

PLANT RESPONSES TO CLIMATE IN THE CAPE FLORISTIC REGION OF SOUTH AFRICA: EVIDENCE FOR ADAPTIVE DIFFERENTIATION IN THE PROTEACEAE

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Received April 9, 2010

Accepted August 24, 2010

Local adaptation along environmental gradients may drive plant species radiation within the Cape Floristic Region (CFR), yet few studies examine the role of ecologically based divergent selection within CFR clades. In this study, we ask whether populations within the monophyletic white protea clade (*Protea* section *Exsertae*, Proteaceae) differ in key functional traits along environmental gradients and whether differences are consistent with local adaptation. Using seven taxa, we measured trait–environment associations and selection gradients across 35 populations of wild adults and their offspring grown in two common gardens. Focal traits were leaf size and shape, specific leaf area (SLA), stomatal density, growth, and photosynthetic rate. Analyses on wild and common garden plants revealed heritable trait differences that were associated with gradients in rainfall seasonality, drought stress, cold stress, and less frequently, soil fertility. Divergent selection between gardens generally matched trait–environment correlations and literature-based predictions, yet variation in selection regimes among wild populations generally did not. Thus, selection via seedling survival may promote gradient-wide differences in SLA and leaf area more than does selection via adult fecundity. By focusing on the traits, life stages, and environmental clines that drive divergent selection, our study uniquely demonstrates adaptive differentiation among plant populations in the CFR.

KEY WORDS: Adaptation, adaptive radiation, selection—natural.

Natural selection along environmental gradients can result in phenotypic differentiation and local adaptation among wild populations (Endler 1986; Linhart and Grant 1996; Ackerly et al. 2000; Geber and Griffen 2003) and can even drive speciation (Kawecki and Ebert 2004; Schluter 2009). An expected outcome of local adaptation is that traits and environments co-vary, as is commonly observed in both plant and animal taxa (e.g., Clausen et al. 1940; Dobzhansky 1947; Reich et al. 2003). Local adaptation can also contribute to species diversification into adaptive radiations, with each species specializing on only a portion of the natural variation in climate, habitat type, or interacting species (e.g., Hodges 1997;

Schluter 2000; Friar et al. 2006). Yet not all among-population divergence is adaptive, and by extension, neither are all evolutionary radiations generated or maintained through adaptive processes (Schluter 2000; Comes et al. 2008). Among-population and among-species trait differences may also reflect environmental plasticity or nonadaptive historical processes, such as dispersal limitation and random genetic drift, yet efforts to distinguish among these alternatives are rare (but see e.g., Thorpe et al. 2005; Ellis et al. 2006; Comes et al. 2008). If local adaptation currently promotes trait diversity within a species or clade, trait–environment correlations should not only exist, they should also

be promoted and maintained by ecologically based divergent selection (Ackerly et al. 2000; Kawecki and Ebert 2004).

The Cape Floristic Region (CFR) of South Africa is characterized by high plant species richness—linked to extensive evolutionary radiations—and by steep environmental gradients (Schulze 1997; Goldblatt and Manning 2000; Linder 2003). The degree to which these characteristics are associated has attracted significant research interest (e.g., Cowling et al. 1997; Goldblatt et al. 2002; Linder 2003), but the role of environmental adaptation in generating diversity has been investigated for only a few Cape species (Verboom et al. 2004; Ellis and Weis 2006; Latimer et al. 2009). Much of the plant diversity in the CFR occurs in the fynbos, which are semi-arid, nutrient-poor scrublands that burn every ~12–30 years (Van Wilgen 1982). This relatively small region (90,000 km²) has experienced numerous bouts of uplift and erosion over its geological history, resulting in over a dozen mountain ranges of varying elevations (up to 2300 m) that are dissected by arid regions where fynbos species do not occur (Partridge 1997). Associated with the mountain ranges are steep gradients in aridity, temperature, length of growing season, and soil fertility. Furthermore, the timing and amount of rainfall varies across the CFR: in the extreme Western Cape, rainfall is very heavy in the wintertime (May–August), becomes more aseasonal toward the eastern limit of the CFR, and shifts to a summer time peak (November–February) toward the eastern coast of South Africa.

Plants respond to environmental gradients like those in the CFR through morphological and physiological traits affecting local performance and survival, known as functional traits (Geber and Griffen 2003; Reich et al. 2003). Examples of vegetative functional traits include leaf size and shape, stomatal density, photosynthetic capacity, height, and specific leaf area (SLA), that is, leaf area per unit mass. Among these, SLA, or its inverse leaf mass per area, contributes to a major axis of differentiation in leaf functional strategies (i.e., the leaf economics spectrum; Wright et al. 2004). Low SLA leaves tend to be longer-lived, have lower nutrient concentrations, and be less prone to wilting. It follows that SLA and associated traits should differ along drought, cold, and soil fertility gradients (reviewed in Reich et al. 2003). For example, across species, populations, and even biomes, SLA, leaf size, and growth rate generally decrease with increasing drought and nutrient limitation (Fonseca et al. 2000; Li et al. 2000; Lamont et al. 2002; Knight and Ackerly 2003; Ordoñez et al. 2009), as do growth rate and leaf area with altitude/cold stress (Grime 1977; Woodward 1986; Oleksyn et al. 1998). In contrast, light-saturated photosynthetic capacity tends to increase with altitude or drought stress (Gurevitch 1992; Ashton and Berlyn 1994; Benowicz et al. 2000; but see Jonas and Geber 1999). For a few of these traits, changes in selection regimes along drought and elevation gradients have been shown to reinforce trait–environment correlations within species (e.g., Etterson 2004; Byars et al. 2007). Tests of

such relationships are rare, however, and they have yet to be performed within the CFR.

Most studies of adaptive trait variation in the CFR have focused on gradients in aridity and soil fertility, because these are considered the most important environmental stresses in the region (Cowling et al. 1997). For example, species that occur in more arid environments in the CFR produce smaller or narrower leaves (Thuiller et al. 2004; Yates et al. 2010), have lower stomatal density (Richardson and Kruger 1990), or have lower SLA (Latimer 2006) than species in less-arid environments. Although various studies have linked trait variability to key environmental gradients in the CFR, it remains poorly understood how these traits and gradients contributed to within- and among-species diversification (Stock et al. 1997). Reciprocal transplant experiments and common gardens provide some evidence for local adaptation along rainfall gradients (Latimer et al. 2009) and to different soil characteristics (Verboom et al. 2004; Ellis and Weis 2006); however, little work has been done to identify which traits mediate these adaptive responses, and which are most important in population divergence along these clines.

Our overall objective is to determine if among-population differences in vegetative traits are related to natural selection and local adaptation in a representative evolutionary radiation in the CFR: the monophyletic white protea clade (*Protea* sect. *Exsertatae*, Proteaceae). Our tests of adaptive differentiation in the white proteas follow several predictions. First, trait differences among populations should be genetically based, such that they may respond to selection. Second, functional vegetative traits should vary along environmental gradients in ways that broadly match well-established trends from the literature. Third, selection gradients should change in strength and direction along key environmental gradients, and in doing so, they should match ecotypic variation among wild populations. To test these predictions, we measured trait–environment associations and selection gradients in relation to the environment using adult plants in 35 wild populations and their offspring grown in two common gardens. Our specific research questions are as follows:

- (1) Do white protea populations differ in key vegetative functional traits when grown in a common environment? that is, *do traits have a strong genetic component?*
- (2) Are among-population trait differences associated with strong CFR environmental gradients such as drought and cold stress, aridity, and soil fertility? that is, *do traits in wild populations or in common gardens correlate with source environments?*
- (3) Are trait–environment correlations maintained by ecologically based divergent selection as inferred from (a) 35 populations of wild adults that span steep environmental clines and (b) the two common gardens with different

