Bergmann's rule is maintained during a rapid range expansion in a damselfly¹

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Abstract

Climate-induced range shifts result in the movement of a sample of genotypes from source populations to new regions. The phenotypic consequences of those shifts depend upon the sample characteristics of the dispersive genotypes, which may act to either constrain or promote phenotypic divergence, and the degree to which plasticity influences the genotype-environment interaction. We sampled populations of the damselfly *Erythromma viridulum* from Northern Europe to quantify the phenotypic (latitude-body size relationship based on seven morphological traits) and genetic (variation at microsatellite loci) patterns that occur during a range expansion itself. We find a weak spatial genetic structure that is indicative of high gene flow during a rapid range expansion. Despite the potentially homogenising effect of high gene flow, however, there is extensive phenotypic variation among samples along the invasion route that manifests as a strong, positive correlation between latitude and body size consistent with Bergmann's rule. This positive correlation cannot be explained by variation in the length of larval development (voltinism). While the adaptive significance of latitudinal variation in body size remains obscure, geographical patterns in body size in odonates are apparently underpinned by phenotypic plasticity and this permits a response to one or more environmental correlates of latitude during a range expansion.

Keywords: Bergmann's rule, microsatellite, Odonata, phenotype, plasticity, range shift, Zygoptera.

Introduction

A major challenge in the study of global ecological change is to predict ultimate consequences from proximate biotic responses to environmental change. One predictive framework arises from static patterns of clinal variation in body size (and associated life-history traits), a common phenomenon whereby individuals become either larger (Bergmann, 1847, Atkinson & Sibly, 1997, Blackburn *et al.*, 1999) or smaller (Park, 1949, Mousseau, 1997) with increasing latitude, presumably a response to variation in the length of the growth season (Chown & Gaston, 1999) and/or temperature (Atkinson, 1994). For example, most ectotherms exhibit an increase in body size at lower developmental temperatures (the temperature size rule; Atkinson, 1994), albeit with the underlying mechanisms poorly-understood (Atkinson, 1994, Forster *et al.*, 2012). Moreover, under counter-gradient variation (Conover & Schultz, 1995) individuals at high latitudes attempt to compensate for the shorter season by growing faster than low latitude conspecifics; perfect compensation for seasonal time constraints will result in a uniform phenotype, but under- or over-compensation of growth rate will be manifest as apparent converse Bergmann or Bergmann clines respectively. While there is some indication that larger terrestrial arthropods almost uniformly exhibit converse Bergmann clines (Blanckenhorn & Demont, 2004), several reviews of latitude-size relationships in insects have yielded few consistent patterns (Chown & Gaston, 2010, Shelomi, 2012).

A second component of the predictive framework involves the dynamics of species' ranges. Morphological changes in colonists during a range expansion have been associated with selection for increased dispersal ability (Parmesan, 2006, Hill *et al.*, 2011). Since many high latitude species are

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apparently attempting to maintain their fundamental niches, the direction of range expansion correlates with latitude (Hill *et al.*, 2011). The extent to which dispersive phenotypes complement or are antagonistic with the expected latitudinal variation in body size during the process of a range expansion is unclear. For example, larger arthropod species with development times that are relatively long compared with the growth season are susceptible to seasonal time constraints (Chown & Gaston, 1999) and tend to decrease in size with latitude (Blanckenhorn & Demont, 2004). However, the relationship between body size and dispersal ability within arthropod taxa is inconsistent, being either positive (Lepidoptera; Stevens *et al.*, 2012), negative (Odonata: Anisoptera; McCauley, 2010) or variable (Odonata: Zygoptera; Conrad *et al.*, 2002). Some additional complexity to the pattern of phenotypic expression that occurs during a range expansion may be associated with the numbers of individuals that comprise the expanding front. High levels of gene flow can limit the capacity for local adaptation (Lenormand, 2002), while colonisation by relatively few individuals allows founder effects and drift (stochastic processes) to predominate. As the phenotypic response to a heterogeneous environment can determine a species' capacity to shift its range (Gunnarsson *et al.*, 2012) it is important to understand the extent to which trait expression differs from the background level of population differentiation due to neutral, demographic processes (McKay & Latta, 2002, Brommer, 2011).

