintained. Although reinforcement is a controversial subject in speciation, Noor (1995) proposed that it was important in the mating behaviour of *D. pseudoobscura* and *D. persimilis*. Because species that live in sympatry, such as *D. teissieri* and *D. yakuba* have similar long song cycles, this would suggest that character

STATE

D. erecta (~48s)	**III**
D. orena (~47s)	**III**
D. teissieri (~72s)	**I**
D. yakuba * (~66s)	**I**
D. melanogaster (~55)	**II**
D. mauritiana (~40s)	**IV**
D. simulans (~42s)**	**IV**
D. sechellia (~48s)	**III**

STATE

I ?
II ?
III ?
IV ?

COMMON

ANCESTOR

Song rhythm = ~40-50s ?
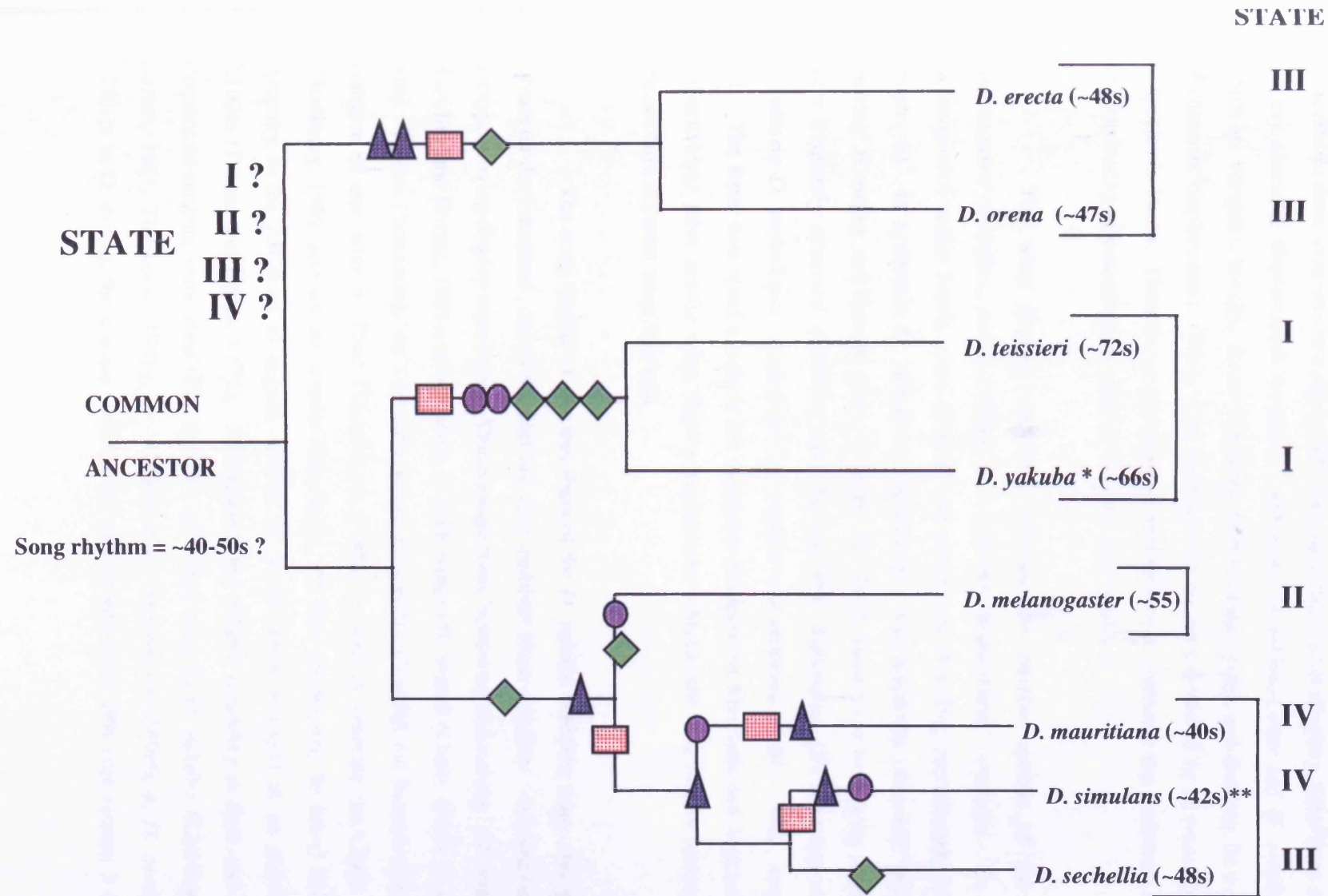
Figure 9.2.2: The phylogenetic tree of the melanogaster subgroup, taken from Nei (1983). The number shown next to each species is the species-specific song period. The (*) by *D. yakuba* is to designate that the period shown is the Thud pulse type song period, since Thud constitutes the majority song in a *D. yakuba* courtship. The (**) designates the period of *D. simulans* taken from Kyriacou and Hall's data (1980; 1986).

KEY:

▲ - STATE I   ⬤ - STATE III

▦ - STATE II   ◆ - STATE IV

Single step changes that the common ancestor might have taken in order to create the different observed

displacement is not occurring. Furthermore, such species *D. simulans*, *D. mauritiana*, and *D. sechellia* show very similar song cycles, but since they live in allopatry, there is no need for any character displacement to occur. However, *D. melanogaster* and *D. simulans*, which are sympatric species, show distinctly different song cycles, and this may be a case of character displacement. Thus, song cycles could not be considered to be contributing to species isolation. Therefore, other song components and courtship characteristics may be contributing to sustaining the species barriers (see below).

The wing display repertoire amongst the member species of the *D. melanogaster* complex, could reflect a 'character displacement' scenario. In *D. melanogaster*, males hardly ever scissor and never row (Cowling and Burnet, 1981; chapter 3). In sympatric *D. simulans*, 'scissoring' was frequently observed, but no 'rowing' (Cowling and Burnet, 1981; Chapter 3). In *D. mauritiana* both wing displays were frequently observed (Cowling and Burnet,1981; Robertson, 1983; section 4.1), whereas in *D. sechellia* a 'scissoring-like' motion was observed (Cobb, 1990; section 4.1). The latter two species, which are allopatric (endemic to Mauritius and Seychelles, respectively), have similar wing display repertoires, whereas the two former sympatric species have different wing displays.

The wing display of the members of the *D. yakuba* complex, may also reflect 'character displacement', due to the fact all four members show a further 'individualism' in the type of wing display repertoires. These range from 'scissoring and rowing' (*D. teissieri*) (Cowling and Burnet, 1981), to vibration of both wings (*D. orena*) (Cobb, 1990), to single wing vibration ('scissoring-like') or both wings (*D. erecta*) (Cowling and Burnet, 1981), to vibration of one wing in Thud (Thackeray, 1989), or both to generate the Clack song (Thackeray, 1989, and see my results Chapter3). All four species may be found living in sympatry in the Afrotropical region, except *D. orena*, which is found at an altitude of 2,100m (Tsacas and David, 1978). Futhermore, the greater variability in their sine song frequencies-ranging from total the absence of a sine song in *D. yakuba* (Cowling and Burnet, 1981; Thackeray, 1989), to ~130Hz in *D. teissieri*, to ~180Hz in *D. orena*, to ~270Hz in *D. erecta*, the greater variability seen in their mean IPIs (see section 3.4 and

4.3), and the broadness of distribution of their song periods (see Figure 4.5.1), may again reflect 'character displacement'.

Many species of *Drosophila* will hybridize, at least under laboratory conditions. The overall picture that emerges from Table 5.1 is that, even if all the participating species in the crosses are members of the same subgroup and/or complex, interspecific hybridisation is achieved with great difficulty; hybrids are often sterile, at least as males (Haldane, 1922; Sturtevant, 1920; David *et al*, 1974; von Schilcher *et al.*, 1975; Lemeunier, 1979; Cowling and Burnet, 1981, Lachaise *et al.*, 1986; Lee and Watanabe, 1987). Barriers to experimental hybridisation between species may be either pre- or postzygotic. Prezygotic isolation is usually assessed by some sort of a mating choice test. The degree of isolation can be measured by observations of matings (Futuyma, 1989). Since mating behaviour in the laboratory is essentially the same as that of wild flies (Krebs and Bean, 1991), varying rearing substrates is likely to have an influence on the composition of volatile cuticular hydrocarbons-which constitute the pheromones exuded by the females (Ferveur *et al.*, 1997), or even other environmental factors that may influence the IPI production both qualitatively and quantitatively (Noor and Aquadro, 1997). Etges (1992) reported that by changing the host plants for feeding *D. mojavensis*, certain physiological shifts can occur, such as cuticular hydrocarbons. Contact pheromones in this species which can be correlated with altered behavioural responses are due to the adaptation of this species to its new host plant. So it is feasible, that by harmonising the rearing substrates of flies of the different species of the *melanogaster* subgroup as in the laboratory, will probably increase interspecific matings. Culturing *D. sechellia* on its natural substrate, the maritime rubacious shrub *Morinda citrifolia* (Lemeunier and Ashburner, 1984), might reduce the interspecific mating ability of this species, but make rearing this species under laboratory conditions easier.

Another way that the degree of isolation could be measured is to dissect females and look for sperm or by the appearance of zygotes (Coyne & Orr, 1989). The hybrid sterility, which may range from slight to complete, may be caused by cytological and genetic factors such as incompatibility of genes of one of the parental species with the

genome of the other species that may lead to lethal physiological distrurbances (Coyne & Orr, 1989; O'Neill & Karr, 1990). In addition, interspecific hybrids are less successful in courtship than individuals of pure species, when species-specific behavioural patterns and species-specific stimuli play an important role (Orr et al., 1990). Interspecific hybrids differ from individuals of the parental species not only in morphology (Coyne et al., 1991), but, in addition, usually in the level of fertility and viability (Coyne et al., 1991). The majority of crosses give a sterile F$_1$, with a sex ratio heavily biased in favour of females, or, if both sexes are present, they are represented in approximately equal frequency, and then the males are typically sterile ('Haldane's' rule, Haldane, 1922; David, 1976; Cowling & Burnet, 1981; Cobb, 1986). Pantazidis and Zouros (1988) discovered that by replacing a D. arizonae autosomal complement in D. mojavensis males carrying the Y-chromosome, normal spermatogenesis could be restored. Therefore, it may be possible that there exist some autosomal complement that could be activated, within the autosomes of the different species of the subgroup. Recently, Davies et al. (1996) have isolated a strain of D. simulans, that produces fertile hybrid females, in crosses with D. melanogaster. This strain may identify some of the genetic components involved in both pre- and post-reproductive isolation in these species.

9.2 Locomotor activity

The locomotor activity of all eight member species of the melanogaster subgroup and their interspecific hybrids were investigated. Two conditions were used, constant darkness (DD), in order to reveal the period of the endogenous influences on this behaviour, and LD12, in order to examine the patterns of activity within each day. Members of the melanogaster complex, with the possible exception of D. mauritiana, show broad levels of activity, with no morning or evening peaks (see Chapter 7). Even species that are sympatric, such as D. simulans and D. melanogaster show similar activity profiles. Therefore, it is clear that in this case character displacement is not occurring. However, in the yakuba complex, where D. teissieri and D. yakuba live together, D. teissieri shows prominent morning and evening peaks, whereas D. yakuba exhibits a more melanogaster-like (broad) activity profile. In this case, character displacement, caused by

reinforcement, may be occurring, and thus avoiding sterile interspecific hybridisation. Thus the sex-linked *per* may not be the sole contributor to the individuality of the circadian locomotor activity. It is therefore possible that other aspects, such as the relative amounts of activity and inactivity, during the day- and night-time respectively, may be contributing to the maintainence of species barriers. Thus, it is conceivable, that several other autosomal loci such as *psi*-2, *psi*-3 (Jackson, 1983), and *tim* (Myers *et al.*, 1995; Gekakis *et al.*, 1995) may also affect locomotor activity, and therefore sustaining 'species individualism'.

All species and their hybrids exhibited free-running rhythms with very similar periods, ranging from between 23 and 25 hours. The absolute amounts of activity in each species/strain/hybrid was very different though. Even though any further statistical analysis has proved impossible for reasons explained in Chapter 7, several inferences could be made for the mode of inheritance of the circadian locomotor activity from the standardised data:

1) The overall pattern of activity of the hybrids resemble the paternal profile, which suggests autosomal influence;
2) The levels of activity are usually intermediate between the two parental levels, which implies autosomal control;
3) The amount of activity around the evening peak generally follows the paternal parent, suggesting autosomal control, although X chromosome involvement cannot be ruled out;
4) The amount of activity around the morning peak generally follows the paternal participant profile, again implying autosomal factors.

The experiments performed in LD cycles were also informative, showing that the pattern of activity within each daily cycle was unique in each species, and similar to the maternal participant in the interspecific hybrids. In particular, the phasing of activity occurring during the light transitions, and the proportion of activity occurring during the lights-on period were shown to be diagnostically distiguishing features in the locomotor activity proflies of each species. However, in the hybrids the phasing of activity with respect to the light transitions, and the levels of absolute activity revealed that probably autosomal rather than Y-chromosome factors may be involved. It is possible that other

autosomally located genes may also be involved in the locomotor activity patterns, such as *tim* (Myers *et al.*, 1995; Gekakis *et* al., 1995). Hence, any species variability in the locomotor activity may reflect species-specific variation in such genes. My results in DD are not consistent with the sex-linked *per* locus having significant influence over the circadian locomotor activity patterns, which contradicts Petersen's (1988) and Hennessey's (pers. comm) work, and possible reasons for this were discussed in Chapter 7.

So , why the activity patterns of the hybrids show maternal influence in LD, and autosomal involvement in DD? So far there is no explanation for the observed discrepancies between the two light conditions. Nonetheless it seems reasonable to speculate that an LD cycle has a direct effect on the locomotor activity profiles. A similar effect can be appreciated when considering the activity patterns of per^{01} flies, in DD and LD conditions. In DD, per^{01} flies do not exhibit any distinguishable pattern of activity (Konopka and Benzer, 1971). However, in LD, these flies are more active during the light phase than during the dark phase, with increased spurts of activity around the two light transitions, suggesting that even if per^{01} flies are not able to measure time endogenously, there is a considerable effect on the locomotor activity patterns in LD (reviewed by Kyriacou, 1990). The activity profiles of the hybrids in LD, suggest that this effect may possibly be mediated by as yet unknown loci, located on the X chromosome; and in DD conditions, some other unknown gene(s) may be involved.

9.3 Future lines of investigation:

The effect of producing rhythmically oscillating IPIs on mating success has been examined in *D. melanogaster*, by using artificially-simulated songs (Kyriacou and Hall, 1982; 1986; Greenacre *et al.*, 1993). The mating speed of wingless males, in the presence of various 'IPI' regimes was found to be at its optimum when a 55s rhythm was superimposed on the *D. melanogaster* mean IPI of 35ms (Kyriacou and Hall, 1982). Moreover, Byrne and Kyriacou (pers. comm.) reported that the mean burst duration is critical in the mating speeds between *D. arizonae* and *D. mojavensis*, in simulator

173

experiments. The same avenue could be pursued in order to study the function of any song cycles in the other species of the *melanogaster* subgroup. Specifically, it would be interesting to investigate the ~70s song cycles found in *D. yakuba* pulse song IPIs (see Chapter 3). It would be prudent and more thorough to not only synthetically generate two independent cycles, i.e., to simulate the Clack and the Thud rhythms, but, furthermore to generate each song type with different periods (within the 60-100s range) in different phases with respect to each other, and different mean burst duration and mean interburst intervals. Only then it would be possible to 'recreate', as close as possible to the real scenario the complex 'cocktail' of the Clack and Thud pulse song types produced by *D. yakuba*.

9.4 Conclusion:

Ultradian rhythms in the courtship song of *Drosophila* (Kyriacou and Hall, 1980) have been investigated. These rhythms are seen to be under the control of the *per* gene which is located on the X chromosome. It is also known that *per* affects circadian locomotor activity (Konopka and Benzer, 1971). Therefore it may have an important role to play in the behavioural activities of *Drosophila*. The potential for evolutionary significant changes occurring at this locus is clear (Peixoto *et al.*, 1992; 1993), since any changes in courtship or locomotor activity behaviours may potentially result in the creation of an isolating mechanism between populations, resulting eventually in the creation of new species. The experiments described here represent a small step towards widening our perception of the evolutionary repercussions of biological rhythms in behaviour.

APPENDICES

APPENDICES

FOR CHAPTER 3

APPENDIX 3.1 :

CLEAN and VdB analyses, with Monte Carlo simulations, results of Kyriacou and Hall's (1980) plus some unpublished songs .

GENOTYPE:	SPECTRAL	ANALYSIS			
	CLEAN		VdB		Observations
per^L	1st PEAK	2nd PEAK	1st PEAK	2nd PEAK	BINS FILLED
Long1	75.14	x	74.77	x	15/28
Long2b	65.32	x	67.23	x	20/30
Long4	82.55	x	81.63	x	23/37
Long4b	74.19	x	71.43	x	18/26
Long6	80.65	x	x	x	22/30
Long7	95.24	x	95.24	x	23/35
Long8	76.32	x	75.47	x	24/32
Long9	93.75	x	94.12	x	32/35
Long10	88.57	x	93.02	x	28/32
Long11	105.26	x	105.26	x	16/29
LongA	80.65	x	80.00	x	20/26
per^{01}					
Arr1	83.74	x	86.02	x	33/36
Arr3	378.05	x	470.59	x	30/33
Arr5	23.08	153.06	22.79	x	32/32
Arr6	x	x	41.88	27.49	7/9
Arr8	x	x	67.23	52.98	15/29
Arrb1	225.81	x	27.40	x	25/29
Arrb2	29.30	x	29.96	x	22/32
Arrb3	20.83	x	21.00	x	24/30
Arrb4	34.38	22.49	35.87	22.47	14/27
Arrb5	20.06	x	60.61	x	14/28
per^S					
Shortb6	39.64	x	40.20	x	29/33
Short5	45.45	x	45.71	x	22/33
ShortC	46.30	x	45.98	x	11/14
Shortb1	x	x	74.77	38.28	13/18
Shortb2	x	x	34.78	x	16/23
Shortb3	43.91	x	43.96	x	28/34
Shortb4	39.02	x	39.41	x	16/17
Shortb5	x	x	43.96	x	25/31
Shortb7	40.62	x	41.24	x	28/31
YShort1	47.62	x	47.62	x	22/27
Yshort2	39.25	x	38.65	x	18/25

3.1:

SPECTRAL	ANALYSIS			
SPECIES/ STRAIN	**CLEAN**		**VdB**		**Observations**
D. simulans	1st PEAK	2nd PEAK	1st PEAK	2nd PEAK	BINS FILLED
SIM2	62.87	x	62.50	x	12/24
SIM4	36.53	x	36.70	x	27/36
SIM5	35.43	x	36.53	x	8/13
SIM8	33.58	x	34.04	x	26/34
SIM9	33.52	x	33.90	x	38/51
SIM1	32.74	x	22.41	x	38/51
SIMD5	31.98	x	32.00	x	18/37
D. melanogaster- **OREGON**					
ORG3	54.24	x	54.79	x	33/34
ORGYM1	63.41	x	62.50	x	24/30
ORGYM2	55.81	x	56.34	x	16/30
ORGYrec2	59.17	x	59.26	x	20/27
ORGYsnm1	82.19	x	56.74	81.63	14/26
ORG2CL	44.59	x	44.94	x	25/35
ORG10	54.46	x	54.05	x	20/24
ORG1CL	60.81	x	62.99	x	20/20
ORG4	47.62	x	50.31	x	12/15
ORG6	54.47	x	52.63	x	27/30
ORG7	52.73	x	53.69	x	30/31
ORG9	60.61	x	60.61	x	17/30
D. melanogater- **Canton-S**					
CSY2	60.98	x	59.26	x	11/12
CSY4	53.30	x	54.42	x	18/23
CSb5	54.31	x	54.42	x	29/30
CSYb3	58.14	x	59.70	x	21/23
CSYb4	56.00	x	56.74	x	28/29
CSYb6	58.62	x	60.61	x	15/19
CSYb7	46.51	x	45.45	x	22/22
CS1CL	62.50	x	62.50	x	10/13
CS2CL	64.29	x	65.57	x	25/29
CS4CL	78.49	x	62.02	x	8/10
CS6	52.00	x	51.95	x	10/15
CS7	56.21	x	56.34	x	21/21
CS9	57.23	x	57.97	x	19/24
CSb1	52.81	x	53.69	x	34/34
CSb2	59.26	x	58.39	x	17/17
CSb3	50.36	x	49.69	x	29/29
CSb4	53.03	x	53.33	x	30/30
CSb6	54.26	x	54.42	x	30/30

Shaded-in values are used to determine mean genotype periods shown in table 3.1.1.

APPENDIX 3.2a:

Spectral analyses of *D. yakuba* song using CLEAN and VdB (with Monte Carlo simulations), and 250 and 50ms cut-off points.

SPECTRAL ANALYSIS						
YAKUBA		**CLEAN**		**VdB**		**Observations**
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
CUT Off POINTS : 250 & 50 ms						
Yk1t1l2	Overall	34.92	85.77	87.91	50.63	36/43
	Thud	66.12	x	66.12	x	22/39
	Clack	57.58	x	57.97	x	22/40
Yk2t1l2	Overall	75.42	45.15	76.92	x	43/56
	Thud	31.18	70.13	31.25	x	28/56
	Clack	23.52	21.82	23.67	21.86	26/51
Yk3t1l2	Overall	33.01	54.31	33.20	x	35/36
	Thud	34.07	21.17	34.04	21.16	20/36
	Clack	53.50	26.37	54.79	37.21	18/35
Yk4t1r1	Oerall	406.98	76.19	533.33	72.73	36/37
	Thud	x	x	533.33	37.38	34/37
	Clack	471.43	x	533.33	x	25/36
Yk5t1r1	Overall	24.27	x	23.95	x	19/34
	Thud	x	x	150.94	x	7/16
	Clack	x	x	x	x	17/34
Yk6t1r2	Overall	386.36	x	470.59	20.67	27/37
	Thud	71.88	x	72.73	35.87	26/30
	Clack	395.35	x	470.59	x	11/37
Yk7t1r1	Overall	42.55	x	42.55	21.45	21/36
	Thud	x	x	83.33	275.86	6/26
	Clack	x	x	42.33	x	20/36
Yk9t7l2	Overall	61.96	x	25.72	65.57	26/45
	Thud	62.14		25.81	65.57	26/45
	Clack	x	x	x	x	x
Yk20t1r1	Overall	40.79	32.53	500.00	25.97	36/37
	Thud	32.32	x	25.48	31.87	30/37
	Clack	24.75	22.35	22.60	x	22/37
Ya1l1t10	Overall	29.29	26.08	29.52	25.89	35/59
	Thud	42.35	29.20	142.86	29.63	32/59
	Clack	22.61	x	22.54	65.04	7/49
Ya12t9r	Overall	622.22	36.18	727.27	31.28	61/77
	Thud	78.55	31.14	173.91	31.37	39/77
	Clack	763.16	x	888.89	250.00	37/71
Yic2t13	Overall	88.76	x	37.91	84.21	20/34
	Thud	83.80	29.30	83.33	29.74	17/34
	Clack	20.80	x	20.89	150.94	6/30
Yam2t9r	Overall	490.74	x	470.59	x	38/66
	Thud	490.74	x	470.59	x	37/66
	Clack	x	x	x	x	x
Y1e2s1	Overall	43.60	36.00	43.01	36.70	35/38
	Thud	87.38	x	87.91	119.40	30/38
	Clack	44.33	x	43.72	333.33	10/35

3.2a:

SPECTRAL ANALYSIS					
YAKUBA		**CLEAN**		**VdB**	**Observations**
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
CUT Off POINTS : 250 & 50 ms					
Y3e2s1	Overall	892.86	27.62	888.89	195.12	43/44
	Thud	20.02	781.25	888.89	35.24	41/44
	Clack	26.12	x	30.19	25.64	14/39
Y8e2s1	Overall	600.00	x	666.67	x	42/44
	Thud	617.65	x	727.27	x	42/44
	Clack	439.02	x	533.33	50.31	11/40
Y9e2s1	Overall	90.91	30.98	91.95	28.37	37/38
	Thud	95.74	23.87	23.87	97.56	36/36
	Clack	22.73	62.50	22.60	37.56	24/38
Y10e2s2	Overall	59.27	x	100.96	x	29/41
	Thud	x	x	100.00	29.96	29/41
	Clack	23.09	57.50	57.14	23.46	26/41
Yy1t13r	Overall	29.48	x	29.85	x	25/29
	Thud	x	x	x	x	19/29
	Clack	29.93	x	30.30	x	13/25
Yy1t14	Overall	57.89	92.17	58.39	98.77	41/43
	Thud	57.14	x	57.55	102.56	33/43
	Clack	22.49	x	22.60	37.38	19/40
Y1t14l2	Overall	33.22	30.05	33.20	52.29	33/43
	Thud	32.55	62.50	61.54	x	29/43
	Clack	28.95	64.68	29.41	62.99	19/32
Yy2t14l	Overall	20.08	55.76	x	x	26/35
	Thud	63.83	x	64.52	x	21/34
	Clack	54.11	x	51.95	41..03	16/31
Yy3t14l	Overall	105.77	54.19	108.11	55.94	44/47
	Thud	143.52	x	131.15	x	32/47
	Clack	x	x	106.67	69.57	24/47
Yy3t14l2	Overall	x	x	533.33	83.33	44/48
	Thud	36.16	28.89	380.95	36.70	32/48
	Clack	21.93	x	21.92	20.62	23/44
Yy3t18	Overall	26.20	x	55.56	25.97	29/39
	Thud	26.17	x	55.56	25.97	28/39
	Clack	x	x	x	x	x
Yy4t14	Overall	103.81	26.52	105.26	x	48/51
	Thud	104.26	20.62	106.67	47.90	44/51
	Clack	155.41	22.22	170.21	55.56	27/51
Yy4t14l	Overall	33.02	34.28	43.24	142.86	47/51
	Thud	42.61	26.41	42.78	26.58	25/48
	Clack	x	x	800.0	62.50	38/51
Yy5t14l	Overall	64.75	21.37	66.12	21.05	31/43
	Thud	30.92	371.79	31.25	26.76	28/33
	Clack	25.26	371.79	26.27	24.02	18/31

3.2a:

SPECTRAL ANALYSIS						
YAKUBA		**CLEAN**		**VdB**		**Observations**
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
CUT Off POINTS : 250 & 50 ms						
Yy5t14l2	Overall	25.62	21.37	400.00	25.81	34/46
	Thud	71.17	x	21.45	71.43	29/46
	Clack	68.03	x	400.00	68.23	25/46
Yy6t13	Overall	x	x	23.32	x	14/21
	Thud	x	x	x	x	12/21
	Clack	25.74	x	60.15	x	5/16
Yy6t14l	Overall	87.04	157.53	25.89	87.91	40/52
	Thud	73.72	x	71.43	98.77	31/45
	Clack	512.82	x	40.40	34.78	22/52
Yy7t14l	Overall	x	x	22.73	27.59	2433
	Thud	x	x	22.54	27.59	22/33
	Clack	x	x	190.48	22.47	3/11
Yy8t13r	Overall	35.50	x	36.53	156.86	32/39
	Thud	x	x	150.94	615.38	32/39
	Clack	x	x	28.67	x	8/21
Yy9t13r	Overall	45.78	x	45.98	61.54	38/40
	Thud	59.01	58.64	45.71	60.15	35/40
	Clack	45.45	x	45.45	26.40	21/40
Yy10t10	Overall	102.89	24.26	24.24	103.90	50/59
	Thud	92.59	24.20	24.46	93.02	28/57
	Clack	30.82	24.11	31.01	x	22/52
Yy10t13	Overall	31.36	22.76	571.43	31.75	30/38
	Thud	22.53	x	22.54	x	26/38
	Clack	31.91	x	32.39	615.58	8/35
Yyh1t10	Overall	84.75	40.13	86.96	42.33	41/59
	Thud	84.75	x	86.02	41.24	31/59
	Clack	28.48	x	28.37	170.21	9/49

Shaded-in values are the Clean values used to determine the mean song periods

APPENDIX 3.2b:

Spectral analyses of *D. yakuba* song using CLEAN and VdB (with Monte Carlo simulations), and 350 and 50ms cut-off points.