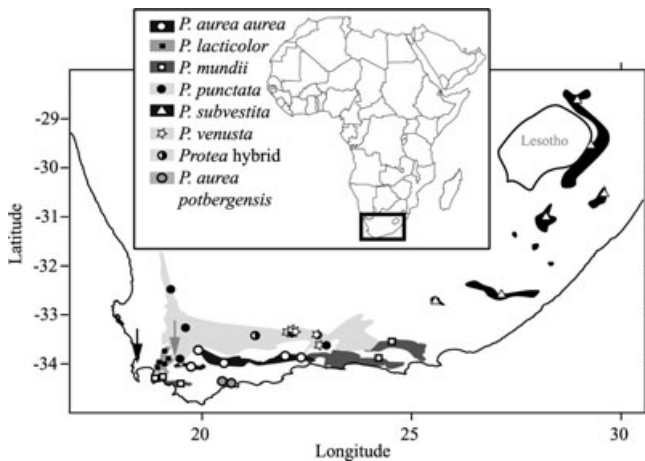


Figure 1. Locations of the 35 white protea populations sampled in South Africa between February 2008 and May 2009. The black arrow points to the Kirstenbosch garden, and the gray arrow points to the Jonaskop garden.

environments? that is, *do local selection gradients in wild populations co-vary with environment, and do selection regimes differ between gardens?*

Materials and Methods

STUDY SPECIES

We investigated trait–environment correlations and local selection gradients across all six species in the white protea clade, including both subspecies of *Protea aurea*: *Protea aurea* (Burm. f.) Rourke ssp. *aurea*, *P. aurea* ssp. *potbergensis* (Rourke) Rourke, *P. lacticolor* Salisb., *P. mundii* Klotzsch, *P. punctata* Meisn., *P. subvestita* N.E. Br., and *P. venusta* Compton. A recent phylogenetic analysis of South African members of the genus *Protea* found 99% posterior support for monophyly of white proteas (Valente et al. 2010). White proteas are broad-leaved, sclerophyllous shrubs, and all are endemic to the CFR except *P. subvestita*, which occurs in the Eastern Cape, Kwa-Zulu Natal, and Lesotho (Rebello 2001). Species distributions are largely allopatric and differ partially in altitude and climate (Latimer et al. 2009; Fig. 1), although overlap along environmental axes is significant (Latimer 2006). For example, *P. mundii* and *P. aurea* occur at relatively low altitudes (up to 1300 m), *P. lacticolor* is intermediate (600–1500 m), and *P. punctata*, *P. venusta*, and *P. subvestita* all occupy relatively high altitude sites (1200 to over 2000 m; Rebello 2001). Among the white protea taxa, only *P. punctata* and *P. venusta* co-occur. These two species hybridize readily in the wild, and most others hybridize readily in cultivation.

The seven white protea taxa share a suite of key life-history traits. They are all long-lived, evergreen perennials and most grow upright to over 4 m in height. Only *P. venusta* has a lateral growth habit, forming low-lying, dense mats. All species take

at least 3 years to reach reproductive maturity, after which annual flower production is linked to their sympodial growth pattern. White proteas are pollinated mainly by sugarbirds and sunbirds (Rebello et al. 1984; Rebello 2006), and their seed set tends to be low, with fewer than 15% of florets in an inflorescence producing plump, seed-containing achenes (henceforth seeds; Carlson and Holsinger 2010). Seeds are stored aboveground in serotinous infructescences (henceforth seed heads) until they are released following fire, and they usually germinate during the next rainy season (Rebello and Rourke 1986). Because fire kills adults, populations re-establish from seed into roughly even-aged stands.

SAMPLING DESIGN

Starting in February 2008, we sampled a total of 35 populations, consisting of five populations each of *P. mundii*, *P. lacticolor*, *P. aurea* subsp. *aurea*, and *P. venusta*, six each of *P. subvestita* and *P. punctata*, two of *P. aurea* subsp. *potbergensis*, and a single hybrid population (Fig. 1; Table S1). Sites were chosen to cover most of the range of each species, using the extensive database of Proteaceae localities compiled by the Protea Atlas Project (Rebello 2001, 2006).

Within each population, we sampled 11–40 adult plants (mean $n = 21$ plants; total $N = 688$). Individuals were selected approximately 10 m apart along transects through or near the population center. On each plant, we measured the total height, the number of seed heads, and the number of branching events on the tallest stem, which serves as a proxy for plant age (± 2 years; T. Rebello, pers. comm.). We estimated the mean annual growth increment by dividing plant height by our proxy for age. We also collected five to eight intact seed heads (≥ 2 years old), and two fully expanded, mature leaves from the plant's most recent growth. On one of the fresh leaves, we measured leaf length, width, and area using a LiCor 3100 leaf area meter (Lincoln, NE), dried the leaf for two weeks at 60°C, and then weighed it. We used these data to calculate leaf length:width ratio (LWR) and SLA (leaf area divided by leaf mass in cm^2/g). We estimated stomatal density (number of stomata per mm^2) on the other fresh leaf using a light microscope and cellophane tape leaf peels from the abaxial leaf surface (following Dunlap and Stettler 2001). Finally, we measured the wood density on a subset of sampled white protea plants ($n = 504$; including all populations except LW and JK), using $\sim 1 \text{ cm}^3$ sections of 3-year-old sapwood that were air-dried, peeled, and rehydrated under a vacuum. The mass of water displaced by the sample was converted to wet volume, and wood density was calculated as the mass of the dried sample (at 75°C for ~ 48 h) divided by its wet volume (following Martinez-Cabrera et al. 2009).

Seed heads were dried at low humidity until opening, after which we removed and counted the number of plump, intact seeds per seed head. Many seed heads were infested with seed-eating

larvae (over 60% of seed heads in some populations; Carlson and Holsinger, 2010), but even those that were infested often contained some intact seeds. We stored all potentially viable seeds at room temperature for use in germination trials and common gardens.

We sowed 3690 white protea seeds in a greenhouse at Kirstenbosch Botanical Garden, Cape Town, in the early rainy season (May) of 2008. Up to 15 seeds from each of eight maternal plants per population were planted into multitrays (24 cups per tray) containing a sandy soil mix (1 loam: 8 bark: 3 sand). Pots were checked for germination and watered every other day for nine weeks, by which time most viable seeds are expected to germinate (Rebelo and Rourke 1986). At the end of the trial, we calculated the mean number of days until germination per maternal plant. We had no seeds from *P. punctata* population JK by May 2008, so this population was not included in germination trials or the common gardens.

In July 2008, we transplanted seedlings from the greenhouse into two common gardens (see Fig. 1 for locations). One was at Kirstenbosch Botanical Gardens (175 m elevation; ~1130 mm rainfall per year) and the other 100 km due east on Jonaskop mountain in the Riviersonderend range (944 m; ~350 mm rainfall per year; Schulze 2007). The climate conditions at each garden were within the range of those experienced by natural populations, with Jonaskop tending to have colder winters, stronger drought, lower soil fertility, and a smaller winter peak in rainfall than Kirstenbosch. We selected up to 43 of the greenhouse-reared seedlings per population (mean $n = 33$), and randomly divided them between the two gardens, with the stipulation that maternal half-siblings were equally represented in each garden, typically by three seedlings each (170 maternal families total). By six months post-transplant, however, high mortality within each garden had reduced a third of maternal lines to just one seedling. As a result, the family structure was not included in our analyses. Three populations had very poor germination (*P. subvestita* RN, and *P. venusta* MJV and KSV) and were each represented by six or fewer seedlings. Seedlings were planted ~25 cm apart in a completely randomized design within gardens, measured for initial height within the first month, and protected from trampling or mammalian herbivory with wire mesh cages around each garden.

Six months after transplant, we removed cages, measured seedling height and collected a single leaf per plant (most seedlings had 10–30 leaves). On each seedling leaf, we measured SLA, leaf area, LWR, and stomatal density using the methods described above for wild plants. We calculated 6 month growth rate as the height in January 2009 minus the initial height. We recorded seedling survival in both gardens at the end of the first dry season (4 months later), which marks the end of a high mortality period for white protea seedlings at both sites (J. E. Carlson et al., unpubl. data). Because we were interested in survival probability as it was related to leaf traits, individuals that died prior to

leaf measurement were excluded from selection gradient analyses (Kirstenbosch $n = 322$; Jonaskop $n = 284$).