Odonata (dragonflies and damselflies) are excellent model organisms to study the processes that occur during range expansions as many species are altering their ranges in response to environmental warming (Hickling *et al.*, 2005, Hassall & Thompson, 2008a, Hassall & Thompson, 2010). Moreover, body size is a key life history parameter for odonates, for example affecting clutch size (Cordero, 1991), fitness (Michiels & Dhondt, 1991, but cf. Thompson & Fincke, 2002), and trophic ecology (Thompson, 1978). Studies that quantify latitudinal gradients in odonate morphology are limited. Hassall (in press) demonstrated that temperature and time stress generate U-shaped relationships between size and latitude (*i.e.* converse-Bergmann clines and Bergmann clines at lower and higher latitudes, respectively) in *Calopteryx maculata*, supporting Johansson's (2003) research on *Enallagma cyathigerum* but raising doubts about whether the higher latitude response was driven solely by changes in voltinism. At a more regional scale, Hassall *et al.* (2009) found the strength of latitudinal variation in body size to differ between zygopteran species in the UK (~51°N to 56.6°N). Northern individuals of *Coenagrion puella* were heavier than their southern counterparts (with no significant correlation between wing length and latitude), and while a similar trend was apparent for *Pyrrosoma nymphula* neither body mass nor wing length significantly correlated with latitude. The contrast may be explained by relative position within a species' range, with *C. puella* collected from its northern range margin where there are both semivoltine and univoltine populations, while samples of *P. nymphula* were at the middle of its latitudinal range and are semivoltine. Hassall *et al.* (2008b) noted a difference in range expansion, with *C. puella* a more recent coloniser than *P. nymphula*. Odonates thus exhibit complex patterns of geographic variation in body size and can exhibit a weak "Bergmann cline" at higher latitudes. Previous work has implicated a genetic component to phenotypic variation in *E. cyathigerum* (De Block *et al.*, 2008), however whether clinal variation in body size is affected by conditions of high gene flow, for example during a rapid range expansion, remains to be determined.

The zygopteran *Erythromma viridulum* (Charpentier) is a coenagrionid damselfly that inhabits ponds, lakes and ditches. *E. viridulum* is bivoltine at the southern edge of its range in Greece (Galletti & Pavesi, 1983) and semivoltine in the UK (Keat, 2007), but no data exist for other European populations. *E. viridulum* has made an extensive range expansion into north-western Europe in the last 30 years (Ketelaar, 2002) and is the first recorded example of a migrant damselfly establishing colonies in the British Isles (Dewick & Gerussi, 2000). Until the 1980s, this species was absent from northern France and was recorded infrequently in the Netherlands (Askew, 1988), but by the mid 1990s, *E. viridulum* had colonised northern continental Europe. Large numbers of *E. viridulum* were observed at a few sites in

south-east UK in 1999 (Dewick & Gerussi, 2000) and in southern UK in 2000 (Cham, 2001). This species subsequently expanded its British range at roughly 30 km yr^{-1} (Fig. 1). Concomitant with these observations, genetic studies have revealed high levels of gene flow among UK populations (Watts *et al.*, 2010). The early records and sampling of this invasion permitted a uniquely-detailed investigation of how high levels of gene flow impacts phenotypic variation at the forefront of an expanding range margin.

We quantify morphological variation along the northwest edge of *E. viridulum*'s range to identify whether a latitude-body size relationship is maintained during a rapid range expansion. It might be expected that the recently-established populations at the leading edge of the range expansion would exhibit similar morphology due to constraints imposed by high levels of gene flow. We examine the ability of latitude, temperature, location and the level of genetic divergence among populations to explain the pattern of morphological variation and find that variation in body size is primarily influenced by latitude and is not constrained during a rapid range expansion.