SPECTRAL ANALYSIS						
YAKUBA		**CLEAN**		**VdB**		**Observations**
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
CUT Off POINTS : 350 & 50 ms						
Yk1t1l2	Overall	34.92	85.77	87.91	50.63	36/43
	Thud	66.12	x	66.12	52.29	22/39
	Clack	57.58	x	57.97	x	22/43
Yk2t1l2	Overall	75.42	45.08	76.92	22.22	43/56
	Thud	31.18	69.77	31.25	x	30/51
	Clack	23.52	21.82	23.67	21.86	27/51
Yk3t1l2	Overall	33.01	54.31	33.20	x	35/36
	Thud	34.07	21.17	34.04	21.16	20/36
	Clack	53.50	26.37	54.79	37.21	18/35
Yk4t1r1	Overall	406.98	76.75	500.00	76.19	37/37
	Thud	x	x	533.33	37.38	34/37
	Clack	500.00	x	533.33	x	25/37
Yk5t1r1	Overall	24.27	x	23.95	x	19/34
	Thud	x	x	150.94	x	7/16
	Clack	x	x	x	x	17/34
Yk6t1r2	Overall	386.36	x	470.59	20.67	27/37
	Thud	71.88	x	72.73	35.87	26/37
	Clack	404.76	x	470.59	x	12/37
Yk7t1r1	Overall	21.38	43.37	43.48	x	22/36
	Thud	x	x	82.47	275.86	8/26
	Clack	x	x	43.24	x	21/36
Yk9t7l2	Overall	62.14	21.06	66.12	55.56	27/45
	Thud	61.96	20.96	65.57	55.56	27/45
	Clack	x	x	x	x	x
Yk20t1r1	Overall	40.79	x	40.79	32.53	36/38
	Thud	32.32	x	32.32	x	30/38
	Clack	24.75	22.35	24.75	22.35	28/38
Ya1lt10	Overall	29.29	26.08	29.29	26.08	38/59
	Thud	42.35	29.20	42.35	29.20	33/59
	Clack	22.67	x	22.67	x	11/49
Ya12t9r	Overall	735.29	110.13	651.16	36.08	63/77
	Thud	31.29	x	78.55	31.14	41/77
	Clack	644.74	228.97	763.16	x	38/71
Yic2t13	Overall	88.86	x	38.17	88.76	20/34
	Thud	83.80	x	83.80	x	20/34
	Clack	20.80	x	20.80	x	8/30
Yam2t9r	Overall	20.08	470.59	490.74	26.88	42/66
	Thud	461.54	x	473.21	x	42/66
	Clack	x	x	x	x	x
Yle2s1	Overall	44.01	x	44.01	36.14	36/39
	Thud	46.88	x	46.88	x	31/39
	Clack	44.33	x	44.33	x	13/35

3.2b:

SPECTRAL ANALYSIS						
YAKUBA		**CLEAN**		**VdB**		**Observations**
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
CUT Off POINTS : 350 & 50 ms						
Y3e2s1	Overall	892.86	213.68	164.06	35.96	43/44
	Thud	757.58	24.18	36.08	160.31	41/44
	Clack	291.67	26.83	26.12	x	15/39
Y8e2s1	Overall	600.00	x	600.00	x	42/44
	Thud	617.65	x	617.65	x	42/44
	Clack	439.02	x	439.02	x	11/41
Y9e2s1	Overall	90.91	28.04	91.95	28.37	37/38
	Thud	96.26	23.87	96.26	23.87	36/38
	Clack	22.73	62.50	22.73	62.50	24/38
Y10e2s2	Overall	59.27	x	59.27	x	30/41
	Thud	x	x	x	x	29/41
	Clack	23.09	57.50	23.09	57.50	26/41
Yy1t13r	Overall	29.48	x	29.85	x	25/29
	Thud	x	x	222.22	62.99	19/29
	Clack	29.93	x	29.93	x	14/26
Yy1t14	Overall	57.89	92.17	57.39	x	41/43
	Thud	57.14	x	57.55	x	33/43
	Clack	22.49	x	22.60	x	19/40
Y1t14l2	Overall	33.22	30.05	33.22	x	33/43
	Thud	32.55	62.50	61.54	x	29/43
	Clack	64.68	28.95	28.95	62.99	19/32
Yy2t14l	Overall	20.08	55.76	x	x	26/35
	Thud	63.83	x	63.65	x	26/35
	Clackx	54.11	x	51.95	x	16/31
Yy3t14l	Overall	105.77	54.19	108.11	55.94	44/47
	Thud	143.52	x	131.15	x	32/47
	Clack	x	x	106.67	69.57	24/47
Yy3t14l2	Overall	x	x	533.33	83.33	44/48
	Thud	36.16	28.89	36.70	28.89	32/48
	Clack	21.93	x	21.92	x	23/44
Yy3t18	Overall	31.03	28.08	26.20	x	29/39
	Thud	23.87	x	26.17	x	28/39
	Clack	x	x	x	x	x
Yy4t14	Overall	103.81	26.52	105.26	x	48/51
	Thud	104.26	20.62	106.67	47.90	44/51
	Clack	22.22	155.41	22.35	170.21	29/51
Yy4t14l	Overall	33.02	34.28	33.25	x	47/51
	Thud	42.61	26.41	42.78	26.58	25/48
	Clack	x	x	800.00	62.50	38/51
Yy5t14l	Overall	64.75	21.37	66.12	21.05	31/43
	Thud	30.92	371.79	31.25	26.76	28/33
	Clack	25.26	371.79	26.27	24.02	18/31

3.2b:

SPECTRAL ANALYSIS					
YAKUBA		**ClEAN**		**VdB**	**Observations**
Song	Pulse Type	1st Peak	2nd Peak	1st Peak	2nd Peak	Bins Filled
CUT Off POINTS : 350 & 50 ms					
Yy5t14l2	Overall	25.62	21.37	400.00	25.81	34/46
	Thud	71.17	x	21.45	71.43	29/46
	Clack	68.03	x	400.00	25.81	25/46
Yy6t13	Overall	x	x	23.32	x	14/21
	Thud	x	x	x	x	12/21
	Clack	x	x	60.15	x	7/16
Yy6t14l	Overall	105.77	54.19	87.04	25.74	43/52
	Thud	143.52	x	73.72	157.53	32/45
	Clack	x	x	512.82	x	24/52
Yy7t14l	Overall	x	x	x	x	24/33
	Thud	36.16	28.89	x	x	23/33
	Clack	21.93	x	x	x	4/11
Yy8t13r	Overall	35.50	x	36.53	x	32/39
	Thud	x	x	150.94	615.38	32/39
	Clack	x	x	x	x	9/21
Yy9t13r	Overall	45.78	x	45.78	58.64	38/40
	Thud	59.01	45.45	61.54	45.71	35/40
	Clack	45.45	x	45.45	26.40	21/40
Yy10t10	Overall	102.89	24.26	24.24	103.90	50/59
	Thud	92.59	24.20	24.46	93.02	28/57
	Clack	30.82	24.11	31.01	x	22/52
Yy10t13	Overall	31.36	22.76	571.43	31.75	30/38
	Thud	22.53	x	22.54	x	26/38
	Clack	31.91	x	32.39	615.58	8/35
Yyh1t10	Overall	84.75	40.13	86.96	42.33	41/59
	Thud	84.75	x	86.02	41.25	31/59
	Clack	28.48	x	170.21	28.37	9/49

APPENDICES

FOR CHAPTER 4

APPENDIX 4.1:

CLEAN and VdB, with Monte Carlo simulations, spectral analyses of *D. teissieri* Sweden songs, using the lower 55 and 10ms cut-off points.

	SPECTRAL		ANALYSIS		
SPECIES	**Clean**		**Van der Berg**		**Observations**
	1ST PEAK	**2ND PEAK**	**1ST PEAK**	**2ND PEAK**	**BINS FILLED**
D. tessieri	CUT Off POINTS : 55 & 10 ms				
Te1t12l2	36.28	30.86	36.53	31.25	28/57
Te2t1l11	88.36	40.51	90.91	50.96	17/47
Te2t11r1	24.46	x	23.05	x	14/20
Te2t6r1	37.14	32.44	37.21	23.67	30/60
Te3t11r	72.70	24.48	72.02	x	30/79
Tei1t6r2	89.93	20.63	62.50	89.89	33/47
Tei2t6r2	86.10	21.61	86.96	21.68	29/59
Tei2t7r1	46.76	127.45	46.78	125.00	20/41
Tei3t7r1	69.21	26.85	70.80	x	60/78
Tei3r1t7	53.16	26.18	52.98	39.80	21/44
Tei3t1l11	258.62	94.34	320.00	112.68	17/34
Tei6t6r2	101.35	x	103.90	x	13/19

APPENDICES

FOR CHAPTER 5

APPENDIX 5.2.1:

One-way ANOVA between the periods of *D. yakuba*, *D. mauritiana* and their reciprocal
interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	3	2040.810	47	563.0552	3.624528	0.019590

Newman-Keuls *a posteriori* test between the periods of *D. yakuba*, *D. mauritiana* and their
reciprocal interspecific hybrids (p-values are given).

	D. yakuba (Thud + Clack)	*D. mauritiana*	*D. yakuba*(f) x *D. mauritiana*(m)	*D. mauritiana*(f) x *D. yakuba* (m)
D. yakuba (Thud + Clack)		0.099060	0.416296	0.347708
D. mauritiana			**0.026089**	0.252098
D. yakuba(f) x *D. mauritiana*(m)				0.191122

KEY : BOLD LETTERS = SIGNIFICANT RESULTS

APPENDIX 5.4.1:

One-way ANOVA between the mean IPIs of *D. simulans, D. mauritiana* and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	2	273.8587	23	78.76285	3.47703	0.047933

Newman-Keuls *a posteriori* test between the mean IPIs of *D. simulans, D. mauritiana* and their interspecific hybrids (p-values are given).

	D. simulans	*D. mauritiana*	*D. simulans*(f) x *D. mauritiana*(m)
D. simulans		**0.044747**	0.124559
D. mauritiana			0.345463

APPENDIX 5.4.2:

One-way ANOVA between the mean SSFs of *D. simulans, D. mauritiana* and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	2	229.8366	7	42.39455	5.421371	0.037821

Newman-Keuls *a posteriori* test between the mean SSFs of *D. simulans, D. mauritiana* and their interspecific hybrids (p-values are given).

	D. simulans	*D. mauritiana*	*D. simulans*(f) x *D. mauritiana*(m)
D. simulans		**0.048342**	0.877454
D. mauritiana			**0.026266**

KEY : BOLD LETTERS = SIGNIFICANT RESULTS

APPENDIX 5.5.1:

One-way ANOVA between the mean IPIs of *D. teissieri*, *D. mauritiana* and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	2	755.7551	40	40.6645	18.58513	0.000002

Newman-Keuls *a posteriori* test between the mean IPIs of *D. teissieri*, *D. mauritiana* and their interspecific hybrids (p-values are given).

	D. teissieri	*D. mauritiana*	*D. teissieri*(f) x *D. mauritiana*(m)
D. teissieri		0.000125	0.010366
D. mauritiana			0.0067061

APPENDIX 5.5.2:

One-way ANOVA between the modal IPIs of *D. teissieri*, *D. mauritiana* and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	2	369.595	40	12.18769	30.32526	0.00000

Newman-Keuls *a posteriori* test between the modal IPIs of *D. teissieri*, *D. mauritiana* and their interspecific hybrids (p-values are given).

	D. teissieri	*D. mauritiana*	*D. teissieri*(f) x *D. mauritiana*(m)
D. teissieri		0.000133	0.000122
D. mauritiana			0.022322

APPENDIX 5.5.3:

One-way ANOVA between the mean SSFs of *D. teissieri*, *D. mauritiana* and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	2	1453.937	8	34.00816	42.75260	**0.000054**

Newman-Keuls *a posteriori* test between the mean SSFs of*D. teissieri*, *D. mauritiana* and their interspecific hybrids (p-values are given).

	D. teissieri	*D. mauritiana*	*D. teissieri*(f) x *D. mauritiana*(m)
D. teissieri		**0.000243**	0.055843
D. mauritiana			**0.000369**

APPENDIX 5.5.4:

One-way ANOVA between the periods of *D. teissieri*, *D. mauritiana* and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
STRAIN	2	2761.923	26	439.1862	6.288728	0.005920

Newman-Keuls *a posteriori* test between the periods of *D. teissieri*, *D. mauritiana* and their interspecific hybrids (p-values are given).

	D. teissieri	*D. mauritiana*	*D. teissieri*(f) x *D. mauritiana*(m)
D. teissieri		**0.006759**	0.223193
D. mauritiana			**0.044922**

KEY : BOLD LETTERS = SIGNIFICANT RESULTS

APPENDICES

FOR CHAPTER 6

APPENDIX 6.1:

Regression of Mean Intrapulse Frequency(IPF) vs Position of IPF
in Burst for the ten longest bursts of song

$$\boxed{\text{EQUATION : IPF=bx+a} \quad \text{where x=time}}$$

Species/Individual songs	Slope (b)	Intercept (a)	r-value	F-value	p-value
melanogaster COMPLEX					
melanogaster	- 2.0905	300.898	0.625	6.41	0.030*
MEL1	- 1.8350	307.782	0.429	2.26	0.164
MEL2	- 3.3150	292.800	0.629	5.91	0.038*
MEL3	- 1.8980	304.740	0.409	2.41	0.146
mauritiana Sweden	- 2.4324	269.698	0.939	82.28	0.000*
MAUR1	- 1.3800	267.460	0.815	27.71	0.006*
MAUR2	- 5.4300	301.000	0.949	125.7	0.000*
MAUR3	- 0.1800	238.840	0.167	0.34	0.568
mauritiana France	0.3906	250.648	0.234	0.58	0.463
MAUR4	1.2395	245.810	0.488	4.38	0.055
MAUR5	- 0.8399	257.404	0.343	1.34	0.274
mauritiana Indiana	2.0289	376.322	0.531	5.11	0.042*
MAUR6	0.7530	395.220	0.170	0.39	0.545
MAUR7	2.9250	361.540	0.471	3.99	0.066
simulans Florida	- 0.0206	408.871	0.000	0.00	0.983
SIMFLO1	2.2260	390.860	0.055	0.13	0.741
SIMFLO2	4.3500	390.600	0.425	1.33	0.293
SIMFLO3	- 1.4170	434.450	0.050	0.10	0.767
SIMFLO4	- 1.0800	402.950	0.520	0.74	0.480
sechellia Cambridge	- 6.9231	401.284	0.932	52.75	0.000*
SECHEL1	- 3.9990	351.970	0.748	12.66	0.005*
SECHEL2	- 3.6210	345.610	0.508	2.78	0.134
SECHEL3	- 5.9110	443.280	0.637	5.48	0.047*
SECHEL4	-13.3600	472.670	0.875	26.02	0.000*
yakuba COMPLEX					
erecta France	- 0.1738	389.688	0.0632	0.040	0.848
EREC1	1.8232	354.990	0.5130	4.99	0.042*
EREC2	- 2.1940	447.510	0.3520	1.42	0.261
EREC3	1.0110	413.480	0.2430	0.87	0.366
EREC4	- 1.9200	270.210	0.3100	1.16	0.304
EREC5	- 1.6170	434.880	0.2970	0.87	0.376
teissieri Sweden	0.7880	433.041	0.2530	0.55	0.481
TEISS1	0.3830	427.330	0.0774	0.06	0.819
TEISS2	0.3656	435.171	0.1000	0.07	0.797
TEISS3	8.1740	400.293	0.9300	38.42	0.001*
TEISS4	- 2.0870	459.110	0.3560	1.02	0.346
TEISS5	0.1550	434.600	0.0316	0.01	0.936
orena France	- 9.1700	639.090	0.8720	25.50	0.000*
ORE1	- 10.293	590.910	0.7420	9.78	0.014*
ORE2	- 6.507	599.63	0.5210	2.99	0.122
ORE3	- 4.425	551.43	0.5504	4.35	0.640
ORE4	- 0.974	787.22	0.8070	8.81	0.012*

6.1:

Species/Individual songs		Slope (b)	Intercept (a)	r-value	F-value	p-value
yakuba Strains						
Strain	**Pulse type**					
yakuba Lamto3						
	THUD	- 3.7680	333.164	0.6440	4.97	0.061
	CLACK	0.2099	322.806	0.0548	0.02	0.089
YL1	THUD	- 6.0750	359.660	0.6530	5.19	0.057
	CLACK	- 4.3340	302.690	0.5890	4.26	0.730
YL2	THUD	- 1.6680	309.050	0.2280	0.39	0.554
	CLACK	- 1.9620	336.319	0.5030	2.37	0.168
yakuba France						
	THUD	- 2.5759	310.935	0.7040	9.83	0.011*
	CLACK	- 5.2982	365.600	0.8330	18.17	0.003*
YF1	THUD	- 3.8690	303.367	0.7321	11.57	0.007*
	CLACK	- 2.7170	302.490	0.4960	2.60	0.145
YF2	THUD	- 1.6350	321.410	0.3070	0.94	0.358
	CLACK	- 8.3810	430.470	0.8580	22.35	0.000*
yakuba S.T.						
	THUD	0.6276	290.745	0.2790	0.85	0.375
	CLACK	- 0.7120	426.310	0.1090	0.10	0.765
YS1	THUD	- 1.2166	272.064	0.4360	3.53	0.080
	CLACK	2.4750	426.460	0.1224	0.07	0.797
YS2	THUD	2.0964	304.122	0.5920	4.84	0.055
	CLACK	- 3.2690	425.140	0.3960	0.74	0.437
yakuba Malawi						
	THUD	- 0.3125	288.108	0.1840	0.28	0.610
	CLACK	- 0.1545	366.870	0	0.00	0.975
YM1	THUD	- 1.7400	281.160	0.6260	5.15	0.053
	CLACK	- 0.6770	362.890	0.0548	0.02	0.902
YM2	THUD	- 1.1146	310.120	0.3860	2.46	0.139
	CLACK	- 0.5270	371.410	0.0632	0.03	0.862
yakuba Ivory Coast						
	THUD	- 1.1570	348.268	0.3020	1.00	0.342
	CLACK	0.7730	415.310	0.1610	0.31	0.655
YIC1	THUD	- 2.3380	344.073	0.5150	3.60	0.087
	CLACK	4.5800	433.030	0.5920	4.32	0.071
YIC2	THUD	0.0250	352.430	0	0.00	0.987
	CLACK	- 2.7180	396.430	0.3330	1.00	0.347
INTRASPECIFIC HYBRIDS						
yakuba S.T.(f) x *yakuba* Lamto3(m)						
	THUD	- 0.9750	320.080	0.2080	0.37	0.562
	CLACK	- 4.8520	499.173	0.7290	9.11	0.017*
YSYL1	THUD	2.8820	291.120	0.5130	2.85	0.130
	CLACK	-11.597	537.290	0.8540	21.59	0.000*
YSYL2	THUD	- 1.825	330.900	0.3290	1.09	0.323
	CLACK	- 1.890	461.090	0.2790	0.68	0.434
yakuba Lamto3(f) x *yakuba* Ivory Coast(m)						
	THUD	- 4.4195	320.866	0.9340	54.4	0.000*
	CLACK	2.1206	425.007	0.7060	7.93	0.023*
YLYIC1	THUD	- 5.5279	322.213	0.8920	31.18	0.000*
	CLACK	1.4410	428.070	0.0774	0.51	0.494
YLYIC2	THUD	- 2.3810	315.780	0.5540	3.99	0.077
	CLACK	4.6560	414.861	0.7450	7.48	0.034*
yakuba Malawi(f) x *yakuba* S.T.(m)						
	THUD	- 0.6020	392.148	0.1820	0.34	0.572
	CLACK	- 1.8020	458.090	0.2950	0.76	0.409
YMYS1	THUD	0.6180	426.630	0.1140	0.12	0.741
	CLACK	- 0.3770	444.290	0.0548	0.03	0.876
YMYS2	THUD	- 1.6240	356.710	0.2550	0.63	0.448
	CLACK	- 2.1620	468.010	0.2850	0.61	0.459

6.1:

Species/Individual songs	Slope (b)	Intercept (a)	r-value	F-value	p-value
INTERSPECIFIC HYBRIDS					
simulans Florida(f) x *mauritiana* Sweden(m)	- 7.2544	549.890	0.8470	20.26	0.000*
SMHYB1	- 8.3240	570.570	0.6900	7.26	0.027
SMHYB2	- 6.2610	615.230	0.4670	2.23	0.174
SMHYB3	- 7.1700	463.860	0.6470	5.77	0.043*
teissieri Sweden(f) x *mauritiana* Sweden(m)	- 1.6750	549.460	0.1840	0.28	0.608
TMHYB1	- 1.8410	549.610	0.2050	0.35	0.568
TMHYB2	1.1860	523.530	0.1000	0.08	0.783
TMHYB3	- 4.9270	569.330	0.2881	0.73	0.418
mauritiana Sweden(f) x *yakuba* Ivory Coast(m)	- 0.7960	435.367	0.2350	0.58	0.465
MSYICHYB1	- 1.9150	468.060	0.3000	0.90	0.369
MSYICHYB2	- 1.2600	472.130	0.1643	0.25	0.631
MSYICHYB3	1.3930	363.210	0.2950	0.95	0.352
mauritiana Sweden(f) x *yakuba* Lamto3(m)	- 0.2021	413.756	0.0949	0.09	0.762
MSYLHYB1	1.3570	381.830	0.1414	0.18	0.679
MSYLHYB2	3.1800	374.850	0.6595	7.70	0.020*
MSYLHYB3	- 2.4500	472.910	0.6511	6.62	0.030*
MSYLHYB4	- 1.0220	415.479	0.2664	0.77	0.402
yakuba France(f) x *teissieri* Sweden(m)	0.38000	409.629	0.0316	0.06	0.816
YFTSHYB1	- 1.5780	365.340	0.3420	1.59	0.232
YFTSHYB2	0.5210	425.730	0.0200	0.002	0.889
YFTSHYB3	1.4300	444.349	0.3610	1.80	0.205
yakuba France(f) x *mauritiana* France(m)	- 1.4210	413.470	0.3950	1.85	0.203
YFMFHYB1	0.1010	394.000	0.0000	0	0.959
YFMFHYB2	- 0.2090	353.210	0.0447	0.03	0.868
YFMFHYB3	- 2.9189	483.439	0.7036	9.80	0.011*
yakuba Malawi(f) x *mauritiana* Sweden(m)	- 0.4900	410.683	0.200	0.29	0.606
YMMS1	- 1.4660	352.170	0.2240	0.42	0.535
YMMS2	0.5120	390.117	0.1265	0.16	0.697
YMMS3	0.7400	453.489	0.2050	0.30	0.598

KEY : * - SIGNIFICANT CORRELATION

APPENDIX 6.2:

Regression of Cycles per Cycles (CPP) vs Position of CPP in Burst for the ten longest bursts of song

