## Project summary

Adaptive radiations, like that of the Galápagos finches, are canonical examples showing the power of natural selection to produce organismal diversity. Indeed, some have argued that most species arise by adaptive radiation. If so, then the species-rich floras of hyperdiverse regions must be dominated by species flocks that are the result of adaptive radiations. Yet not all evolutionary radiations (*sensu* Futuyma) are adaptive. Some, like the radiation of salamanders in the genus *Plethodon* exhibit both high rates of taxonomic diversification and little morphological divergence. The flora of southern Africa includes a remarkable set of highly diverse and largely endemic evolutionary radiations, e.g., the ice plants (> 1500 spp.), irises (~900 spp.), restios (~340 spp.), and proteas (~340 spp). The flora of the Cape region of southern Africa, in particular, is among the richest and most diverse in the world, and leading explanations for its diversity invoke both adaptive and non-adaptive processes to explain the remarkable, repeated radiations. This project focuses on a typical radiation within the Proteaceae and seeks to determine whether diversification has been primarily adaptive or non-adaptive.

***Intellectual merit*:** We propose to use experimental measurements of individual performance in experimental gardens and wild populations to identify traits that govern response to the dominant environmental gradients (functional traits) and to identify traits that are strongly associated with taxonomic identity (taxonomic traits). Focusing on functional traits, we propose to determine whether among-population differences are driven primarily by environmental adaptation or by physical isolation and whether among-species differences arise from the simple extrapolation of within-species trends or through processes different from those that lead to population divergence. Specifically, we propose to answer the following three questions:

1. *Which plant traits are reliable indicators of individual performance and which are reliable indicators of taxonomic identity?*
2. *Is divergence among <u>populations</u> primarily adaptive or non-adaptive?*
3. *Is divergence among <u>species</u> primarily adaptive or non-adaptive?*

To answer these questions the project will also ***develop new methods (1) for comparing patterns of differentiation in morphological/physiological traits and genetic markers and (2) for inferring population phylogenies from allele frequencies***. Software implementing these new methods will be made freely available.

***Broader impacts*:** The project offers unique opportunities for international education, research, and collaboration. Scientists from the South African National Biodiversity Institute (SANBI) will be full collaborators in the project, and participants from the U.S. will have a unique multicultural experience – South Africa is known as "a world in one country" for its mixing of African, European, and Asian cultures. SANBI supports bioregional planning initiatives with coordinated conservation implementation, and a comprehensive threatened species action program. It brings together scientists, professionals from conservation NGOs and GOs, as well as members of the public to forge conservation policy and effect implementation, and the project will be part of these efforts. Finally, we will focus recruiting efforts on students from underserved groups, using our existing network of contacts in the University of Connecticut at nearby "majority minority" middle schools and high schools.

# TABLE OF CONTENTS

For font size and page formatting specifications, see GPG section II.C.



*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

## Results from prior NSF support

***DEB-0424767*:** $19,374 – 09/01/04-08/31/07 (PI Robin Chazdon, co-PI Holsinger): "Collaborative Research: Causes and consequences of tree colonization patterns in wet tropical forests."
*Research results*: Genetic analysis of individuals in the founding generation of a secondary forest showed that more than 50% of the genes present were contributed by only two parents (Sezen et al. 2005). Many seedlings and saplings are derived from the founding generation, although some long-distance dispersal events have been detected (Sezen et al. submitted). Several to many generations will be required before patterns of genetic diversity are similar to those in primary forests.
*Broader impacts*: As part of a larger project studying regeneration of secondary forests near La Selva, Costa Rica, this project is supporting one Ph.D. dissertation (a Turkish male).
***DEB-0089801*:** $227,500 – 04/01/01-03/31-05 (PI Silander, co-PI Gelfand): "Stochastic modeling for geographic diversity in plant species richness in South Africa."
*Research results:* Twenty journal articles related to this project have been published or are in press. More have been submitted or are in manuscript (marked with ** in Literature Cited). Gelfand et al. (2005) developed a spatially explicit hierarchical Bayesian model for species distribution patterns. Latimer et al. (2006) used this model to explain presence/absence of two closely related species (*Protea punctata* and *P. mundii*) as a function of twenty-eight environmental variables.
*Broader impacts*: This project partially supported two Ph.D. dissertations (Statistics: Asian female – EEB: white male, NSF Graduate Research Fellow) and two post-doctoral associates (white male, Hispanic female). Three undergraduates were supported. Part of this research was done in cooperation with CapeNature, the major conservation agency in the region.
***Relationship to DEB-0516320*:** $450,000 – 09/01/05-08/31/08 (PI: Silander, co-PI Gelfand). Work supported by DEB-0516320 (1) extends the models to predict local abundances rather than presence/absence, (2) characterizes climate variability, trends, and extremes at a fine spatial scale, and (3) develops models for population-level performance in response to climate variation. Work proposed here will take advantage of new environmental data layers and measures of population performance produced by DEB-0516320, but other aspects of the projects are non-overlapping.
***Relationship to OISE-0623341*:** $149,923 – 09/15/06-09/14/09 (PI: Silander, co-PIs Holsinger, Jones, Schlichting). In collaboration with faculty from the University of Cape Town, this NSF-IRES project will train students, primarily undergraduates, through field-based research on ecological and evolutionary processes in the Cape Floristic Region. Each year 3-4 students will devote several weeks to supervised research on pelargoniums, geophytes, or proteas. We expect to engage students in work related to the project proposed here in one of the three years OISE-0623341 is active.

## Response to panel

We believe the proposal has been substantially improved as a result of suggestions made by previous panels and reviewers. We summarize changes made in response to the most recent comments below.
*1. The proposal does not describe and delineate the taxonomic and functional traits.* We now provide a comprehensive list of all traits we propose to measure (Table 1), and we propose to use the results of our experiments to distinguish taxonomic from functional traits. The traits we propose to analyze are derived from recent taxonomic monographs of the group and from principles of plant physiological ecology. We will assess the relationship between *all* trait values and taxonomic identity, and we will identify taxonomic traits as those in which individual differences are statistically associated with differences in taxonomic identity. Similarly, we will measure the relationship between *all* trait values and individual performance, and we will identify functional traits as those in which inidividual differences are statistically associated with differences in individual performance (p. 6). Any traits identified as both taxonomic traits and functional traits will be treated

separately in later analyses. By using the results of our experiments to distinguish taxonomic and functional traits we avoid *a priori* decisions about the category to which traits are assigned.

## Responses to reviewers

*1. The methodology for objective 3 involves assumptions that are not spelled out.* The proposal now makes clear (a) that the method for reconstructing population phylogenies reflects recency of common ancestry, including common ancestry through gene flow (p. 12) and (b) that interpreting the joint history of trait evolution for taxonomic and functional traits depends only on where character-state changes are mapped on a tree, not on whether the taxonomic traits are neutral (p. 14).

*2. The brief window of the experiments samples only a small portion of environmental variability.* We agree, and we address this point in describing our studies of the relationship between trait values and individual performance. In particular, DEB-0516320 provides a population-level context to judge the impact of climate extremes, allowing us to relate short-term measurements of individual performance to performance under climate extremes (details provided below – p. 6).

*3. One of the requirements for the project is an adequate species phylogeny.* In fact, one fundamental question we address is whether among-species differences arise from extrapolation of among-population differences (pp. 3, 11). Thus, we propose to construct an adequate *population* phylogeny, not just an adequate species phylogeny.

*4. The contrast between adaptive and non-adaptive radiations is a false dichotomy*. We agree that both adaptive and non-adaptive processes will be involved in most radiations. We do not agree that the contrast is a false dichotomy, although they do represent ends of a continuum. In an adaptive radiation differential adaptation is the driving force for diversification. Thus, in an adaptive radiation functional trait differentiation will substantially exceed differentiation in neutral markers. In a non-adaptive radiation functional trait differention will be equal to or less than differentiation in neutral markers. We now make this distinction clear (p. 9).

*5. The distribution of associated species (a "phytometer" approach) could provide more information.* A "phytometer" approach is implicitly included in our methods. The random "site" and "population" effects in our statistical models account for *all* unmeasured background variables, not just the distribution of associated species (p. 7).

*6. Having a specialist in ecophysiology would strengthen the proposal.* We agree that a specialist in ecophysiology is important. Indeed, one of our South African collaborators, Guy Midgley, is a highly regarded plant ecophysiologist (e.g., Agenbag et al. 2004a,b,c; Huntley et al. 2004).

## Project Description

### Introduction

> ***Adaptive radiation*** is…the differentiation of a single ancestor into an array of species that inhabit a variety of environments and that differ in traits used to exploit those environments … ***It is regarded as the hallmark of adaptive evolution and may well be the most common syndrome in the origin and proliferation of taxa*** (Schluter 2000, p. 1; emphasis added).

If Schluter is right, if adaptive radiation is "the most common syndrome in the origin and proliferation of taxa," then the species-rich floras of hyperdiverse regions, like the Neotropics or southeastern Asia, must be dominated by species flocks that result from adaptive radiations. In fact, Linder (2005) argues that the diversity in most vascular plant floras is accounted for by a few species-rich lineages and that most lineages are species-poor. Clearly, "the central question is when, and why, the evolution of these species flocks started" (Linder 2005, p. 536). ***Why did the evolution of these species flocks start?*** is a question because radiations need not be adaptive. For example, the 46 species of *Plethodon* found in eastern North America show remarkable morphological uniformity relative to other plethodontid salamanders in spite of rates of taxonomic diversification comparable

to those of the most dramatic adaptive radiations (Kozak et al. 2006 and references therein). *Plethodon* appears to be an example of an evolutionary radiation (Futuyma 1998, p. 117) that is not an adaptive radiation.