Methods

Sample collection and processing

Between 2002 and 2006, 777 adult male *E. viridulum* were captured from a total of 21 sites: 12 in southern UK and 9 (from 4 countries: Germany, Belgium, Netherlands and France) in northern continental Europe (Fig. 1, Table 1). While it is impossible to discriminate immigrants from residents at any site, we assume that most, if not all, of the individuals that were sampled had emerged from the water body at which they were caught. Despite the recent immigration of *E. viridulum* from the continent, coenagrionid damselflies rarely disperse more than 1-2 km when they reside within a suitable habitat matrix (Conrad *et al.*, 1999, Watts *et al.*, 2004). If immigrants were present in the samples they likely constituted a small proportion of the individuals and thus the patterns here are assumed to represent a developmental response to the local environment. Seven phenotypic traits were measured on all specimens to the nearest 0.1 mm using either digital dial callipers or a light microscope and eyepiece micrometer: head width, wing length, wing width, thorax length, thorax width, tibia length and abdomen length. A subsample of 713 specimens from the 12 largest-samples were genotyped at 10 microsatellite loci (Keat *et al.*, 2005) with genotyping procedures provided in Watts *et al.* (2010), as incorporating data from the other sites with relatively few genotypes (mean sample size $n=9$) would introduce noise. Temperature data were extracted from the 30 arc-seconds ($\sim 1 \text{ km}$) resolution WorldClim dataset (Hijmans *et al.*, 2005) to provide estimates of mean annual temperature (Temp_{ANN}) and mean winter temperature (Temp_{WIN} , the mean temperature of December, January, and February) for every site.

Data analyses

As all phenotypic traits were significantly correlated ($r>0.5$, $p<0.001$ in all cases), a principal components analysis (PCA) was used to summarise the overall variation in body size. The first principal component (PC1) explained 92% of the variation and was negatively correlated with all traits: PC1 was "absolute" transformed ($-PC1$) to give a positive measure of overall body size, hereafter "SIZE". We tested for a correlation between latitude and mean population SIZE in (i) the full sample ($n=21$), (ii) the UK sites only ($n=12$), and (iii) the European sites only ($n=9$). We then identified the subset of variables that best predicted variation in body size using generalised linear mixed effects models (GLMMs), with the individual as the unit of replication, constructed in the nlme package (Pinheiro *et al.*, 2013) in R (R Development Core Team, 2013); SIZE was the response variable examined against four predictor variables, latitude, Temp_{ANN} , Temp_{WIN} , and location, and with Site always included as a random effect. While latitude as a predictor has no biological relevance *per se*, odonate body size is influenced by photoperiodic time stress which varies systematically with latitude (Hassall, in press). The eight potential models (including the null model with a floating intercept) were compared using AIC in the AICcmodavg (Mazerolle, 2012) package in R (R Development Core Team, 2013) to select the most parsimonious

model. We also examined for a latitude-location interaction (*i.e.* SIZE~latitude+location+latitude×location) within the GLMM framework to test for a difference in the latitude-size pattern between the UK and continental Europe. We evaluated the potential for a confounding effect of multicollinearity among predictors using variance inflation factors (VIFs) calculated for the models' fixed effects.

To examine the effect of genetic divergence among populations upon phenotypic variation, we transformed the pattern of phenotypic variation to the measure " P_{ST} ", a measure of phenotypic differentiation among pairs of populations that is analogous to F_{ST} , a measure of genetic distance among samples using methods described by McKay & Latta (2002). In its original derivation, phenotypic divergence " Q_{ST} " requires an estimate of additive genetic variance that should be obtained from common garden experiments (McKay & Latta, 2002); since such extensive larval breeding was not possible, the measure of phenotypic divergence P_{ST} includes non-additive effects (Brommer, 2011). Components of trait variance are partitioned within and among populations, with the proportion of the total variance among populations acting as a measure of the differentiation in the trait(s) between populations. Where P_{ST} is greater than F_{ST} (for neutral loci) the trait has diversified more than would be expected purely on the basis of gene flow and genetic drift implying a selective response to the environment.