EQUATION : CPP=bx+a   where x=time

Species/Individual songs	Slope (b)	Intercept (a)	r-value	F-value	p-value
melanogaster COMPLEX					
melanogaster	0.03627	1.96213	0.9290	63.79	0.000*
MEL1	0.05301	1.70024	0.8456	25.14	0.000*
MEL2	0.02764	2.20510	0.3990	1.51	0.254
MEL3	0.00282	2.08450	0.0316	0.01	0.922
mauritiana Sweden	0.05852	1.66669	0.8440	27.32	0.000*
MAUR1	0.05903	1.47610	0.6820	6.95	0.030*
MAUR2	0.05542	2.12420	0.6900	9.09	0.013*
MAUR3	0.03655	1.54430	0.5215	2.99	0.122
mauritiana France	0.01424	2.47550	0.2830	0.86	0.375
MAUR4	0.02198	2.44270	0.3332	1.00	0.347
MAUR5	0.01465	2.47380	0.2324	0.51	0.494
mauritiana Indiana	0.01683	2.15240	0.4990	3.98	0.069
MAUR6	-0.03182	2.79090	0.3098	0.96	0.354
MAUR7	-0.01091	2.59270	0.1897	0.34	0.575
simulans Florida	0.011679	1.40023	0.4701	2.27	0.170
SIMFLO1	0.04875	1.34140	0.7950	12.01	0.010*
SIMFLO2	0.00767	1.32770	0.1095	0.09	0.779
SIMFLO3	0.01460	1.46622	0.3674	1.09	0.330
SIMFLO4	0.00873	1.40890	0.1049	0.08	0.785
sechellia Cambridge	0.10593	2.42557	0.9430	64.75	0.000*
SECHEL1	0.11238	3.00100	0.5718	3.89	0.084
SECHEL2	0.13807	2.39690	0.8798	24.02	0.000*
SECHEL3	0.00458	2.47220	0.0447	0.01	0.919
SECHEL4	0.17436	1.81330	0.9274	55.15	0.000*
yakuba COMPLEX					
erecta France	0.02560	2.73880	0.0447	0.02	0.888
EREC1	0.02926	2.29500	0.3911	2.53	0.134
EREC2	-0.02210	2.76020	0.2510	0.41	0.547
EREC3	0.03427	2.75250	0.6900	12.70	0.003*
EREC4	0.00929	2.55080	0.1414	0.20	0.665
EREC5	0.00258	2.73880	0.0447	0.02	0.888
teissieri Sweden	0.00895	1.70684	0.5590	3.65	0.092
TEISS1	- 0.00060	1.52960	0.0316	0.000	0.946
TEISS2	0.03158	1.61407	0.8781	23.57	0.000*
TEISS3	-0.01763	1.54421	0.3886	1.070	0.341
TEISS4	0.00363	1.95028	0.0774	0.050	0.838
TEISS5	0.02281	1.91000	0.3886	1.420	0.268
orena France	0.04982	1.36593	0.8930	31.68	0.000*
ORE1	0.03939	1.40333	0.8081	15.07	0.005*
ORE2	0.06970	1.11670	0.6848	7.060	0.029*
ORE3	0.03182	1.54318	0.8614	28.82	0.000*
ORE4	0.02358	1.49340	0.7668	11.41	0.010*

6.2:

Species/Individual songs		Slope (b)	Intercept (a)	r-value	F-value	p-value
yakuba Strains						
Strain	Pulse type					
yakuba Lamto3						
	THUD	-0.00233	2.3449	0.0316	0	0.953
	CLACK	0.10373	2.0081	0.6840	6.17	0.042*
YL1	THUD	-0.00747	2.4647	0.0632	0.04	0.854
	CLACK	0.11633	2.0351	0.6090	4.71	0.062
YL2	THUD	0.00455	2.2880	0.0837	0.07	0.793
	CLACK	0.12788	1.8467	0.9343	55.10	0.000*
yakuba France						
	THUD	0.02101	1.6232	0.5270	3.85	0.078
	CLACK	0.10882	1.9741	0.8600	22.81	0.000*
YF1	THUD	0.17572	1.7376	0.8683	21.42	0.000*
	CLACK	0.05727	2.3214	0.6041	4.43	0.068
YF2	THUD	-0.00465	1.8287	0.0775	0.06	0.814
	CLACK	0.04091	1.4455	0.6550	6.76	0.029*
yakuba S.T.						
	THUD	0.03933	1.6873	0.4470	2.50	0.145
	CLACK	-0.02287	1.7464	0.4712	2.34	0.165
YS1	THUD	0.02383	1.6666	0.1225	0.06	0.816
	CLACK	0.00882	1.8618	0.1414	0.31	0.587
YS2	THUD	0.05067	1.6441	0.3873	1.58	0.240
	CLACK	-0.06721	1.8453	0.5329	1.98	0.218
yakuba Malawi						
	THUD	0.01292	1.8581	0.2680	0.62	0.453
	CLACK	0.08323	1.4626	0.8760	19.88	0.004*
YM1	THUD	-0.00103	2.0525	0	0.01	0.943
	CLACK	0.08241	1.5667	0.6641	6.30	0.036*
YM2	THUD	-0.00221	2.0625	0.0447	0.02	0.880
	CLACK	0.06909	1.4600	0.8355	18.51	0.003*
yakuba Ivory Coast						
	THUD	0.03421	2.0106	0.7400	12.10	0.006*
	CLACK	0.03467	2.0573	0.4540	2.08	0.188
YIC1	THUD	0.02832	1.9909	0.4775	2.96	0.116
	CLACK	0.00894	1.7183	0.1449	0.17	0.690
YIC2	THUD	0.03636	2.0470	0.4494	2.53	0.143
	CLACK	0.03697	2.4267	0.3755	1.31	0.285
INTRASPECIFIC HYBRIDS						
yakuba S.T.(f) x *yakuba* Lamto3(m)						
	THUD	0.01636	2.1600	0.3100	0.85	0.384
	CLACK	0.01576	2.5933	0.3320	0.99	0.349
YSYL1	THUD	0.00788	2.1067	0.0837	0.06	0.817
	CLACK	0.01212	2.6533	0.1581	0.21	0.660
YSYL2	THUD	-0.00035	2.1773	0	0.00	0.988
	CLACK	-0.00909	2.7000	0.1449	0.17	0.690
yakuba Lamto3(f) x *yakuba* Ivory Coast(m)						
	THUD	0.03773	1.9650	0.6620	6.24	0.037*
	CLACK	0.01030	2.0833	0.2390	0.48	0.507
YLYIC1	THUD	0.03243	1.9830	0.4035	1.93	0.194
	CLACK	-0.00182	1.7400	0.0316	0.01	0.923
YLYIC2	THUD	0.02168	2.0924	0.2846	0.88	0.369
	CLACK	-0.02197	1.9383	0.3975	1.50	0.256
yakuba Malawi(f) x *yakuba* S.T.(m)						
	THUD	0.01129	2.2715	0.1820	0.34	0.573
	CLACK	0.06007	1.4564	0.7100	8.12	0.021*
YMYS1	THUD	0.03427	1.8439	0.4680	2.80	0.125
	CLACK	0.05939	1.2333	0.8283	17.51	0.003*
YMYS2	THUD	0.00175	2.5636	0	0.00	0.949
	CLACK	0.02121	1.8133	0.2569	0.57	0.473

6.2:

Species/Individual songs	Slope (b)	Intercept (a)	r-value	F-value	p-value
INTERSPECIFIC HYBRIDS					
simulans Florida(f) x *mauritiana* Sweden(m)	0.01285	2.2076	0.3420	1.06	0.334
SMHYB1	0.02121	2.23333	0.4889	2.52	0.151
SMHYB2	0.03697	1.72667	0.6395	5.53	0.047*
SMHYB3	-0.01424	2.61330	0.1844	0.29	0.607
teissieri Sweden(f) x *mauritiana* Sweden(m)	0.01758	2.06030	0.2700	0.63	0.449
TMHYB1	-0.00424	2.08333	0.1183	0.11	0.748
TMHYB2	-0.01788	2.19333	0.4848	2.46	0.156
TMHYB3	-0.00061	2.13333	0	0.00	0.969
mauritiana Sweden(f) x *yakuba* Ivory Coast(m)	-0.00285	1.97605	0.0774	0.06	0.819
MSYICHYB1	0.00818	2.05090	0.1581	0.23	0.645
MSYICHYB2	-0.01182	1.98910	0.1643	0.25	0.631
MSYICHYB3	0.01154	1.81670	0.1673	0.29	0.600
mauritiana Sweden(f) x *yakuba* Lamto3(m)	0.02483	1.91360	0.2970	0.96	0.349
MSYLHYB1	0.02313	1.66280	0.3860	1.58	0.240
MSYLHYB2	0.00699	1.72120	0.1449	0.22	0.650
MSYLHYB3	0.01489	1.16708	0.4336	2.09	0.182
MSYLHYB4	0.02483	1.91360	0.2966	0.96	0.349
yakuba France(f) x *teissieri* Sweden(m)	-0.00306	1.27411	0.1790	0.20	0.673
YFTSHYB1	-0.09037	1.69190	0.6782	3.40	0.139
YFTSHYB2	-0.03874	1.56790	0.5206	1.49	0.290
YFTSHYB3	-0.01349	1.13571	0.5000	2.00	0.207
yakuba France(f) x *mauritiana* France(m)	0.00720	1.81052	0.2120	0.47	0.507
YFMFHYB1	0.00082	1.39666	0	0.00	0.949
YFMFHYB2	0.03000	1.52667	0.7457	16.27	0.001*
YFMFHYB3	0.06554	2.23050	0.5376	3.25	0.109
yakuba Malawi(f) x *mauritiana* Sweden(m)	0.01820	1.65069	0.7510	10.37	0.012*
YMMS1	- 0.00788	2.15330	0.1643	0.22	0.650
YMMS2	0.02194	1.16818	0.7134	4.48	0.860
YMMS3	0.02764	1.64773	0.6419	5.61	0.045*

KEY : * - SIGNIFICANT CORRELATION

APPENDIX 6.3:

Regression of Mean Interpulse Interval (IPI) vs Position of IPI
in Burst for the ten longest bursts of song

EQUATION : IPI=bx+a   where x=time

Species/Individual songs	Slope (b)	Intercept (a)	r-value	F-value	p-value
melanogaster COMPLEX					
melanogaster	0.11151	32.9994	0.4500	2.29	0.164
MEL1	0.17360	34.1330	0.2863	0.89	0.368
MEL2	-0.05250	32.8950	0.0707	0.05	0.829
MEL3	0.09598	32.5770	0.3130	1.08	0.323
mauritiana Sweden	-2.46740	57.2350	0.9590	115.40	0.000*
MAUR1	-2.03400	57.1860	0.8462	42.82	0.000*
MAUR2	-0.77360	42.5010	0.8112	30.81	0.000*
MAUR3	-1.82470	58.5540	0.9192	81.72	0.000*
mauritiana France	-1.43050	44.3610	0.9350	63.11	0.000*
MAUR4	-0.00300	46.0150	0	0.00	0.974
MAUR5	-2.00780	59.6690	0.9083	70.68	0.000*
mauritiana Indiana	-2.61900	64.1490	0.9560	129.57	0.000*
MAUR6	-1.85540	59.1880	0.9160	73.19	0.000*
MAUR7	-2.65550	64.8950	0.9455	118.67	0.000*
simulans Florida	0.40680	66.5790	0.2323	0.40	0.549
SIMFLO1	-0.25470	73.4100	0.1000	0.07	0.797
SIMFLO2	-1.69380	63.4510	0.6760	5.89	0.046*
SIMFLO3	0.20770	70.0890	0.1000	0.07	0.798
SIMFLO4	1.14900	53.3870	0.4219	1.52	0.257
sechellia Cambridge	-0.46450	100.035	0.4850	2.15	0.186
SECHEL1	-0.57090	100.005	0.4099	2.02	0.185
SECHEL2	-1.64970	115.306	0.6731	8.28	0.016*
SECHEL3	0.54940	97.0450	0.4324	2.30	0.160
SECHEL4	-3.06180	112.212	0.8591	28.20	0.000*
yakuba COMPLEX					
erecta France	-0.91260	51.0904	0.9150	46.71	0.000*
EREC1	-1.28770	50.1750	0.9284	62.45	0.000*
EREC2	-0.48850	47.5070	0.7456	12.52	0.005*
EREC3	-1.43580	56.3533	0.9757	200.28	0.000*
EREC4	-0.52990	43.7630	0.4754	2.92	0.188
EREC5	-0.36530	53.0450	0.2646	0.76	0.405
teissieri Sweden	0.20007	20.2320	0.8510	18.37	0.004*
TEISS1	0.22026	18.2141	0.6797	7.71	0.021*
TEISS2	0.60000	21.2020	0.7842	12.78	0.007*
TEISS3	-0.33024	20.9730	0.7846	12.85	0.007*
TEISS4	-0.27490	24.7380	0.5089	3.84	0.076
TEISS5	0.39876	17.7108	0.9317	52.69	0.000*
orena France	-1.52600	66.8010	0.7250	7.76	0.027*
ORE1	-1.64470	66.0640	06481	5.79	0.043*
ORE2	-0.03090	52.3000	0	0.00	0.970
ORE3	-0.97890	72.4020	0.4074	1.59	0.243
ORE4	-1.74330	69.0590	0.7655	11.32	0.010*

6.3:

Species/Individual songs		Slope (b)	Intercept (a)	r-value	F-value	p-value
yakuba Strains						
Strain	**Pulse type**					
yakuba Lamto3						
	THUD	3.5810	107.300	0.5540	2.66	0.154
	CLACK	- 0.0360	111.363	0.0000	0	0.983
YL1	THUD	- 1.2350	134.360	0.2449	0.71	0.419
	CLACK	- 2.1390	121.850	0.3742	1.14	0.320
YL2	THUD	1.11600	106.849	0.2665	0.61	0.457
	CLACK	1.61300	104.246	0.3808	1.69	0.222
yakuba France						
	THUD	- 3.0250	145.900	0.5200	3.33	0.101
	CLACK	- 1.77100	129.461	0.4580	1.86	0.215
YF1	THUD	- 2.2003	139.730	0.4970	4.93	0.042*
	CLACK	- 4.3535	144.987	0.8706	28.21	0.000*
YF2	THUD	- 2.4500	143.840	0.4701	3.13	0.105
	CLACK	3.0500	106.780	0.4561	2.37	0.158
yakuba S.T.						
	THUD	- 2.9509	114.690	0.8570	24.88	0.000*
	CLACK	- 2.8980	153.469	0.6750	5.87	0.046*
YS1	THUD	- 0.3129	86.9780	0.2490	0.98	0.337
	CLACK	- 9.5200	191.190	0.6870	4.47	0.088
YS2	THUD	- 4.8125	134.367	0.8944	35.89	0.000*
	CLACK	4.2270	114.800	0.4037	0.97	0.369
yakuba Malawi						
	THUD	- 6.9253	162.490	0.9945	209.00	0.000*
	CLACK	- 6.5301	150.802	0.9884	628.25	0.000*
YM1	THUD	- 6.2196	154.649	0.9859	281.62	0.000*
	CLACK	- 4.4704	145.403	0.9170	42.27	0.000*
YM2	THUD	- 4.8539	157.513	0.9428	119.23	0.000*
	CLACK	- 6.3416	149.175	0.9889	361.01	0.000*
yakuba Ivory Coast						
	THUD	- 3.3627	111.106	0.7820	14.17	0.004*
	CLACK	- 0.8162	118.380	0.4610	1.90	0.211
YIC1	THUD	- 0.0393	84.5020	0.0316	0.02	0.896
	CLACK	4.6400	91.1390	0.8367	18.68	0.003*
YIC2	THUD	- 2.4784	111.059	0.8000	28.50	0.000*
	CLACK	- 5.6995	142.851	0.9685	120.57	0.000*
INTRASPECIFIC HYBRIDS						
yakuba S.T.(f) x *yakuba* Lamto3(m)						
	THUD	- 0.9563	99.1860	0.3990	1.33	0.287
	CLACK	- 1.3020	124.159	0.3520	0.99	0.352
YSYL1	THUD	0.7332	83.2630	0.5394	3.29	0.107
	CLACK	- 0.2040	118.807	0.0548	0.02	0.881
YSYL2	THUD	- 1.6512	111.770	0.5718	5.34	0.041*
	CLACK	- 1.6970	126.944	0.4416	1.94	0.201
yakuba Lamto3(f) x *yakuba* Ivory Coast(m)						
	THUD	- 0.5727	100.088	0.3600	1.05	0.340
	CLACK	- 0.1042	140.279	0.0447	0.01	0.912
YLYIC1	THUD	0.6093	95.0770	0.2074	0.45	0.517
	CLACK	- 2.8330	148.600	0.5109	2.47	0.160
YLYIC2	THUD	0.0715	97.8480	0.0447	0.02	0.898
	CLACK	1.5950	136.137	0.4062	1.58	0.244
yakuba Malawi(f) x *yakuba* S.T.(m)						
	THUD	0.5655	95.2360	0.2740	0.73	0.416
	CLACK	2.6232	114.561	0.7060	6.97	0.033*
YMYS1	THUD	1.5598	77.2350	0.7211	11.93	0.005*
	CLACK	2.2930	118.322	0.4472	1.74	0.228
YMYS2	THUD	- 0.2650	112.458	0.0632	0.04	0.853
	CLACK	2.1685	113.676	0.6641	7.09	0.026*

6.3:

Species/Individual songs	Slope (b)	Intercept (a)	r-value	F-value	p-value
INTERSPECIFIC HYBRIDS					
simulans Florida(f) x *mauritiana* Sweden(m)	1.8013	45.3530	0.868	21.31	0.000*
SMHYB1	0.0109	37.1200	0	0.000	0.955
SMHYB2	- 0.4955	63.4370	0.2510	0.67	0.431
SMHYB3	0.6295	50.0720	0.2610	0.66	0.438
teissieri Sweden(f) x *mauritiana* Sweden(m)	- 0.3308	41.5300	0.4380	1.67	0.238
TMHYB1	- 1.1174	49.4950	0.8025	18.12	0.000*
TMHYB2	- 0.6693	40.0560	0.6395	6.93	0.025*
TMHYB3	- 0.0778	38.0860	0.0632	0.03	0.860
mauritiana Sweden(f) x *yakuba* Ivory Coast(m)	0.8087	107.755	0.4560	2.37	0.158
MSYICHYB1	0.2823	95.1770	0.1304	0.18	0.683
MSYICHYB2	1.5040	119.868	0.5514	4.37	0.063
MSYICHYB3	0.1020	110.440	0.0316	0.01	0.927
mauritiana Sweden(f) x *yakuba* Lamto3(m)	0.3697	85.8770	0.3350	1.14	0.313
MSYLHYB1	0.1082	107.686	0.0632	0.004	0.842
MSYLHYB2	- 0.1532	102.701	0.1000	0.11	0.747
MSYLHYB3	0.0952	60.7860	0.0447	0.02	0.886
MSYLHYB4	0.8309	77.0210	0.4637	3.01	0.110
yakuba France(f) x *teissieri* Sweden(m)	- 0.3525	78.7530	0.1580	0.13	0.733
YFTSHYB1	- 0.7515	77.7420	0.3962	1.68	0.228
YFTSHYB2	- 0.1020	77.7010	0.0316	0.00	0.948
YFTSHYB3	- 0.0620	77.5440	0	0.00	0.968
yakuba France(f) x *mauritiana* France(m)	1.0605	47.8400	0.7730	13.40	0.005*
YFMFHYB1	0.0689	37.6070	0.0949	0.09	0.772
YFMFHYB2	1.1423	45.7040	0.7994	17.74	0.002*
YFMFHYB3	0.8790	88.3230	0.2501	0.67	0.433
yakuba Malawi(f) x *mauritiana* Sweden(m)	- 0.6123	44.4070	0.6530	5.20	0.057
YMMS1	0.1580	33.1760	0.4461	2.49	0.146
YMMS2	0.5965	42.1070	0.2627	0.74	0.411
YMMS3	0.3422	41.5570	0.4550	2.62	0.137

KEY : * - SIGNIFICANT CORRELATION

APPENDIX 6.4:

Temporal changes in IPF, CPP and IPI for all the members species of the *D. melanogaster* subgroup. (Note that the individual song trends are presented).

Species/Strain	Intrapulse Frequency (IPF) Overall		Individual songs		Cycles per Pulse (CPP) Overall		Individual songs		Interpulse Interval (IPI) Overall		Individual songs	
	Thud	Clack	Thud	Clack	Thud	Clack	Thud	Clack	Thud	Clack	Thud	Clack
melanogaster(Brighton)	↓		All 3 ↓		↑		All 3 ↑		↑		2↑,1↓	
simulans (Florida)	Slight ↓		2↑,2↓		↑		4↑		↑		2↓,2↑	
mauritiana(Sweden)	↓		3↓		↑		3↑		↓		3↓	
mauritiana (France)	Slight ↑		1↑,1↓		↑		1↑,1↓		↓		2↓	
mauritiana(Indiana)	↑		2↑		↑		2↑		↓		2↓	
sechellia(Cambridge)	↓		All 4↓		↑		3↑,1↓		↓		3↓,1↑	
teissieri (Sweden)	↑		4↓,1↑		↓		2↑,3↓		↑		3↑,2↓	
orena (France)	↓		4↓		↑		4↑		↓		3↓,1↑	
erecta (France)	↓		2↑,3↓		↑		3↑,2↓		↓		5↓	
yakuba Strains	Thud	Clack	Thud	Clack	Thud	Clack	Thud	Clack	Thud	Clack	Thud	Clack
France	↓	↓	↓	↓	↑	↑	↓	↑	↓	↓	↓	1↑,1↓
Ivory Coast	↓	↑	1↑,1↓	1↑,1↓	↑	↑	2↑	1↑,1↓	↓	↓	1↑,1↓	1↑,1↓
Lamto3	↓	↑	2↓	1↑,1↓	↓	↑	1↑,1↓	2↑	↑	↓	1↑,1↓	1↑,1↓
Malawi	↓	↓	2↓	2↓	↑	↑	2↓	2↑	↓	↓	2↓	2↓
S.T.	↑	↓	1↑,1↓	1↑,1↓	↑	↓	2↑	1↑,1↓	↓	↓	2↓	1↑,1↓
yakuba Intraspecific Hybrids												
Malawi(f) x S.T.(m)	↓	↓	1↑,1↓	2↓	↑	↑	2↑	2↑	↑	↑	1↑,1↓	2↑
S.T.(f) x Lamto3(m)	↓	↓	1↑,1↓	1↑,1↓	↑	↑	1↑,1↓	1↑,1↓	↓	↓	1↑,1↓	2↓
Lamto3(f) x Ivory Coast(m)	↓	↑	2↓	2↑	↑	↑	2↑	2↓	↓	↓	2↑	1↑,1↓

KEY: ↑ = Increase, ↓ = Decrease

APPENDIX 6.5

APPENDIX 6.5

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃

A IPF (Hz)

y = -1.421x + 413.470

B CPP

y = 0.0072x + 1.81052

C IPI(ms)

y = 1.0605x + 47.840*

POSITION
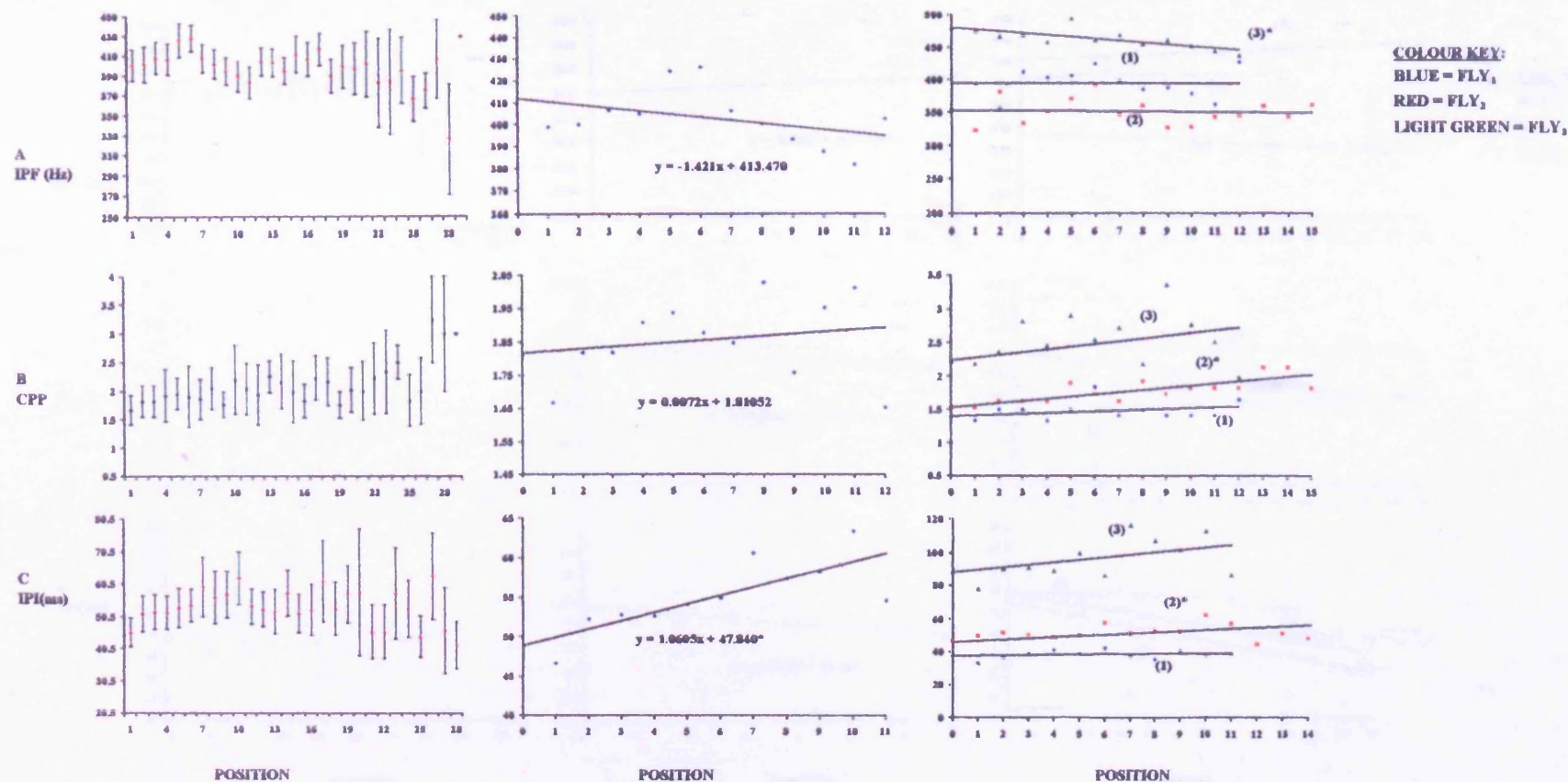
POSITION

POSITION

Figure 6.18 : *D. yakuba* France(f) x *D. mauritiana* France(m)-Hybrid : Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃

A IPF (Hz)

B CPP

C IPI(ms)