In vascular plants, the most thoroughly studied evolutionary radiations may be the Hawaiian silverswords (Carr et al. 1989; Robichaux et al. 1990; Friar et al. 2006), the Hawaiian lobeliads (Givnish et al. 1995, 2004), and the columbines (Hodges 1997; Hodges and Arnold 1995). Each of these seems to be an adaptive radiation, with individuals occupying a wide range of habitats in silverswords and lobeliads and using a wide diversity of pollinators in columbines. But ***the most spectacular plant evolutionary radiations are in southern Africa***, e.g., ice plants (Aizoaceae subfamily Ruschioideae, > 1500 spp. in < 9 million years: Klak et al. 2004), irises (Iridaceae subfamilies Ixioideae and Nivenioideae, ~900 spp. in ~20 million years: Goldblatt et al. 2002), restios (Restionaceae, ~340 spp. in ~28 million years: Linder 2003; Linder and Hardy 2004), and proteas (Proteaceae, ~360 spp. in 10-37 million years: Reeves 2001).

Within southern Africa, the Cape Floristic Region (CFR) provides especially rich opportunities to investigate the contribution of adaptive and non-adaptive processes to evolutionary radiations. The flora is exceptionally diverse (~9000 species in an area the size of Maine, comparable to the most diverse floras of the world: Latimer et al. 2005), and its ecology is well-known (Cowling 1995; Cowling and Heijnis 2001). In contrast to the best studied examples of radiation in plants, radiations in the CFR appear to involve both adaptive (edaphic or climatic specialization) and non-adaptive (limited dispersal) mechanisms (Cowling et al. 1996; Goldblatt and Manning 2000; Linder 2003). In *Argyroderma*, for example, adaptation appears to have followed allopatric differentiation (Ellis et al. 2006). Radiations within the Proteaceae of the CFR are particularly attractive for further analysis.

Not only do members of the Proteaceae have many life-forms (from prostrate, creeping vine to large shrubs or small trees), occur in many habitats, and exhibit a broad functional diversity (e.g., leaf size and shape; pollination and dispersal syndrome) (Rebelo 2003), but the existing data on species distributions are very rich (>60,000 geo-referenced sites, > 250,000 records of species presence, absence, and abundance and ecological site attributes: http://protea.worldonline.co.za/default.htm). The extent, detail, and accuracy of these distributional data allow us to easily identify sampling locations that span a range of climatic, edaphic, and topographic possibilities, quickly surmounting the first hurdle that any experimental study of evolutionary radiations must cross: locating suitable samples. Similarly, the quality and extent of environmental data for the CFR is comparable to that available anywhere in the developed world.

***We propose to determine whether diversification in a representative clade of Proteaceae in the CFR has been primarily adaptive or primarily non-adaptive.*** Schluter (2000, p. 31) argues that "adaptive radiation is a statement about the 'fit' of organisms to their diverse environments and not about the historical sequence of steps that produced this fit." Thus, we seek first to determine whether most trait differences among-population and among-species differences "fit" the organisms to their environment. And to the extent we find such a fit, we also propose to investigate its causes. We propose to determine whether among-population differences are driven primarily by environmental adaptation or by physical isolation and whether among-species differences arise from the extrapolation of within-species trends or through distinct processes associated with speciation. To address this broad question we propose to answer the following specific questions:

1. Which plant traits are reliable indicators of individual performance and which are reliable indicators of taxonomic identity, i.e., which traits are ***functional traits***, which are ***taxonomic traits***, and which are both?
2. Is divergence among <u>populations</u> primarily adaptive or non-adaptive? Specifically, is there greater among-population differentiation in ***functional traits*** than predicted from variation in ***genetic markers*** we presume to be neutral?

3. Is divergence among <u>species</u> primarily adaptive or non-adaptive? Specifically, are patterns of divergence among species in ***taxonomic traits*** consistent with those predicted from variation in ***functional traits*** (adaptive divergence) or with those predicted from ***genetic markers*** (non-adaptive divergence)?

### *Choice of focal taxa within South African Proteaceae*

"Expansion to new resources and environments is a dominant theme in adaptive radiation" (Schluter 2000, p. 49). We focus on the white proteas (*Protea* section *Exsertae*) as an exemplar of CFR radiations. White proteas are broadly distributed through the CFR (Figure 1), and their populations span a wide range of environments found in the CFR. If "expansion to new resources" plays a major role in diversification within any CFR plant group, it is likely to play a role in the white proteas.

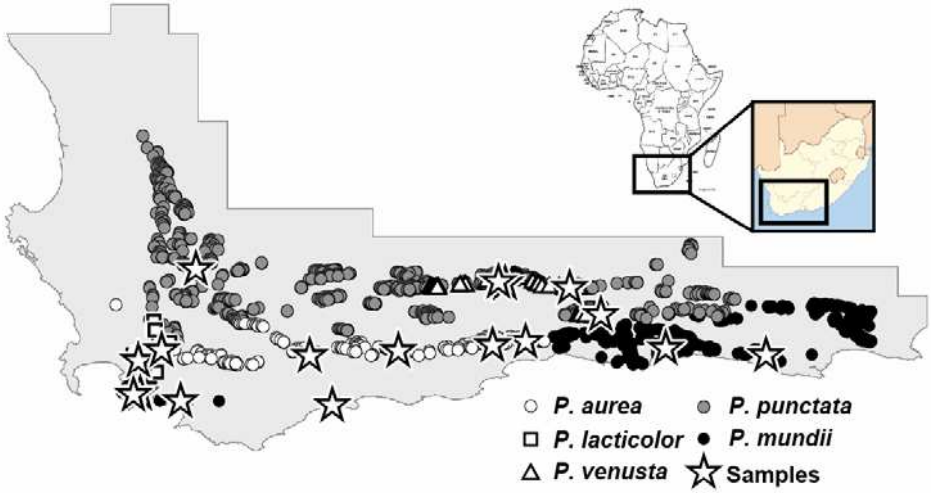


**Figure 2**: Distribution of white protea populations in the CFR. Stars identify the location of 17 populations from which tissue samples have already been collected and from which cuttings are being established in the gardens at Kirstenbosch.

White proteas are morphologically distinct from other *Protea*s, with hairless styles longer than the involucral bracts and a slender perianth that coils into the base of the flowerhead on opening (Rebelo 2003). Our phylogenetic analysis of the *trn*L-F spacer region, *rps*16, the *atp*B-*rbc*L spacer region, and *cp*GS for all sequences of South African Proteaceae available in Genbank (3500bp, 94 species) combined with 138 AFLP markers and 13 insertions/deletions (from Reeves 2001) provides substantial support for monophyly of the white proteas (Figure 2). Limited variation within the clade suggests a recent and rapid radiation. Indeed, the clade including the 5 South African genera in subfamily Proteoideae (ca. 360 spp.; Hoot and Douglas 1998; Rourke 1998) may be only 10 my old (Reeves 2001), suggesting that the white proteas may be as little as 1-2 my old.

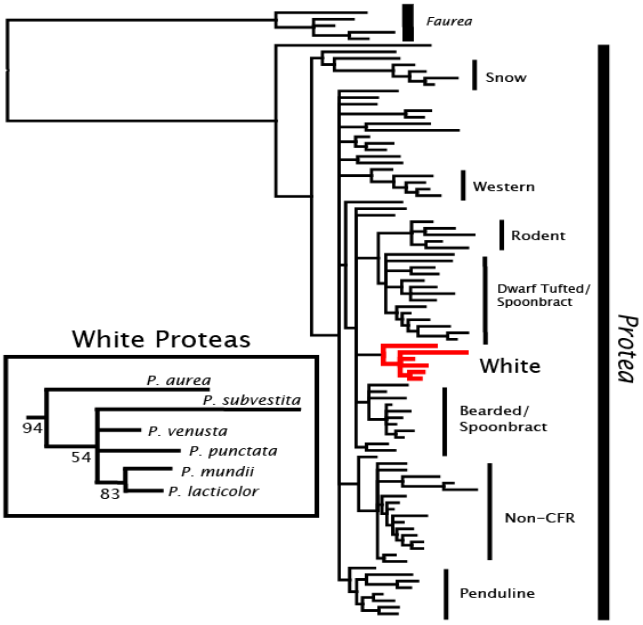


**Figure 1**: Molecular consensus phylogeny of the genus *Protea* based on data from Reeves (2001). Values below nodes in inset are posterior probabilities. *Faurea* and *Protea* are sister within subfamily Proteoideae (Hoot and Douglas 1998).

Our experiments will include all six members of the white protea clade, including *P. subvestita* which is found in eastern South Africa outside the CFR. Monophyly of the white proteas is necessary for us to determine whether species arose through processes different from those that led to among-population differences and whether diversification has led to "an array of [populations and] species that inhabit a variety of environments" (Schluter 2000, p. 1), the definition of an adaptive radiation.

## Research plan and objectives

### *1. Which plant traits show adaptive differentiation among populations and which indicate taxonomic identity?*

Silander et al. (2006a,b), Gelfand et al. (2005, 2006), and Latimer et al. (2006) used a suite of 28 environmental variables to predict local presence/absence (1×1 minute grid) of several groups of CFR Proteaceae, including the white proteas (Figure 3). For example, Latimer et al. (2006) showed that heat units (degree days) is negatively associated with presence of *P. mundii*, while January maximum temperature, summer soil moisture days, and enhanced vegetation index are positively associated with its presence. In *P. aurea*, interannual CV for precipitation and July minimum temperature are negatively associated with presence, while roughness, elevation, potential evapotranspiration, frost season length, enhanced vegetation index, and soil fertility are positively associated with presence. Furthermore, Latimer (2006) has shown through a series of reciprocal transplants that *P. aurea*, *P. mundii*, and *P. punctata* perform best, in terms of biomass accumulation and/or survival, in sites close to the median of the environmental axes along which they occur, especially the axes of total rainfall, rainfall seasonality, and temperature. In short, distribution of white proteas is strongly influenced by gradients in water and nutrient availability.