Genetic differentiation between all pairs of samples (F_{ST}) was calculated using FSTAT v.2.9.3 (Goudet, 2001). To quantify the effect of spatial structuring upon genetic and phenotypic variation, the matrices of pairwise F_{ST} and P_{ST} were correlated against a matrix of the linear distances separating sample pairs (In km; Rousset, 1997) using Mantel tests. A third Mantel test was used to test for a correlation between the matrix of F_{ST} and the corresponding matrix of P_{ST} to determine whether there is any relationship between the level of genetic and phenotypic divergence among samples. The significance of each Mantel test was calculated by permutation procedure (n=999 permutations).

Results

E. viridulum exhibited substantial variation in body size among sites, with individual wing length varying from 15.9 to 20.6 mm and abdomen length varying from 20.9 to 26.9 mm. Body size (SIZE) was positively associated with latitude in the data set as a whole (Pearson's correlation, $r=0.717$, $p<0.001$, $n=21$) continental European populations ($r=0.664$, $p=0.051$, $n=9$) and in the UK ($r=0.671$, $p=0.017$, $n=12$) (Fig. 2). The best-fit model describing variation in body size involved latitude, location and the latitude×location interaction, although the explanatory power was similar to the same model without that interaction ($\Delta AICc=1.63$, Table 2). After centring the latitude predictor, VIFs for the predictors in the top model of SIZE~latitude+location+latitude×location were 3.117, 1.139 and 2.919, respectively – well below the guideline value of 10 where multicollinearity is thought to interfere with models (Kutner *et al.*, 2005) and providing confidence in the parameterisation of our models (which are presented with uncentred predictors for ease of interpretation). Body size was marginally greater in the UK than in the older populations in continental Europe (hence the selection of the location term in the two best models; Tables 2 and 3), although there is no conspicuous difference to the latitude-size relationship between the populations from the UK and continental Europe that would be indicative of a fundamental location effect (Fig. 2). Indeed, while the location and latitude×location interaction terms contributed to improved fit in the two top models (Table 2), neither were statistically significant (Table 3) which suggests that any location effects are minor. There was no evidence for a sawtooth cline in the relationship between size and latitude that might indicate a change in voltinism across the study area (Fig. 2).

The level of genetic differentiation among all pairs of populations was low, with average $F_{ST}=0.032\pm0.003$ (SE) and all pairwise values of $F_{ST}<0.09$. Distance separating the sample sites had no significant effect on the amount of genetic differentiation between sites ($r=0.148$, $p=0.202$, Fig. 3a), which is consistent with the recent range expansion. Mean $P_{ST}=0.264\pm0.033$ (SE) was an order of magnitude greater than

F_{ST} and accordingly, pairwise estimates of phenotypic variation (P_{ST}) among locations generally were greater than the corresponding estimates of F_{ST} (a pattern consistent with other studies, Brommer, 2011). We did not find a significant relationship between P_{ST} and the distance separating populations ($r=-0.186$, $p=0.904$, Fig. 3b), or between genetic (F_{ST}) and phenotypic (P_{ST}) differentiation among sample locations ($r=0.036$, $p=0.372$, Fig. 3c).

Discussion

Body size is an important biological trait, influencing behaviour, physiology and fitness. Like many animals, *E. viridulum* body size varies with latitude, with a general increase in size at high latitudes, an apparent Bergmann cline. We demonstrate that this latitudinal variation in body size occurs during this species' extensive northwards range expansion and under conditions of high gene flow that might have been expected to constrain morphology. In fact emerging adults of the newly-founded populations in the UK almost immediately (*i.e.* within a few generations) fit into the "appropriate place" on a latitudinal cline in body size. The lack of evidence for a shift in voltinism in our sample populations implies that this pattern may occur within a group of populations exhibiting the same mode of voltinism.