$y = 0.380 + 409.629$

$y = -0.003064x + 1.27411$

$y = -0.3528x + 78.753$

POSITION   POSITION   POSITION
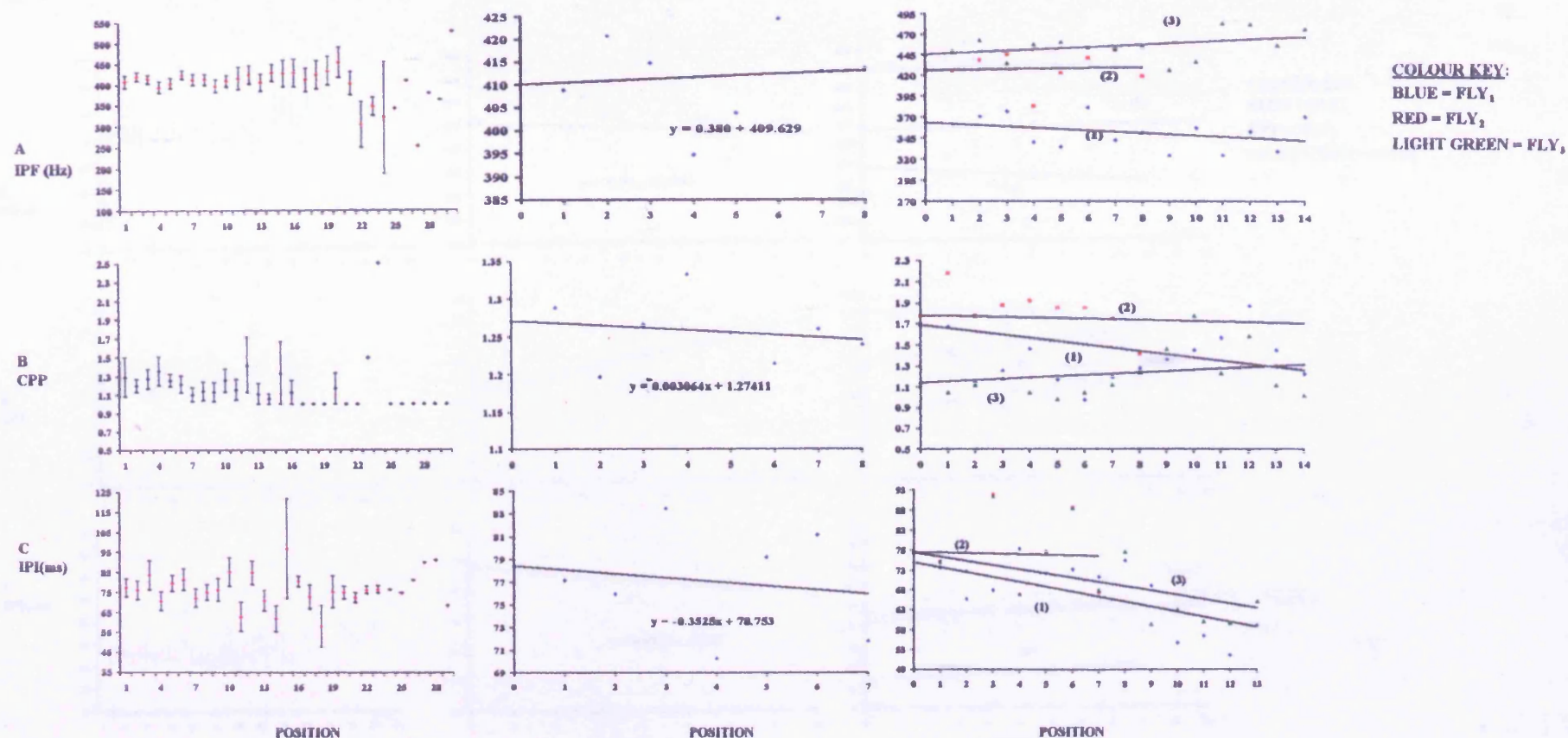
Figure 6.20 : *D. yakuba* France(f) x *D. teissieri* Sweden(m)-Hybrid: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY$_1$
RED = FLY$_2$
LIGHT GREEN = FLY$_3$

A
IPF (Hz)

y = -0.49x + 410.683

B
CPP

y = 0.018199x + 1.65069*

C
IPI(ms)

y = -0.6123x + 44.407

POSITION
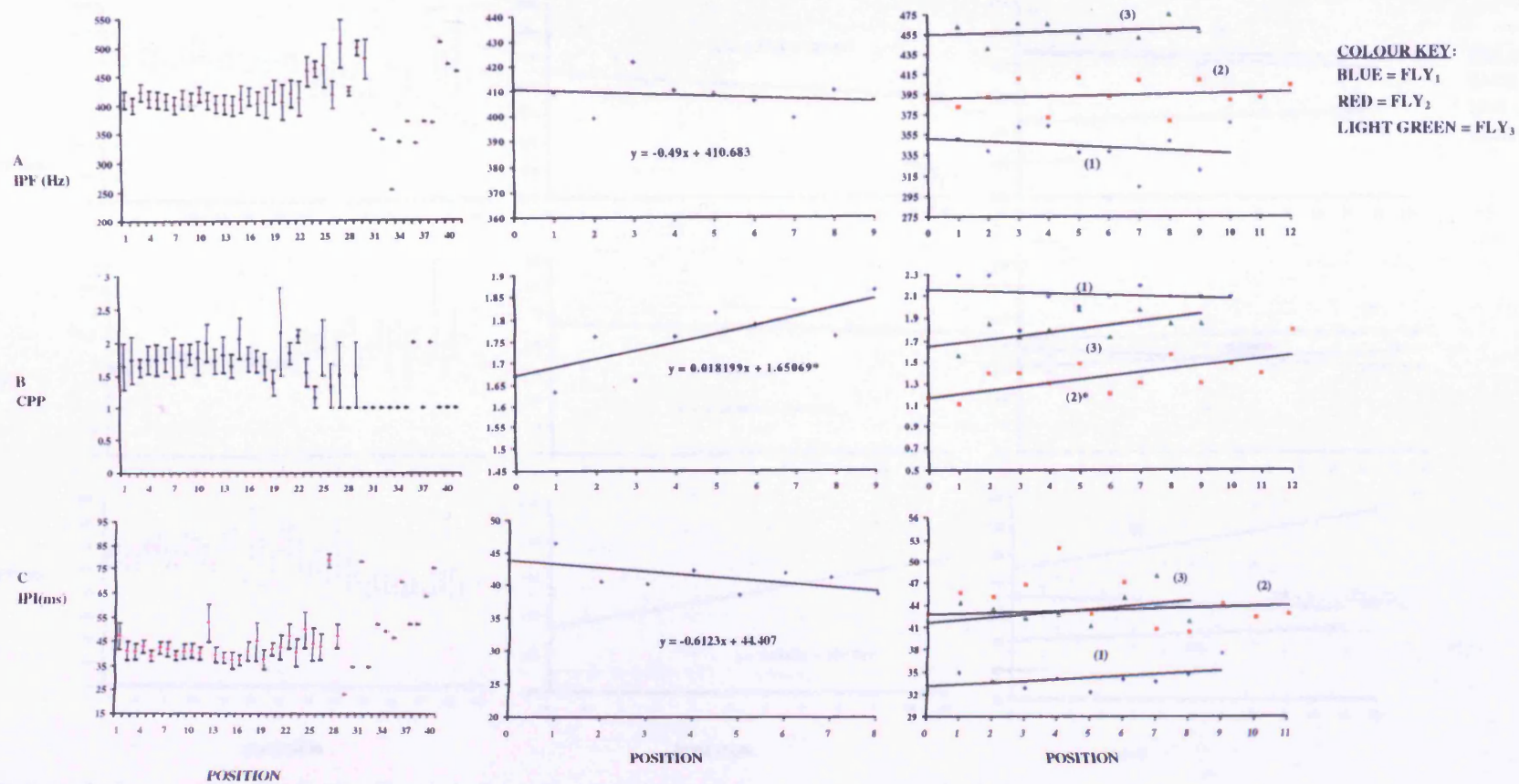
POSITION

POSITION

Figure 6.19 : *D . yakuba* Malawi(f) x *D . mauritiana* Sweden(m)-Hybrid: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃
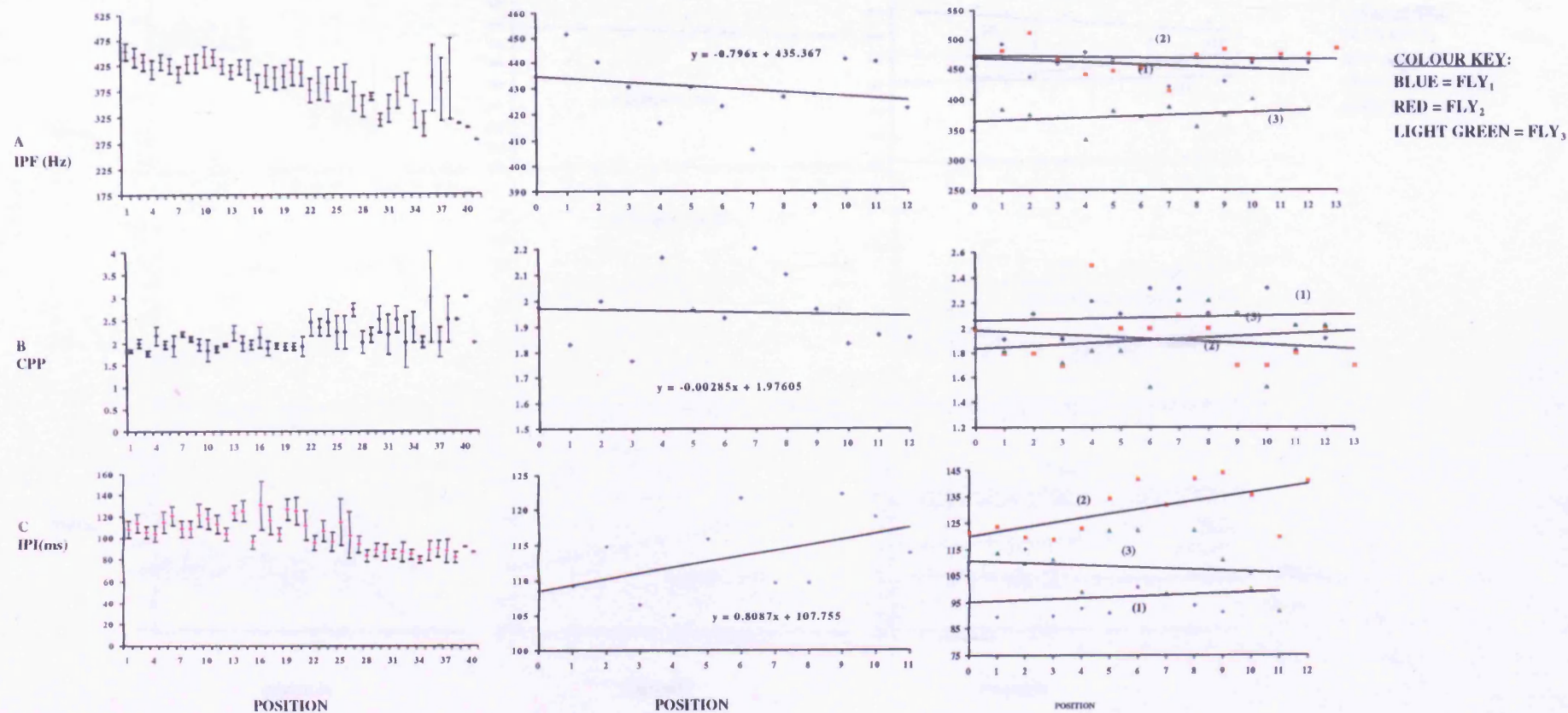
Figure 6.21 : *D . mauritiana* Sweden(f) x *D . yakuba* Ivory Coast(m): Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).
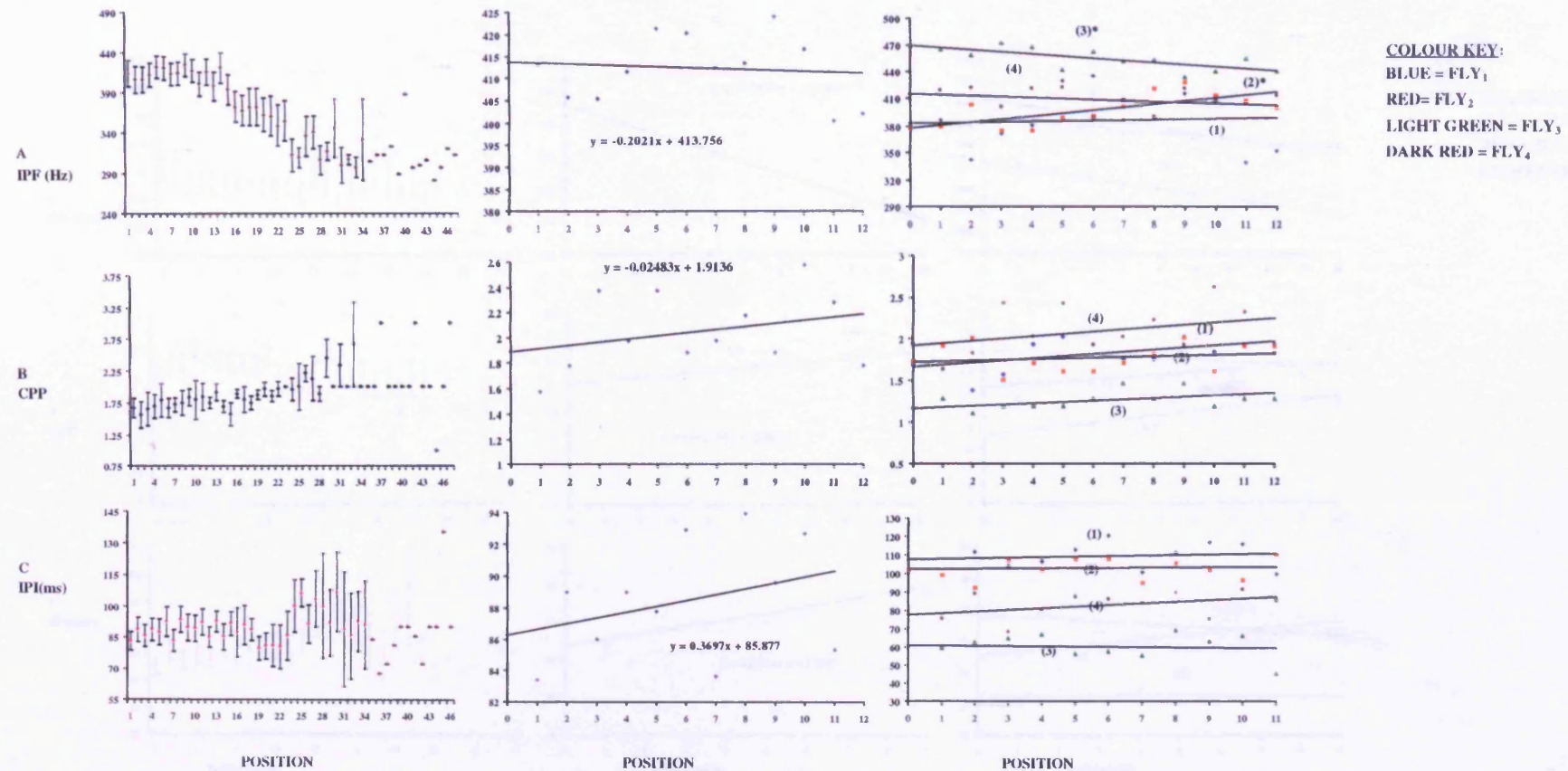
Figure 6.22 : *D . mauritiana* **Sweden(f) x** *D . yakuba* **Lamto3(m)-Hybrid: Song elements versus pulse position in burst**

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY$_1$
RED = FLY$_2$
LIGHT GREEN = FLY$_3$
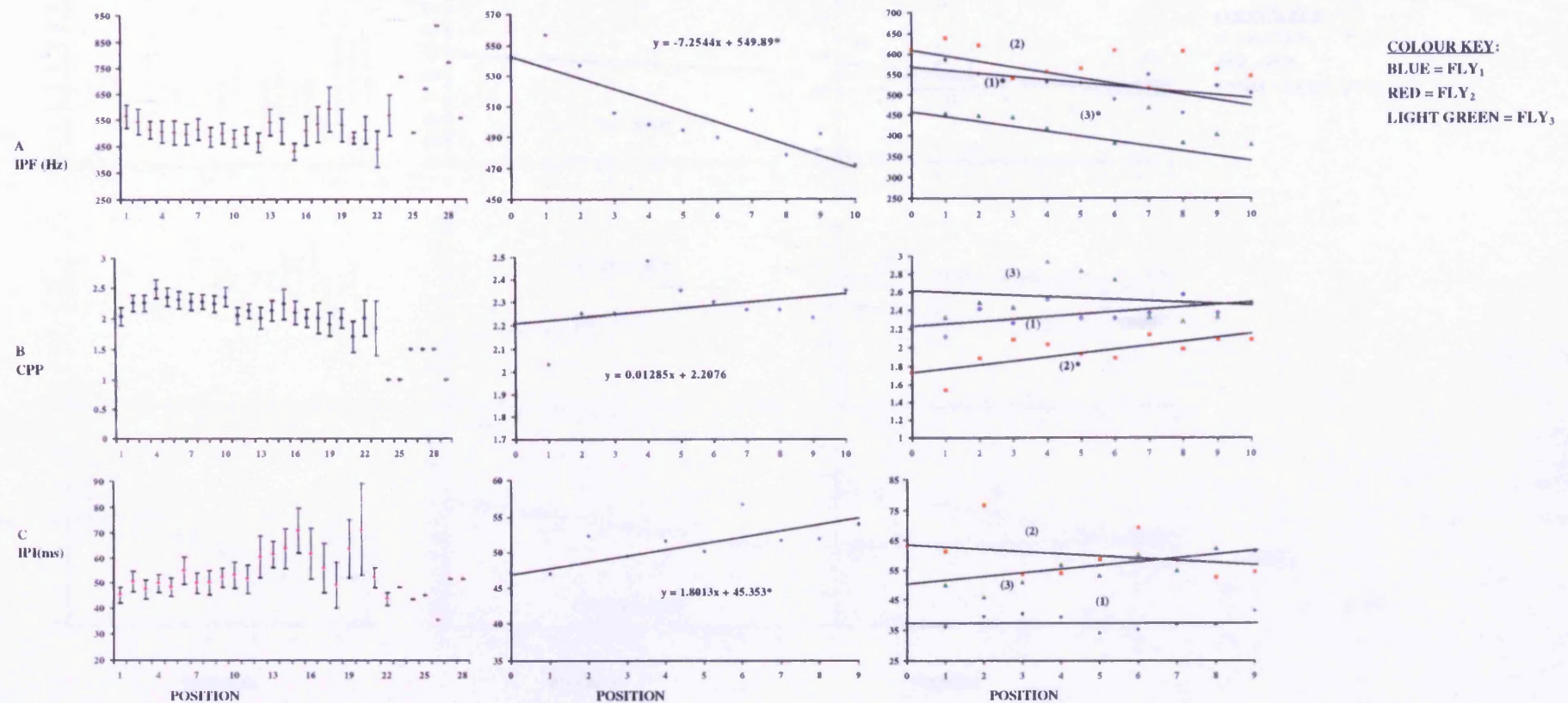
Figure 6.23 : *D . simulans* Florida(f) x *D . mauritiana* Sweden(m)-Hybrid: Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT -   REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

COLOUR KEY:
BLUE = FLY₁
RED = FLY₂
LIGHT GREEN = FLY₃

A
IPF (Hz)

$y = -1.675x + 549.46$

B
CPP

$y = 0.01758x + 2.0603$

C
IPI(ms)

$y = -0.3308 + 41.530$

POSITION
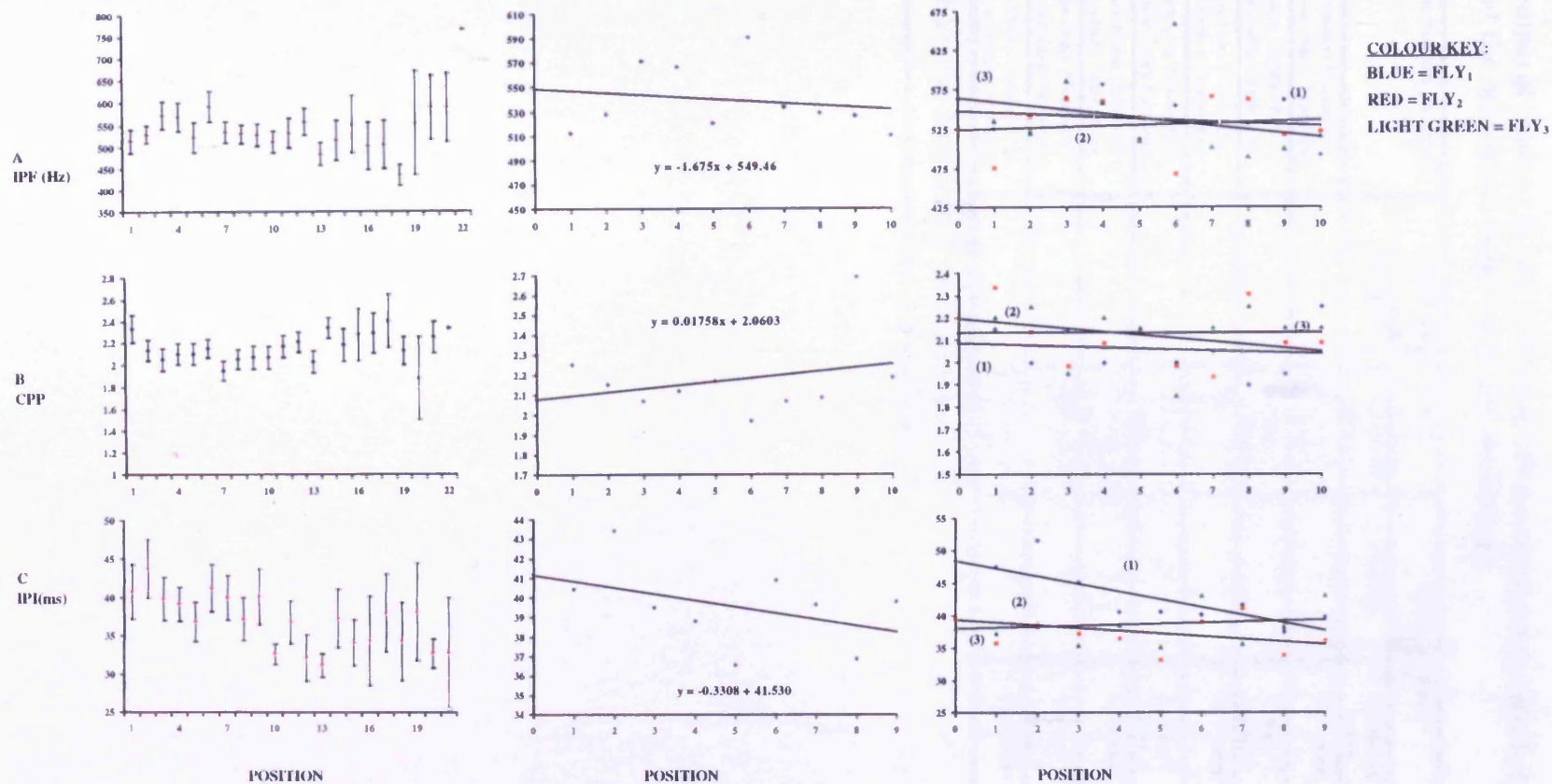
POSITION

POSITION

Figure 6.24 : *D . teissieri* Sweden(f) x *D . mauritiana* Sweden(m)-Hybrid : Song elements versus pulse position in burst

TOP ROW : A - INTRAPULSE FREQUENCY (IPF)
MIDDLE ROW : B - CYCLES PER PULSE (CPP)
BOTTOM ROW : C - INTERPULSE INTERVAL (IPI)

COLUMN: LEFT - MEAN OF ELEMENT VERSUS PULSE NUMBER
MIDDLE - SPECIES REGRESSION FOR ELEMENT MEANS
RIGHT - REGRESSION FOR ELEMENT IN INDIVIDUAL SONG
ASTERISKS DENOTE SIGNIFICANT REGRESSIONS (SEE APPENDICES 6.1, 6.2 & 6.3).

APPENDIX 6.6:

Temporal changes in IPF, CPP and IPI for the interspecific hybrid songs. (Note that the individual song trends are presented).

Interspecific genotypes	Intrapulse Frequency (IPF)		Cycles per Pulse (CPP)		Interpulse Interval (IPI)	
	Overall	Individual songs	Overall	Individual songs	Overall	Individual songs
yakuba France(f) x *mauritiana* France(m)	↓	2 songs ↓ 1 song ↑	↑	All 3 songs ↑	↑	All 3 songs ↑
yakuba Malawi(f) x *mauritiana* Sweden(m)	↓	2 songs ↑ 1 song ↓	↑	2 songs ↑ 1 song ↓	↓	All 3 songs ↑
yakuba France(f) x *teissieri* Sweden(m)	↑	2 songs ↑ 1 song ↓	↓	All 3 songs ↓	↓	All 3 songs ↓
mauritiana Sweden(f) x *yakuba* Ivory Coast(m)	↓	2 songs ↓ 1 song ↑	↓	2 songs ↑ 1 song ↓	↑	All 3 songs ↑
mauritiana Sweden(f) x *yakuba* Lamto3(m)	↓	2 songs ↑ 2 songs ↓	↑	All 4 songs ↑	↑	3 songs ↑ 1 song ↓
simulans Florida(f) x *mauritiana* Sweden(m)	↓	All 3 songs ↓	↑	2 songs ↑ 1 song ↓	↑	2 songs ↑ 1 song ↓
teissieri Sweden(f) x *mauritiana* Sweden(m)	↓	2 song s ↓ 1 song ↑	↑	All 3 songs ↓	↓	All 3 songs ↓

KEY: ↑ = Increase, ↓ = Decrease

198

SUMMARY FOR APPENDICES 6.7, 6.8 & 6.9:

In *D. mauritiana* France, half of the songs were shown to have shorter mbd and mibi for the first half of the song and half were shown to have shorter mbd and mibi for the second half of the song. In *D. mauritiana* Sweden, the great majority of songs examined showed shorter mbd and ibi for the first half of the song, while in *D. mauritiana* Indiana, the mbd and ibi of the second half of the song was shorter than those of the first half (Appendix : 6.7). In *D. yakuba* strains, the only parental strain that was shown to have shorter mbd and ibi in second half of the song was Malawi. Furthermore, all the *yakuba* intraspecific hybrids were shown to have a mbd and ibi shorter in the first half of the song (Appendix 6.8). In *D. erecta* and *D. orena* strains examined, both the mbd and ibi were shown to be shorter for the first half of the song than the second half (Appendix : 6.8). Upon examining the results of the various interspecific hybrids, the majority of the songs were shown to have a shorter mbd and ibi for the first half of the songs, while others had shorter mbd and ibi in the course of the second half, such as the *D. mauritiana*(f) x *D. yakuba*(m) interspecific genotype (see Appendix : 6.9).