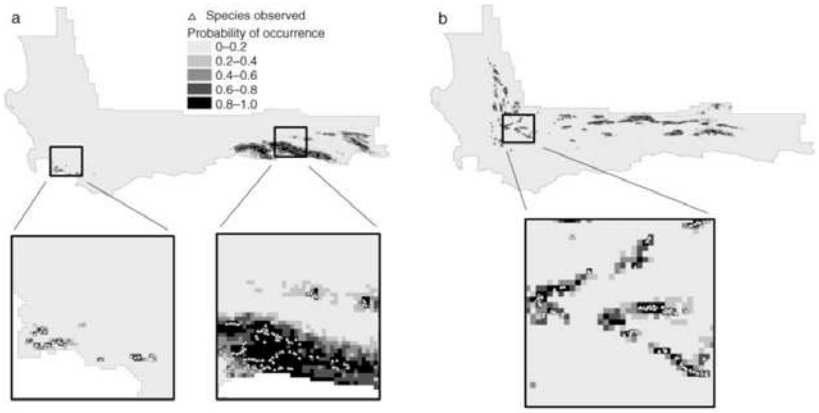


**Figure 3**: Predicted and observed distribution of (a) *P. punctata* and (b) *P. mundii.* Darker shades correspond to a higher probability of occurrence. Circles indicate known points of occurrence.

A central tenet of plant physiological ecology is that individual-level anatomical, morphological, and physiological traits mediate plant performance (growth, reproduction, mortality) in response to climatic and environmental variation (Weiher et al. 1999; Lambers et al. 2000; Geber and Griffen 2003; Ho et al. 2005; Ellis and Weis 2006). Thus, we propose to use a combination of experimental and observational analyses to identify those plant traits with the strongest relationship to individual performance (growth, reproduction, survival). Traits listed in Table 1 include both a variety of traits that physiological ecologists have identified as important mediators of individual plant responses to environmental variation and a variety that have been used in recent taxonomic monographs to distinguish members of the white proteas from one another and from closely related *Protea* clades. It

| | | | | |
|---|---|---|---|---|
| *Specific leaf area | *Stomatal density | *Wood density | Water use efficiency | Tissue elastic modulus |
| *Leaf N content | *Leaf P content | Root allocation | Nutrient use efficiency | Leaf chlorophyll content |
| Light harvesting efficiency | Electron transport efficiency | *Stem pubescence | *Leaf pubescence | *Leaf l/w ratio |
| *Leaf length | *Flowerhead length | *Flowerhead width | *Involucral bract color | *Involucral bract pubescence |
| *Involucral bract length | *Perianth length | *Perianth tube length | *Style tip bent/straight | *Pollen presenter shape |
| *Stem/leaf angle | *Leaf base angle | *Perianth pubescence | *Involucral bract shape | *Leaf color |

**Table 1**: Plant traits to be measured in white proteas. Traits marked with * will be measured in wild populations as well as in experimental gardens

is reasonable to regard traits with a strong relationship to individual performance as ***functional traits***, because differences in those traits presumably reflect differences in physiological function that lead to differences in individual performance. Such traits are likely to be subject to natural selection, and among population or among species differences in these traits are candidates for adaptive differentiation (Ackerly 2004; Westoby et al. 2002; Geber and Griffen 2003). We will use the same set of observations to identify those traits with the strongest relationship to taxonomic identity. It is reasonable to regard traits with a strong relationship to taxonomic identity as ***taxonomic traits***, because differences in these traits presumably reflect the historical events associated with speciation.

Some traits, e.g., leaf shape and leaf pubescence, may be strongly related both to individual performance and to taxonomic identity. Such traits are simultaneously ***functional*** and ***taxonomic traits***. Any such traits will be treated separately from purely ***functional*** and purely ***taxonomic traits*** in reconstruction of trait evolutionary histories (p. 13).

***Experimental design*:** We will establish two common gardens to measure trait values and individual performance under controlled experimental conditions. Establishing more than two gardens is logistically intractable, given the time and expenses involved with establising a large number of replicates within each garden. We have chosen gardens at Kirstenbosch and at the mid-elevation site of the Jonaskop transect (cf. Agenbag 2004a-c), because they provide environmental conditions broadly representative of those within the CFR (Table 2) and because previous work has shown that plants grown in more extreme locations often fail to survive.[1] At each location we will grow samples of *Protea mundii*, *P. punctata*, *P. venusta*, *P. subvestita*, *P. lacticolor*, and *P. aurea* collected from wild populations. Each species will be represented by 6-8 clonally propagated individuals (cuttings) from each of 5 populations chosen to represent the range of climates in which that species is found (a subset of the populations sampled for molecular markers, p. 8, because establishing individuals from more populations is not feasible). We will use mixed-effect modeling (see details of statistical analysis below) for simultaneous analysis of performance in all individuals, a total sample size of at least 360 individuals (2 experimental sites × 6 species × 5 populations × 6 individuals). The analysis will be replicated across years, with each individual measured in both years and year of observation included as a random effect. We will use linear discriminant analysis to identify traits strongly associated with taxonomic identity. Taxonomic identity will be verified by local experts (see attached letter).

| Environmental Variable | Kirstenbosch | Jonaskop (950 m site) | Range (17 sites) |
|---|---|---|---|
| Roughness (alt. range in m) | 645 | 268 | (25) 207-629 |
| Elevation (m) | 141 | 944 | 171-1967 |
| Potential evapotranspiration | 1769 | 1628 | 444-2071 |
| Frost season length (days) | 10 | 86 | 9-180 |
| Heat units (degree days) | 8.25 | 0.45 | 0-15.1 |
| Mean annual precip. (mm) | 1131 | 720 | 448-3345 |
| C.V. precipitation | 16.7 | 26 | 15-32 |
| Jan. maximum temp. (C) | 26.2 | 24.1 | 18-30 |
| July minimum temp. (C) | 7.8 | 4.4 | -3-8 |
| Rainfall seasonal concentration | 45 | 39 | 3-54 |
| Summer soil moisture days | 26.2 | 16.6 | 11-47 |
| Winter soil moisture days | 102.6 | 81.8 | 38-110 |
| Enhanced vegetation index | 169 | 92 | 52-205 |
| Pct. high-fertility soils | 33 | 0 | 0-100 |
| Pct. fine-texture soils | 3 | 0 | 0-63 |
| Pct. acidic soils | 67 | 100 | 0-100 |

**Table 2.** Key environmental variables at experimental sites. Range is for 17 sample sites used in preliminary analysis of specific leaf area (p. 6).

In addition to measurements from experimental gardens, we will measure a subset of traits on individuals in wild populations sampled for molecular markers (marked with a * in Table 1). We will use the same statistical approaches to identify traits strongly associated with individual performance

[1]Transplant survival of *P. mundii*, *P. aurea*, *P. punctata*, and *P. subvestita* is significantly lower at the 744m and 1250m sites than at the mid-elevation site on the Jonaskop transect (Latimer 2006).

in these analyses. The wild populations included in these analyses will span almost the entire range of environments in the CFR. Thus, we propose to substitute variation in space for variation in time, partially compensating for restricted sampling of climate extremes in a 3-year common garden study. Our analysis of wild populations will focus only on the relationship between *phenotype* and fitness. Thus, trait plasticity will not affect the validity of any relationships we identify.

We will measure each of the traits mentioned in Table 1, except root allocation and wood density, on every individual during the three years of the study. Root allocation and wood density will be measured after a destructive harvest in year 3. For leaf-level traits we will select fully expanded leaves formed in the year of measurement. Specific leaf area will be measured by weighing oven dried leaves to the nearest 0.1mg and measuring the area of individual leaves with a digital scanner. Instantaneous water use efficiency will be measured at light saturation with a LiCor 6400. Tissue elastic modulus will be estimated from the relationship between measurements of leaf water potential (Scholander pressure bomb; Soil Moisture Corporation, Santa Barbara, CA) and leaf water content (% dry weight; Tyree and Jarvis 1982; Robichaux et al. 1986). We will measure leaf light harvesting and electron transport efficiencies with an Optisciences OS1Fl fluorometer, and leaf chlorophyll content with a SPAD cholophyll meter. We will measure flowerhead, bract, and involucral traits with digital calipers to the nearest millimeter. As indices of performance, we will measure growth (height, internode length, biomass accumulation), reproduction (number of flower heads), and survival at the end of each season. Once established, white proteas are killed primarily as a result of fire, and lifetime reproductive success depends directly on aboveground biomass. Thus, differences in growth are directly related to differences in lifetime fitness. Weather data will be derived from stations already established at the two experimental sites or from high-resolution interpolations derived from DEB-0516320 (p. 1).

***Our experiments will identify traits likely to show adaptive differentiation by identifying those with a strong relationship to individual performance, i.e., functional traits, and traits likely to reflect patterns of evolutionary history by identifying those with a strong association with taxonomic identity, i.e., taxonomic traits.***

***Statistical analysis of experimental results*:** We will identify ***functional traits*** as that subset of traits in Table 1 for which we detect an effect of trait value on individual performance in either experimental gardens or in wild populations. Statistical analysis of the data will be straightforward. Using environment data for each season and experimental location, a random site effect to reflect unmeasured environmental covariates at each experimental location, a random population effect to reflect shared history of individuals collected from the same site, and trait data, we will construct a mixed-effect linear (growth and reproduction) or logistic (survival) regression of individual performance on environmental covariates and traits. This approach allows us to distinguish collection site effects, plastic responses, and genetic differences among populations. We will use standard model choice criteria (Gelfand and Ghosh 1998; Spiegelhalter et al. 2002) to identify traits with an important influence on individual performance at each site. We will identify ***taxonomic traits*** as that subset of traits in Table 1 with significant loadings on the major axes of a linear discriminant analysis, with species treated as the groups to be discriminated. Some traits may be identified as *both* functional and taxonomic traits. We will treat traits falling into each of the three categories – pure functional, pure taxonomic, both functional and taxonomic – separately in all analyses.

## 2. Is divergence among <u>populations</u> primarily adaptive or non-adaptive?

The analyses just described allow us to identify those traits that are strongly associated with individual performance, with taxonomic identiy, or both. But they do not allow us to determine whether trait differences among populations arose through historical accidents associated with genetic drift, through adaptive divergence caused by natural selection, or both. To determine whether among-population functional trait differences represent adaptations to the dominant ecological

gradients, we must determine whether patterns of variation in functional traits match those predicted by those gradients or whether they simply reflect patterns of historical relationship as revealed by presumably neutral genetic markers.