While few consistent latitude-body size patterns have been identified in insect taxa (Blanckenhorn & Demont, 2004, Chown & Gaston, 2010, Kivelä *et al.*, 2011, Shelomi, 2012), predicting the latitude-body size response of odonates will be affected by the semi-aquatic life history. For example, Forster *et al.* (2012) showed a trend towards a negative relationship between temperature and body size in large aquatic species, but a more positive (and more variable) relationship in large terrestrial species. Structural growth occurs only during the aquatic (larval) period in Odonata. This may explain why the clear increase in body size of adult *E. viridulum*, and other odonates (Johansson, 2003, Hassall, in press), at higher latitudes contrasts with the typical pattern exhibited by large terrestrial insect taxa (Blanckenhorn & Demont, 2004) but is consistent with the broader pattern described by Forster *et al.* (2012). In the case of *E. viridulum*, the apparent speed of response appears to suggest that phenotypic plasticity during larval growth drives the latitude-size relationship. However, does the broader pattern in Odonata and other aquatic insects imply greater selection on the aquatic phase? Even so, the body-size response of odonates is somewhat counterintuitive as small size at high latitudes is an expected response to a shorter larval growth season (Mousseau, 1997, Blanckenhorn & Demont, 2004). Since the impact of seasonal time constraints upon growth will be more severe in more northerly populations, one mechanism by which individuals may complete development and emerge as viable adults is to extend the duration of the larval phase (Johansson, 2003, Cabanita & Atkinson, 2006). For organisms with a flexible larval developmental period, an interaction between shifts in voltinism and growth season is predicted to manifest as a sawtooth cline in body size whereby size decreases with latitude until the growth season is so short that there is an obligate extension to the larval period and concomitant increase in size (Roff, 1980). A sawtooth cline was not apparent from Johansson's (2003) study of *E. cyathigerum*, most likely because the large distances (*ca.* 2,000 km) separating high latitude samples (from Belgium, Sweden and Norway) coincided with shifts in voltinism. The finer spatial resolution of our sampling (populations separated by up to ~400 km) addresses this issue, but still provides no evidence for a sawtooth cline in body size. It is hard to argue that larval developmental period in our sample of continental European populations was different to the UK populations, which are semivoltine (Keat, 2007). At least for *E. viridulum*, a two-year developmental period may relax the effects of seasonal constraints compared with an attempt to complete development within a single growth season. With this in mind, it is notable that univoltine adult *E. cyathigerum* decreased in size with an increase in latitude between 42°N and 57°N (Johansson, 2003). The larval stage is an important component in determining the rate of range expansion (*e.g.* Crozier, 2004), and variation in latitudinal body-size relationships among arthropod taxa may reflect the ability of the immature phase to tolerate (cold) winters and thus develop over successive growth seasons.

Latitude encompasses a range of abiotic components that affect growth, particularly temperature and photoperiod, and odonate larvae certainly are capable of marked physiological responses to these factors (De Block & Stoks, 2003, De Block *et al.*, 2008). Like Johansson (2003), we observe a strong effect of latitude, but in contrast to that study we found no effect of temperature. Indeed there is a contrast between laboratory experiments that have shown body size in ectotherms to increase at lower developmental temperatures (larger size at lower temperatures, Atkinson, 1994) and the complex latitude-body size relationships exhibited by empirical systems (Chown & Gaston, 2010), presumably due to additional drivers of body size. Temperature determines the length of the growing season for many insects through thermal thresholds for development, although where range expansions maintain a thermal niche the effect on body size may not be important. Where photoperiod determines the length of the growing season, movement of individuals into a new latitudinal zone with shifting climate space may result in shorter growing seasons at a given temperature regime. Decreasing photoperiods reduce odonate size in laboratory experiments when temperature is kept constant (Johansson & Rowe, 1999), and with photoperiod and temperature working synergistically to reduce body size when the latter is increased (De Block & Stoks, 2003). Such an effect of photoperiod (reduced growth season) is not observed here, implying for example some adaptive changes to any new photoperiod regimes (Harada *et al.*, 2005), a response to some other correlate(s) of latitude, or over-compensation in growth rate.