We also measured light-saturated photosynthetic rate on a subset of seedlings at Kirstenbosch only. Photosynthesis measurements were taken on 171 plants in full sun, between 0800 and 1000 h on clear days from January to March 2008. We clamped a fully expanded upward-facing leaf, still attached to the plant, into the chamber of a LiCor 6400XT with CO₂ mixing system and a red/blue LED light source (Lincoln, NE). We maintained conditions inside the chamber at values close to external conditions, with PAR at $1500 \mu\text{mol} \times \text{m}^{-2} \times \text{s}^{-1}$, CO₂ concentration at $400 \mu\text{mol} \times \text{mol}^{-1}$, and temperature at 25–26°C. Mean relative humidity was 36% (9% SD). We took the average of at least two measurements per plant—often measuring multiple leaves or on different days—and then we calculated photosynthetic rate per unit area (A_a). We also calculated photosynthetic rate per unit mass (A_m), but we only report results for A_a because patterns of variation along environmental gradients were the same.

ENVIRONMENTAL DATA

We quantified climatic differences among white protea populations and common gardens using a suite of GIS climate layers from the South African Atlas of Climatology and Agrohydrology (Schulze 2007; see Appendix S1). Our focal layers were chosen to reflect climate gradients known to affect plant traits and survival in the CFR (Cowling et al. 1997; Thuiller et al. 2004; Yates et al. 2010) and were useful in predicting white protea species distributions (Latimer et al. 2006; Latimer 2006). Our first axis was PPTCON, which is an index of rainfall concentration throughout the year. Low values of PPTCON represent even precipitation throughout the year, and high values represent winter or summer peaks in precipitation. Our second and third climate axes, COLDPKA and DRYPCA, were the first axes of principal components analyses on two winter temperature layers or two dry season rainfall layers, respectively (described in Appendix S1). Low values of COLDPKA are associated with longer, colder winters (more days below 10°C), and high values with warmer, milder winters. COLDPKA was also strongly correlated with elevation ($r = -0.85$). Low values of DRYPCA are associated with longer, more severe drought during the driest 3 months, and high values are associated with a milder, moister dry season. The Pearson's product moment correlations (r) between focal variables were 0.37 for COLDPKA and DRYPCA, -0.32 for DRYPCA and PPTCON, and -0.08 for PPTCON and COLDPKA.

We used field-collected soil samples to describe a fourth environmental gradient, that of soil fertility. At the beginning, midpoint, and end of each sampling transect, we collected soil from two depths, 0–15 cm and 15–30 cm, and bulked samples from the same depth. We sampled soil at both depths in each common garden as well. We dried all soil samples for 1 week at 60°C and

had them analyzed for extractable macro-nutrients at BEM Labs (Somerset West, SA). We averaged nutrient values from the two soil depths and performed a PCA on the amount of nitrogen (%), phosphorous (mg/kg), and potassium (mg/kg) in the soil. The first axis of the PCA was used as our soil fertility axis, FERTPCA, explaining 58% of variation (respective nutrient loadings were 0.66, 0.49, and 0.57). Soil fertility was not strongly correlated with any of the three climate axes ($r = -0.02$ to -0.22).

ANALYSES OF TRAIT VARIATION AND GENETIC EFFECTS

Do traits have a strong genetic component?

We used traits of plants grown in the two common gardens to determine the extent to which among-population trait differences are genetically influenced. We estimated variance components for each of the four seedling leaf traits, the light-saturated photosynthetic rate (A_a), and the 6-month growth rate in a random effects ANOVA. The four leaf traits were log transformed to improve the normality of residuals. We calculated the percent variance explained by species, population nested in species, garden, and the interactions based on variance parameter estimates. Significance was assessed using the type three analysis of variance (Proc MIXED method = TYPE3; Littell et al. 2006). Unless otherwise indicated, we performed all analyses in SAS version 9.1.3 (Cary, NC) using Proc MIXED or Proc GLIMMIX and Kenward-Roger adjustments for degrees of freedom (Littell et al. 2006).

Do traits in wild populations or common gardens correlate with source environments?

We performed a series of multiple regressions to examine relationships among leaf traits and environmental gradients in the wild and in their offspring in common gardens. For the wild adult analyses, we used each of four wild adult leaf traits—SLA, LWR, stomatal density, and leaf area—as the sole response variable in a multiple linear regression with the remaining three leaf traits and the four environmental axes as the fixed effects. Because our primary focus was on relating leaf traits to environment axes, we analyzed all species and populations together, and we included species and populations as random rather than fixed effects. We used the same multiple regression models on each common garden dataset, except that all leaf traits were from garden seedlings and environmental axes were from the parental population. Prior to analyses, all seedling and adult leaf traits were log-transformed as predictor and response variables to improve the normality of residuals. The four leaf traits and the four environmental axes were then standardized to a mean of zero and standard deviation of one within datasets, allowing us to compare the magnitude of multiple regression coefficients within and among analyses. Because we used identical models, we could interpret shared relationships between wild and gardens as further evidence that trait–

environment correlations among wild populations had a strong genetic component.

We performed a second set of multiple regressions on growth, germination, and photosynthetic characteristics of wild plants or their offspring. Each response variable was regressed separately on the four environmental axes and the four leaf traits, using adult leaf traits for adult analyses, and seedling leaf traits for seedling analyses. The adult response variables were mean annual growth increment, wood density, and per-adult averages of time to germinate. The response variables from common garden seedling were 6-month growth rate in each garden and A_a in Kirstenbosch. Mean annual growth increment was included as an additional fixed effect for all adult analyses except for its own, and seedlings growth rate was a fixed effect in the photosynthesis analysis. All response and predictor variables were standardized, but only the leaf traits were log transformed. Random effects were species and population nested within species.

ANALYSES OF SELECTION GRADIENTS

Do local selection gradients in wild populations co-vary with environment?

To investigate the relationships between local selection gradients and local environments, we developed a hierarchical Bayesian model in which the direct selection coefficients on traits within wild populations were regressed on the four environmental axes (details in Appendix S2). This model incorporates uncertainty about local selection gradients into the regression of selection gradients on environmental axes. Our direct selection coefficients were the standardized partial regression coefficients from a multiple regression of four focal traits on a fitness component (Lande and Arnold 1983). Our focal traits were stomatal density, SLA, LWR, and leaf area, and our primary fitness component was realized fecundity per plant (seed heads \times seeds per seed head). Our secondary fitness component was seed heads per plant, which represents a plant's reproductive investment, largely independent of pollination rates and seed predation. We also examined whether the relationships between each fitness component and mean annual growth increment varied with environment, but did so separately to avoid over-fitting the within-population multiple-regression models. Prior to all analyses, we log-transformed leaf traits across all populations and then standardized leaf traits and growth within populations to a mean of zero and a standard deviation of one. Fitness measures were standardized to a mean of one (Lande and Arnold 1983). We simultaneously estimated selection gradients and their relationships with environmental axes in a single Bayesian model using Gaussian priors (mean = 0, SD = 1) on all regression coefficients and a uniform prior (0, max.sd) with max.sd set to four times the observed maximum within population standard deviation for fitness (Gelman et al. 2004). We report posterior means and 95% credible intervals for each parameter based

on 5000 Markov chain Monte Carlo (MCMC) samples from five independent chains (burnin = 1000, sample = 25,000, thin = 25). MCMC sampling was implemented in WinBUGS version 1.3 (Spiegelhalter et al. 2002), and standard convergence diagnostics (Gelman and Rubin 1995) indicated that the chains had converged satisfactorily (G-R statistic < 1.01 for all parameters).

Do selection regimes differ between gardens?