APPENDIX 6.7:

Mean Burst Duration & Mean Interburst Interval for songs of members of the *melanogaster* complex

SPECIES:	Mean burst duration for the entire song(ms)	Mean burst duration for the first half of the song (ms)	Mean burst duration for the second half of the song(ms)	Mean Interburst Interval for the entire song(s)	Mean Interburst Interval for the first half of the song (s)	Mean Interburst Interval for the second half of the song (s)
melanogaster						
MB5t10r1	435.99	359.42	512.56	1.6602	1.2897	2.0307
MB4t10r1	444.81	377.39	512.23	1.2760	0.9657	1.5963
MB3t10r1	352.33	321.98	382.68	2.1625	1.8374	2.4876
MB2t10r1	370.81	296.78	446.84	1.7535	1.4883	2.0187
MB1t10r1	383.58	301.48	465.68	1.3841	0.9793	1.7889
simulans						
SI3t10r1	457.76	528.47	387.05	18.2027	11.5649	24.9405
SI2t10r1	772.48	894.78	650.18	14.2071	17.6745	10.7357
SI1t10r1	901.73	674.57	1128.99	16.1608	19.0431	13.2785
SI1t10l2	555.71	784.87	325.55	16.6755	21.7859	11.5651
SI5E2S1	476.81	328.57	625.05	7.6102	5.9763	9.2451
mauritiana						
MISC3t13l1	559.06	561.32	556.80	3.5769	3.5837	3.5701
MISC1t14l2	674.19	683.24	665.14	13.3707	13.5897	13.1517
MAS1t13r1	725.67	654.98	796.36	8.6014	7.9853	9.2175
MAS5t10r1	449.49	358.38	540.60	4.7294	3.5873	5.8715
MAS4t7l2	332.13	328.51	335.75	18.1718	15.7548	20.5888
MAS1t6r1	316.35	320.24	312.46	10.9660	11.6521	10.2799
MA4t10r1	325.64	325.07	326.21	1.8189	1.8175	1.8203
MAS1t6l2	390.75	396.51	384.99	5.5587	5.5478	5.5696
MA6t10r1	357.67	358.97	356.37	5.3813	5.4128	5.3498
MAU3t8r1	325.16	328.64	321.68	2.7071	2.7195	2.6947
MAU2t8l2	390.78	396.54	385.02	7.1286	7.3214	6.9358
MAU3t8l2	441.55	399.52	483.58	5.8436	5.7548	5.9324
MAU5t7l2	333.49	331.59	335.39	35.3339	33.8534	36.8144
sechellia						
S6SE11R1	981.48	954.87	1008.09	3.7262	3.1548	4.2976
S2SE11R1	1257.23	1058.72	1455.74	4.7623	3.9857	5.5389
S5SE11R1	1005.84	857.64	1154.04	7.6804	7.2398	8.1210
S8SE11R2	907.82	785.46	1030.18	5.6678	4.2368	7.0988
S7SE11R2	902.43	871.02	933.84	10.1428	12.3254	7.9602
S1SE11R2	1187.65	987.23	1388.07	13.6154	12.3654	14.8654
SE1SE6S2	963.79	945.86	981.72	9.1626	8.2165	10.1087
S11S11R2	1316.65	1534.67	1098.63	26.6141	32.1548	21.0734

200

APPENDIX 6.8:

Mean Burst Duration & Mean Interburst Interval for songs of members of the *yakuba* complex

SPECIES:	Mean burst duration for the entire song(ms)	Mean burst duration for the first half of the song (ms)	Mean burst duration for the second half of the song(ms)	Mean Interburst Interval for the entire song(s)	Mean Interburst Interval for the first half of the song (s)	Mean Interburst Interval for the second half of the song (s)
teissieri						
TEl6t6r2	252.51	242.89	362.13	4.9885	3.6548	6.3222
TE1t6r2	123.31	103.97	142.65	4.1956	3.8654	4.5258
TEl2t6r2N	209.04	193.63	224.45	3.9358	3.6978	4.7738
TEl3t7r1	161.94	117.89	205.99	3.7450	3.2985	4.1915
TE3t11r1	274.02	139.65	408.39	3.9554	3.5874	4.3234
TE2t11r1	327.81	205.98	449.64	3.6580	3.5963	3.7197
TEl2t7r1	167.62	154.39	180.85	5.7290	4.4698	6.9882
TEl1t6r1	206.90	165.43	248.37	3.5588	2.8965	4.2211
erecta						
EL2t13l1	750.41	625.31	875.51	16.3972	14.3267	18.4677
EL1t13l1	704.66	583.65	825.67	9.1148	8.1392	10.0904
EL2t12l1	720.26	589.58	850.94	7.4428	5.2354	9.6502
EL5t13l1	488.56	495.23	481.89	18.0391	13.9867	22.0915
EL1t11r2	744.07	523.12	965.02	16.3344	14.9832	17.6856
EL3t12l1	866.96	625.63	1108.21	5.8470	4.6321	7.0613
EL1t13r2	1308.87	1253.83	1363.91	16.9150	15.8623	17.9677
EL10E2S2M1	1135.80	1025.98	1245.62	23.5732	19.9545	27.1919
EL4E2S2M1	1376.28	1234.35	1518.21	17.5560	15.4387	19.6733
EL3E2S2M1	1247.87	1170.32	1325.42	16.1597	14.5246	17.7948
EL5E2S2M1	1310.52	1196.65	1424.39	17.8212	15.9852	19.6572
orena						
OR2t12l1	591.65	456.28	727.02	4.4701	3.8597	5.0805
OR3t11r2	651.46	498.76	804.16	10.4021	9.3258	11.4784
OR3t12l1	759.32	695.34	823.30	2.9997	1.9872	4.0122
OR4t12l1	477.80	398.62	556.98	2.2684	1.9564	2.5804
OR1t12l1	693.29	708.65	677.93	4.3230	3.5217	5.1243
OR3SE10r1	628.67	594.35	662.99	2.5276	2.3798	2.6754
OR7SE1l1	946.72	835.64	1057.80	4.6136	4.7513	4.4759
OR5SE10r2	695.69	556.98	834.40	5.4085	4.4386	6.3784
OR8SE1l1l	974.49	867.54	1081.44	3.9538	3.1287	4.7789
OR6SE10r2	943.40	987.53	899.27	2.9732	3.1054	2.8410
OR1SE10r2	586.04	832.64	339.44	3.1746	4.2568	2.0924
OR4SE10r2	843.69	839.62	847.76	3.4798	3.3897	3.5699
OR2SE10r2	702.48	698.32	706.64	1.1824	1.0987	1.2661
yakuba						
Yam1t5r2	1408.55	1596.21	1220.89	18.3405	15.2381	21.4429
Yam2t9r2	1458.59	1365.89	1551.29	11.7863	11.8594	11.7132
Yam3t9r2	1456.50	1385.32	1527.68	11.2452	11.3896	11.1008
Yic2t13r1	2197.28	2054.36	2340.20	11.3563	10.9632	11.7494
Yic1t13r1	2359.03	2285.36	2432.70	7.6757	7.5893	7.7621
Yk7t1r1	1403.40	1324.58	1482.22	5.0609	4.8263	5.2955
Yk2t1l2	1128.25	836.92	1419.58	2.9466	2.3154	3.5778
Yk1t1l2	1147.68	925.64	1369.72	2.6807	2.4587	2.9027
Yk6t1r2	1239.60	1256.31	1222.89	2.7904	2.9548	2.6260
Yk3t1l2	1720.87	1756.25	1685.49	2.4564	2.5398	2.3730
Yk20t1r1	1508.23	1510.56	1505.90	1.6364	1.6378	1.6342
Yk4t1r1	1686.60	1523.87	1849.33	1.3787	1.4695	1.2879
Yal2t9r1	900.38	789.32	1011.44	3.2215	3.1405	3.3025
Yal1t10l1	1430.44	1489.65	1371.23	5.4844	4.9682	6.0006
Yal3t9r1	1230.73	1268.32	1193.14	4.4626	3.2985	5.6267
Yst1t11r1	1597.89	1652.63	1543.15	12.2732	13.1456	11.4008
Yst1t10r2	1306.51	1185.79	1427.23	10.3236	11.3625	9.2847
Yst3t11r1	1484.89	1372.34	1597.44	15.9640	16.5843	15.3437
Yak10e2s2m1	2738.20	2614.83	2861.57	3.5157	3.5032	3.5282
Yak2e2s2	3519.46	3284.36	3754.56	1.6927	1.6234	1.7620
Yak8e2s2m1	4023.62	2689.24	5358.00	1.1962	1.0658	1.3266
Yak9e2s2m1	3411.55	3258.65	3564.45	1.4069	1.3256	1.4882

6.8:

SPECIES:	Mean burst duration for the entire song(ms)	Mean burst duration for the first half of the song (ms)	Mean burst duration for the second half of the song(ms)	Mean Interburst Interval for the entire song(s)	Mean Interburst Interval for the first half of the song (s)	Mean Interburst Interval for the second half of the song (s)
Intraspecific Hybrids						
Yal3Yic2t14l2	2209.76	1987.54	2431.98	3.1338	3.3216	2.9460
Yal3Yic3t14l2	2859.89	2967.43	2752.35	2.3206	2.5413	2.0999
Yal3Yic1t14l2	2531.31	2168.35	2894.27	2.3187	2.3814	2.2560
Yal3Yic1t13r2	1985.59	1798.32	2172.86	4.3321	4.1235	4.5407
Yal3Yic1t10r1	1038.61	1125.38	951.84	5.2166	5.2368	5.1964
YamYst2t10r2	1519.31	1482.32	1556.30	2.6805	2.5632	2.7978
YamYst1t10r1	1138.61	1093.24	1183.98	5.2166	5.3614	5.0718
YstYal35t13r1	1908.73	2015.63	1801.83	6.1427	8.2149	4.0705
YstYal34t13r1	1807.96	1623.54	1992.38	2.9854	2.4879	3.4829
YstYal34t14l1	2047.36	2083.54	2011.18	2.7494	2.8741	2.6247
YstYal36t14l1	1708.63	1759.85	1657.41	3.6068	3.8476	3.3660
YstYal35t14l1	1557.54	1560.32	1554.76	3.7962	3.9855	3.6045
YstYal37t13r1	1606.60	1756.59	1456.61	6.8724	6.9238	6.8210
YstYal33t13r1	1939.72	1087.32	2792.12	4.8734	3.6985	6.0483
YstYal38t13r1	1661.59	2052.36	1270.82	5.1699	4.9852	5.3546
YstYal37t14l1	1370.77	1423.52	1318.02	6.8962	7.1206	6.6718
Yal3Yst2t14l1	1276.87	1364.87	1188.91	5.7773	6.2147	5.3399
Yal3Yst2t13r2	1674.59	1458.23	1890.95	10.0080	11.5712	8.4448
Yal3Yst1t14l1	1119.80	1257.28	1033.32	6.5001	8.2436	4.5766

APPENDIX 6.9:

Mean Burst Duration & Mean Interburst Interval for songs of the hybrid genotypes

HYBIRID:	Mean burst duration for the entire song (ms)	Mean burst duration for the first half of the song(ms)	Mean burst duration for the second half of the song(ms)	Mean Interburst Interval for the entire song(s)	Mean Interburst Interval for the first half ofthe song (s)	Mean Interburst Interval for the second half of the song (s)
yakuba(f) x *mauritiana* (m)						
HY2t1011	669.24	485.29	853.19	9.2031	6.2587	12.1475
HY7t612	414.91	403.25	426.57	6.8490	4.8752	8.8228
HY1t10r2	472.79	481.23	464.35	9.6084	10.2548	8.9620
HY4t5r1	576.21	575.89	576.53	4.5391	4.5232	4.5550
HYB1t612	907.23	765.41	1049.05	4.4034	3.6254	5.1814
HYB5t5r2	910.48	924.86	896.10	5.1912	5.4682	4.9142
HYB7t5r2	676.86	830.04	523.68	7.2513	5.2178	7.2848
HYB8t511	622.64	548.36	696.92	1.6246	1.2483	2.0009
HYB3t511	805.24	745.23	865.25	1.1089	1.0956	1.1222
HYB6t5r2	679.10	758.12	600.08	12.8978	14.3267	11.4693
HY4t10L1	788.70	780.21	797.13	1.8586	1.5954	2.1208
HYB3t612	638.08	632.54	643.62	8.4628	8.1296	8.7960
HYB2t5r2	947.79	936.81	958.77	10.1218	9.2354	11.0082
yakuba(f) x *teissieri*(m)						
HYB4t711	684.90	548.63	821.17	10.4696	9.8752	11.0640
HYB9t612	476.26	480.23	472.29	6.6453	6.7214	6.5692
HYB6t711	633.71	610.56	656.86	15.9063	15.7523	16.0603
HYB8t612	531.30	529.24	531.36	6.5112	5.9621	7.0603
HYB6t612	588.30	586.54	590.06	7.5103	7.4283	7.5923
mauritiana(f) x *yakuba*(m)						
MY4t15r2	1423.61	1623.54	1223.68	5.4803	5.6423	5.3183
MY7t1512	1141.76	1235.64	1047.88	2.4759	2.5842	2.3676
MY6t15r1	2949.31	3021.54	2877.08	4.8514	4.9583	4.7445
MY2t1511	1389.61	1354.56	1424.66	4.1386	3.9652	4.3120
MY3t14r2	1226.87	1358.34	1095.40	10.4731	11.8623	9.0839
MY8t1511	1289.49	1314.23	1264.75	3.6168	3.8521	3.3815
MY8t15r1	2295.58	2054.86	2536.30	7.2075	6.9854	7.4296
simulans(f) x *mauritiana*(m)						
SM1t1711	509.62	452.31	552.93	9.3900	6.5241	12.6529
SM2se2s2m1	633.35	584.32	682.38	10.4317	8.3269	12.5365
SM4se2s2m1	779.96	658.71	901.21	7.2263	5.3658	9.0868
SM3se2s2m1	952.07	752.36	1151.78	4.7793	3.9852	5.5734
SM1se2s2m1	499.39	328.56	670.22	4.6184	4.0128	5.2240
teissieri(f) x *mauritiana*(m)						
TM1se2s1m2	528.79	496.37	561.21	4.6099	3.2564	5.9634
TM9se2s2m2	527.64	493.25	562.03	3.8443	3.1642	4.5244
TM3se2s1m2	520.64	483.63	557.65	4.0436	2.5948	5.4924
TM6se2s1m2	462.95	398.67	527.23	5.6501	4.6387	6.6615
TM5se2s1m2	435.12	395.21	475.03	6.8130	4.6329	8.9931
TM18se2s2m2	545.71	436.85	654.57	11.5418	8.9632	14.1204
TM1se2s2m1	545.83	368.27	723.39	9.9106	6.5342	13.2870
TM7se2s1m2	548.73	487.36	610.10	4.3857	2.5986	6.1728

APPENDIX 6.10:

Two-way ANOVA for MBD for the first and second part of song between the different species of the *D. melanogaster* subgroup and their interspecific hybrids.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	12	9514228	129	1072191	8.873630	0.000000
Part A or B	1	811136	129	779337	1.040803	0.309544
Interaction: Genotypes x Part A or B	12	264427	129	779337	0.339298	0.980327

Newman-Keuls *a posteriori* test for the mbd between the members of the *D. melanogaster* subgroup and their interspecific hybrids (p-values are given). Note that only significant results are given.

	melanogaster	simulans	mauritiana	sechellia	teissieri	erecta	orena	yakuba	yakuba(f) x mauritiana(m)
D. melanogaster									
D. simulans									
D. mauritiana									
D. sechellia									
D. teissieri									
D. erecta									
D. orena									
D. yakuba	0.001511	0.08203	0.001879	0.041735	0.000214	0.037464	0.008068		
yakuba x teissieri								0.006278	
teissieri x mauritiana									
simulans x mauritiana									
yakuba x mauritiana								0.008880	
mauritiana x yakuba			0.027785						0.065615

KEY: BOLD LETTERS - SIGNIFICANT RESULTS

APPENDIX 6.11:

Two-way ANOVA for IBI for the first and second part of song between the different species of the *D. melanogaster* subgroup and their interspecific hybrids.

Effect	df Effect	Ms Effect	df Error	MS Error	F	p-level
Genotypes	12	266.5058	129	143.27368	6.15861	0.00000
Part A or B	1	51.3965	129	3.25092	15.80984	0.000116
Interaction: Genotypes x A or B	12	12.5476	129	3.25092	3.85972	0.000048

Newman-Keuls *a posteriori* test between the ibi of the members of the *D.melanogaster* subgroup and their interspecific hybrids (p-values are given).

	melanogaster		simulans		mauritiana		sechellia		teissieri		erecta		orena		yakuba		yakuba x teissieri		teissieri x mauritiana		simulans x mauritiana		yakuba x mauritiana		mauritiana x yakuba
	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI1	IBI2	IBI2
melanogaster(A)																									
melanogaster(B)	0.461																								
simulans (A)	0.000																								
simulans(B)			0.000		0.169																				
mauritiana (A)	0.000		0.000																						
mauritiana (B)			0.000		0.000	0.937																			
sechellia (A)	0.000		0.000			0.685																			
sechellia(B)			0.000		0.000		0.933	0.724																	
teissieri(A)	0.053		0.000			0.000	0.000																		
teissieri(B)			0.019		0.000		0.000		0.000	0.518															
erecta(A)	0.000		0.042		0.000		0.005		0.000																
erecta(B)			0.000		0.002		0.000		0.000		0.04	0.000													
orena(A)	0.030		0.000		0.000				0.000		0.998	0.000													
orena(B)				0.049	0.000				0.000			0.81	0.000	0.720											
yakuba(A)	0.000		0.000			0.003	0.000			0.300	0.000			0.355											
yakuba(B)		0.002	0.000		0.000			0.001		0.74			0.000		0.666	0.934									
yakuba xteissieri(A)									0.000								0.004								
yakuba xteissieri(B)										0.00							0.000	0.835							
teissieri x mauritina (A)			0.000							0.575															
teissieri x mauritiana(B)					0.521					0.01															
simulans xmauritiana (A)			0.000		0.003																0.0				
simulans xmauritiana(B)				0.000		0.948																			
yakuba x mauritiana(A)					0.003												0.996						0.030		
yakuba x mauritiana(B)						0.020											0.750							0.290	
mauritiana x yakuba(A)			0.002														0.998						0.876		
mauritiana x yakuba(B)					0.000												0.723							0.605	0.987

KEY : BOLD LETTERS = SIGNIFICANT RESULTS   SHADED-IN = NON-SIGNIFICANT RESULTS

APPENDIX 6.12:

One-way ANOVA for % courtship vigour (arcsin-corrected) for the entire song between the different species of the *D. melanogaster* subgroup and their interspecific hybrids.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	12	0.137597	129	0.015093	9.116560	0.000000

Newman-Keuls *a posteriori* test for the % courtship vigour (arcsin-corrected) for the entire song between the members of the *D. melanogaster* subgroup and their interspecific hybrids (p-values are given).

	melanogaster	*simulans*	*mauritiana*	*sechellia*	*teissieri*	*erecta*	*orena*	*yakuba*(f) x *teissieri*(m)	*yakuba*(f) x *mauritiana*(m)	*mauritiana*(f) x *yakuba*(m)
D. melanogaster										
D. simulans										
D. mauritiana										
D. sechellia										
D. teissieri										
D. erecta										
D. orena										
yakuba x *teissieri*					0.773625					
simulans x *mauritiana*		0.995774	0.978840							
teissieri x *mauritiana*			0.794858		0.987436					
D. yakuba	0.118640	**0.000501**	**0.001093**	**0.037026**	**0.000459**	**0.001470**	0.099258	**0.001376**	**0.047273**	0.24425
yakuba x *mauritiana*			0.435760							
mauritiana x *yakuba*			0.052017							0.430652

KEY: BOLD LETTERS - SIGNIFICANT RESULTS

APPENDICES

FOR CHAPTER 7

APPENDIX 7.1:

One-way ANOVA for the mean periods between the different species of the *D. melanogaster* subgroup and their interspecific hybrids in DD.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	11	4.731971	440	0.464965	10.17705	0.000000

Newman-Keuls *a posteriori* test for the mean periods between the members of the *D. melanogaster* subgroup and their interspecific hybrids (p-values are given).

	melanogaster	simulans	mauritiana	sechellia	teissieri	erecta	orena	yakuba	yakuba(f) x mauritiana(m)
D. melanogaster									
D. simulans									
D. mauritiana									
D. sechellia	0.000024	0.003892	0.001136		0.003751	0.004665	0.000632	0.000860	
D. teissieri									
D. erecta									
D. orena									
D. yakuba									
yakuba(f) x mauritiana(m)			0.994294					0.844087	
mauritiana(f) x yakuba(m)			0.971415					0.983324	0.974245
yakuba(f) x teissieri(m)					0.004091			0.002455	
simulans(f) x mauritiana(m)		0.820356	0.737729						

KEY: BOLD LETTERS - SIGNIFICANT RESULTS

APPENDIX 7.2:

One-way ANOVA for the mean periods between the different parental strains and their interspecific hybrids in DD.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	13	0.819735	271	0.360305	2.275116	0.007376

Newman-Keuls *a posteriori* test for the mean periods between the different parental strains and their interspecific hybrids (p-values are given).

	yakuba France	*yakuba* Malawi	*yakuba* Lamto3	*mauritiana* France	*mauritiana* Sweden	*mauritiana* Isofemale72	*simulans* Florida	*teissieri* Sweden
yakuba F.(f) x *mauritiana* F. (m)	0.904375			0.809891				
yakuba M.(f) x *mauritiana* S.(m)		**0.007969**			**0.013902**			
yakuba L3(f) x *mauritiana* Iso.72(m)			0.488569			0.298375		
mauritiana S. (f) x *yakuba* L3(m)			0.672525		0.976669			
yakuba F.(f) x *teissieri* S.(m)	**0.037999**							**0.045118**
simulans F.(f) x *mauritiana* S.(m)					0.774811		0.817486	

KEY: BOLD LETTERS - SIGNIFICANT RESULTS

APPENDICES

FOR CHAPTER 8

APPENDIX 8.0:

Various components of locomotor activity in L:D conditions

SPECIES/STRAIN	1	2	3	4	5	6	7	8
melanogaster (Brighton)	416.575	0.626	0.374	0.707	0.206	0.288	0.160	0.306
simulans (Florida)	239.577	0.592	0.408	0.804	0.134	0.272	0.239	0.216
mauritiana STRAINS	137.886	0.579	0.421	0.820	0.201	0.279	0.322	0.228
FRANCE	104.574	0.680	0.320	0.589	0.228	0.273	0.345	0.189
SWEDEN	135.925	0.749	0.251	0.468	0.193	0.268	0.261	0.292
INDIANA	129.571	0.360	0.640	1.839	0.086	0.312	0.014	0.476
MG17	164.448	0.671	0.329	0.607	0.177	0.262	0.383	0.113
MG71	12.604	0.492	0.508	1.119	0.115	0.212	0.005	0.570
ISOFEMALE 72	342.599	0.301	0.699	2.378	0.235	0.320	0.518	0.126
ISOFEMALE 102	107.884	0.961	0.039	0.220	0.457	0.210	0.428	0.058
ISOFEMALE 152	110.219	0.760	0.240	0.451	0.178	0.557	0.143	0.226
ISOFEMALE 197	47.841	0.684	0.316	0.613	0.219	0.221	0.391	0.139
DAVID 75	180.929	0.130	0.870	0.405	0.501	0.143	1.656	0.246
DAVID 105	96.877	0.871	0.129	0.307	0.159	0.185	0.284	0.083
sechellia (Cambridge)	73.610	0.755	0.245	0.435	0.039	0.504	0.903	0.003
yakuba complex								
yakuba STRAINS	263.513	0.620	0.380	0.721	0.232	0.207	0.199	0.295
FRANCE	454.688	0.596	0.404	0.782	0.176	0.192	0.151	0.228
MALAWI	136.524	0.607	0.393	0.753	0.282	0.313	0.284	0.457
LAMTO 3	235.115	0.961	0.039	0.219	0.231	0.182	0.173	0.576
IVORY COAST	252.734	0.619	0.381	0.723	0.349	0.344	0.285	0.188
S.T.	90.224	0.359	0.641	1.850	0.249	0.066	0.179	0.392
SUBTAOME	117.704	0.439	0.561	1.350	0.314	0.077	0.146	0.070
JAPAN	183.048	0.570	0.430	0.850	0.080	0.165	0.116	0.160
EL8	19.667	0.286	0.714	2.540	0.587	0	0.064	0.975
INDIANA	138.657	0.576	0.424	0.835	0.113	0.207	0.230	0.105
LAMTO 5	72.167	0.865	0.135	0.315	0.622	0.116	1.265	0.005
teissieri (Sweden)	342.748	0.490	0.510	1.123	0.146	0.357	0.408	0.195
orena (France)	11.246	0.678	0.322	0.593	0	1.487	0.168	0.227
erecta (France)	203.714	0.459	0.541	1.259	0.134	0.479	0.417	0.182
INTERSPECIFIC HYBRIDS 0.272								
yakuba France(f) x *mauritiana* France(m)	697.996	0.774	0.226	0.431	0.193	0.184	0.236	0.272
yakuba Malawi(f) x *mauritiana* Sweden(m)	269.185	0.480	0.520	1.162	0.422	0.205	0.124	1.376
yakuba Lamto 3(f) x *mauritiana* Iso 72(m)	115.518	0.853	0.147	0.329	0.187	0.394	0.208	0.654
yakuba France(f) x *teissieri* Sweden(m)	100.437	0.847	0.153	0.181	0.217	0.284	0.492	0.021
mauritiana Sweden(f) x *yakuba* Lamto3(m)	155.095	0.650	0.350	0.590	0.203	0.289	0.248	0.259
mauritiana Iso 72(f) x *yakuba* Lamto3(m)	104.228	0.844	0.156	0.342	0.213	0.265	0.166	1.746
simulans Florida (f) x *mauritiana* Sweden(m)	183.649	0.908	0.092	0.101	0.332	0.939	0.179	0.617