The latitude-size relationship is essentially similar for the older populations from continental Europe and in the recent UK colonists (Fig. 2), and presumably is adaptive. A key issue is whether any phenotypic response relies upon standing genetic variation or is plastic. In contrast to the effect of latitude *per se* (Table 1), the pattern of phenotypic variation exhibited by *E. viridulum* is decoupled from the distance separating populations (Fig. 3b) and the amount of genetic differentiation between areas (Fig. 3c). The implication is that an environmental correlate of latitude elicits a strong selective pressure on body size and that *E. viridulum* is capable of mounting an appropriate phenotypic response despite the potential homogenising effect of high gene flow. Of course, gene flow may increase the efficacy of selection under some circumstances (Holt & Gomulkiewicz, 1997). However, interpreting the action of selection using estimates of P_{ST} is complicated when the additive genetic component to the trait is unknown (Brommer, 2011). While many species are assumed to have a substantial additive genetic component to their body size (Brommer, 2011) this does not appear to be the case for odonates; Lowe *et al.* (2009) failed to find significant heritability for body size in *Coenagrion puella*. More relevant would be the role of phenotypic plasticity in facilitating colonisation of novel habitats (Knop & Reusser, 2012). For many odonates larval development rate appears to be quite plastic, with individuals capable of altering their developmental period in response to local conditions (Watts *et al.*, 2005, De Block *et al.*, 2008, Watts & Thompson, 2012); delayed larval development has been observed in other aquatic insect taxa (Cabanita & Atkinson, 2006). Conversely, a genetic component to latitudinal variation in body mass in *E. cyathigerum*, consistent with the size variation described by Johansson (2003), was manifest through variation in growth rates among areas and an interaction with development time (De Block *et al.*, 2008).

Under conditions of high gene flow during a rapid range expansion odonates are capable of sufficient development phenotypic plasticity that ultimately determines adult body size to allow an appropriate response to a new environment (Fig. 2; see also Watts *et al.*, 2010). Such plasticity could also facilitate subsequent improved adaptation through selection at colonised areas. Studies of fitness components in odonates generally have emphasised the importance of stabilising selection (Fincke, 1982, Stoks, 2000) for male body size and yet the apparent optimal body size varies considerably with latitude. It would be interesting to quantify the fitness consequences of variation in body size throughout a species' range. Such large-scale patterns in the extent of phenotypic variation, the underlying drivers in the field, and their adaptive and ecological consequences remain to be uncovered.

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Tables

Table 1 – Sites from which male *Erythromma viridulum* were collected in 2005 and 2006. “Label” gives the label of the points in the map in Fig. 1 and * indicates sites for which genetic data are available.

Country	Site name	Founded	Label	Lat (°N)	Long (°E)	Temp _{WIN} (°C)	Mean size (-PC1)	SE	n
Belgium	Boves	<2002	BVS	49.844	2.383	3.63	-9.111	1.940	6
	Wijvenheide Eutroof Ven, Zonhoven	<2002	WEV	50.985	5.315	2.73	-1.513	1.194	5
	Wijvenheide Grote Vijver, Zonhoven	<2002	WGV	51.014	5.349	2.70	-13.510	1.740	10
France	Blangy-Tronville*	<2002	BLT	49.880	2.423	3.60	-8.997	1.933	24
	Le Marais de Guines	<2002	LMD	50.879	1.877	4.53	-9.094	1.597	9
Germany	Braunschweig*	<2002	BRN	52.264	10.526	0.87	7.098	2.511	43
Netherlands	Alphen Ann Den Rijn*	<2002	UAD	52.129	4.658	2.97	-3.516	1.368	34
	Het Vinne, Zoutleeuw	<2002	HET	50.833	5.104	2.90	-11.013	1.826	5
	Koudekerke*	<2002	KDK	51.483	3.555	3.70	-4.872	1.750	19
UK	Beaulieu Heath	2004	BLH	50.844	-1.455	4.97	-12.252	2.619	4
	East Ruston*	2002	EAR	52.800	1.471	4.50	5.673	1.667	64
	Fingringhoe	2002	FGH	51.663	0.963	4.10	10.025	2.540	5
	Lowestoft*	2004	LWS	52.504	1.733	4.27	15.109	1.687	84
	Maldon 2*	2002	ML2	51.745	0.716	3.90	-4.094	2.339	98
	Morton Pond*	2004	MOR	50.671	-1.141	4.80	-2.873	1.817	55
	Parsonage Peat Pond*	2004	PPP	50.690	-1.204	4.53	-6.480	1.466	42
	Priory Country Park*	2002	PCP	52.129	-0.428	3.80	0.901	1.578	103
	Salcott-cum-Virley*	2002	SCV	51.786	0.814	3.83	-0.432	1.984	58
	Silver End	2002	SVE	51.841	0.636	3.70	1.120	2.019	7
	Thorpe-Le-Soken	2002	TLS	51.849	1.179	4.23	2.512	1.637	14
	Wrest Park*	2004	WRP	52.003	-0.409	3.67	-5.177	1.179	88