To evaluate whether natural selection favored different individuals in each garden, we measured garden-specific selection gradients and differentials on seedling leaf traits and growth rate. Our primary interest was to estimate directional selection in each garden, so we did not include quadratic effects to test for stabilizing or diversifying selection. To estimate direct selection on traits—that is, selection gradients—we used separate multiple logistic regressions in each garden to examine the fixed effects of seedling SLA, LWR, leaf area, stomatal density, and growth rate on seedling survival at 10 months. We standardized all variables except survival, and we log-transformed leaf traits before standardization. Species and population were random effects, and the environmental axes were covariates. We converted logistic regression coefficients to selection gradients by adapting the method described in Janzen and Stern (1998). Additional details on the models and conversions are in the Appendix S3. To determine whether differences between gardens were significant, we performed a separate selection gradient analysis on the combined garden dataset with the fixed effects of seedling leaf traits, growth rate, garden, and the trait by garden interactions.

The use of individual plants rather than genotypic means may introduce bias in selection gradient analyses because variation in microsite quality may produce apparent correlations between traits and fitness in the absence of selection on those traits (i.e., Rausher 1992). We could not use family means because of high mortality, so we used a statistical approach to account for potential spatial autocorrelation associated with microsite quality within gardens. We reran the analyses with a spatial covariance structure and the plant's location within garden as a random effect (exponential spatial model; Littell et al. 2006; Spigler and Chang 2008). Because the results from the spatial model were indistinguishable from the original model, only the original results are presented.

We estimate cumulative selection on traits in each garden—that is, selection differentials—using an approach similar to that used for selection gradients (Details in Appendix S3). These estimates were nevertheless almost identical to the differences in mean trait value between the two time steps, so these “raw” differentials were used instead. We calculated raw differentials by subtracting the mean trait value at 6 months minus a recalculated mean based on survivors at 10 months, using log-transformed, standardized traits. We tested the significance of the selection

differentials using *t*-tests comparing the pre- and post-selection means within gardens. Because we measured A_a on only a small subset of seedlings, we estimated its selection differential but not selection gradient.

Results

TRAIT VARIATION AND GENETIC EFFECTS

Do traits have a strong genetic component?

Differences among white protea seedlings in the common gardens were partially explained by their source population and species, revealing significant genetic effects on all traits measured (Table 1). The local environment of each garden also affected seedling traits, as evidenced by significant differences between gardens in almost all traits ($P < 0.04$ for each; Table 1). Seedlings tended to grow faster, produce larger leaves, and have fewer stomata per square millimeter in the Kirstenbosch garden than in the Jonaskop garden (Table 1). Only for SLA did we fail to detect between garden differences, suggesting that environmental plasticity was very low in this trait. When analyses were restricted to the 32 maternal families with 100% survival (to control for differential mortality between gardens), we still found significant garden effects on stomatal density ($P = 0.008$), growth rate ($P = 0.008$), leaf area ($P = 0.003$), but none on SLA or LWR ($P > 0.3$ for both), confirming that between-garden differences in most traits were largely due to environmental plasticity rather than habitat filtering.

Do traits in wild populations or common gardens correlate with source environments?

Among-population differences in adult leaf traits were significantly correlated with three major environmental gradients: dry season drought stress, winter cold stress, and rainfall concentration ($P < 0.05$ for all; Fig. 2A, coefficients and summary figure in Table S2 and Fig. S1). Leaf area, SLA, and stomatal density were each significantly correlated with at least one axis (Figs. 3A, B and C), and these relationships were independent of trait covariances or other trait-environment correlations. We detected no associations between LWR and any environmental axis, although there was weak evidence that leaves tended to be broader (i.e., low LWR) at high soil fertility ($P = 0.07$; Figs. 2A and 3D).

The analyses on common garden plants revealed that most correlations in the wild were also present in at least one garden, and many new correlations were present only in the gardens (Figs. 2A, B and C; Table S2 and Fig. S1). Two correlations were significant in all three datasets, both with leaf area. Leaves were significantly smaller in adults and their common garden offspring if they came from aseasonal sites or sites with harsher

Table 1. Variance in seedling traits explained by species, population, garden effects, and interactions. Each garden contained seedlings from 32 populations encompassing seven white protea taxa. Leaf traits and growth rates were measured 6 months post-transplant on 284 plants in Jonaskop (J) and 325 in Kirstenbosch (K). Photosynthesis was measured in Kirstenbosch only, on 171 plants. Each plant trait was the response variable in a variance components analysis in PROC MIXED (method=type 3; Littell et al. 2006). Bold values are associated with statistical significance.

Source of variation	SLA (cm ² × g ⁻¹)		Leaf area (cm ²)		LWR		Stomatal density (stomata × mm ⁻²)		6-month growth rate (mm)		A _a (μmol CO ₂ m ⁻² × s ⁻¹)	
	Percentage of variance	P-value	Percentage of variance	P-value	Percentage of variance	P-value	Percentage of variance	P-value	Percentage of variance	P-value	Percentage of variance	P-value
Species	24.2	0.0007	31.0	0.0004	36.7	0.0003	9.2	0.006	25.5	<0.0001	33.3	0.0002
Population(species)	13.0	0.0002	20.3	<0.0001	18.9	<0.0001	3.1	0.03	9.8	<0.0001	7.2	0.04
Garden	0.0	0.65	11.7	<0.0001	3.2	0.03	35.9	<0.0001	28.5	<0.0001	-	-
Garden × species	0.0	0.54	0.6	0.11	3.0	0.004	1.1	0.14	0.9	0.13	-	-
Garden × pop(sp.)	0.6	0.35	0.0	0.97	0.0	0.75	0.0	0.61	0.0	0.51	-	-
Residual	62.2	-	36.5	-	38.2	-	50.7	-	35.2	-	59.5	-
Mean trait value (Raw mean ± SE)	J: 79.8 ± 0.8 K: 79.9 ± 0.9	J: 2.66 ± 0.85 K: 3.94 ± 0.14	J: 2.90 ± 0.03 K: 2.96 ± 0.03	J: 57.0 ± 0.7 K: 45.6 ± 0.6	J: 45.3 ± 1.5 K: 75.8 ± 2.1	J: 3.52 ± 0.16						

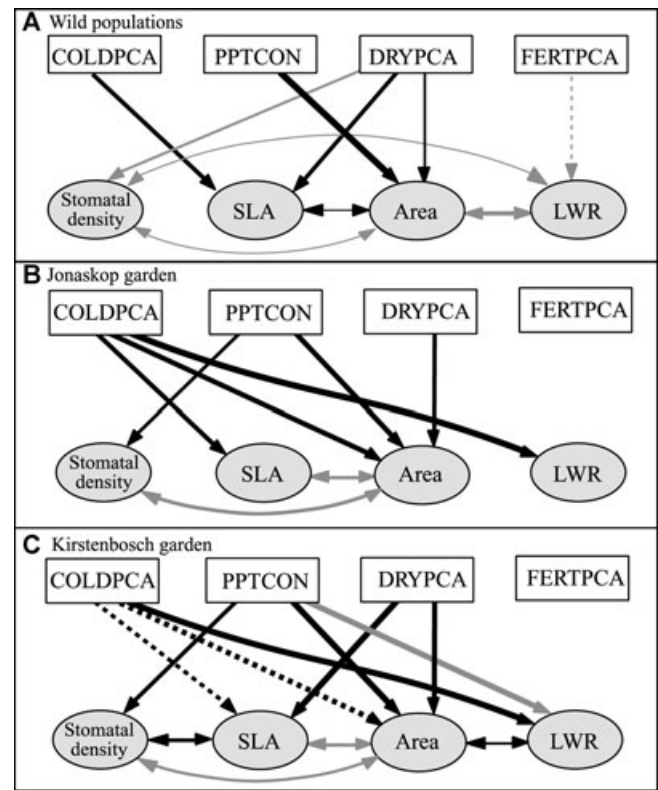


Figure 2. Analyses of trait–environment correlations based on white protea plants measured in the wild (A), in the Jonaskop garden (B), and in the Kirstenbosch garden (C). For analysis (A), traits were measured on 688 adult plants in 35 populations, and environmental data for each population were extracted from GIS layers. For analyses (B) and (C), traits were measured on 284 and 325 seedlings, respectively, after 6 months in the garden, and the environmental data were associated with each seedling’s source population environment. Solid arrows indicate significant correlations ($P \leq 0.05$), and dotted arrows, marginally significant correlations ($P < 0.08$). Black arrows show positive correlations, and gray, negative correlations. The thickness of the arrow is directly proportional to the slope of the regression line. Species and populations were included as random effects in all models, and in all cases, they accounted for significant trait variation (based on AIC comparisons).