KEY:
1. Total Activity (Mean Total Activity per fly over 48 bins, TA)
2. Day Activity ÷ Total Activity (D/T)
3. Night Activity ÷ Total Activity (N/T)
4. Night Activity ÷ Day Activity (N/D)   see Chapter 2
5. 4 bins after lights-on (Startle On) ÷ Day Activity (SON)
6. 4 bins before lights-off (Anticipation Off) ÷ Day Activity (ANTOFF)
7. 4 bins before lights-on (Anticipation On) ÷ Night Activity (ANTON)
8. 4 bins after lights-off (Startle Off) ÷ Night Activity (SOFF)

APPENDIX 8.1a:

One-way ANOVA for Total Activity between the different species of the *D. melanogaster* subgroup.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	7	1096990	692	394464.1	2.7809692	0.007391

APPENDIX 8.1b:

One-way ANOVA for Day Activity between the different species of the *D. melanogaster* subgroup.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	7	362008.3	692	73060.83	4.95448	0.000017

Newman-Keuls *a posteriori* test between the Day Activity of the members of the *D. melanogaster* subgroup (p-values are given).

	erecta	*orena*
melanogaster	0.010264	0.012049

APPENDIX 8.1c:

One-way ANOVA for the Startle-On response between the different species of the *D. melanogaster* subgroup.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	7	0.265635	692	0.036808	7.216719	0.000000

Newman-Keuls *a posteriori* test for the Startle-On response between the members of the *D. melanogaster* subgroup (p-values are given). Note that only significant result are shown.

	melanogaster	simulans	sechellia	teissieri	erecta	mauritiana	yakuba
sechellia	0.008374						
orena	0.000494	0.029822		0.023532	0.016157	0.000568	0.000087
mauritiana			0.008395				
yakuba			0.001653				

APPENDIX 8.1d:

One-way ANOVA for the Anticipation-Off response between the different species of the *D. melanogaster* subgroup.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	7	2.177805	692	0.045875	47.47283	0.000000

Newman-Keuls *a posteriori* test for the Anticipation-Off response between the members of the *D. melanogaster* subgroup (p-values are given). Note that only significant result are shown.

	melanogaster	simulans	sechellia	teissieri	erecta	orena
sechellia	0.001400	0.001035				
teissieri			0.037248			
erecta	0.003175	0.003432		0.04037		
orena	0.000017	0.000026	0.000009	0.000008	0.000022	
mauritiana			0.001198		0.003428	0.000020
yakuba			0.000031		0.000052	0.000032

APPENDIX 8.1e:

One-way ANOVA for the Anticipation-On response between the different species of the *D. melanogaster* subgroup.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	7	1.022367	692	0.96894	10.55138	0.00000

Newman-Keuls *a posteriori* test for the Anticipation-On response between the members of the *D. melanogaster* subgroup (p-values are given). Note that only significant result are shown.

	melanogaster	simulans	sechellia	teissieri	erecta
sechellia	0.000032				
teissieri	0.030062	0.000017	0.000024		
erecta	0.029106		0.000010		
orena			0.000026	0.029357	0.030420
mauritiana			0.000008		
yakuba			0.000020		

APPENDIX 8.1f:

One-way ANOVA for the Startle-Off response between the different species of the *D. melanogaster* subgroup.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	7	0.226265	692	0.084319	2.683447	0.009536

Newman-Keuls *a posteriori* test for the Startle-Of response between the members of the *D. melanogaster* subgroup (p-values are given). Note that only significant result are shown.

	melanogaster	erecta	yakuba
sechellia	0.006218	0.044587	0.007965

APPENDIX 8.2a:

One-way ANOVA for the Night/Day Activity ratio between the different *D. maurtiana* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	10	43.94639	240	3.273543	13.42472	0.00000

Newman-Keuls *a posteriori* test for the Night/Day Activity ratio between the different *D. mauritiana* (p-values are given). Note that only significant results are shown.

	France	Sweden	MG17	MG71	Isofemale 72	Isofemale 152	Isofemale 197	Isofemale 102	David 75	David 105
Indiana	0.000017	0.000020	0.000032	0.000009	0.000022	0.000026	0.000008	0.000015	0.000012	0.00001

APPENDIX 8.2b:

One-way ANOVA for the Startle-On between the different *D. maurtiana* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	10	0.097232	240	0.0310770	3.128753	0.000892

Newman-Keuls *a posteriori* test for the Startle-On between the different *D. mauritiana* strains (p-values are given). Note that only significant results are shown.

	France	Sweden	MG17	MG71	Isofemale 72	Isofemale 152	Isofemale 197	Indiana	David 105
Isofemale 102	0.012439	0.008592	0.008195	0.000370	0.005872	0.006066	0.016009	0.000121	0.004618
David 75	0.004445	0.001932	0.001513	0.000047	0.003134	0.001162	0.004651	0.000024	0.000747

213

APPENDIX 8.2c:

One-way ANOVA for the Anticipation-Off between the different *D. maurtiana* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	10	0.135136	240	0.044509	3.036157	0.001219

Newman-Keuls *a posteriori* test for the Anticipation-Off between the different *D. mauritiana* strains (p-values are given). Note that only significant results are shown.

	France	Sweden	MG17	MG71	Isofemale 72	Isofemale 197	Indiana	Isofemale 102	David 75	David 105
Isofemale 152	0.014760	0.019456	0.023109	0.004658	0.012641	0.007220	0.026998	0.006107	0.000586	0.003312

APPENDIX 8.2d:

One-way ANOVA for the Anticipation-On between the different *D. maurtiana* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	10	0.135136	240	0.044509	3.036157	0.001219

Newman-Keuls *a posteriori* test for the Anticipation-On between the different *D. mauritiana* strains (p-values are given). Note that only significant results are shown.

	France	Sweden	MG17	MG71	Isofemale 72	Isofemale 152	Isofemale 197	Indiana	Isofemale 102	David 105
Isofemale 72				0.023511						
Indiana					0.012115					
David 75	0.00002	0.00003	0.000017	0.000012	0.000009	0.000010	0.000008	0.000015	0.000022	0.000026

214

APPENDIX 8.2e:

One-way ANOVA for the Startle-Off between the different *D. maurtiana* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Species	10	0.328588	240	0.069897	4.701053	0.000004

Newman-Keuls *a posteriori* test for the Anticipation-On between the different *D. mauritiana* strains (p-values are given). Note that only significant results are shown.

	MG17	MG71	Indiana
MG71	0.016374		
Isofemale 72		0.019562	
Isofemale 152			
Isofemale 197		0.021652	
Indiana	0.047865		
Isofemale 102		0.004048	0.015243
David 75			
David 105		0.007964	0.026682

APPENDIX 8.3a:

One-way ANOVA for the Night/Day Ratio between the different *D. yakuba* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Strains	9	7.874258	219	1.353914	5.815923	0.000000

Newman-Keuls *a posteriori* test for the Night/Day ratio between the different *D. yakuba* strains (p-values are given). Note that only significant results are shown.

	Indiana	France	Ivory Coast	Japan	S.T.	Lamto3	Lamto5	Subtaome	Malawi
EL8	0.000034	0.000028	0.000044	0.000029	0.000042	0.000010	0.000012	0.007741	0.000019
Lamto3								0.016310	
Lamto5								0.0200010	

APPENDIX 8.3b:

One-way ANOVA for the Startle-On response between the different *D. yakuba* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Strains	9	0.213679	219	0.052074	4.107284	0.000067

Newman-Keuls *a posteriori* test for the Startle-On response between the different *D. yakuba* strains (p-values are given). Note that only significant results are shown.

	Indiana	France	Japan	S.T.	Lamto3
EL8	0.002566	0.014526	0.001006		0.047322
Lamto5	0.001142	0.007643	0.000422	0.035414	0.029038

APPENDIX 8.3c:

One-way ANOVA for the Anticipation-Off response between the different *D. yakuba* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Strains	9	0.166924	219	0.033489	4.9844961	0.000004

Newman-Keuls *a posteriori* test for the Anticipation-Off response between the different *D. yakuba* strains (p-values are given). Note that only significant results are shown.

	Ivory Coast	Malawi
EL8	0.007928	0.020268

APPENDIX 8.3d:

One-way ANOVA for the Anticipation-On response between the different *D. yakuba* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Strains	9	0.289905	219	0.050217	5.773060	0.000000

Newman-Keuls *a posteriori* test for the Anticipation-On response between the different *D. yakuba* strains (p-values are given). Note that only significant results are shown.

	Indiana	EL8	France	Ivory Coast	Japan	S.T.	Lamto3	Subtaome	Malawi
Lamto5	0.000008	0.000012	0.000026	0.000009	0.000010	0.000017	0.000020	0.000032	0.000022

APPENDIX 8.3e:

One-way ANOVA for the Startle-Off response between the different *D. yakuba* strains.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Strains	9	0.666440	219	0.075939	8.775963	0.000000

Newman-Keuls *a posteriori* test for the Startle-Off response between the different *D. yakuba* strains (p-values are given). Note that only significant results are shown.

	Indiana	France	Ivory Coast	Japan	S.T.	Lamto3	Lamto5	Subtaome	Malawi
EL8	0.000035	0.000127	0.000063	0.000047	0.006466	0.040020	0.000012	0.000011	0.013676
Lamto 3	0.022402						0.002619	0.012445	
Lamto 5									0.031349

APPENDIX 8.4a:

One-way ANOVA for the Day Activity between the different species and their interspecific hybrids.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	14	558058.3	504	113408.4	4.920782	0.000000

Newman-Keuls *a posteriori* test for the Day Activity between the different species and their interspecific hybrids (p-values are given). Note that only significant results are shown.

	D. yakuba France(f) x *D. mauritiana* France(m)
D. yakuba France	0.043025
D. mauritiana France	0.019481

APPENDIX 8.4b:

One-way ANOVA for the Startle-On response between the different species and their interspecific hybrids.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	14	558058.3	504	113408.4	4.920782	0.000000

Newman-Keuls *a posteriori* test for the Startle-On response between the different species and their interspecific hybrids (p-values are given). Note that only significant results are shown.

	D. yakuba Malawi(f) x *D. mauritiana* Sweden(m)
D. yakuba Malawi	0.061250
D. mauritiana Sweden	0.0077034

KEY: BOLD LETTERS - SIGNIFICANT RESULTS

APPENDIX 8.4c:

One-way ANOVA for the Anticipation-On response between the different species and their interspecific hybrids.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	14	0.340690	504	0.069484	4.903166	0.000000

Newman-Keuls *a posteriori* test for the Anticipation-On response between the different species and their interspecific hybrids (p-values are given). Note that only significant results are shown.

	D. yakuba France(f) x *D. teissieri* Sweden(m)	*D. mauritiana* Isofemale 72(f) x *D. yakuba* Lamto 3(m)	*D. yakuba* Lamto 3(f) x *D. mauritiana* Isofemale 72(m)
D. yakuba France	0.016817		
D. yakuba Lamto 3		0.936035	0.923849
D. mauritiana Isofemale 72		0.015069	0.043371

KEY: BOLD LETTERS - SIGNIFICANT RESULTS

APPENDIX 8.4d:

One-way ANOVA for the Startle-Off response between the different species and their interspecific hybrids.

Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
Genotypes	14	0.340690	504	0.069484	4.903166	0.000000

Newman-Keuls *a posteriori* test for the Startle-Off response between the different species and their interspecific hybrids (p-values are given). Note that only significant results are shown.

	D. mauritiana Isofemale 72(f) x *D. yakuba* Lamto 3(m)	*D. yakuba* Malawi(f) x *D. mauritiana* Sweden(m)
D. yakuba Malawi		0.015752
D. mauritiana Sweden		0.000898
D. mauritiana Isofemale 72	0.000023	

REFERENCES

REFERENCES

Albagli, O., Dhordain, P., Deweindt, C., Lecocq, G., and Leprince, D. (1995). The BTB/POZ domain: a new protein-protein interaction motif common to DNA- and actin-binding proteins. Cell Growth Differ. **6**, 1193-1198.

Alexander, B.D. (1962). Evolutionary change in cricket acoustic communication. Evolution **16**, 443-467.

Alt, S., Ringo, J., Talyn, B., Bray, W., and Dowse, H. (1997). The *period* gene controls courtship song cycles in *D. melanogaster*. (in press).

Antoch, M.P., Song, E.-J., Chang, A.-M., Vitaterna, M.H., Zhao, Y., Wilsbacher, L.D., Sangoram, A.M., King, D.P., Pinto, L.H., and Takahashi, J.S. (1997). Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. Cell **89**, 14-26.

Anthony, C., and Jallon, J.-M. (1982). The chemical basis for sex recognition in *Drosophila melanogaster*. J. Insect Physiol. **28**, 873-880.

Anthony, C., Davis, T.L., Carlson, D.A., Penichi, J.-M., and Jallon, J.-M. (1985). Compared behavioural responses of male *Drosophila melanogaster* (Canton-S) to natural and synthetic aphrodisiacs. J. Chem. Ecol. **11**, 1617-1629.

Ashburner, M., Bodmer, M., and Lemeunier, F. (1984). On the evolutionary relationships of *Drosophila melanogaster*. J. Ins. Physiol. **30**, 873-880.

Ashburner, M. (1989). '*Drosophila* : A Laboratory Handbook'. Eds. Cold Spring Harbour Laboratory Press.

Ayer, S., and Benyajati, C. (1990). Conserved enhancer and silencer elements responsible for differential *Adh* transcription in *Drosophila* cell lines. Mol.Cel.Biol. **10**, 3512-3523.

Baker, B.S., and Ridge, K.A. (1980). Sex and the single cell. I. On the action of major foci affecting sex-determination in *Drosophila melanogaster*. Genetics **94**, 383-423.

Bargiello, T.A., Jackson, F.R., and Young, M.W. (1984). Restoration of circadian behavioural rhythms by gene transfer in *Drosophila*. Nature **312**, 752-754.

Bargiello, T.A., and Young, M.W. (1984). Molecular genetics of a biological clock in *Drosophila*. Proc. Natl. Acad. Sci. USA **81**, 2142-2146.

Barinaga, M. (1995). Bisexual Fruit flies point to brain courtship centres. Science **267**, 791-792.

Bastock, M., and Manning, A. (1955). The courtship of *Drosophila melanogaster*. Behaviour **8**, 85-111.

Baylies, M.K., Bargiello, T.A., Jackson, F.R., and Young, M.W. (1987). Changes in abundance or structure of the *period* gene product can alter periodicity of the *Drosophila* clock. Nature **326**, 390-392.

Begun, D.J., and Aquadro, C.F. (1991). Molecular population genetics of the distal portion of the X chromosome in *Drosophila*: Evidence for genetic hitchhiking of the *yellow-achaete* region. Genetics **129**, 1147-1158.

Bennet-Clark, H.C., and Ewing, A.W. (1969). Pulse interval as the critical parameter of the courtship song of *Drosophila melanogaster*. Anim. Behav. **17**, 755-759.

Berg, M. J. van den (1988). Assortive mating in *Drosophila melanogaster* and among three species of the *melanogaster* subgroup. Ph.D. Thesis, University of Groningen, Holland.

Bixler, A., Jenkins, .J.B., Tompkins, L., and McRobert, S.P. (1992). Identification of acoustic stimuli that mediate sexual behaviour in *Drosophila busckii* (Diptera: Drosophilidae). J. Insect. Behav. **4**, 469-478.

Boake, C.R.B., and Hoikkala, A. (1995). Courtship behaviour and mating success of wild-caught *Drosophila silvestris* males. Anim. Behav. **49**, 1303-1313.

Bodmer, M., and Ashburner, M. (1984). Conservation and change in the DNA sequences coding for alcohol dehydrogenase in sibling species of *Drosophila*. Nature **309**,425-430.

Bourdon, M.A., Oldberg, A., Pierschbacher, M., and Ruoslahti, E. (1985). Molecular cloning and sequence analysis of a chondroitin sulphate proteoglycan cDNA. Proc. Natl .Acad. Sci. USA **82**, 1321-1325.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a mean of altering cell fates and generating dominant phenotypes. Development **118**, 401-415.

Breedlove, S.M. (1994). Sexual differentiation of the human nervous system. Annu. Rev. Psychol. **45**, 389-418.

Burnet, B. and Connolly, K. (1974). Activity and sexual behaviour in *Drosophila melanogaster*. In: J.H.F. van Abeelen (Editior), The Genetics of behaviour, North Holland, Oxfprd, pp: 201-258.

Burtis, K.C., and Baker, B.S. (1989). *Drosophila doublesex* gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides. Cell **56**, 997-1010.

Burtis, K.C. (1993). The regulation of sex determination and sexually dimorphic differentiation in *Drosophila*. Curr. Opin. Cell Biol. **5**, 1006-1014.

Cariou, M.-L. (1987). Biochemical phylogeny of the eight species in the *Drosophila melanogaster* subgroup, including *D. sechellia* and *D. orena*. Genet. Res. Camb. **50**, 181-185.

Carracedo, M.C., Casares, P., Izquierdo, J.I., and Piñeiro, R. (1991). Receptivity and sexual maturation of *Drosophila melanogaster* females in relation to hybridisation with *D. simulans* males. Genome **29**, 334-339.

Carson, H.L., Clayton, F.E., and Stalker, H.D. (1967). Karyotypic stability and speciation in Hawaiian *Drosophila*. Proc. Natl. Acad. Sci. USA **57**, 1280-1285.

Casares, P., Carrecedo, M.C., Piñeiro, R., and San Miguel, E. (1992). Genetic basis for female receptivity in *Drosophila melanogaster*: A diallel study. Heredity **69**, 400-411.

Charalambous, M. (1990). Genetics of song and female preference in the grasshopper *Chorthippus brunneus* (Orthoptera Acrididae): Sexual selection and the mate recognition system. Ph.D. Thesis, University of East Anglia.

Charalambous, M., Butlin, R.K., and Hewitt, G..M. (1994). Genetic variation in male song and female song preference in the grasshopper, *Chorthippus brunneus* (Orthoptera: Acrididae). Anim. Behav. **47**, 399-411.

Charlesworth, B., Coyne, J.A., and Barton, N. (1987). The relative rates of Evolution of Sex-chromosomes and autosomes. Am. Nat. **130**, 113-146.

Charlesworth, B. (1991). The evolution of sex chromosomes. Science **251**, 1030-1033.

Citri, Y., Colot, H.V., Jacquier, A.C., Yu, Q., Hall, J.C., Baltimore, D., and Rosbash, M. (1987). A family of unusually spliced and biologically active transcripts is encoded by a *Drosophila* clock gene. Nature **326**, 42-47.

Cline, T.W. (1984). Autoregulatory functioning of a *Drosophila* gene product that establishes and maintains the sexually determined state. Genetics **107**, 231-77.

Cobb, M., Connolly, K., and Burnet, B. (1985). Courtship behaviour in the *melanogaster* species sub-group of *Drosophila*. Behaviour **95**, 203-231.

Cobb, M., Burnet, B., and Connolly, K. (1986). The structure of courtship in the *Drosophila melanogaster* species sub-group. Behaviour **97**, 182-212.

Cobb, M., Connolly, K., and Burnet, B. (1987). The relationship between locomotor activity and courtship behaviour in the *melanogaster* species subgroup of *Drosophila*. Anim. Behav. **35**, 705-713.

Cobb, M., Burnet, B., and Connolly, K. (1988). Sexual isolation and courtship behaviour in *D. simulans* and *D. mauritiana* and their interspecific hybrids. Behav. Genet. **18**, 211-225.

Cobb, M., Burnet, B., Blizard, R., and Jallon, J.-M. (1989). Courtship in *Drosophila sechellia*: its structure, functional aspects and relationship to that of the other members of the *D. melanogaster* species subgroup. J. Ins. Behav. **2**, 63-89.

Cobb, M., Burnet, B., Blizard, R., and Jallon, J-M. (1990). Altered mating behaviour in a Carsonian population of *Drosophila sechellia*. Evolution **44**, 2057-2068.

Cobb, M., and Jallon, J.-M. (1990). Phermones, mate recognition and courtship stimulation in the *Drosophila melanogaster* species subgroup. Anim. Behav. **39**, 1058-1067.

Colot, H.V, Hall, J. C., and Rosbash, M. (1988). Interspecific comparison of the period gene of Drosophila reveals large blocks of non-conserved coding DNA. EMBO Journal **7** (12), 3929-3937.

Cook, R. (1980). The extent of visual control in the courtship tracking of *Drosophila melanogaster*. Biol. Cybernet. **37**, 41-51.

Cook, S.E., Vernon, J.G., Bateson, M., and Guildford, T. (1994). Mate choice in the polymorphic African swallowtail butterfly, *Papillo dardanus*: male-like females may avoid sexual harassment. Anim. Behav. **47**, 389-397.

Crosthwaite, S.K., Dunlap, J.C., and Loros, J.J. (1996). *Neurospora wc-*1 and *wc-*2: Tanscription, photoresponses, and the origins of circadian rhythmicity. Science **276**, 763-769.

Costa, R., Peixoto, A.A., Thackeray, J.R., Dalgleish, R., and Kyriacou, C.P. (1991). Length polymorphism in the Threonine-Glycine- encoding repeat region of the period gene in Drosophila. J. Mol. Evol. **32**, 238-246.

Costa, R., Peixoto, A.A., Barbujani, G., and Kyriacou, C.P. (1992). A latitudinal cline in a *Drosophila* clock gene. Proc. R. Soc. Lond. [Biol.] **250**, 43-49.

Cowling, D.E., and Burnet, B. (1981). Courtship songs and genetic control of their acoustic characteristics in sibling species of the Drosophila melanogaster subgroup. Anim. Behav. **29**, 924-935.

Coyne, J.A. (1983). Genetic basis of differences in genital morphology among three sibling species of *Drosophila*. Evolution **37**, 1101-1118.

Coyne, J.A., and Kreitman, M. (1986). Evolutionary genetics of two sibling species, *D. simulans* and *D. sechellia*. Evolution **40**, 673-91.

Coyne, J.A., and Milstead, B. (1987). Long-distance migration of *Drosophila*. III. Dispersal of *D. melanogaster* alleles from a Maryland orchard. Am. Nat. **130**, 70-82.

Coyne, J.A., and Orr, H.A., (1989). '*In speciation and its consequences*'. Eds Otto, D. & Endler, J. (Sinauer, Sunderland, Massachusetts), 180-207.

Coyne, J.A., and Orr, H.A., (1989). Patterns of speciation in *Drosophila*. Evolution **43**, 262-281.

Coyne, J.A., and David, J.R. (1991). Genetics of morphological differences and hybrid sterility between *Drosophila sechellia* and its relatives. Genet. Res. **57**, 113-122.

Coyne, J.A. (1992). Genetics and Speciation. Nature **355**, 511-515.

Coyne, J.A., and Oyama, R. (1995). Localisation of pheromonal sexual dimorphism in *Drosophila melanogaster* and its effect on sexual isolation. Proc. Nat. Sci. U.S.A. **92**, 9505-9509.

Crossley, S.A. (1986). Courtship sounds and behaviour in the four species of the *Drosophila bipectinata* complex. Anim. Behav. **34**, 1146-1159.

Crossley, S.A. (1988). Failure to confirm rhythms in *Drosophila* courtship song. Anim. Behav. **36**, 1098-1109.

Crossley, S.A. (1989). Reply to Kyriacou and Hall 1989. Anim. Behav. **37**, 860-861.

Crow, J.F. (1948). Alternative hypothesis of hybrid vigour. Genetics **33**, 477-487.

Crow, J.F. (1952). '*Dominance and Overdominance*', in Gowen, J. W. (Ed.), Heterosis, Iowa State College, Ames, Iowa, USA, 282-297.

Davis, A.W., Roote, J., Morley, T., Sawamura, K., Herrmann, S., and Ashburner, M. (1996). Rescue of hybrid sterility in crosses between *D. melanogaster* and *D. simulans*. Nature **380**, 157-159.

Darwin, C. (1859). On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London.

David, J.R., McEvey, S.F., Solignac, M, and Tsacas, L. (1989). *Drosophila* communities on Mauritius and the ecological niche of *D. mauritiana* (Diptera, Drosophilidae). J. Afr. Zool. **103**, 107-116.

David, J., Bocquet, C., Lemeunier, F., and Tsacas, L. (1976). Persistance of male sterility in strains issued from hybrids between two sibling species: *D. simulans* and *D. mauritiana*. Genetics **62**, 93-100.

Davis, AW., Roote, J., Morley, T., Sawamura, K., Herrmann, S., and Ashburner, M. (1996). Rescue of hybrid sterility in crosses between *D. melanogaster* and *D. simulans*. Nature **380**, 157-159.

Doherty, J.A., and Gerhardt, H. (1984). Acoustic communication in hybrid tree frogs: sound production by males and selective phonotaxis by females. J. Comp. Physiol. **154**, 319-330.

Dover, G.A. (1987). DNA turnover and the molecular Clock. J. Mol. Evol. **26**, 47-58.

Droney, D.C. (1994). Tests of hypotheses for lek formation in a Hawaiian *Drosophila*. Anim. Behav. **47**, 351-361.

Dudai, Y. (1985). Genes, enzymes and learning in Drosophila. Trends in Neurosci. **8**, 18-21.

Dushay, M.S., Rosbash, M., and Hall, J.C. (1989). The *disconnected* visual system mutations in *D. melanogaster* drastically disrupt circadian rhythms. J. Biol. Rhythms **4**, 1-27.

Dyson-Hudson,V.R.D. (1956). The daily activity rhythm of *Drosophila subobscura* and *D. obscura*. Ecology **37**, 562-567.

Easteal, S., and Oakshott, J.G. (1985). Estimating divergence times of *Drosophila* species from DNA sequence comparisons. Mol.Biol.Evol. **2**, 87-91.

Edery, I., Rutila, J.E., and Rosbash, M. (1994). Phase-shifting of the circadian clock by induction of the *Drosophila period* protein. Science **263**, 237-239.

Eisses, K.T., Van Dijk, H., and Van Delden, W. (1979). Genetic differentiation within the *melanogaster* species group of the genus *Drosophila* (Sophophora). Evolution **33**, 1063-1068.