***Functional trait analyses*:** Comparison of variation in functional traits and genetic markers is hampered by the plasticity of many functional traits. Because we want to determine whether differences in plant functional traits represent adaptive responses to environmental differences, ***we must assess the underlying genetic contribution to functional trait differences***. The white proteas are moderately long-lived, woody perennials and will not grow from seed to flower during the period of this project, so standard quantitative genetics approaches (e.g., parent-offspring regression, sib analysis) are not feasible. Thus, we propose to use classic common garden techniques to minimize the influence of plasticity on our results. Specifically, our analyses will focus on traits measured on clonally propagated individuals in the relatively uniform conditions of our experimental gardens at Kirstenbosch and Jonaskop (p. 7).

***Molecular analyses*:** Among-population differences in functional traits may have arisen by natural selection, because of their strong influence on individual performance. ***Detecting the effect of selection requires some standard of comparison. Microsatellites and AFLPs provide a convenient standard*** for several reasons. First, many, perhaps most, microsatellites are located in non-coding DNA, meaning that differences are likely to be selectively neutral and patterns of variation are likely to reflect primarily the processes of drift, mutation, and migration.[2] Second, the number of repeat differences between microsatellite alleles is related to their time since divergence (Estoup et al. 2002), making them a useful marker of evolutionary history. Third, microsatellite loci typically harbor a large number of co-dominant alleles, and they are readily multiplexed on automated DNA sequencers, making genotyping rapid and reliable. We also propose to use AFLP markers (Vos et al. 1995) as an independent source of molecular data to assess the hypothesis of selective neutrality for the molecular markers (p. 9). Although AFLPs are dominant markers, they provide substantial power to discriminate among populations because of the large number of polymorphic loci they reveal (Gerber et al. 2000). We have used them in other studies at the University of Connecticut, identifying 140-200 polymorphic loci in a palm (*Iriartea deltoidea*: Sezen et al. 2005; Sezen et al. 2007), legumes (*Desmodium* spp.: Skogen unpub.), and barberries (*Berberis* spp.: Lubell unpub.).

***Population sampling*.** We will collect leaf samples for DNA extraction from 20-30 individuals from each of 15 geographically reprepsentative populations of the most widespread white proteas (*P. aurea*, *P. mundii*, and *P. punctata*) and also from each of 8 geographically representative populations of the remaining white proteas. To reduce duplication of effort, sample sites will be chosen from those used for population performance analyses in DEB-0516320 (p. 1). Samples of 20-30 individuals per population are necessary to ensure that the samples are representative of the local genetic composition. DNA extractions will be performed in the molecular facility at SANBI following standard CTAB extraction protocols (Doyle and Doyle 1988). Restriction digests, PCR amplification, labeling, and fragment analysis (with an ABI-3100 automated capillary sequencer) will be done at the University of Connecticut. Fragment analyses will use the ROX 400HD size marker (Applied Biosystems). Initial automated scoring with Genescan/Genotyper will be verified with visual inspection of chromatograms for fragments of 50-400bp.

***Microsatellite markers*.** To date we have identified 6 microsatellite loci with predicted melting temperatures between 50°C and 60°C and with C/G content greater than 50%. Preliminary analyses (4 loci, 74 individuals, 5 sites) of *Protea punctata* revealed between 10 and 13 alleles per locus, providing some discrimination among populations (p. 10). These loci also cross-amplify readily in *P. aurea* and *P. mundii*.

---

[2] Some human genetic diseases and morphological variation in dog breeds are important exceptions: Cummings and Zoghbi 2000; Fondon and Gardner 2004.

***AFLP markers.*** We will use *Mse*I-*Eco*R1 for double digestion of DNA samples followed by PCR amplification with fluorescent primers using three selective nucleotides at their 3' ends. We have screened six primer pairs, and a preliminary survey of *P. punctata* and *P. mundii* with one of them revealed more than 200 polymorphic bands (*Mse*I: 5'GATGAGTCCTGAGTAA**-CAC**3'; *Eco*R1: 5'GACTGCGTACCAATTC**-ACA**3').

***Statistical analyses*:** Comparisons of morphological and molecular diversity commonly use comparisons of $F_{st}$ and $Q_{st}$ (McKay and Latta 2002; Merilä and Crnokrak 2001; O'Hara and Merilä 2005). $F_{st}$, Wright's statistic of population differentiation, is typically defined as the correlation between uniting gametes within a population. If a large number of populations are exchanging genes, it is approximately equivalent to the proportion of genetic diversity due to allele frequency differences among populations (Song et al. 2006). $Q_{st}$ is an analogously defined statistic for quantitative traits, the proportion of additive genetic diversity due to differences among populations (Spitze 1993). *If* the genetic markers from which $F_{st}$ is estimated are evolving according to the same process as the loci underlying the traits from which $Q_{st}$ is estimated and *if* there is no dominance or epistasis in the quantitative traits, then the two statistics should be indistinguishable. If $Q_{st} > F_{st}$, diversifying selection is implicated. If $Q_{st} < F_{st}$, stabilizing selection is implicated. An adaptive radiation occurs when natural selection is the predominant force causing differences among populations. When it is, among population differentiation in functional traits will be greater than would be predicted on the basis of mutation and drift alone. Thus, we will have preliminary evidence that the radiation has been adaptive if $Q_{st} > F_{st}$ for most or all of the functional traits we identify.

We will perform approximate $Q_{st}/F_{st}$ comparisons as an initial step in the analysis, including the approach for multilocus/multitrait comparison developed by Kremer et al. (1997). Our comparisons are approximate because we must assume that dominance and epistatic contributions to the genetic variance are negligible.[3] We regard $Q_{st}/F_{st}$ comparisons only as an initial step in the analysis because estimates of $F_{st}$ may differ substantially among markers, making the appropriate standard of comparison uncertain (Holsinger and Wallace 2004). More importantly, $Q_{st}/F_{st}$ comparisons do not allow us to identify environmental covariates with an important influence on the structure of variation we find for morphological or molecular markers, although we will explore extensions of Foll and Gaggiotti (2006) that may allow us to overcome this limitation. In short, $Q_{st}/F_{st}$ comparisons will not allow us to determine whether "excess" variation in functional traits is associated with environmental gradients in the way expected if such variation is adaptive. Thus, ***we propose to develop a method that allows us simultaneously to compare the pattern of variation for functional traits and genetic markers and to determine the association of environmental covariates with those patterns.***

Specifically, we will use individual assignment (Pritchard et al. 2000; Chikhi et al. 2001; Paetkau et al. 2004) to compare patterns of functional trait and molecular marker diversity by identifying environmental covariates explaining the composition of populations. If we let $\boldsymbol{Y}_{ijk}$ be a vector of molecular marker or functional trait data for the *j*th individual in the *k*th experimental location from the *i*th collection site,[4] then $f(\boldsymbol{Y}_{ijk}|\pi_i)$ is the probability of selecting an individual with those characteristics from collection site *i*, where $\pi_i$ is the frequency vector for markers or traits in that population. We assume that each population is composed of a mixture of frequency vectors and that differences among populations arise from differences in the magnitude of the component associated with each vector. We seek to explain site-to-site differences in those magnitudes. Specifically,

[3] Our measurements will be based on clonal replicates, not on sibships or parent-offspring combinations, which can not be produced during the lifetime of this project. Thus, our estimate of $Q_{st}$ will be based on broad-sense heritabilities.

[4] Note that if $\boldsymbol{Y}_{ij}$ is a vector of functional trait data, it will correspond to traits measured on individuals in an experimental garden. Thus, it reflects genetic variation in the functional trait.

suppose there are $P$ components in the mixture, and let $f(\theta_p)$ be the probability density associated with the frequency vector of markers or traits in component $p$. Then $\pi_i$ comes from a mixture distribution, $\pi_i = \Sigma_p w_{ip} f(\theta_p)$, where $w_{ip}$ is the weight of component $p$ in population $i$, $\Sigma_p w_{ip} = 1$. We will model the weights as $\text{logit}(w_{ip}) = X_i^T \beta_p + \phi_i$, where $X_i^T$ is a vector of environmental covariates, $\beta_p$ is the corresponding vector of regression coefficients, and $\phi_i$ is a random effect associated with each collection site. The spatial random effects, $\phi_i$, may vary across the $P$-1 logits. ***If among population differences in a functional trait are selectively neutral***, then either

1. ***None of the regression coefficients explaining assignment for that trait will be distinguishable from zero***, i.e., there will be no relationship between the genetic differences in functional traits among populations and the environmental variables associated with those populations, or
2. ***The set of regression coefficients for that trait will be indistinguishable from the set of regression coefficients for genetic markers,*** i.e., the pattern of genetic differences in functional traits is consistent with the pattern of differences in genetic markers, suggesting that the differentiation reflects purely historical, non-adaptive processes.

Model choice criteria (Gelfand and Ghosh 1998) will be used to identify the best choice of $P$ for trait and for molecular marker data and to determine which, if any, of the regression coefficients differ from zero. We will use posterior comparison (Holsinger and Wallace 2004) to determine whether regression coefficients differ between molecular markers and functional traits. If most or all functional traits have regression coefficients that differ from those for molecular markers, we have evidence that natural selection is responsible for most differentiation in functional traits. We will perform the same analyses for traits identified as pure taxonomic traits, i.e., those that are strong indicators of taxonomic identity and unrelated to individual performance. Among population differences in such traits should be selectively neutral, meaning either (1) or (2) should hold. The analysis of pure taxonomic traits is an internal control for the suitability of our method.