dry seasons ($P < 0.05$ for both PPTCON and DRYPCA). The associations between SLA and winter cold stress or dry season drought stress were also persistent across gardens and the wild. Seedlings whose parents experienced colder winters or more intense drought had lower SLA in at least one of the two gardens (COLDPCA: Jonaskop $P = 0.05$, Kirstenbosch $P = 0.07$; DRYPCA: Jonaskop $P = 0.69$, Kirstenbosch $P = 0.01$). Finally, the positive correlations between stomatal density and PPTCON and between LWR and COLDPCA were shared only between the gardens ($P \leq 0.05$ for all; Figs. 2B and C, Table S2 and Fig. S1). In sum, the home environment predicted trait variation in adults

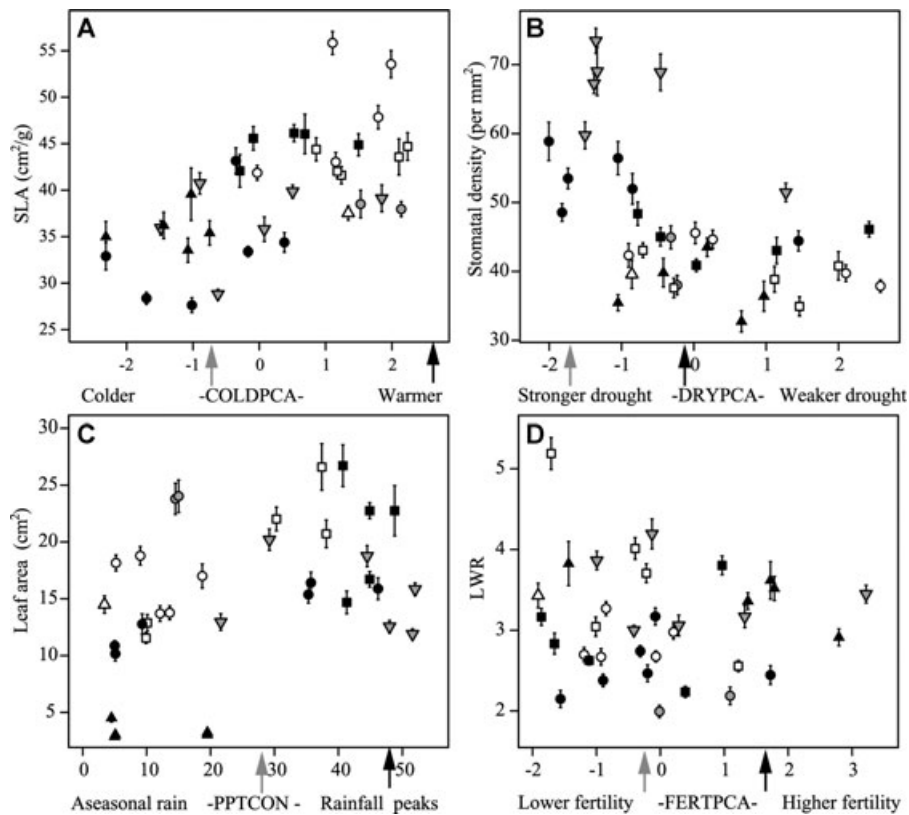


Figure 3. Per-population means of four adult functional traits along four environmental gradients: (A) SLA versus COLDPCA, (B) stomatal density versus DRYPCA, (C) leaf area versus PPTCON and (D) LWR versus FERTPCA. Graphed data are raw means ± 1 SE. The gray and black arrows indicate the locations of the Jonaskop and Kirstenbosch common gardens, respectively, along each environmental gradient. White circles are *P. aurea aurea*, gray circles are *P. aurea potbergensis*, black boxes are *P. lacticolor*, white squares are *P. mundii*, black circles are *P. punctata*, gray upside-down triangles are *P. subvestita*, black triangles are *P. venusta*, and the white triangle is a *Protea* hybrid. All relationships depicted are significant in a multiple regression, except LWR along the FERTPCA axis, which is marginally significant at $P = 0.07$.

as well as their common garden offspring, confirming that most trait–environment correlations were genetically influenced and not simply a result of plasticity.

Growth, germination, and photosynthetic rates of white proteas were also strongly related to the home or source climate (Table 2A and B; Fig. S1). Plants in sites with lower COLDPCA values (colder winters) grew more slowly as adults, and, similarly, seedlings that were sourced from lower COLDPCA populations grew more slowly and had a higher maximum photosynthetic rate (A_a) in Kirstenbosch. Adults from sites with seasonally heavy rainfall (high PPTCON) produced seeds that were slower to germinate in the greenhouse, and the seedlings sourced from those sites also grew more slowly. In both gardens, seedlings from higher fertility parental environments grew more slowly than those from lower fertility parental environments.

We detected numerous intertrait correlations within adult plants and within seedlings. From the adult analyses, plants with denser wood tended to be slower-growing and have lower SLA (Table 2A). Furthermore, larger adult leaves tended to be broader (lower LWR), have fewer stomata per square millimeter, and have

higher SLA (Fig. 2A). In contrast, larger seedling leaves tended to be narrower (Kirstenbosch only), have fewer stomata per square millimeter, and have lower SLA (Figs. 2B and C). Also from the seedling analyses, seedlings that grew more quickly generally produced larger leaves (Table 2B). Finally, A_a from Kirstenbosch was positively correlated with leaf area and negatively correlated with SLA (Table 2B).

SELECTION GRADIENTS

Do local selection gradients in the wild co-vary with environment?

We detected relationships between selection gradients on leaf area, LWR, and SLA with one or more of the environmental axes, although the slope of the SLA relationship was barely distinguishable from zero (Table 3). Selection on growth rate, in contrast, did not vary significantly along any of the environmental axes (based on 95% symmetric credible intervals; results not shown). There were three main patterns of divergent fecundity selection on leaf traits along the environmental clines. First, plants with larger leaves produced more seed heads than those with smaller

Table 2. Results of multiple regressions using standardized leaf traits and environmental axes to predict (A) adult growth in the wild and germination in the greenhouse, and (B) light-saturated photosynthetic rate and 6-month growth rate in the common gardens. In (A), all trait data except germination were collected from adult plants in 35 wild populations of seven white protea taxa. Time to germination was measured on their seeds sown in greenhouse at Kirstenbosch. In (B), leaf traits and growth rate were measured on seedlings after 6 months in common gardens. In both (A) and (B), environmental effects were from the home or parental population, and random effects were species and population. The species and population effects accounted for significant variation in all variables (based on AIC comparisons), except that only population was significant for A_a . Bold values are associated with statistical significance.

(A) Wild adults	Mean annual growth=increment height/ \sim year, $n=688$			Wood density=dry mass/wet volume, $n=500$			Average days to germination of offspring, $n=233$		
	df	β	<i>P</i> -value	df	β	<i>P</i> -value	df	β	<i>P</i> -value
SLA	674	-0.06	0.12	488	-0.17	0.002	183	-0.05	0.61
Leaf area	607	0.08	0.16	422	0.08	0.29	60	-0.12	0.33
LWR	678	-0.03	0.34	479	0.09	0.08	110	-0.04	0.61
Stomata	670	0.1	0.01	489	0.02	0.75	123	-0.04	0.66
Growth rate	-	-	-	465	-0.13	0.04	134	-0.07	0.49
DRYPCA	27	-0.06	0.62	25	-0.02	0.88	25	0.18	0.08
COLDPCA	30	0.3	0.03	28	0.07	0.6	30	-0.19	0.11
PPTCON	30	0.03	0.86	31	-0.18	0.27	22	0.5	0.002
FERTPCA	28	-0.04	0.65	26	0.01	0.88	29	0.12	0.15