Engelmann, W., and Mack, W. (1978). Different oscillators control the circadian rhythm of eclosion and activity in *Drosophila*. J. Comp. Physiol. **127**, 229-237.

Etges, W.J. (1992). Premating isolation is determined by larval substrates in cactophilic *Drosophila mojavensis*. Evolution **46**, 1945-1950.

Ewer, J., Hamblen-Coyle, M., Rosbash, M., and Hall, J.C. (1991). Requirement for *PERIOD* gene expression in the adult and not during development for locomotor activity rhythms of imaginal *Drosophila melanogaster*. J. Neurogen. **7**, 31-73 .

Ewing, A.W., and Bennet-Clarke, H.C. (1968). The courtship songs of *Drosophila*. Behaviour **31**, 288-301.

Ewing, A.W. (1969). The genetic basis of sound production in *Drosophila pseudoobscura* and *D. persimilis*. Anim. Behav. **17**, 555-560.

Ewing, A.W. (1977). Communication in Diptera. In: How Animals Communicate (Ed. by T.A. Sebeok), p:403-17. Bloomington & London: Indiana University Press.

Ewing, A.W. (1983). Functional aspects of *Drosophila* courtship. Biol. Rev. **58**, 275-292.

Ewing, A.W., and Miyan, J.A. (1986). Sexual selection, sexual isolation and the evolution of song in the *Drosophila repleta* group of species. Anim. Behav. **34**, 421-429.

Ewing, L.S., and Ewing, A.W. (1987). Courtship in *Drosophila melanogaster* in large observation chambers: the influence of reproductive state. Behaviour **101**, 288-301.

Ewing, A.W. (1988). Cycles in the courtship song of male *Drosophila melanogaster* have been demonstrated. Anim. Behav. **36**, 1091-1097.

Falconer, D.S. (1971). Improvement ofo litter size in a strain of mice at a selection limit. Genet. Res. **17**, 215-235.

Fejes, E. (1990). A 268bp upstream sequence mediates the circadian clock-regulated transcription of the wheat Cab-1 gene in transgenic plants. Plant Mol. Biol. **15**, 921-932.

Feldman, J.F., and Hoyle, M.N.(1973). Isolation of circadian clock mutants of *Neurospora crassa*. Genetics **75**, 605-613.

Ferveur, J.-F., Störtkuhl, K.F., Stocker, R.F., and Greenspan, R.J. (1995). Genetic feminization of brain structures and changed sexual oreintation in male *Drosophila*. Science **267**, 902-905.

Ferveur, J.-F., Cobb, M., Boukella, H., and Jallon, J.-M. (1996). World-wide variation in *Drosophila melanogaster* sex pheromone: genetic bases and behavioural consequences. Genetica **97**, 73-80.

Ferveur, J.-F., and Sureau, G. (1996). Simultaneous influence on male courtship of stimulatory and inhibitory pheromones produced by live sex-mosaic *Drosophila melanogaster*. Proc. R. Soc. London series B- Biol. Sci. **263**, 967-973.

Ferveur, J.-F., Savarit, F., O'Kane, C.J., Sureau, G., Greenspan, R.J., and Jallon, J.-M. (1997). Genetic feminization of pheromones and its behavioural consequences in *Drosophila* male.

Folkard, S.S., Hume, K.I., Minors, D.S., Waterhouse, D.M. and Watson, F.L. (1985). Independence of the circadian rhythm in alertness from the sleep/wake cycle. Nature **313**, 678-679.

Ford, M.J., Yoon, C.K., and Aquadro, C. (1994). Molecular evolution of the *period* gene in *Drosophila athabasca*. Molec. Biol. Evol. **11**, 169-182.

Futuyma, D.J. (1989). '*In speciation and its consequences*'. Eds Otto,D. & Endler, J. (Sinauer, Sunderland, Massachusetts), p:557-578.

Gailey, D.A., Jackson, F.R. and Siegel, R.W. (1984). Conditioning mutations in *Drosophila melanogaster* affect experience-dependent behavioural modification in courting males. Genetics **106**, 613-623.

Gailey, D.A., and Hall, J.C. (1989). Behaviour and cytogenetics of *fruitless*, in *Drosophila melanogaster*. Different courtship defects caused by separate, closely linked lesions. Genetics **121**, 773-785.

Gailey, D.A., Taylor, B.J., and Hall, J.C. (1991). Elements of the *fruitless* locus regulate development of the muscle of Lawrence, a male-specific structure in the abdomen of *Drosophila melanogaster* adults. Development **113**, 879-990.

Gailey, D.A., Ohshima, S., Santiago, S.J.-M., Montez, J.M., Arellano, A.R., Robillo, J., Villarmo, C.A., Roberts, L., Fine E., Villella, A., and Hall, J.C. (1997). The muscle of Lawrence in *Drosophila*: A case of repeated evolutionary loss. Proc. Natl. Acad. Sci. U.S.A. **94**, 4543-4547.

Galton, F. (1865). Hereditary talent and character. Macmillans Magazine **12**, 155-166, 318-327.

Gekakis, N., Saez, L., Delahayebrown, A.M., Myers, M.P. Seghal, A., Young, M.W., and Weitz, C.J. (1995). Isolation of *timeless* by *per* protein interaction, defective interaction between *timeless* protein and long-period mutant *per*^{L}. Science **270**, 811-815.

Gill, K.S. (1963). A mutation causing abnormal courtship and mating behaviour in males of *Drosophila melanogaster*. (Abstract) Am. Zool. **3**, 507.

Glover, D.M. (1991). Mitosis in the *Drosophila* embryo-in and out of control. Trends in Genet. **7**(4), 125-132.

Good, D.S. (1993). Evolution of behaviours in *Drosophila melanogaster* in high temperatures: Genetic and Environmental effects. J. Ins. Physiol. **39**, 537-544.

Gorczyca, M., Hall, J.C., and Mackey, M.C. (1987). The INSECTAVOX, an integrated device for recording and amplifying courtship songs. Dros. Info. Serv. **66**, 157-160.

Govind, S., and Steward, R. (1991). Dorsoventral pattern formation in *Drosophila*: signal transduction and nuclear targeting. Trends in Genet. **7**, 119-125.

Grant, P.R. (1984). Recent research on the evolution of land birds of the Galapagoes. J. Biol. Soc. Limn. of London **21**, 113-136.

Greenacre, M.L. (1990). Genetic analysis of courtship behaviour and biological rhythms in *Drosophila*. Ph.D Thesis.

Greenacre, M.L., Ritchie, M.G., Byrne, B.C., and Kyriacou, C.P. (1993). Female song preference and the *period* gene in *Drosophila*. Behav. Genet. **23**, 85-90.

Greenspan, R.J. (1995). Undestanding the genetic construction of behaviour. Sci. Am. **272**, 74-79.

Griffith, L.C., Verselis, L.M., Danho, W., and Greenspan, R.J. (1993). Inhibition of calcium/calmodulin-dependent protein kinase in *Drosophila* disrupts behavioural plasticity. Neuron **10**, 501-509.

Grossfield, J. (1966). The influence of light on the mating behaviour of *Drosophila*. Stud. Genet. Univ. Tex. Publ. 6615 **3**, 147-176.

Grossfield, J. (1968). The relative importance of wing utilisation in light dependent courtship in *Drosophila*. Genet. Univ. Tex. Publ. 6818 **4**, 147-156.

Grossfield, J. (1971). Geographic distribution and light-dependent behaviour in *Drosophila*. Proc. Natl. Acad. Sci. **68**, 2669-2672.

Hall, J.C. (1977). Portions of the central nervous system controlling reproductive behaviour in *Drosophila melanogaster*. Behav. Genet. **7**, 291-312.

Hall, J.C. (1978). Courtship among males due to a male-sterile mutation in *Drosophila melanogaster*. Behav. Genet. **8**, 125-141.

Hall, J.C. (1978a). Behavioural analysis in *Drosophila* mosaics. In : Genetic mosaics and cell differentiation. Eds by W.J. Gerhing. Springer-Verlag, Heidelberg.

Hall, J.C. (1979). Control of male reproductive behaviour by the central nervous system of *Drosophila*: dissection of a courtship pathway by genetic mosaics. Genetics **92**, 437-457.

Hall, J.C. (1984). Complex brain and behaviour functions disrupted by mutations in *Drosophila*. Dev. Genet. **4**, 355-378.

Hall, J.C. (1990). Genetics of circadian rhythms. Ann. Rev. Genet. **24**, 659- 697.

Hall, J.C., and Kyriacou, C.P. (1990). Genetics of biological rhythms in *Drosophila*. Adv. Ins. Physiol. **22**, 221-298.

Hall, J.C., Hamblen-Coyle, M.J., Moroz, L., Rutila, J.E., Yu, O., Rosbash, M. and Wheeler, D.A. (1992). Circadian rythms of *D. melanogaster* transformed with DNA from the *period* gene of *D. simulans*. *Dros. Inf. Serv.* **71**, 204-207.

Hall, J.C. (1994). The mating of a fly. Science **264**, 1702-1714.

Hall, J.C. (1995). Tripping along the trail to the molecular mechanisms of biological clocks. Trends in Neurosci. **18**, 230-240.

Hall, J.C. (1996). Are cycling gene products as internal zeitgebers no longer the zeitgeist Chronobiology?. Neuron **17**, 799-802.

Halliday, T.R. (1978). *Sexual selection and mate choice.* In behavioural Ecology- an evolutionary approach ed. J. R. Krebs and N. B. Davies. Blackwell scientific Publication.

Hamblen-Coyle, M., Zehring, W.A., Kyriacou, C.P., Reddy, P., Yu, Q., Wheeler, D.A., Zweibel, L.J., Konopka, R.J., Rosbash, M., and Hall, J.C. (1986). Germ-line transformation involving DNA from the period locus in *Drosophila melanogaster*; Overlapping genomic fragments that restore circadian and ultradian rhythmicity to per^0 and *per-* mutants. J. Neurogen. **3**, 249-291.

Hamblen-Coyle, M., Wheeler, D.A., Rutila, J.E., Rosbash, M., and Hall, J.C. (1992). Behaviour of *period*-altered circadian rhythm mutants of *Drosophila* in Light:Dark cycles (Diptera: *Drosophilidae*). J. Ins. Behav. **5**(4), 417-446.

Handler, A.M., and Konopka, R.J. (1979). Transplantation of a circadian pacemaker in *Drosophila*. Nature **279**, 236-238.

Hardeland, R., and Strange, G. (1973). Comparative studies on the circadian rhythms of locomotor activity of 40 *Drosophila* species. J. Interdiscipl. Cycle Res. **4**, 353-359.

Hardin, P.E. (1992). Identification and analysis of a circadian output gene from *Drosophila*. EMBO Workshop on Molecular Chronobiology. Abstr. pp:12.

Hardin, P.E., Hall, J.C., and Rosbash, M. (1992). Behavioural and molecular analyses suggest that circadian output is disrupted by disconnected mutants in *D. melanogaster*. EMBO Journal **11**, 1-6.

Hardin, P.E., Hall, J.C., and Rosbash, M. (1992). Circadian oscillations in *period* gene mRNA levels are transcriptionally regulated. Proc. Natl. Acad. Sci. U.S.A. **89**, 11711-11715.

Hardin, P.E., and Siwicki, K.K. (1995). The multiple roles of *per* in the *Drosophila* circadian clock. Seminars in Neurosciences **7**, 15-25.

Haymer, D.S., and Hartl, D.L. (1982). The experimental assessment of fitness in *Drosophila*, I. Comparative measures of competitive reproductive success. Genetics **102**, 455-466.

Haymer, D.S., and Hartl, D.L. (1983). The experimental assessment of fitness in *Drosophila*, II. A comparison of competitive and non-competitive measures. Genetics **104**, 343-352.

Haymer, D.S., and Marsh, J.L. (1986). Germ line and somatic instability of a *white* mutation in *Drosophila mauritiana* due to a transposable genetic element. Devel. Genet. **6**, 281-291.

Hazelrigg, J. (1987). The *Drosophila white* gene: a molecular update. Trends in Genet. **3**, 43-47.

Heisenberg, M. (1994). Central brain function in insects: genetic studies on the mushroom bodies and complex in *Drosophila*. In Neural Basis of Behavioural Adaptations, K. Schildberger and N. Elsner, eds. (Stuttgart, Germany: Gustav Fischer Verlag), 61-79.

Helfrich-Förster, C. (1996). *Drosophila* rhythms: from brain to behaviour. Sem. in Cell and Devel. Biology **7**, 791-802.

Helversen, D. von, and Helversen, O. von. (1975). Verhaltensgenetische untersuchugen am akustischen kommunikationssystem der feldheuschrecken (Ortherptera: Acridae). I. Der gesang von artbastarden zwischen *Chorthippus biggutulus* und *Ch. mollis*. J. Comp. Physiol. **104**, 273-299.

Hing, A.L.Y., and Carlson, J.R. (1996). Male-male courtship behaviour induced by ectopic expression of the *Drosophila white* gene: role of sensory function and age. J. Neurobiol. **30**, 454-464.

Hoikkala, A. (1985). Genetic variation in tme male courtship sound of *Drosophila littoralis*. Behav. Genet. **15**, 135-142.

Hoikkala, A., and Lumme, J. (1987). The genetic basis of evolution of the male courtship sounds in the *Drosophila virilis* group. Evolution **41**, 827-845.

Hoikkala, A. (1988). The importance of different courtship stimuli in the mating behaviour of european species of the *Drosphila virilis* group. Ann. Zool. Fennici **25**, 257-263.

Hoikkala, A. (1993). The importance of different courtship stimuli in the mating behaviour of different European species of the *Drosophila virilis* group. Ann. Zool. Fennici **25**, 257-263.

Hoikkala, A., Kaneshiro, K.Y., and Hoy, R.R. (1994). Courtship songs of the picture-winged *Drosophila planitibia* subgroup species. Anim. Behav. **47**, 1363-1374.

Hoikkala, A., and Welbergen, P. (1995). Signals and responses of females and males in successful and unsuccessful courtships of three Hawaiian lek-mating *Drosophila* species. Anim. Behav. **50**, 177-190.

Hotta, Y., and Benzer, S. (1976). Courtship in *Drosophila* mosaics: sex-specific foci for sequential action patterns. Proc. Natl. Aca. Sci. USA **73**, 4154-4158.

Hunter-Ensor, M., Ousley, A., and Sehgal, A. (1996). Regulation of the *Drosophila* protein *timeless* suggests a mechanism for resetting the circadian clock by light. Cell **84**, 677-685.

Hutter, P., Roote, J., and Ashburner, M. (1990). A genetic basis for the inviability between sibling species of *Drosophila*. Genetics **124**, 909-920.

Huang, Z.J., Edery, I., and Rosbash, M. (1993). PAS is a dimerization domain common to *Drosophila period* and several transcription factors. Nature **364**, 259.

Hudson, R.R., Kreitman, M., and Aguade, M. (1987). A test of neutral molecular evolution based on nucleotide data. Genetics **116**, 153-159.

Ikeda, H., Takabatake, I., and Sawada, (1980). Variation in courtship sounds among three geographical strains of *Drosophila mercatorum*. Behav. Genet. **10**, 361-375.

Ishida, N. (1991). Diurnal regulation of *per* repeat mRNA in the suprachiasmatic nucleus in the rat brain. Neuroscience Letters **122**, 113-116.

Ito, H., Fujitani, K., Usui, K., Shimizu-Nishikawa, K., Tanaka, S., and Yamamoto, D. (1996). Sexual orientation in *Drosophila* is altered by the *satori* mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. P.N.A.S. USA **93**, 9687-9692.

Izquierdo, J.I., Carracedo, M.C., Piñeiro, R., and Casares, P. (1992). Response to selection for increased hybridisation between *Drosophila melanogaster* females and *D. simulans* males. J. Hered. **83**, 100-104.

Jackson, F.R., Bargiello, T.A., Yun, S-H, and Young, M.W. (1986). Product of *per* locus of *Drosophila* shares homology with Proteoglycans. Nature **320**, 185-188.

Jallon, J.-M., and Hotta, Y. (1979). Genetic and behavioural studies of female sex-appeal in *Drosophila*. Behav. Genet. **8**, 487-502.

Jallon, J.-M. (1984). A few chemical words exchanged by *Drosophila* during courtship and mating. Behav. Genet. **14**, 441-478.

Jallon, J.-M., Anthony, C., Chang Yong, T.P., and Maniar, S. (1986). Genetic factors controlling the production of aphrodisiac substances in *Drosophila*. In 'Advances in Invertebrate Reproduction' 4 (ed. M Porchet, J.C. Andries, and A. Dhainaut), pp. 445-452. Elsevier Science Publishers B.V.

Jallon, J.-M., and David, J.R. (1987). Variations in cuticular hydrocarbons along the eight species of the *Drosophila melanogaster* subgroup. Evolution **41**, 294-302.

Jewett, M.E., Kronauer, R.E., and Czeisler, C.A. (1991). Light-induced suppression of endogenous circadian amplitude in humans. Nature **350**, 59-62.

Johnson, C.H., and Hastings, J.W. (1986). The Elusive Mechanism of the Circadian clock: The quest for the chemical basis of the biological clock is beginning to yield tantalising clues. Am. Scient. **74**, 29-36.

Jones, M.D.R., Cubbin, C.M., and Marsh, D. (1972). Light-on effects and the question of bimodality in the circadian flight activity of the mosquito *Anopheles gambiae*. J. Exp. Biol. **57**, 347-357.

Kalmus, H. (1940). The resistance to dessication of *Drosophila* mutants affecting body colour. Proc. Roy. Soc. B. **130**, 183-201.

Kalmus, H. (1945). Adaptive and selective responses of a population of *D. melanogaster* containing e and e^+ to differences in temperature, humidity, and to selection for developmental speed. J. Genet. **49**, 58-63.

Kawanishi, M., and Watanabe, T.K. (1980). Genetic variation of courtship song of *Drosophila melanogaster* and *D. simulans*. Jap. J. Genet. **55**, 235-240.

Kawanishi, M., and Watanabe, T.K. (1981). Genes affecting courtship song and mating preference in *Drosophila melanogaster*, *Drosophila simulans* and their hybrids. Evolution **35**, 1128-1133.

Kelly, J.K., and Noor, M.A.F. (1996). Speciation by Reinforcement: A model derived from studies of *Drosophila*. Genetics **143**, 1483-1497.

King, D.P., Zhao, Y., Sangoram, A.M., Wilsbacher, L.D., Tanaka, M., Antoch, M.P., Steeves, T.D.L., Vitaterna, M.H., Kornhauser, J.M., Lowrey, P.L., Turek, F.W., and Takahashi, J.S. (1997). Positional cloning of the mouse circadian *Clock* gene. Cell **89**, 1-13.

Kliman, R.M., and Hey, J. (1993). DNA sequence variation at the *period* locus within and among species of the *Drosophila melanogaster* complex. Genetics **133**, 375-387.

Konopka, R.J., and Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **68**, 2112-2116.

Konopka, R.J., and Orr, D. (1979). Effects of a clock mutation on the subjective day-Implications for the membrane model of the *Drosophila* circadian clock. In: Development and neurobiology of *Drosophila*. Plenum Books.

Konopka, R.J., and Wells, S. (1980). *Drosophila* clock mutations affect the morphology of a brain neurosecretory cell group. J. Neurobiol. **11**, 411-415.

Konopka, R.J., Wells, S., and Lee, T. (1983). Mosaic analysis of a *Drosophila* clock mutant. Mol. Gen. Genet. **190**, 284-288.

Konopka, R.J. (1987a). Genetics of biological rhythms in *Drosophila*. Ann. Rev. Genet. **21**, 227-236.

Konopka, R.J. (1987b). Neurogenetics of *Drosophila* circadian rhythms. In "*Evolutionary Genetics of Invertebrate Behaviour*" (ed. M. D. Huettel), p:215-221. Plenum Press, New York.

Konopka, R.J, Smith, R.F., and Orr, D. (1991). Characterisation of *Andante*, a new *Drosophila* clock mutant, and its interactions with other clock mutants. J. Neurogen. **7**, 103-114.

Konopka, R.J, Hamblen-Coyle, M.J., Jamison, C., and Hall, J.C. (1995). An ultrashort clock mutation at the *period* locus of *Drosophila melanogaster* that reveals some new features of the fly's circadian system. J. Biol. Rhythms **4**.

Konopka. R.J.. Kyriacou. C.P., and Hall. J.C. (1997). Mosaic analysis in the *Drosophila* CNS of circadian and courtship song rhythms affected by a *PERIOD* clock mutation . J. Neurogen. (in press).

Kripke. D.F., Risch. S.C., and Janowsky, D. (1983). Bright white light alleviates depression. Psychiatry Research **10**, 105-112.

Krebs. R.A.. and Bean. K.L. (1991). The mating behaviour of *Drosophila mojavensis* on organ pipe and agria cactus. Phyche **98**, 101-109.

Kulkarni, S.J.. and Hall, J.C. (1987). Behavioural and cytogenetic analysis of the *cacophony* courtship song mutant and interacting genetic variants in *Drosophila melanogaster*. Genetics **115**, 461-475.

Kulkarni, S.J., Steinlauf, A.. and Hall, J.C. (1988). The *dissonance* mutant courtship song in *Drosophila melanogaster*: Isolation, behaviour, and cytogenetics. Genetics **118**, 267-285.

Kyriacou. C.P.. and Hall. J.C. (1980). Circadian rhythm mutations in *Drosophila melanogaster* affect short-term fluctuations in the male's courtship song. Proc. Natl. Acad. Sci. USA **77**, 6729-6733.

Kyriacou, C.P., and Hall, J.C. (1982). The function of courtship song rhythms in *Drosophila*. Anim. Behav. **30**, 794-801.

Kyriacou, C.P.. and Hall, J.C.(1984). Learning and memory mutations impair acoustic priming of mating behaviour in *Drosophila*. Nature **308**, 62-65.

Kyriacou. C.P.. and Hall, J.C. (1984). Action potential mutations stop a biological clock in *Drosophila*. Nature **314**, 171-173.

Kyriacou, C.P. (1985). Long-term ebony polymorhisms: A comparison of the Contributions of Behavioural and non-behavioural fitness characters. Behav. Genet. **15**, 165-180.

Kyriacou, C.P., and Hall, J.C. (1986). Interspecific genetic control of courtship song production and reception in *Drosophila*. Science **232**, 494-497.

Kyriacou, C.P., and Hall, J.C. (1988). Comment on Crossley's and Ewing's failure to detect cycles in *Drosophila* mating songs. Anim. Behav. **36**, 1110.

Kyriacou, C.P., and Hall, J.C. (1989). Spectral analysis of *Drosophila* courtship song rhythms. Anim. Behav. **37**, 851-859.

Kyriacou, C.P. (1990). The molecular ethology of the *period* gene in *Drosophila*. Behav. Genet. **20**, 191-211.

Kyriacou, C.P., Oldroyd, M., Wood, J., Sharp, M., and Hill, M.(1990). Clock mutations alter developmental timing in *Drosophila*. Heredity **64**, 395-401.

Kyriacou, C.P., Berg, M.J. van den, and Hall, J.C. (1990). *Drosophila* courtship song cycles in normal and *period* mutant males revisited. Behav. Genet. **20**, 617-644.

Kyriacou, C.P., Greenacre, M.L., Ritchie, M.G., Byrne, B..C., and Hall, J.C. (1992). Genetic analysis of the love song preferences of *Drosophila* females. Amer. Zool. **32**, 31-39.

Kyriacou, C.P, Sawyer, L.A., Piccin, A., Couchman, M.E., and Chalmers, D. (1996). Evolution and population biology of the *period* gene. Sem. in Cell and Devel. Biology **7**, 803-810.

Kyriacou, C.P., Campesan, S., Stanley, R., Demetriades, M.C., and Rosato, E. (1997). Forward and reverse genetic approaches to the study of behaviour in higher eukaryotes. Recent Res. Devel. in Molecular Biol. **1**, 29-40..

Lachaise, D., Lemeunier, F., David, J.R., Tsacas, L., and Ashburner, M. (1986). The reproductive relatioships of *Drosophila sechellia* with *D. mauritiana*, *D. simulans*, and *D. melanogaster* from the Afrotropical region. Evolution **40**, 262-271.

Lawrence, P.A., and Johnston, P. (1984). The genetic specification of pattern in a *Drosophila* muscle. Cell **36**, 775-782.

Lawrence, P.A., and Johnston, P. (1986). The muscle pattern of a segment of *Drosophila* may be determined by neurons and not by contributing myoblasts. Cell **45**, 505-513.

Lee, C., Parikh, V., Itukaichi, T., Bae, K., and Edery, I. (1996). Resetting the *Drosophila* clock by photic regulation of *per* and a *per-tim* complex. Science **271**, 1740-1744.

Lee, W.H., and Watanabe, T.K. (1987). Evolutionary genetics of the *D. melanogaster* subgroup. Jpn J. Genetics **62**, 225-239.

Lemeunier, F., and Ashburner, M. (1976). Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (Sophophora). II. Phylogenetic relationships between six species based upon polytene chromosome banding sequences. Proc. R. Soc. London B. **193**, 275-94.

Lemeunier, F., and Ashburner, M.(1984). Relationships within the *melanogaster* species subgroup of the genus *Drosophila* (Sophophora). IV. The chromosomes of the new species. Chromosoma **89**, 343-351.

Lemeunier, F., David, J.R., Tsacas, L., and Ashburner, M. (1986). The *melanogaster* species group. The Genetics and Biology of *Drosophila* Vol. **3e**, 147-256. Academic Press, London.

Liimatainen, J. (1993). Courtship signals, their importance and inheritance in the species of the *Drosophila virilis* group. ACTA Univ. Ouluensis A **248**.

Lilliefors, H.W. (1967). The Kolmogorov-Smirnov test for normality with mean and variance unknown. J. Amer. Stat. Assoc. **62**, 399-402.

Liu, X., Lorenz, L., Yu, Q., Hall, J.C., and Rosbash, M. (1988). Spatial expression of the period gene in *D. melanogaster*. Genes and Development **2**, 228-238.

Livingstone, M.S. (1981). Two mutations in *Drosophila* affect the synthesis of octapamine, dopamine and serotonin by altering the activities of two amino-acid decarboxylases. Neurosci. Abstr. **7**, 249-259.