To assess the neutrality hypothesis for our molecular markers, ***we will perform separate analyses of microsatellite and AFLP data***. Differences between them would indicate that patterns of variation for at least one set have been influenced by selection. If the analyses show similar patterns of variation, both could have been influenced by selection in the same way. If either analysis shows only the spatial random effect as an important covariate, then it seems reasonable to conclude that those markers are effectively neutral. Thus, if our markers show different patterns of variation, we will be able to identify which set is effectively neutral and which has been affected by selection, allowing us to choose an appropriate standard of comparison for detecting adaptive differentiation in functional traits. In addition, we will use locus-specific estimates of $F_{st}$ (Guo et al. 2007) and coalescent-based comparisons of observed and expected heterozygosities at microsatellite loci (Payseur et al. 2006) to identify AFLP and microsatellite markers that may be subject to selection. These analyses will allow us to exclude from our analyses loci that show a signature of selection.

***Preliminary molecular/functional trait comparisons*:** To assess the feasibility of these methods ***we analyzed variation at 4 microsatellite loci*** in a sample of individuals of *Protea punctata* (74 individuals, 5 populations; Bokkeveld ridge, Bokkeveld ravine, Baviaansberg, Swartberg Pass, Jonaskop). Analysis in `Structure` (Pritchard et al. 2000) strongly supported a classification with 5 genetic components (based on log likelihood at the posterior mean: Figure 5). Populations 1-4 and half of population 5

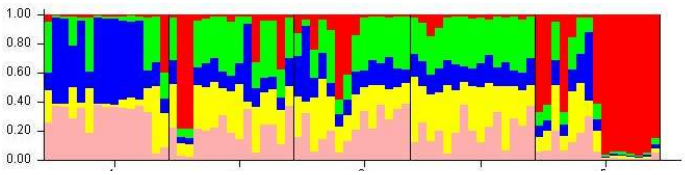


**Figure 4.** `Structure` analysis of microsatellite variation in *P. punctata*. Each vertical bar corresponds to one individual. The proportion of that bar that is a specific color corresponds to the posterior probability that the individual belongs to the corresponding cluster.

(Jonaskop) were relatively similar to one another, although individuals in population 1 (Bokkeveld ridge) have a larger contribution from component 3 (blue) than other populations. Almost half of the individuals at Jonaskop were composed predominantly of component 5 (red), suggesting that this population includes individuals with a history quite different from other populations we studied. ***This preliminary analysis indicates that the level of variability we have already identified is likely to be sufficient to reveal significant structure in patterns of genetic variation.***

***We also analyzed variation in specific leaf area*** ($cm^2$-$g^{-1}$) on field-collected leaves of *P. punctata* (4 sites, 80 individuals), *Protea aurea* (5 sites, 96 individuals), *Protea mundii* (5 sites, 100 individuals), *Protea venusta* (1 site, 20 individuals) and *Protea lacticolor* (2 sites, 39 individuals) using a hierarchical normal mixture model in OpenBUGS v2.1, allowing a different mean and variance for each component and using a mixed-effect logistic model to explain the component composition of the samples from each site (environmental covariates from Table 2, p. 6, except roughness, enhanced vegetation index, soil texture, soil fertility, and soil acidity; random effects for individual, population, and taxon). This analysis classified individuals into 4 components (Figure 6). There were striking differences among species, especially *P. punctata* and *P. venusta* versus the remaining taxa, but there are also differences among populations within species, and among individuals within populations. The best model for these data includes random effects for individual, population, and taxon in addition to heat units, interannual CV of rainfall, and soil fertility. It is not surprising that specific leaf area of field-collected samples was related to these environmental variables, since many authors have shown that specific leaf area shows plastic responses to water availability and soil nutrient status (Knight and Ackerly 2003; Westoby et al. 2002). However, Latimer et al. (2006) also identified these environmental variables as having an important influence on presence/absence of *Protea mundii* and *P. aurea* (p. 5). Moreover, we are able to detect taxon and population effects, even after controlling statistically for direct environmental influences. ***These results suggest that there is a genetic component to differences in specific leaf area among species and among populations within species.***

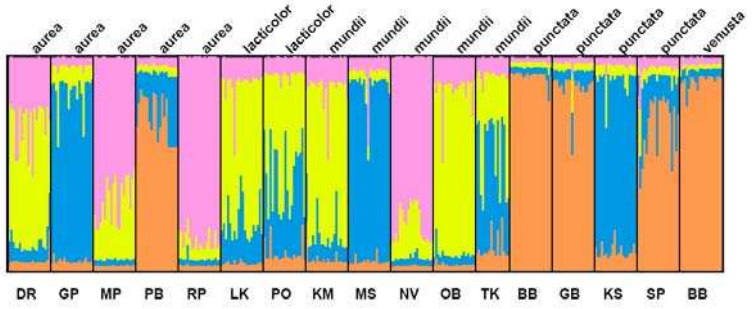


**Figure 5.** Mixture analysis of specific leaf variation. Each vertical bar corresponds to one individual. The proportion of that bar that is a specific color corresponds to the posterior probability that the individual belongs to the corresponding cluster.

## 3. Is divergence among <u>species</u> primarily adaptive or non-adaptive?

Our analyses of individual performance will allow us to identify those traits strongly associated with individual performance (question 1; functional traits). Our analyses of trait variation in relation to taxonomic identity will allow us to identify those traits most closely associated with species identity (question 1; taxonomic traits). Our analyses of functional trait and genetic marker variation within species will allow us to determine whether divergence among populations within species is primarily adaptive or primarily non-adaptive (question 2). But even if divergence among populations is primarily adaptive, divergence among species need not be. Speciation may occur by processes different from those that lead to differentiation among populations. ***We propose to determine whether population divergence and taxonomic diversification are coupled or uncoupled and to unravel the history of taxonomic and functional trait evolution within these taxa*** by (1) determining whether patterns of divergence among species in traits used to distinguish them (taxonomic traits) are consistent with those predicted from variation in functional traits (*adaptive divergence*) or with those

predicted from genetic markers (*non-adaptive divergence*) and (2) using a phylogenetic analysis of relationships among all populations we sample to determine whether changes in functional traits arise predominantly during speciation, suggesting that adaptive differentiation plays an important role in taxonomic diversification.

***Taxonomic trait analyses*:** Many of the traits we propose to measure (Table 1, p. 6) have been used in recent mongraphs to distinguish species within the white proteas, e.g., leaf shape (length:width ratio), leaf pubescence (hairless at maturity *versus* densely hairy margin), and involucral bract color. Operationally, we make the reasonable assumption that divergence in taxonomic traits, i.e., those strongly associated with taxonomic identity (p. 7), serves as a proxy for the unobservable process of speciation or lineage divergence. We will use the assignment method described above to determine whether patterns of variation for pure taxonomic traits, i.e., those that are not also functional traits, are consistent with patterns of variation in molecular markers or pure functional traits. ***If taxonomic divergence is primarily associated with adaptive processes***, then

1. ***Regression coefficients associated with each pure taxonomic trait will be statistically distinguishable from zero***, while random effects associated with collection site and taxon may or may not be statistically distinguishable from zero and
2. ***Regression coefficients associated with taxonomic traits will be statistically indistinguishable from those associated with pure functional traits***.

***If taxonomic divergence is primarily associated with non-adaptive processes*** then

3. ***Regression coefficients associated with many pure taxonomic traits will be statistically indistinguishable from zero***, while random effects associated with collection site and taxon will be statistically distinguishable from zero and
4. ***Non-zero regression coefficients associated with pure taxonomic traits will be statistically indistinguishable from those associated with genetic markers***.

***Phylogenetic analyses*:** Reconstructing the history of trait evolution within a clade requires a phylogeny. In our case we are interested both in the history of diversification among species and in the history of diversification among populations within species. Moreover, to determine whether changes in functional traits are correlated with changes in taxonomic traits we must determine whether those changes are concentrated on the same branches of a phylogenetic tree connecting populations. Thus, ***we propose to develop a phylogenetic hypothesis for the group using populations, rather than individuals or species, as the unit of analysis***. We propose a new approach that accommodates mutational models appropriate for microsatellites (unlike the Brownian motion model underlying CONTML: Cavalli-Sforza and Edwards 1967; Felsenstein 1973, 2004). The coalescent-based method of Nielsen et al. (1998) shares this advantage, but our approach does not require integration across coalescent genealogies. As a result, it is far more computationally efficient. We will explore both our method and the method of Nielsen et al. (1998) in our analyses.

A straightforward extension of results in Wright (1942) shows that the probability distribution for $\pi_k$, the frequency of allele $A_1$ at a biallelic locus in population $k$, is well approximated by a beta distribution with parameters $((1-\theta)/\theta)\pi$ and $((1-\theta)/\theta)(1-\pi)$, where $\pi$ is the allele frequency at this locus in the ancestral population. $\theta$ depends on the mutation rate and divergence time: $(1-(\Phi^2(1-1/2N_e))^t)/(2N_e-\Phi^2(2N_e-1))$ where $N_e$ is the effective population size, $t$ is the number of generations since divergence, $\mu$ is the mutation rate, and $\Phi=(1-\mu)^2$ (Kimura 1955; Holsinger 2006). Because $\theta$ and $\pi$ completely account for the distribution of $\pi_k$, they are sufficient statistics for $N_e$, $\mu$, and t. These results are easily generalized to multiallelic loci with a uniform mutation matrix (a Dirichlet distribution replaces the beta: Holsinger 2006), and we will extend them to a stepwise mutation matrix appropriate for microsatellite loci. While these results assume that there is no migration among populations after they have diverged, results in Slatkin (1991) and Holsinger and Mason-Gamer (1996) show that $\theta$ is best thought of as a measure of relative coalescence time, where

the time to coalescence reflects both recency of common ancestry and the extent of gene flow. Thus, we anticipate that the method is robust to moderate amounts of among-population gene flow, and we will test this expectation as part of our simulation studies. We will use these observations to construct a Bayesian approach for inference of population phylogenies.