(B) Gardens	A_a ($\mu\text{mol CO}_2 \times \text{m}^{-2} \times \text{s}^{-1}$) Kirstenbosch, $n=171$			6-month growth rate of seedlings (mm) Kirstenbosch, $n=325$			6-month growth rate of seedlings (mm) Jonaskop, $n=284$		
	df	β	<i>P</i> -value	df	β	<i>P</i> -value	df	β	<i>P</i> -value
SLA	143	-0.21	0.03	304	-0.06	0.17	273	0.08	0.009
Leaf area	161	-0.31	0.01	210	0.12	0.02	269	0.44	<0.0001
LWR	43	0.05	0.6	277	0.54	<0.0001	273	0	0.92
Stomata	160	-0.11	0.22	313	-0.01	0.81	265	-0.05	0.1
Growth rate	157	0.07	0.48	-	-	-	-	-	-
DRYPCA	11	-0.06	0.63	26	0.03	0.74	23	0.03	0.53
COLDPCA	15	-0.42	0.01	38	0.17	0.05	35	0.1	0.15
PPTCON	13	-0.11	0.37	27	-0.27	0.001	29	0	0.91
FERTPCA	10	0.07	0.52	25	-0.19	0.01	25	-0.16	0.004

Table 3. Posterior means (and symmetric 95% credible intervals) for multiple regressions of local selection gradients on environmental variables of seven white protea taxa. Regression coefficients that are statistically distinguishable from zero are highlighted in bold. Analyses are based on 688 plants; population sample sizes are given in Table S1; Details of statistical tests are in Appendix S2. Bold values are associated with statistical significance.

Effects	DRYPCA		COLDPCA		PPTCON		FERTPCA	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Seed heads								
SLA	-0.050	-0.128, 0.025	0.013	-0.059, 0.089	-0.003	-0.071, 0.066	-0.076	-0.144, -0.001
Leaf area	-0.002	-0.068, 0.072	-0.036	-0.102, 0.030	-0.097	-0.159, -0.034	-0.014	-0.082, 0.052
LWR	-0.002	-0.085, 0.081	-0.003	-0.080, 0.073	0.017	-0.057, 0.091	0.007	-0.065, 0.077
Stomatal density	-0.055	-0.127, 0.015	0.049	-0.019, 0.120	-0.001	-0.067, -0.061	-0.005	-0.07, 0.059
Realized fecundity								
SLA	0.005	-0.100, 0.112	0.017	-0.098, 0.130	0.016	(0.094, 0.130)	-0.050	-0.158, 0.054
Leaf area	-0.053	-0.150, 0.046	-0.001	-0.100, 0.104	-0.065	-0.167, 0.042	0.002	-0.093, 0.099
LWR	0.083	-0.018, 0.186	-0.145	-0.253, -0.031	0.036	-0.071, 0.146	-0.157	-0.260, -0.054
Stomatal density	-0.003	-0.111, 0.112	-0.060	-0.180, 0.053	0.007	-0.111, 0.122	0.005	-0.107, 0.107

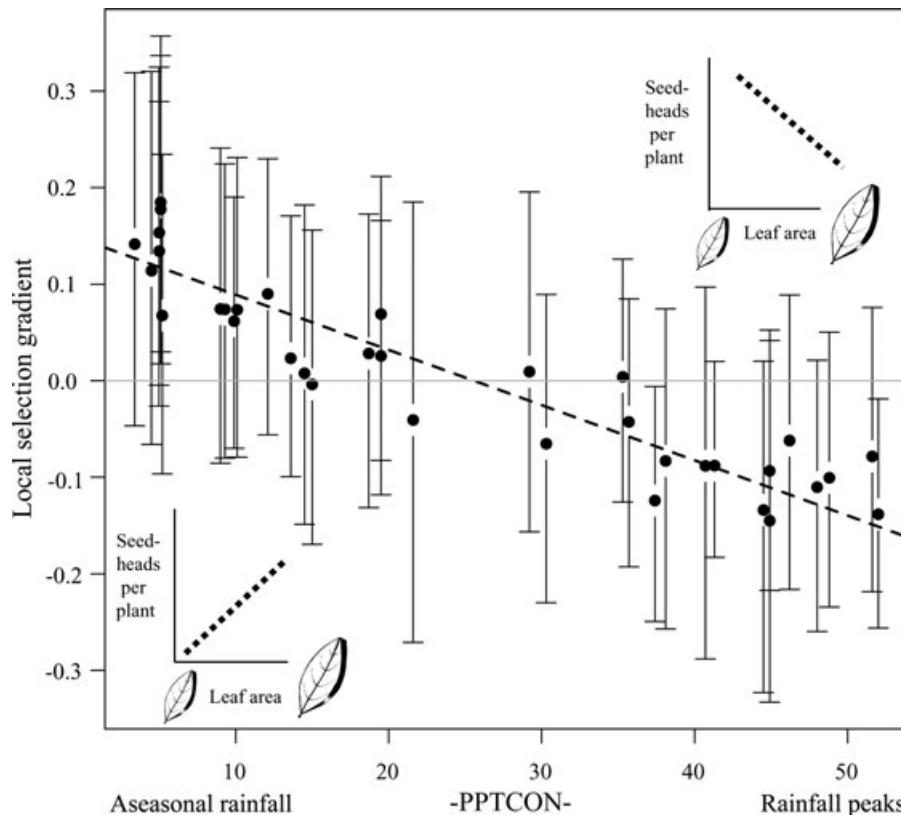


Figure 4. Local selection gradient on white protea leaf area as it varies across the geographical gradient in rainfall concentration. Each dot represents the local selection gradient (i.e., slope) of leaf area versus seed heads per plant within a single white protea population, and the error bars represent the 95% credible intervals around the local selection gradient. The dashed line represents the regression line describing the relationship between local selection gradients and PPTCON. Methods for deriving selection gradients and the regression on PPTCON are in Appendix S2.

leaves in sites with even rainfall throughout the year, whereas in sites with seasonal rainfall peaks, plants with smaller leaves produced more seed heads (Fig. 4). Second, plants with broader leaves (low LWR) had higher realized fecundity than those with narrower leaves in sites with high soil fertility or warmer winters, although there was no detectable relationship between LWR and realized fecundity in sites with lower soil fertility or colder winters. Third, plants with lower SLA produced more seed heads than those with higher SLA at sites with high soil fertility, although there was no detectable selection at sites with low soil fertility (Table 3).

Do selection regimes differ between gardens?

Natural selection via seedling survival favored different seedling traits in each garden (Table 4). In Kirstenbosch, there was positive direct selection on leaf area, that is, seedlings with larger leaves were more likely to survive. In Jonaskop, survival probability was greater for fast-growing seedlings and seedlings with less leaf area per unit mass (lower SLA). When both gardens were analyzed together in a combined analysis, we detected significant differences in selection gradients between gardens for SLA (trait \times garden:

$F_{1,590} = 7.77$, $P = 0.006$), leaf area ($F_{1,590} = 7.52$, $P = 0.006$), and growth rate ($F_{1,590} = 30.1$, $P < 0.0001$), but only marginally significant for LWR ($F_{1,590} = 3.08$, $P < 0.08$). Only the gradients on stomatal density were indistinguishable between gardens ($F_{1,590} = 0.34$, $P = 0.56$). Independent of differences in the traits that were favored, selection gradients and differentials were larger in the Kirstenbosch garden overall, because it had higher seedling mortality between January and June (67% vs. 27% died).

Additional traits were favored through indirect selection in each garden, as evidenced by significant selection differentials but nonsignificant selection gradients (Table 4). In Kirstenbosch, there was indirect selection on growth rate and stomatal density, likely mediated through a phenotypic correlation with leaf area. Similarly, indirect selection favored larger leaves in Jonaskop, probably mediated through SLA and growth rate. We detected no net selection on either SLA or LWR in either garden, and none on stomatal density at Jonaskop (Table 4). There was also no net selection on light-saturated photosynthetic rate in Kirstenbosch ($\beta = 0.028$, $P = 0.55$). In sum, selection favored fairly similar phenotypes in both gardens, although the traits that experienced direct selection differed between gardens.