Logan, I.G., and Rosenberg, J. (1989). A Referee's comment on the identification of cyles in the courtship song of *Drosophila melanogaster*. Anim. Behav. **37**, 860-871.

Lutz, F.E. (1932). Experiments with Orthoptera concerning diurnal rhythms. Am. Mus. Novitates **550**, 1-24.

Manning, A. (1977). The control of sexual receptivity in female *Drosophila*. Anim. Behav. **15**, 239-250.

Massey, F.J., Jr. (1951). The Kolmogorov-Smirnov test for goodness of fit. J. Am. Stat. Assoc. **46**, 68-78.

Matsomoto, A., Motoshige, T., Murata, T., Tomioka, K., Tanimura, T., and Chiba, Y. (1994). Chronobiological analysis of a new clock mutant *Toki*, in *D. melanogaster*. J. Neurogenet. **9**, 141-155.

McClung, C.R., Fox, B.A., and Dunlap, J.C. (1989). The *Neurospora* clock gene frequency shares a sequence element with the *Drosophila* clock gene period. Nature **339**, 558-562.

McKeown, M. (1994). Sex determination and differentiation. Dev. Genet. **15**, 201-204.

McRobert, S.P., and Tompkins, L. (1985). The effect of *transformer*, *doublesex*, and *intersex* mutations on the sexual behaviour of *Drosophila melanogaster*. Genetics **108**, 103-110.

Menaker, M., Takahashi, J.S., and Eskin, A. (1978). The physiology of circadian pacemakers. Ann. Rev. Physiol. **40**, 501-526.

Miller, A., in ' Biology of *Drosophila*', M. Demerec, Ed. (Hafner, New York, 1950), pp. 420-534.

Moore-Ede, M.C., Sulzman, F.M., and Fuller, C. A. (1982). The clocks that time Us. Harvard Univ. Press.

Murata, M., Matsumoto, A., Tomioka, K., and Chiba, Y. (1995). *ritsu-* a rhythm mutant from a natural-population of *Drosophila melanogaster*. J. Neurogenet. **9**, 239-249.

Myers, M.P., Wagner-Smith, K., Welsey, C.S., Young, M., and Seghal, A. (1995). Positional cloning and sequence analysis of the *Drosophila* clock gene, *timeless*. Science **270**, 805--808.

Myers, M.P., Wager-Smith, K., Rothenfluh-Hilfiker, A., and Young, M.W. (1996). Light-induced degradation of *timeless* and entrainment of the *Drosophila* circadian clock. Science **271**, 1736-1740.

Nambu, J.R., Franks, R.G., Hu, S., and Crews, S.T. (1990). The *single-minded* gene of *Drosophila* is required for the expression of genes important for the development of the CNS Midline cells. Cell **63**, 63-75.

Newby, L.M., and Jackson, F.R. (1991). *Drosophila ebony* mutants have altered circadian activity rhythms but normal eclosion rhythms. J. Neurogen. **7**, 85-101.

Newby, L.M., and Jackson, F.R. (1993). A new biological rhythm mutant of *Drosophila melanogaster* that identifies a gene with an essential embryonic function. Genetics **135**, 1077-1090.

Nissani, M. (1977). Gynandromorph analysis of some aspects of sexual behaviour of *Drosophila melanogaster*. Anim. Behav. **25**, 555-566.

Noor, M.A.F. (1995). Speciation driven by natural selection in *Drosophila*. Nature **375**, 674-675.

Noor, M.A.F., and Aquadro, C.F. (1997). The courtship songs of *Drosophila pseudoobscura* and *D. persimilis*. I. Analysis of variation among species, populations, and individuals (submitted).

O'Kane, C.J., and Gehring, W.J. (1987). Detection *in situ* of genomic regulatory elements in *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. **84**, 9123-9127.

O'Neall, S.L., and Karr, T.L. (1990). Bidirectional incompatibility between conspecific population of *D. simulans*. Nature **348**, 178-180.

Ohnishi, S., Kawanishi, M., and Watanabe, T.K. (1983). Biochemical phylogenetics of *Drosophila*: Protein differences detected by two-dimensional electrophoresis. Genetica **6**, 55-63.

Pantazidis, A.C., and Zouros, E. (1988). Location of an autosomal factor causing sterility in *Drosophila mojavensis* males carrying the *Drosophila arizonensis* Y chromosome. Heredity **60**, 299-304.

Pantazidis, A.C., Galanopoulos, V.K., and Zouros, E.   An autosomal factor from *Drosophila arizonae* restores normal spermatogenesis in *D. mojavensis* males carrying the *D. arizonae* Y chromosome.

Paterson, H.E.H. (1985).   The recognition concept of species.   In: *Species and Speciation*. (Ed. by E. S. Vrba), pp:21-29.   Pretoria: Transval Museum.

Pawson, T., and Bernstein, A. (1990).   Receptor tyrosine kinase: genetic evidence for their role in *Drosophila* and mouse development.   Trends in Genet.   **6**, 350-356.

Peixoto, A.A., Costa, R., Wheeler, D.A., Hall, J.C., and Kyriacou, C.P. (1992). Evolution of the Threonine-Glycine repeat region of the *period* gene in the *melanogaster* species subgroup of *Drosophila*.   J. Mol. Evol.   **35**, 711-719.

Peixoto, A.A., Campesan, S., Costa, R., and Kyriacou, C.P. (1993).   Molecular evolution of a repetitive region within the *per* gene of *Drosophila*.   Mol. Biol. Evol.   **10**, 127-139.

Peixoto, A.A., Smith, L.A., and Hall, J.C. (1997).   Genomic organization and evolution of alternative exons in a *Drosophila* calcium channel gene.   Genetics   **145**, 1003-1013.

Pelandakis, M., Higgins, D.G., and Solignac, M. (1991).   Molecular phylogeny of the subgenus *Sophophora* of *Drosophila* derived from large subunit of ribosomal RNA sequences.   Genetica   **84**, 87-94.

Piñeiro, R., Carracedo, M.C., Izquierdo, J.I., and Casares, P. (1993).   Bidirectional selection for female receptivity in *Drosophila melanogaster*.   Behav. Genet.   **23**(1), 77-83.

Pirotta,V., Hadfield, C., and Pretorius, G.H.J. (1983).   Microdissection and cloning of the white locus and the 3B1-3C2 region of the *Drosophila* X chromosome.   EMBO J. **2**, 927-934.

Pittendrigh, C.S. (1960). Circadian rhythms and the circadian organisation of living systems. Cold Spring Harbour Symp. Quant. Biol. **25**, 159-184.

Pittendrigh, C.S. (1965). On the mechanism of entrainment of a circadian rhythm by light cycles. In Circadian Clocks ed. J. Aschoff, p:277-297. North Holland, Amsterdam.

Pittendrigh, C.S., and Daan, S. (1976). A functional anlysis of circadian pacemakers in nocturnal rodents. IV Entrainment: pacemaker as clock. J. Comp. Physiol. **106**, 291-331.

Power, J.M., Ringo, J.M., and Dowse, H.B. (1995). The efects of *period* mutations and light on the activity rhythms of *Drosophila melanogaster*. J. Biol. Rhyt. **10**, 267-280.

Reddy, P., Zehring, W.A., Wheeler, D.A., Pirotta, V., Hadfield, C., Hall, J.C., and Rosbash, M. (1984). Molecular analysis of the *period* locus in *Drosophila melanogaster* and identification of a transcript involved in biological rhythms. Cell **38**, 701-710.

Redman, J., Armstrong, S., and Ng, K.T. (1983). Free-running activity rhythms in the rat: Entrainment by melatonin. Science **219**, 1089-1092.

Reeve, E.C.R. (1955a). Inbredding with homozygotes at a disadvantage. Ann. Human Genet. **19**, 332-346.

Ritchie, M.G., and Gleason, J.M. (1995). Rapid evolution of courtship song pattern in *Drosophila willistoni* sibling species. J. Evol. Biol. **8**, 463-479.

Ritchie, M.G., and Kyriacou, C.P. (1994). Reproductive isolation and the *period* gene of *Drosophila*. Mol. Ecol. **3**, 595-599.

Ritchie, M.G., Yates, V.H., and Kyriacou, C.P. (1994). Genetic variability of the interpulse interval of courtship song among some European populations of *Drosophila melanogaster*. Heredity **72**, 459-464.

Rio, D.C. (1990). Molecular mechanisms regulating P-element transposition. Ann. Rev. Genet. **24**, 543-578.

Roberts, R.C. (1960). The effects of litter size of crossing lines of mice inbred without selection. Genet. Res. **1**, 239-252.

Roberts, S.K. de F. (1974). Circadian rhythms in cockroaches. Effect of the optic lobe lesions. J. Comp. Physiol. **88**, 21-30.

Robertson, A. (1952). The effect of inbreeding on the variation due to recessive genes. Genetics **37**, 189-207.

Robertson, H.M. (1983). Mating behaviour and the evolution of *Drosophila mauritina*. Evolution **37**, 1283-1293.

Romer, F. , in 'Recent Advances in Comparative Anthropods Morphology, Physiology and Development', A.P. Gupta, Ed. (New Brunswick, N.J., Rutgers Univ. Press, 1991), pp. 542-566.

Rosato, E., Peixoto, A.A., Barbujani, G., Costa, R., and Kyriacou, C. P. (1994). Molecular polymorphism in the *period* gene of *Drosophila simulans*. Genetics **138**, 693-707.

Rosato, E., Peixoto, A.A., Gallippi, A., Kyriacou, C.P., and Costa, R. (1996). Mutational mechanisms, phylogeny, and evolution of a repetitive region within a clock gene of *Drosophila melanogaster*. J.Mol. Evol. **42**, 392-408.

Rosato, E., Peixoto, A.A., Costa, R., and Kyriacou, C.P. (1997). Linkage disequilibrium, mutational analysis and selection in the repetitive region of the clock gene. *period*, in *Drosophila melanogaster*. Genet. Res. (in press).

Rutila, J.E., Edery, I., Hall, J.C., and Rosbash, M. (1992). The analysis of new short-period circadian rhythm mutants suggests features of *D. melanogaster period* gene function. J. Neurogen. **8**, 101-113.

Rutila, J.E., Hongkui, Z., Le, M., Curtin, K.D., Hall, J.C., and Rosbash, M. (1996). The *tim*[SL] mutant of the *Drosophila* rhythm gene *timeless* manifests allele-specific interactions with *period* gene mutants. Neuron **17**, 921-926.

Ryner, L.C., Goodwin, S.F., Castrillon, D.H., Anand, A., Villella, A., Baker, B.S., Hall, J.C., Taylor, B.J., and Wasserman, S.A. (1996). Control of male sexual behaviour and sexual orientation in *Drosophila* by the *fruitless* gene. Cell **87**, 1079-1089.

Sinchez, L., and Nöthiger, R. (1982). Clonal analysis of *Sex-lethal*, a gene neede for female sexual development in *Drosophila melanogaster*. Wilhelm Roux' Arch. **191**, 211-214.

Satokangas, P., Liimatainen, J.O., and Hoikkala, A. (1994). Songs produced by the females of the *Drosophila virilis* group of species. Behav. Genet. **24**, 263-272.

Sauman, I., and Reppert, S.M. (1996a). Circadian clock neurons in the silkmoth *Antheraea pernyi*: Novel mechanisms of *period* protein regulation. Neuron **17**, 889-900.

Sauman, I., and Reppert, S.M. (1996b). Molecular characterisation of pro-thoracicotropic hormone (PTTH) from the giant silkmoth *Antheraea pernyi*: developmental appearance of PTTH-expressing cells and relationship to circadian clock cells in central brain. Devel. Biol. **178**, 418-429.

Saunders, D. S. (1982). Insect clocks. 2ND Edition. Pergamon, Oxford.

Sawyer, L.A., Hennessey, M.J., Peixoto, A.A., Rosato, E., Parkinson, H., Costa, R., and Kyriacou, C.P. (ms submitted). Threonine-Glycine repeat length within the *period* gene modulates circadian temperature compensation in *Drosophila melanogaster*.

Scavarda, N.J., and Hartl, D.L. (1984). Interspecific DNA transformation in *Drosophila*. Proc. Natl. Acad. Sci. USA **81**, 7515-7519.

Schilcher, F. von. (1976a). The role of auditory stimuli in the courtship of *Drosophila melanogaster*. Anim. Behav. **24**, 18-26.

Schilcher, F. von. (1976b). The function of pulse song and sine song in the courtship of *Drosophila melanogaster*. Anim. Behav. **24**, 622-625.

Schilcher, F. von. (1977). A mutation which changes courtship song in *Drosophila melanogaster*. Behav. Genet. **7**, 251-259.

Schilcher, F. von, and Hall, J.C. (1979). Neural topology of courtship song in sex mosaics of *Drosophila melanogaster*. J. Comp. Physiol. **129**, 85-95.

Schluter, D. (1994). Experimental evidence that competition promotes divergence in adaptive radiation. Science **266**, 798-801.

Schluter, D., and McPhail, J.D. (1992). Ecological character displacement and speciation in sticklebacks. Am. Natur. **140**, 85-108.

Scott, D. (1986). Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. Proc. Nat. Sci. U.S.A. **83**, 8429-8433.

Scott, D., and Jackson, L. (1988). Interstrain comparison of male-predominant antiaphrodisiacs in *Drosophila melanogaster*. J. Ins. Physiol. **34**, 863-871.

Scott, D., Richmond, R.C., and Carlson, D.A. (1988). Pheromones exchanged during mating: a mechanism for mate assessment in *Drosophila*. Anim. Behav. **36**, 1164-1173.

Sehgal, A., Price, J.L., Man, B., and Young, M.W. (1994). Loss of circadian behavioural rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. Science **263**, 1603-1606.

Sheridan, A.K. (1981). Crossbreeding and heterosis. Ann. Breed. Abstr. **49**, 131-144.

Shin, H-S., Bargiello, T.A., Clark, B.T., Jackson, F.R., and Young, M.W. (1985). An unusual coding sequence from a *Drosophila* clock gene is conserved in vertebrates. Nature **317**, 445-448.

Siegfried, E., Ambrosio, L., and Perrimon, N. (1990). Serine/Glycine protein kinases in *Drosophila*. Trends in Genet. **6**, 357-362.

Smith, R.F., and Konopka, R.J. (1981). Circadian clock phenotypes of chromosome aberrations with a breakpoint at the *per* locus. Mol. Gen. Genet. **183**, 243-251.

Smith, R.F., and Konopka, R.J. (1982). Effects of dosage alterations at the *per* locus on the period of the circdian clock of *Drosophila*. Mol. Gen. Genet. **185**, 30-36.

Solignac, M., and Monnerot, M. (1986). Race, formation, speciation, and introgression within *D. simulans*, *D.mauritiana*, and *D. sechellia* inferred from mitochondrial DNA analysis. Evolution **40**, 531-539.

Spieth, H.T. (1952). Mating behaviour within the genus *Drosophila*. Bull. Am. Mus. Nat. Hist. **99**, 401-474.

Stanewsky, R., Fry, T.A., Reim, I., Saumweber, H., and Hall, J.C. (1996). Bioassaying putative RNA-binding motifs in a protein encoded by a gene that influences

courtship and visually mediated behaviour in *Drosophila: In vitro* mutagenesis of *nonA*. Genetics **143**, 259-275.

Suvanto, L., Hoikkala, A., and Liimatainen, J.O. (1994). Secondary courtship songs and inhibitory songs of *Drosophila virilis*-group males. Behav. Genet. **24**, 85-94.

Swaab, D.F., and Hofman, M.A. (1995). Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation. Trends Neurosci. **18**, 264-270.

Tajima, F. (1989). Statistical Method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**, 585-595.

Takahashi, J.S. (1991). Circadian rhytms:from gene expression to behaviour. Current Op. Neurobiol. **1**, 556-561.

Taylor, B.J. (1992). Differentiation of a male-specific muscle in *Drosophila melanogaster* does not require the sex-determining genes *doublesex* and *intersex*. Genetics **132**, 179-191.

Taylor, B.J., Villella, A., Ryner, L.C., Baker, B.S., and Hall, J.C. (1994). Behavioural and neurobiological implications of sex-determining factors in *Drosophila*. Dev. Genet. **15**, 275-296.

Taylor, B.J., and Knittel, L.M. (1995). Sex-specific differentiation of a male-specific abdominal muscle, the Muscle of Lawrence, is abnormal in hydroxyurea-treated and in *fruitless* male flies. Development **121**, 3079-3088.

Thackeray, J.R., and Kyriacou, C.P. (1991). Molecular evolution in the *D. yakuba period* locus. J. Mol. Evol. **31**, 389-401.

Throchmorton, L.H. (1975). The phylogeny, ecology and geography of *Drosophila*. In handbook of Genetics ed. R. C. King, 421-469.

Timblin, C., Battey, J. and Kuehl,W.M. (1990). Application for PCR technology to subtractive cDNA cloning: identification of genes expressed specifically in murine plasmacytoma cells. Nuc. Acid Res. **18**, 1587-1993.

Tomaru, M., and Oguma,Y. (1994a). Differences in courtship song in the species of the *Drosophila auraria* complex. Anim. Behav. **47**, 133-140.

Tomaru, M., and Oguma,Y. (1994b). Genetic basis and evolution of species-specific courtship song in the *Drosophila auraria* complex. Genet. Res. Camb. **63**, 11-17.

Tomaru, M., Matsubayashi, H., and Oguma,Y. (1995). Heterospecific interpulse intervals of courtship song elicit female rejection in *Drosophila biauraria*. Anim. Behav. **50**, 905-914.

Tompkins, L., Hall, J.C., and Hall, L.M. (1980). Courtship-stimulating volatile compounds from normal and mutant *Drosophila*. J. Ins. Physiol. **26**, 689-697.

Tompkins, L., and Hall, J.C. (1981). *Drosophila* males produce a phermone which inhibits courtship. Z. Naturforsch. **36c**, 694-696.

Tompkins, L., and Hall, J.C. (1983). Identification of brain sites controlling femlae receptivity in mosaics of *Drosophila melanogaster*. Genetics **103**, 179-195.

Tompkins, L., Gross, A., Hall, J.C., Gailey, D.A., and Siegel, R.W. (1982). The role of female movement in the sexual behaviour of *Drosophila melanogaster*. Behav. Genet. **12**, 295-307.

Tompkins, L., Siegel, R.W., Gailey, D.A., and Hall, J.C. (1983). Conditioned courtship in *Drosophila* and its mediation by association of chemical cues. Behav. Genet. **13**, 565-578.

Tompkins, L. (1984). Genetic analysis of sex appeal in *Drosophila*. Behav. Genet. **14**, 411-440.

Tompkins, L. (1984). The effect of *Sex-lethal* mutations on the sexual behaviour of *D. melanogaster*. Genetics **107**, 107-115.

Tompkins, L. (1985). Genetic control of sexual behaviour in *Drosophila melanogaster*. Genetics **108**, 57-63.

Truman, J.W. (1974). Physiology of insect rhythms IV. Role of the brain in the regulation of the flight rhythm of the giant Silkmoth. J. Comp. Physiol. **95**, 281-296.

Tsacas, L. (1971). *D. teissieri*, nouvelle espèce africaine du groupe *melanogaster* et notre sur deux autres espèces nouvelles pour l' Afrique. Bull. Soc. Entomol. Fr. **76**, 35-45.

Tsacas, L., Lachaise, D., and David, J.R. (1981). Composition and Biogeography of the Afrotropical Drosophilid Fauna. In: The genetics and biology of *Drosophila* (eds. M. Ashburner, H.L. Carson and J.N. Thompson, Jr.), Vol. **3a**, p:197-259, Academic Press London.

Tsacas, L. (1984). Nouvelle données sur la biogéographie et l'évolution du groupe *Drosophila melanogaster* en Afrique. Description de six nouvelles espèces (Diptera, Drosophilidae). Ann. Soc. Entomol. Fr. (N.S.) **16**, 517-540.

Tsacas, L., and Bächli, G. (1981). *Drosophila sechellia*, n. sp., huitième espèce du sous-groupe *melanogaster* des Iles Sichelles (Diptera, Drosophilidae). Rev. Fr. Entomol. **3**, 146-150.

Tsacas, L., and David, J.R. (1974). *Drosophila mauritiana*, n. sp. du groupe *melanogaster* de l' ile Maurice (Diptera, Drosophilidae). Bull. Soc. Entomol. Fr. **79**, 42-46.

Tsacas, L., and David, J.R. (1978). Une septiàme espàce appartenant au sous-groupe *Drosophila melanogaster* Meigen: *Drosophila orena* spec. nov. du Cameroun (Diptera: Drosophilidae). Beitr. Entomol. (Berlin) **28**, 179-182.

Tsacas, L., and Lachaise, D. (1974). Quatre nouvelles espàces de la Càte-d' Ivoire du genre *Drosophila*, groupe *melanogaster*, et discussion de l' origine du sous-groupe *melanogaster* (Diptera: Drosophilidae). Ann. Univ. Abidjan E. **7**, 193-211.

Tully, T. (1984). *Drosophila* learning: behaviour and biochemistry. Behav. Genet. **14**, 527-557.

Tully, T., and Quinn, W.G. (1985). Classical conditioning and retention in normal and mutant *D. melanogaster*. J. Comp. Physiol. **157**, 263-277.

Turek, F.W. (1985). Circadian neural rhythms in mammals. Ann. Rev. Physiol. **47**, 49-64.

Turek, F.W., and Losee-Olsen, S. (1986). A benzodiazepine used in the treatment of insomnia phase-shifts the mammalian circadian clock. Nature **321**, 167-168.

Van Gelder, R.N., and Krasnow, M.A. (1996). A novel circadianly expressed *D. melanogaster* gene dependent on the *period* gene for its rhythmic expression. EMBO Journal **15**, 1625-1631.

Venard, R., and Jallon, J.-M. (1980). Evidence for an aphrodisiac pheromone of female *Drosophila*. Experientia **36**, 211-212.

Villella, A., and Hall, J.C. (1996). Courtship anomalies caused by *doublesex* mutations in *Drosophila melanogaster*. Genetics **143**, 331-344.

Vosshall, L.B., and Young, M.W. (1992). Circadian behaviour defects of eye, optic lobe, and brain mutants of *Drosophila melanogaster*. In Young, M.W. (ed.), *Molecular Genetics of Biological Rhythms*. Marcel Dekker, New York.

Vosshall, L.B., Price, J.L., Seghal, A., Saez, L., and Young, M.W. (1994). Block in nuclear localisation of *period* protein by a second clock mutation, *timeless*. Science 263, 1606-1609.

Vuoristo, M., Isoherranen, E., and Hoikkala, A. (1996). Female wing spreading as acceptance signal in the *Drosophila virilis* group of species. J. Insect Behav. 9, 505-515.

Watanabe, T.K., and Kawanishi, M. (1979). Mating preference and direction of evolution in *Drosophila*. Science 205, 906-907.

Watterson, G.A. (1975). On the number of segregating sites in general models without recombination. Theoretical Population Biology 7, 256-276.

Wehr, T.A., and Wirz-Justice, A. (1982). Circadian rhythm mechanisms in affective illness and in antidepressant drug action. Pharmcopsychiatry 15, 31-39.

Welbergen, P., van Dijken, F.R,. and Scharloo, W. (1987). Collation of courtship behaviour of sympatric species of *Drosophila melanogaster* and *Drosophila simulans*. Behaviour 101, 253-274.

Welbergen, P., Spruijt, B, and van Dijken, F.R. (1992). Mating speed and interplay between male and female courtship responses in *Drosophila melanogaster* (Diptera: Drosophilidae). J. Ins. Behav. 5, 229-244.

Wheeler, D.A., Fields, W.L., and Hall, J.C. (1988). Spectral analysis of courtship songs: *D. melanogaster*, *D. simulans*, and their interspecific hybrids. Behav. Genet. 18, 675-703.

Wheeler, D.A., Kulkarni, S.J., Gailey, D.A., and Hall, J.C. (1989). Spectral analysis of courtship songs in behavioural mutants of *Drosophila melanogaster*. Behav. Genet. **19**, 503-528.

Wheeler, D.A., Kyriacou, C.P., Greenacre, M.L., Yu, Q., Rutila, J.E., Rosbash, M., and Hall, J.C. (1991). Molecular transfer of a species-specific courtship behaviour from *Drosophila simulans* to *Drosophila melanogaster*. Science **251**, 1082-1085.

White, J., Yeats, A., and Skipworth, G. (1994). *Tables for statisticians*. 3rd Edition, Stanley Thornes, (Publishers) LTD.

Winer, B.J. (1971). Statistical Principles in Experimental Design. Second Edition. Publ. Mc-Graw-Hill and Kogakusha.

Winfree, A.T. (1987). The timing of Biological clocks. Scientific American Library, Inc.

Yoon, C.K. (1991). Molecular and behavioural evolution in the semispecies of *Drosophila athabasca*. Ph.D. Thesis Cornell University, U.S.A.

Yoon, C.K., and Aquadro, C.K. (1994). Mitichondrial DNA variation among the *Drosophila athabasca* semispecies and *Drosophila affinis*. J. Hered. **85**, 421-426.

Young, M. (1992). New clock mutants in *Drosophila*. EMBO Workshop on Molecular Chronobiology, Abstr. pp. 11.

Yu, Q., Jacquier, A.C., Citri, Y., Hamblen, M., Hall, J.C., and Rosbash, M. (1987a). Molecular mapping of point mutations in the *period* gene that stop or speed up biological clocks in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **84**, 784-788.

Yu, Q., Colot, H.V., Kyriacou, C.P., Hall, J.C., and Rosbash, M. (1987b). Behaviour modification by *in vitro* mutagenesis of a variable region within the *period* gene of *Drosophila*. Nature **326**, 765-769.

Zehring, W.A., Wheeler, D.A., Reddy, P., Konopka, R.J., Kyriacou, C.P., Rosbash, M., and Hall, J.C. (1984). P-element transformation with *period* locus DNA restores rhythmicity to mutant, arrhythmic *Drosophila melanogaster*. Cell **39**, 369-376.

Zeng, H., Qian, Z., Myers, M.P., and Rosbash, M. (1996). A light-entrainment mechanism for the *Drosophila* circadian clock. Nature **380**, 129-135.

Zhang, S.-D., and Odenwald, W.F. (1995). Misexpression of the *white* (*w*) gene triggers male-male courtship in *Drosophila*. Proc. Natl. Acad. Sci. USA **92**, 5525-5529.