Given a vector of allele counts at $l$ loci in population $k$, $\boldsymbol{x}_k = (x_{1k},\ldots,x_{lk})$, the first-stage likelihood is product binomial, $P(\boldsymbol{x}_k|\boldsymbol{\pi}_k)$, where $\boldsymbol{\pi}_k$ is the (unknown) vector of allele frequencies. Any topology induces a prior for each $\boldsymbol{\pi}_k$ that depends on the $\theta$s associated with each branch and the vector of allele frequencies in the common ancestral population, $\boldsymbol{\pi}_0$. Given priors on the $\theta$s and $\boldsymbol{\pi}_0$ the posterior on all parameters is tedious to specify, but straightforward to calculate. The priors on the $\theta$s may be further specified in terms of the effective population size along the lineage corresponding to each $\theta$, the number of generations ($t$) on each branch, and a common mutation rate across the phylogeny ($\mu$ may vary across loci). Estimates of $t$ will be relative to a specified baseline. Our approach relies on logic similar to Felsenstein's CONTML except that it allows us to estimate $N_e$ and $t$ along each branch and $\mu$ at each locus.

***Preliminary results*:** To explore the feasibility of this approach we performed a small-scale simulation study in which we generated 2-allele, 50-locus data sets according to a drift-mutation process with a specified phylogeny. For these initial simulations we used a three-population phylogeny (A,(B,C)) with constant population sizes on each branch. We denoted the time from the split at the base of the phylogeny to the split between B and C $t_1$ and the time from the split between B and C to the time of data collection $t_2$. We sampled 25 individuals from each population generated by this process and estimated the posterior distribution of the $\theta$s and $\boldsymbol{\pi}_0$ for each of the three possible topologies. We then calculated the likelihood of the data at the posterior mean of the parameters as a measure of goodness of fit and recorded the number of times the highest likelihood was associated with each topology. The results are presented in Table 3. For the set of simulation conditions considered, the likelihood of each phylogeny calculated at the posterior mean provides a reasonable criterion with which to recognize the true phylogeny.

| | (A,(B,C))* | (B,(A,C)) | (C,(A,B)) |
|---|---|---|---|
| N=100, $\mu$=0.0001, $t_1$=100 | | | |
| $t_2$=110 | 10 | 0 | 0 |
| $t_2$=125 | 10 | 0 | 0 |
| $t_2$=150 | 10 | 0 | 0 |
| $t_2$=200 | 6 | 1 | 3 |
| N=1000, $\mu$=0.00001, $t_1$=500 | | | |
| $t_2$=550 | 10 | 0 | 0 |
| $t_2$=625 | 10 | 0 | 0 |
| $t_2$=750 | 10 | 0 | 0 |
| $t_2$=1000 | 10 | 0 | 0 |

*"true" phylogeny

**Table 3.** Phylogeny estimates from simulated allele frequency data. 50 loci. 10 replicates. Columns correspond to alternative rooted phylogenies, denoted in Newick format.

Enumerating all possible topologies and evaluating the likelihood at the posterior mean for each is possible only when there are a small number of populations or when there are a small number of alternative evolutionary scenarios to be considered. In addition to more extensive validation of the above approach to inference for small numbers of populations (up to 5) and with a wider range of mutation rates and populations sizes, we will develop branch-swapping methods like those used in phylogenetic inference with DNA sequences (Felsenstein 2004; Larget and Simon 1999; Lewis et al. 2005) to make phylogenetic inference possible for larger numbers of populations.

***Interpreting evolutionary history*:** If most or all taxonomic traits are simultaneously functional traits, we have presumptive evidence that taxonomic and functional diversification are associated. In the more likely case that many taxonomic traits are not functional traits, our historical analysis of trait evolution will allow us ***to determine whether differences in functional traits arise at the same time as differences in taxonomic traits***. Ideally inference from the population phylogeny would proceed from the full posterior, but because of computational challenges we will initially focus on the majority-rule consensus tree derived from the posterior.

Specifically, we will determine whether divergence in pure functional traits is correlated with divergence in pure taxonomic traits, a strong indication that adaptive differentiation plays an

important role in taxonomic diversification. In the case of discrete taxonomic traits, e.g., involucral bract shape, we will compare the consequences of using parsimony, likelihood, and Bayesian approaches for character state reconstruction. We will then calculate an index for the magnitude of change along each branch (e.g, % change in each continuous character + number of changes in discrete characters), and use the Spearman rank correlation coefficient to measure the correlation in change along each branch between the functional and taxonomic traits. We will determine whether the observed correlation is larger than expected through randomizations in which we construct a large number of randomly rearranged trees with the same total branch length. This approach uses the same logic as Maddison's (1990) concentrated changes test for correlated evolution, which is a conservative approach for detecting correlated evolutionary change (Lorch and McEadie 1999). This test does not assume that all taxonomic traits are neutral. If changes in functional traits are concentrated on branches that correspond to species differences, then we have evidence that adaptation is associated with speciation.

### *4. Software development*

Implementation of the new analytical methods described in this proposal will require development of new software, specifically software for individual assignment with environmental covariates for phenotypic and genetic data and for inference of population phylogenies from microsatellite or AFLP data. Our analytical methods will be applicable to a broad range of evolutionary questions associated with understanding processes of population and species divergence. As a result, we will devote considerable effort to developing user-friendly software to implement these analyses. The resulting analytical routines will either be incorporated into `Hickory` (originally developed with support from an unrelated grant from the National Institutes of Health: http://darwin.eeb.uconn.edu/hickory/hickory.html) or released as standalone packages. In either case, source code and executables (Windows, Linux) will be made freely available under terms of the GNU General Public License.

## Broader impacts

This project includes a diverse and complementary group of individuals, disciplines and institutions. As a consequence it offers unique opportunities for education and research to the students, post-doc, and scientists involved. The project will involve senior scientists from two institutions and two countries: the University of Connecticut and the South African National Biodiversity Institute (SANBI), an NGO. It brings together a unique combination of experts in evolutionary biology, ecology, botany, and conservation biology. Annual meetings in Cape Town for all participants will ensure project cohesion. They will be scheduled to correspond with visits to field/experimental sites to minimize international travel costs for U.S. participants. All personnel will be fully engaged in all aspects of the project. Group meetings will follow the successful model of NCEAS working groups, a model we became familiar with as a result of previous NCEAS-supported collaboration on spatial modeling of species distributions. Research exchange opportunities among institutions (US and South Africa) are built into the project for the graduate students, post-doc, and scientists. The South African experience will provide a unique multicultural experience – South Africa is known as "a world in one country" for its mixing of African, European, and Asian cultures

To enhance communication among project members we will use an open source enterprise collaboration suite (e.g., eGroupWare: http://www.egroupware.org/) hosted on one of the servers available for use with this project (see the description of computer facilities available in the *Facilities and Equipment* section). In addition, we will develop a standards-compliant public web site that describes the findings and implications of the project to encourage communication and to enhance our educational outreach: K-university as well as the public at large. We will model the new site on

two public outreach web sites we have developed: Rebelo's protea project web site: http://protea.worldonline.co.za, and Silander's IPANE web site: http://www.IPANE.org

This project also offers a unique opportunity for linking scientific findings to conservation applications. The mission of the South African National Biodiversity Institute (SANBI) is "To promote the sustainable use, conservation, appreciation and enjoyment of the exceptionally rich biodiversity of South Africa, for the benefit of all people." SANBI supports bioregional planning initiatives with coordinated conservation implementation, and a comprehensive threatened species action program. It brings together scientists, professionals from conservation NGOs and GOs, as well as members of the public to forge conservation policy and effect implementation. Its public outreach program includes local (indigenous) community empowerment through environmental education and awareness programs. The participation of SANBI in this project will allow us to directly link the science in this project with conservation application.

One of our major goals is to recruit underserved graduate and undergraduate students to the project and to careers in evolutionary biology. We will build on the success of local initiatives in which we are already involved. Silander was awarded a competitive grant by the University of Connecticut in 2005 and 2006 specifically to develop novel ways to recruit underserved students from HBCU, HACU and AIHEC institutions. In July 2005 and June 2006 he ran a 4-day workshop on Biodiversity Science for 14 faculty mentors of underserved students from 18 HBCU, HACU and AIHEC institutions across the US. The specific objective was to develop sustainable relationships with underserved undergraduate institutions in the fields of evolutionary biology and ecology. Success of the workshop exceeded all expectations. We now have a sustained network in place and we will continue this workshop throughout the life of the project. Indeed, in May 2006 the University hired 2 participants from the 2005 workshop to develop a 3-year undergraduate recruitment initiative. As in the past, we anticipate, on a competitive basis, some Multicultural Graduate Fellowship support from the University during this grant. In addition, we will make a special effort to recruit students from underrepresented groups for the field-based training course associated with OISE-0623341 (p. 1) in the year or years its focus is associated with this project.

To develop a local pipeline of underserved students with interests in evolutionary biology and ecology we have established educational links with K-12 schools in Hartford, CT: Bulkeley High School, Two Rivers Science Magnet School (TRSMS) and the Greater Hartford Academy of Math & Science (all "majority minority" schools; one of Holsinger's Ph.D. students teaches biology at the Academy of Math & Science). We are currently testing a Biodiversity Science curriculum we developed for middle schools, particularly focusing on urban and underserved schools. If the grant proposed here is funded, we will seek supplementary RET and RAMHHS funds to connect teachers and students with the project's research and outreach and internship opportunities, as well as to connect with schools we have contact with in South Africa. Chris Lennard, a post-doc on another grant of Silander's, will facilitate school connection in South Africa; as part of his Ph.D. support at UCT he was the school-university liaison in greater Cape Town for collaborative environmental studies in underserved schools. SANBI has established connections with the University of the Western Cape (now and historically a university catering to "formerly under-represented students"). We will look here for local field assistants. UWC also provides additional multicultural opportunities for collaboration. Other initiatives include: (1) coordinating our efforts with the ESA-SEEDS, a minority recruiting program initiated in collaboration with the UNCF; Silander is on the SEEDS Fellowship Committee; and (2) providing internship and research opportunities for undergraduates in UConn's Louis Stokes Alliance for Minority Participation (LSAMP) Program, and UConn's NSF-AGEP initiative.