Table 4. Selection gradients and differentials on seedling leaf traits and growth rate of white proteas in two common gardens. Fitness was measured as seedling survival at 10 months post-transplant. Leaf traits were log transformed and standardized, and growth at 6 months was standardized but not transformed. Selection gradients were from a multiple regression on binary survival data, with species and population as random effects and environmental axes as covariates (see Methods for details). Coefficients were converted to differentials and gradients partially following Janzen and Stern (1998). In regression analyses, none of the environmental covariates were significant ($P \geq 0.11$), and models were not improved by either random effect (based on AIC comparisons) except for the species effect at Jonaskop. Bold values are associated with statistical significance.

Fixed effects	Selection gradients				Selection differentials			
	Kirstenbosch		Jonaskop		Kirstenbosch		Jonaskop	
	Estimate	<i>P</i> -value	Estimate	<i>P</i> -value	Estimate	<i>P</i> -value	Estimate	<i>P</i> -value
SLA	-0.024	0.74	-0.017	0.0002	-0.133	0.19	-0.079	0.35
LWR	-0.058	0.89	0.024	0.95	-0.016	0.88	0.126	0.16
Leaf area	0.389	0.003	-0.007	0.46	0.494	<0.0001	0.223	0.004
Stomatal density	-0.061	0.34	-0.006	0.21	-0.230	0.03	-0.123	0.10
Growth rate	0.097	0.12	0.180	<0.0001	0.396	0.008	0.227	0.0002

Do selection gradients in the gardens or wild match trait-environment correlations?

The selection gradients of each garden differed in a few key aspects, and these differences largely matched heritable trait-environment correlations, given the environmental differences between gardens (Table 5). Plants with lower SLA and faster growth were favored in the drier, colder, and less-fertile garden (Jonaskop), and plants with larger leaves were favored in the moister, warmer, and more-fertile garden (Kirstenbosch).

Thus, divergent selection on SLA and leaf area matched among-population variation in relation to drought, rainfall concentration, and/or cold. The different selection regimes on growth rate also matched trait-environment correlations with PPTCON and FERT-PCA, but mismatched that with COLDPCA.

Patterns of divergent selection among populations in the wild, in contrast, generally did not match to expectations based on ecotypic variation observed in the same plants and their offspring (Table 5). Of the four examples of selection gradients co-varying

Table 5. Summary table of significant trait-environment correlations and selection gradients in the gardens and wild for white proteas. A match between the trait-environment correlation and a selection gradient suggests that divergent selection promotes differentiation along environmental clines. Jonaskop has colder winters, stronger drought, low and aseasonal rainfall, and lower soil fertility relative to Kirstenbosch (see arrows in Fig. 3). *W*=Adult plants measured in the wild; *J*=seedlings in Jonaskop; *K*=seedlings in Kirstenbosch. *ns*=nonsignificant. An asterisk indicates a marginally significant effect at $P < 0.08$.

Trait	Trait-environment correlations (corr.)				Selection gradients in gardens		Selection gradients in wild	
	COLD PCA	PPT CON	DRY PCA	FERT PCA	Differences between gardens	Match corr.	Differences along environmental clines	Match corr.
Stomatal density (stomata/mm ²)	ns	+	-	ns	ns	—	ns	—
SLA (cm ² /g)	+	ns	+	ns	Lower SLA favored in harsher garden (<i>J</i>)	Yes	Lower SLA favored in more fertile sites	ns corr.
Leaf area (cm ²)	+	+	+	ns	Higher leaf area favored in milder garden (<i>K</i>)	Yes	Lower leaf area favored where rainfall peaks	No
LWR	+	-	ns	-	ns	—	Broader leaves favored in more fertile/hotter sites	Yes*/No
Growth rate	+	-	ns	-	Faster growth favored in harsher garden (<i>J</i>)	Yes/No	ns	—
<i>A_a</i>	+	ns	ns	ns	—	—	—	—
Average time to germination	ns	+	ns	ns	—	—	—	—

with environment, only one matched a trait environmental correlation: that of LWR along the fertility gradient. Wild adult selection gradients involving leaf area and PPTCON as well as LWR and COLDPCA were in the opposite direction of standing ecotypic variation, and selection on SLA along fertility gradients was unexpected, because SLA and FERTPCA were not significantly correlated in the gardens or the wild.

Discussion

TRAIT VARIATION AND GENETIC EFFECTS

Our results demonstrate that white protea populations differ in key functional traits along the steep environmental gradients of the CFR, and that some of these differences may be maintained by ecologically based divergent selection on traits conferring higher seedling survival. The extreme environmental heterogeneity of the CFR has often been implicated as an important force shaping plant functional traits (Stock et al. 1997), and here we show how this process may occur in white proteas, particularly regarding the effects of aridity and cold stress on SLA, leaf area, stomatal density, and growth rate. Our results generally align with well-documented patterns of functional trait variation from the literature (i.e., Reich et al. 2003) with only a few notable exceptions, as we describe below.

Winter cold stress

Of the four environmental axes, the severity of winter cold stress at a site was correlated with the greatest number of functional traits of white proteas. Seedlings sourced from colder sites had lower SLA, leaf area, length:width ratio, growth rates, and had higher light-saturated photosynthetic rates at Kirstenbosch. All of the above correlations match patterns seen in the literature, particularly given that COLDPCA is tightly correlated with elevation and length of growing season (winter days above 0°C; $r = 0.94$). Numerous studies have demonstrated heritable, fitness-related differences in leaf morphology and height along altitudinal clines using common gardens or reciprocal transplants (Woodward 1986; Oleksyn et al. 1998; Byars et al. 2007) including seminal work by Clausen et al. (1940). Slow growth, low SLA, and small, long-lived leaves are likely favored at cold, high elevation sites because resource conservation is at a premium, that is, nutrients are more limiting due to slower decomposition, water is often inaccessible as ice or snow, and strong winds increase evaporative water loss (Grime 1977; Reich et al. 2003). Furthermore, slow growing white proteas tend to have denser wood (this study), and denser wood is more resistant to cavitation caused by drought or freezing (Reich et al. 2003). High elevation populations of white proteas (i.e., *P. venusta*, *P. punctata*, or *P. subvestita*) undoubtedly experience most or all of these stresses, given that they are often found on exposed, rocky slopes and may occasionally be subject to below-freezing temperatures during up to 6 months of the year.

The shorter growing seasons experienced by high elevation white proteas may also explain why their offspring had a higher light-saturated photosynthetic rate in the Kirstenbosch garden. A similar pattern of increasing photosynthetic rate with decreasing growing season length has been observed in *Picea abies*, (Oleksyn et al. 1998), *Achillea lanulosa* (Gurevitch 1992), *Alnus sinuata*, and *Betula papyrifera* (Benowicz et al. 2000). Such differences may be adaptive because plants at high elevations have a smaller window during which temperatures are suitable for growth, and plants that compensate with increased photosynthetic efficiency or capacity may have a selective advantage in those sites (Arntz and Delph 2001). Even so, photosynthetic rate has also been observed to decrease with elevation (e.g., Jonas and Geber 1999), suggesting that increased rates may not always be the favored ecophysiological response at high elevations.

Dry-season drought stress

Patterns of heritable trait variation in SLA, leaf area, and growth rate along our index of dry season drought stress were also in agreement with predictions from the literature. The relationships between drought and functional leaf morphology and physiology are extremely well studied worldwide (e.g., Arntz and Delph 2001; Niinemets 2001; Wright et al. 2004; Ordoñez et al. 2009), and have also been explored within the CFR (Stock et al. 1997; Lamont et al. 2002; Thuiller et al. 2004; Yates et al. 2010). On a regional level, there is overwhelming evidence that SLA and leaf area decrease with increasing drought stress/aridity among populations of the same species (e.g., Li et al. 2000; Etterson 2004; Ramirez-Valiente et al. 2009) among communities (e.g., Fonseca et al. 2000; Hallik et al. 2009), and among species using phylogenetically independent contrasts (Wright and Westoby 1999; Knight and Ackerly 2003; Preston and Ackerly 2003). The main adaptive function of dense or thick leaves (i.e., low SLA) in drought-prone environments is that they are more resistant to wilting (Cunningham et al. 1999; Niinemets 2001), and that they last longer, which conserves resources (Reich et al. 2003). Drought-mediated selection on leaf size and growth are also likely related to resource conservation. For example, small, narrow leaves have less surface area from which water may be lost, and their thinner boundary layers allow for more heat shedding in the absence of transpirational cooling (Givnish 1987; Fonseca et al. 2000; Yates et al. 2010). In sum, most patterns of white protea trait variation along the drought stress gradient may be interpreted as adaptive.