Table 2 – Comparison of models explaining variation in body size in *Erythromma viridulum* using Akaike's information criterion controlling for small sample sizes (AICc). Temp_{WIN}=mean winter temperature, Temp_{ANN}=mean annual temperature, Location=factor describing the sampling site as either in the UK or in continental Europe. The "Null" model has a floating intercept. w_i (the Akaike weight) gives the likelihood of each model given the set of models that are tested.

Model	K	AICc	Δ AICc	w_i
Latitude+Location+Latitude×Location	6	5355.0	0	0.54
Latitude+Location	5	5356.6	1.63	0.24
Latitude	4	5358.5	3.57	0.09
Latitude+Temp _{ANN} +Location	6	5359.5	4.49	0.06
Latitude+Temp _{WIN} +Location	6	5360.9	5.97	0.03
Latitude+Temp _{ANN}	5	5361.8	6.85	0.02
Latitude+Temp _{WIN} +Temp _{ANN} +Location	7	5362.1	7.16	0.01
Latitude+Temp _{WIN}	5	5363.1	8.17	0.01
Latitude+Temp _{WIN} +Temp _{ANN}	6	5364.2	9.19	0.01
Temp _{ANN} +Location	5	5365.4	10.42	0
Location	4	5366.7	11.72	0
Temp _{WIN} +Temp _{ANN} +Location	6	5367.5	12.52	0
Temp _{WIN} +Location	5	5368.5	13.5	0
Temp _{WIN} +Temp _{ANN}	5	5371.0	16.05	0
Null	3	5372.1	17.16	0
Temp _{ANN}	4	5373.5	18.53	0
Temp _{WIN}	4	5376.6	21.61	0

Table 3 – Variation in overall body size ("SIZE", summarised by -PC1 from principal component analysis) in male *Erythromma viridulum* as determined by the best-fit general linear mixed effects model (GLMM). Location=a factor describing the site as either in the UK or in continental Europe.

	Estimate	SE	DF	t	p
Intercept	-162.810	75.096	756	-2.168	0.031
Latitude	3.110	1.468	17	2.118	0.049
Location	-63.565	107.694	17	-0.590	0.563
Latitude×Location	1.270	2.093	17	0.607	0.552

Figure legends

Figure 1 – (a) The geographic context of the study, (b) the pattern of spread of *Erythromma viridulum* through the UK, and (c) locations of sampling sites for *E. viridulum* males. For coordinates and sample sizes see Table 1.

Figure 2 – Variation between SIZE (first principle component [-PC1] describing seven morphometric traits) and latitude across 21 samples of *Erythromma viridulum* males from locations northwest Europe. Open symbols represent UK populations and closed symbols represent populations from continental Europe, and error bars are ± 1 SE.

Figure 3 – Relationships between (a) genetic distance (F_{ST}) and geographic distance, (b) phenotypic distance (P_{ST}) and geographic distance, and (c) pairwise genetic distance (F_{ST}) and pairwise phenotypic distance (P_{ST}) (dashed line indicates $P_{ST}=F_{ST}$) for 12 populations of the zygopteran *Erythromma viridulum* from northern Europe. See text for statistics.