## Literature cited


Ackerly, D. D. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* 74:25-44.

Agenbag, L., K. J. Esler and G. Midgley. 2004a. Studies along an environmental gradient reveal how biome boundaries may respond to climate change. *Proceedings of the 10th MEDECOS Conference*, ed. M. Arianoutsou and V.P. Papanastasis. Millpress, Rotterdam, Netherlands.

Agenbag, L., K. J. Esler and G. Midgley. 2004b. An environmental gradient reveals how climate change influences phenophases in Fynbos growth forms. In *Proceedings of the 10th MEDECOS Conference*, ed. M. Arianoutsou and V.P. Papanastasis. Millpress, Rotterdam, Netherlands.

Agenbag, L., M. Rutherford, G. Midgley and K. J. Esler. 2004c. Fundamental and realized niches of Fynbos and karoo seedlings as revealed by reciprocal transplants. In *Proceedings of the 10th MEDECOS Conference*, ed. M. Arianoutsou and V.P. Papanastasis. Millpress, Rotterdam, Netherlands.

**Agarwal, D., A.E. Gelfand, and J. Silander, 2002. Investigating Tropical Deforestation Using Two Stage Spatially Misaligned Regression Models. *Journal Agricultural, Biological and Environmental Statistics* 7, 420-439.

**Agarwal, D.K., J.A. Silander, Jr., A.E. Gelfand, R.E. Dewar and J.G. Mickelson, Jr. 2005. Tropical Deforestation in Madagascar: Analyses using hierarchical, spatially explicit, Bayesian regression models. *Ecological Modelling* 185: 105-131.

**Barber, J. J., A.E. Gelfand and J. A. Silander, Jr. 2006. Modeling map positional error to infer about true feature location. Submitted to *Journal of Statistical Planning and Inference*.

**Barber, J. J., and A. E. Gelfand. 2006. Hierarchical spatial modeling for estimation of population size. Submitted to *Ecological and Environmental Statistics*.

**Barber, J.J., A. E. Gelfand and D. W. Nychka. 2006. Space-time modeling of precipitation events with misaligned reporting times. In manuscript.

**Banerjee, S. B.P. Carlin and A.E. Gelfand. 2004 Hierarchical Modeling and Analysis for Spatial Data, Chapman-Hall (CRC), Boca Raton, FL.

Carr, G. D., R. H. Robichaux, M. S. Witter, and D. W. Kyhos. 1989. Adaptive radiation of the Hawaiian silversword alliance (Compositae-Madiinae): a comparison with Hawaiian picture-winged *Drosophila*. In *Genetics, Speciation, and the Founder Principle*, ed. L. V. Giddings, K. Y. Kaneshiro, and W. W. Anderson, pp. 79-97. Oxford University Press, Oxford, UK.

Cavalli-Sforza, L. L. and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 32:550-570.

Chikhi, L., M. W. Bruford, and M. A. Beaumont. 2001. Estimation of admixture proportions: A likelihood-based approach using Markov chain Monte Carlo. *Genetics* 158:1347-1362.

**Clark, J.S. and A. Gelfand (eds). 2006. *Applications of Computational Statistics in the Environmental Sciences: Hierarchical Bayes and MCMC Methods*. Oxford University Press, Oxford, UK (In press).

Cowling, R.M. 1995. Patterns and determinants of biodiversity components in Cape Fynbos. *Conservation of Biodiversity in Africa*, ed. In L.A. Bennum, R.A. Aman and S.A. Crafter. Centre for Biodiversity, National Museum of Kenya, Nairobi, Kenya.

**Cowling, R.M. and C.E. Heijnis. 2001. The identification of Broad Habitat Units as biodiversity entities for systematic conservation planning in the Cape Floristic Region. *South African Journal of Botany* 67:15-38.

**Cowling, R.M. and A. T. Lombard. 2002. Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. *Diversity and Distributions* 8, 163–179

**Cowling, R.M. and R.L. Pressey. 2003 Introduction to systematic conservation planning in the Cape Floristic Region. *Biological Conservation* 112:1-13.

**Cowling, R.M., F. Ojeda, B. B. Lamont, P.W. Rundel and R. Lechmere-Oertel. 2006. Rainfall reliability, plant reproductive traits and regional-scale diversity in fire-prone Mediterranean-climate ecosystems. *Global Ecology and Biogeography* 14: 509-519.

Cowling, R. M., P. W. Rundel, B. B. Lamont, M. K. Arroyo, and M. Arianoutsou. 1996. Plant diversity in Mediterranean-climate regions. *Trends in Ecology & Evolution* 11:362-366.

Cummings, C. J. and H. Y. Zoghbi. 2000. Fourteen and counting: unraveling trinucleotide repeat diseases. *Human Molecular Genetics* 9:909–916.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin* 19:11-15.

Ellis, A. R., and A. E. Weis. 2006. Coexistence and differentiation of "flowering stones": the role of local adaptation to soil microenvironment. *Journal of Ecology* 94:322-335.

Ellis, A. R., A. E. Weis, and B. S. Gaut. 2006. Evolutionary radiation of "stone plants" in the genus *Argyroderma* (Aizoaceae): unraveling the effects of landscape, habitat, and flowering time. *Evolution* 60:39-55.

Estoup, A., P. Jarne, and J.-M. Cornuet. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology* 11:1591-1604.

** Etienne, R.S., A.M. Latimer, J.A. Silander, Jr. and R.M. Cowling 2006. Limitations of species abundance data for disclosing information on speciation rate and dispersal. *Science* (in press).

Evans, M. E. K., D. J. Hearn, W. J. Hahn, J. M. Spangle, and D. L. Venable. 2005. Climate and life-history evolution in evening primroses (*Oenothera*, Onagraceae): a phylogenetic comparative analysis. *Evolution* 59:1914-1927.

Felsenstein, J. 1973. Maximum-likelihood estimation of evolutionary trees from continuous characters. *American Journal of Human Genetics* 25:471-92.

Felsenstein, J. 2004. *Inferring Phylogenies*. Sinauer Associates, Sunderland, MA.

Foll, M., and O. Gaggiotti. 2006. Identifying the environmental factors that determine the genetic structure of populations. *Genetics* 174:875-891.

Fondon, J. W., 3rd, and H. R. Garner. 2004. Molecular origins of rapid and continuous morphological evolution. *Proceedings of the National Academy of Sciences USA* 101:18058-18063.

Friar, E. A., L. M. Prince, E. H. Roalson, M. E. McGlaughlin, J. M. Cruse-Sanders, S. J. De Groot, and J. M. Porter. 2006. Ecological speciation in the East Maui-endemic *Dubautia* (Asteraceae) species. *Evolution* 60:1777-1792.

Futuyma, D. J. 1998. *Evolutionary Biology*, 3rd ed. Sinauer Associates, Sunderland, MA.

Gelfand, A. E., and S. K. Ghosh. 1998. Model choice: A minimum posterior predictive loss approach. *Biometrika*. 85:1-11.

**Gelfand, A.E., A.M. Schmidt, S. Wu, J.A. Silander, Jr., A. Latimer and A.G. Rebelo. 2005. Modeling species diversity through species level hierarchical modeling. *Applied Statistics: Journal of the Royal Statistical Society, Section C* 54(1):1-20

**Gelfand, A.E., J.A. Silander, Jr., S. Wu, A. Latimer, P. Lewis, Anthony G. Rebelo and M. Holder. 2006. Explaining species distribution patterns through hierarchical modeling. *Bayesian Analysis* 1:41-92.

**Gelfand, A., J.S. Clark, and B. Carlin. 2006. Elements of Bayesian Inference. In *Applications of Computational Statistics in the Environmental Sciences: Hierarchical Bayes and MCMC Methods*, ed. J.S. Clark and A. Gelfand. Oxford University Press, New York, NY (In press).

Geber, M. A., and L. R. Griffen. 2003. Inheritance and selection on plant functional traits. *International Journal of Plant Sciences* 164(suppl. 3):S21-S42.

Gerber, S., S. Marriette, R. Streiff, C. Bodenes, and A. Kremer. 2000. Comparison of microsatellite and amplified length polymorphism markers for parentage analysis. *Molecular Ecology* 9:1037-1048.

Gittenberger, E. 1991, What about non-adaptive radiation? *Biological Journal of the Linnean Society* 43:263–272.

Givnish, T. J., R. A. Montgomery, and G. Goldstein. 2004. Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: light regimes, static light responses, and whole-plant compensation points. *American Journal of Botany* 91:228-246.

Givnish, T. J., K. J. Sytsma, W. J. Hahn, and J. F. Smith. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). In *Hawaiian Biogeography: Evolution on a Hot-Spot Archipelago*, ed. W. L. Wagner and V. A. Funk, pp. 288-337. Smithsonian Institution Press, Washington, DC.

Goldblatt, P. and J. C. Manning. 2000. *Cape Plants: A Conspectus of the Cape Flora of Southern Africa*. National Biodiversity Institute, Pretoria, South Africa.

Goldblatt, P., V. Savolainen, O. Porteous, L. Sostaric, M. Powell, G. Reeves, J. C. Manning, T. G. Barraclough, and M. W. Chase. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* 25:341-360.

Guo, F., D. K. Dey, and K. E. Holsinger. 2007. On detecting divergent or stabilizing selection using patterns of variation at SNP loci. *Journal of the American Statistical Association* (submitted).

Hilton-Taylor, C.. 2000. *2000 IUCN Red List of Threatened Species*. IUCN, Gland, Switzerland and Cambridge, UK.

Ho, M. D., J. C. Rosas, K. M. Brown, and J. P. Lynch. 2005. Root architectural tradeoffs for water and phosphorous acquisition. *Functional Plant Biology* 32:737-748.

Hodges, S. A. 1997. Rapid radiation due to a key innovation in columbines (Rannunculaceae: *Aquilegia*). In *Molecular Evolution and Adaptive Radiation*, ed. T. J. Givnish and K. J. Sytsma, pp. 391-406. Cambridge Univeristy Press, Cambridge, UK.

Hodges, S. A., and M. L. Arnold. 1995. Spurring plant diversification: are floral nectar spurs a key innovation? *Proceedings of the Royal Society of London, Series B* 262:343-348.

Holsinger, K. E. 2006. Divergence of population subject to mutation and drift. (manuscript available at http://darwin.eeb.uconn.edu/notes/divergence.pdf).

Holsinger, K. E., P. O. Lewis, and D. K. Dey. 2002. A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology* 11:1157-1164.

Holsinger, K. E., and R. J. Mason-Gamer. 1996. Hierarchical analysis of nucleotide diversity in subdivided populations. *Genetics* 142:629-639.

Holsinger, K. E. and L. E. Wallace. 2004. Bayesian approaches for the analysis of population structure: an example from *Platanthera leucophaea* (Orchidaceae). *Molecular Ecology* 13:887-894.

Hoot, S. B., and A. W. Douglas. 1998. Phylogeny of the Proteaceae based on *atp*B and *atp*B-*rbc*L intergenic spacer region sequences. *Australian Systematic Botany* 11:301-320.

Kimura, M. 1955. Random genetic drift in a multi-allelic locus. *Evolution* 9:419-435.

Knight, C. A., and D. D. Ackerly. 2003. Evolution and plasticity of photosynthetic thermal tolerance, specific leaf area, and leaf size: congeneric species from desert and coastal environments. *New Phytologist* 160:337-347.

Kozak, K. H., D. W. Weisrock, and A. Larson. 2006. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: *Plethodon*). *Proceedings of the Royal Society of London, Series B* 273:539-546.

Kremer, A., A. Zanetto, and A. Ducousso. 1997. Multilocus and multitrait measures of differentiation for gene markers and phenotypic traits. *Genetics* 145:1229-1241.

Klak, C., G. Reeves, and T. Hedderson. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature* 427:63-65.

Lambers, H., F. S. Chapin III, and T. L. Pons. 2000. *Plant Physiological Ecology*. Springer-Verlag, Berlin.

Larget, B. and D. L. Simon. 1999. Markov chain monte carlo algorithms for Bayesian analysis of phylogenetic trees. *Molecular Biology & Evolution* 16:750-759.

**Latimer, A. M. 2006. *Environmental and geographical controls on species distributions: a case study on proteas of the Cape Floristic Province*. Ph.D. Dissertation, University of Connecticut.

**Latimer, A. M., J. A. Silander, Jr., and R. M. Cowling. 2005. Neutral ecological theory reveals isolation and rapid speciation in a biodiversity hot spot. *Science* 309:1722-1725.

**Latimer, A.M., J.A. Silander Jr , A.E. Gelfand , A.G. Rebelo and D.M. Richardson. 2004. Quantifying threats to biodiversity from invasive alien plants and other factors: a case study from the Cape Floristic Region. *South African Journal of Science* 100: 81-86.

**Latimer, A. M., S. Wu, A. E. Gelfand, and J. A. Silander, Jr. 2006. Building statistical models to analyze species distributions. *Ecological Applications* (in press).

**Laurie, H. and J.A. Silander, Jr. 2002. Geometric constraints and spatial pattern of species richness: critique of range-based null models. *Diversity and Distributions*. 8:351-364.

**Laurie, H., T. Rebelo J.A. Silander, Jr and W. Smit. 2006. Spatial variation in power-law species-area curves for a single clade in a single biome. (manuscript for *Diversity and Distribution*)

Lewis, P. O., M. T. Holder, and K. E. Holsinger. 2005. Polytomies and Bayesian phylogenetic inference. *Systematic Biology* 54:241-253.

Linder, H. P. 2003. The radiation of the Cape flora, southern Africa. *Biological Reviews* 78:597-638.

Linder, H. P. 2005. Evolution of diversity: the Cape flora. *Trends in Plant Sciences* 10:536-541.

Linder, H. P. and C. R. Hardy. 2004. Evolution of the species-rich Cape flora. *Proceedings of the Royal Society of London, Series B* 359:1623-1632.

Little, R. J. A. and D. B. Rubin. 1987. *Statistical Analysis with Missing Data*. John Wiley & Sons, New York, NY.

Lorch, P. D., and J. A. McEadie. 1999. Power of the concentrated changes test for correlated evolution. *Systematic Biology* 48:170-191.

Maddison, W. 1990. A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution* 44:539-557.

Martins, E. 1994. Estimating rates of change from comparative data. *American Naturalist* 144:193-209.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution* 17:285.

Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14:892-903.

Nielsen, R., J. L. Mountain, J. P. Huelsenbeck, and M. Slatkin. 1998. Maximum-likelihood estimation of population divergence times and population phylogeny in models without mutation. *Evolution* 52:669-677.

O'Hara, R. B., and J. Merila. 2005. Bias and precision in QST estimates: problems and some solutions." *Genetics* 171:1331-1339.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13:55-65.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature*, 401:877-884.

Pagel, M., A. Meade, and D. M. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53:673-684.

Paradis, E. and J. Claude. 2002. Analysis of comparative data using generalized estimating equations. *Journal of Theoretical Biology* 218:175-185.

Payseur, B. A., A. D. Cutter, and M. W. Nachman. 2006. Searching for evidence of positive selection in the human genome using patterns of microsatellite variability. *Molecular Biology & Evolution* 19:1143-1153.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

**Rebelo, A. G. 2003. *Proteas: A Field Guide to the Proteas of Southern Africa*. 2nd edition. Fernwood Press, Vlaeberg, SA.

**Rebelo, A.G. 2004. The Protea Atlas Project. http://protea.worldonline.co.za/default.htm

**Rebelo, A. G. 2006. The Fynbos Biome. In: Mucina, L. & Rutherford, M.C. (eds) Vegetation Map of South Africa, Lesotho and Swaziland: An Illustrated Guide. Strelitzia. (in press).

Reeves, G. 2001. Radiation and macroevolutionary ecology of the African genus *Protea*. Ph.D. dissertation, Imperial College of Science, Technology, and Medicine, London, UK.

Robichaux, R. H., G. D. Carr, M. Liebman, and R. W. Pearcy. 1990. Adaptive radiation of the Hawaiian silversword alliance (Compositae-Madiinae): ecological, morphological, and physiological diversity. *Annals of the Missouri Botanical Garden* 77:64-72.

Robichaux, R. H., K. E. Holsinger, and S. R. Morse. 1986. Turgor maintenance in Hawaiian *Dubautia* species: the role of variation in tissue osmotic and elastic properties. In *On the Economy of Plant Form and Function*, ed. T. J. Givnish, pp. 353-380. Cambridge University Press, Cambridge, UK.

Rourke, J. P. 1982. *The Proteas of Southern Africa*. Centaur, Johannesburg.

Rourke, J. P. 1998. A review of the systematics and phylogeny of the African Proteaceae. *Australian Systematic Botany* 11:267-285.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford, UK.

Schluter, D., T. Price, A. O. Mooers, and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiation. *Evolution* 51:1699-1711.

*Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2005. Genetic consequences of tropical second-growth forest regeneration. *Science* 307:891.

*Sezen, U. U., R. L. Chazdon, and K. E. Holsinger. 2007. Multi-generational genetic analysis of tropical second-growth forest regeneration in a canopy palm. *Ecology* (submitted).

**Silander, J.A., A.M. Latimer, and A.E. Gelfand. 2006. Using Bayesian methods to predict species distribution patterns *BioScience* (invited manuscript).

**Silander Jr., J. A., A. Latimer, S. Wu, A. E. Gelfand, A. G. Rebelo, R. M. Cowling, A. M. Schmidt, W Smit and H. Laurie. 2006a. Explaining species distributions: Bayesian hierarchical models for South African proteas. (manuscript).

**Silander Jr., J. A., A. Latimer, S. Wu, A. E. Gelfand, A. G. Rebelo, R. M. Cowling, A. M. Schmidt, W Smit and H. Laurie. 2006b. Bayesian hierarchical models of biodiversity: examples from South African proteas. (manuscript).

Slatkin, M. 1991. Inbreeding coefficients and coalescence times. *Genetical Research* 58:167-175.

Song, S., D. K. Dey, and K. E. Holsinger. 2006. Hierarchical models with migration, mutation, and drift: implications for genetic inference. *Evolution* (in press).

Spiegelhalter D J, Best N G, Carlin B P and van der Linde A (2002) Bayesian measures of model complexity and fit (with discussion). *J. Roy. Statist. Soc. B*. **64**, 583-640.

Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* 135:467-374.

Thomas, C.D., A. Cameron, R.E. Green, M. Bakkenes, L.J. Beaumont, Y.C. Collingham, B.F.N. Erasmus, M. Ferreira de Siqueira, A. Grainger, L. Hannah, L. Hughes, B.

Huntley, A.S. van Jaarsveld, G.F. Midgley, L. Miles, M.A. Ortega-Huerta, A. T. Peterson, O.L. Phillips and S.E. Williams. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Tyree, M. T., and P. G. Jarvis. 1982. Water in tissue and cells. In *Encyclopedia of Plant Physiology, New Series, Vol. 12B*, edited by. O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, pp. 35-77. Springer-Verlag, Berlin.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407-14.

Weiher, E., A. Van Der Werf, K. Thompson, M. Roderick, E. Garnier, and O. Eriksson. 1999. Challenging theophrastus: A common core list of plant traits for functional ecology. *Journal of Vegetation Science* 10:609-620.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33:125-159.

Wright, S. 1942. Statistical genetics and evolution. *Bulletin of the American Mathematical Society* 48:223-246.

**Wu, S. 2004. Statistical Models Developed Toward Explaining Species Diversity. Ph.D. Dissertation, University of Connecticut.

**Wu, S., J. A. Silander, P.O. Lewis, A.E. Gelfand, A. Latimer, and A. G. Rebelo. 2006. Explaining species distribution patterns through hierarchical modeling: phylogenetic and ecological relationships. *Manuscript*

* articles written with support from NSF DEB-0424767

** articles and books written with support from NSF DEB-0089801