Rainfall seasonality

The third environmental axis of PPTCON describes a rainfall gradient specific to regions that, like the CFR, change from strongly seasonal to aseasonal rainfall within a few hundred kilometers. In our study, PPTCON was correlated with stomatal density, leaf

area, LWR, growth rate and the mean number of days between sowing and germination. The latter two correlations are likely related to the timing component of PPTCON, because the timing of growth and germination are likely dependent on when rainfall is most abundant (see also Stanton et al. 2000; Thuiller et al. 2004; Wu et al. 2010). The former three correlations are probably less related to timing, and are instead more related to an environmental covariate of PPTCON, the year-round moisture level at a site. Among our sites, those with higher PPTCON tend to receive more annual rainfall ($r = 0.51$) and have less daily variation in temperature (mean annual daily temperature range, $r = -0.57$), which is indicative of heavier cloud cover or higher humidity (e.g., Dai et al. 1999). These correlations suggest that plants in high PPTCON sites may be less heat- and water-stressed on average, thereby favoring the production of leaves that are larger and broader, because such traits maximize photosynthetic area when risks of overheating and drought stress are low (Givnish 1987; Hallik et al. 2009).

The positive relationship between stomatal density and PPTCON as interpreted above also aligns with early literature predictions that lower stomatal density promotes water conservation in xeric environments (Galston et al. 1980). Many studies have detected a similar trend of decreasing stomatal density with increasing aridity (Ashton and Berlyn 1994; Pääkkönen et al. 1998; Stenström et al. 2002; Yu et al. 2008; Wu et al. 2010); however, the reverse pattern is also fairly common (Clay and Quinn 1978; Dunlap and Stettler 2001; Preston and Ackerly 2003). Even in the present study, relationships involving stomata were inconsistent between wild populations and common gardens. Wild populations that experienced harsher drought (low DRYPCA) had higher stomatal density, and stomatal density was also higher in the drier of the two common gardens. We interpret the negative correlation between stomatal density and DRYPCA in the wild and between gardens as more likely a result of environmental plasticity and indirect selection through leaf area or other correlated traits. It remains unclear, however, if either pattern of plastic or genetic variation is truly adaptive in white proteas, because we detected no direct selection on stomatal density in either garden or within populations in the wild.

Soil fertility

The environmental axis that represented macronutrients in upper rhizosphere soil was a surprisingly poor predictor of trait variation in this study. FERTPCA showed only a marginally significant trend with leaf length:width ratio in the wild, and significant, non-intuitive correlations with growth in both gardens. The absence of strong correlations was unexpected, given that SLA, leaf area, or width, and growth rate have repeatedly been shown to decrease with decreasing soil fertility (Cunningham et al. 1999; Wright and Westoby 1999; Fonseca et al. 2000; Ordoñez et al. 2009). For

the CFR in particular, Stock et al. (1997) suggest that low SLA has evolved in response to low soil nutrients more so than water scarcity. This study, along with other recent work on white proteas (Latimer 2006) suggests that to the contrary, soil fertility has a weak effect on SLA relative to water-limitation and cold stress. Our fertility effects may have been weak because white proteas occur on a limited range of soil fertilities, relative to the range across habitats in the CFR. Even so, Lamont et al. (2002) similarly demonstrated that SLA and other functional traits were more strongly correlated with rainfall than fertility gradients across sites spanning much broader gradients in South Africa and Australia.

Genetic and plastic effects

Environmental clines and functional traits are expected to covary if populations are locally adapted (Endler 1986); however, trait–environment correlations may result from either genetic differences, environmental plasticity, or both. Our findings suggest that most white protea leaf traits and growth rate are shaped by both plastic and genetic effects, which is consistent with most studies on heritability and cross-gradient trait variation in plants (e.g., Dudley 1996; Li et al. 2000; Etterson 2004; Byars et al. 2007; Latta and McCain 2009; Ramirez-Valiente et al. 2009). In our study, genetic effects were made evident by (1) the presence of trait–environment correlations in at least one common garden, if not also in the wild and (2), the fact that species and population effects in the common gardens accounted for significant variation in all measured traits. Environmental plasticity in white protea traits was demonstrated by (1) two trait–environment relationships that were apparent in the wild but not in the gardens, and (2), differences in mean trait values between Kirstenbosch and Jonaskop that were independent of differential mortality. Only SLA was invariant between gardens, which was surprising because it has been shown to have high plasticity in other species (e.g., Maron et al. 2007), even when variation also has a significant heritable component (Agrawal et al. 2008). It may be that plastic differences in SLA will emerge in older protea seedlings, or that SLA is simply less plastic than other traits. Upcoming work will focus on variation in plasticity among white protea species, and whether plasticity in some traits may also contribute to local adaptation, given that environmental conditions can vary widely within populations and over time (see also Arntz and Delph 2001).

SELECTION GRADIENTS AND TRAIT DIVERGENCE

Selection gradients on white proteas changed in strength and direction along certain environmental gradients in the wild and between gardens, and in some cases, these changes reflected local adaptation (Kawecki and Ebert 2004). Our strongest examples of adaptive differentiation involved the disparate selection regimes on SLA and leaf area between gardens. To the extent that our two-garden comparison reflects a broader trend, we suggest

that survival selection via drought and cold stress could currently promote among-population differentiation in SLA and leaf area. Similar results found elsewhere suggest that our inferences are reasonable (Dudley 1996; Byars et al. 2007). For example, selection gradients on SLA in *Chamaecrista fasciculata* changed from positive to negative across three common gardens that were increasingly drought-prone (Etterson 2004). Selection on growth rate also differed between our gardens in accordance with trait variation along the FERTPCA and PPTCON axes; however, these differences mismatched growth variation along the COLDPCA gradient. Although this result was unexpected and the underlying mechanisms are unclear, this study is not the first to reveal inconsistent and difficult to explain variation in selection regimes on plants (e.g., Etterson 2004).

None of the four local selection gradients on traits of wild adults matched heritable ecotypic variation, and neither did they match any of the trait–environment correlations mentioned previously. This paradoxical result suggests to us that fecundity selection plays a minor role in maintaining adaptive differences in these traits of white proteas, particularly in relation to survival selection on seedlings. Natural selection in outdoor gardens of *Avena barbata* was also found to operate more through traits influencing survival than those influencing fecundity, relative to selection in a well-watered greenhouse (Latta and McCain 2009). Researchers studying the Cape flora have long thought that the seedling stage is the most influential in determining species distributions and population characteristics (e.g., Bond 1984), yet this study is the first to compare selection on the same traits of seedlings and adults.

Although such garden and wild comparisons are suggestive, our conclusions are limited because we measured selection via seedling survival only in common gardens and not in the wild. Selection gradients detected in our common gardens will not precisely match those found in the wild, because both the environmental context and the range of phenotypic variation differed from those in any populations in the wild. For example, wild populations had a much smaller range of trait variation, and they contained fewer poorly adapted phenotypes relative to our common gardens, in part because the garden gene pool was much larger and early-life mortality was buffered in the greenhouse. As a result, survival selection on seedlings in the wild may be less pronounced than what we detected between gardens. Nonetheless, the advantage of measuring selection gradients on a more-diverse gene pool is that it reflects selection prior to local adaptation. This type of common garden experiment is often used for studies of selection on novel mutations, hybrids, on phenotypes confronted with climate change or range expansion (e.g., Schemske and Bradshaw 1999; Moeller and Geber 2005; Maron et al. 2007), or as for this study, for inferring how selection may have shaped young or incipient species in new habitats.

Our study focuses on adaptive differentiation among populations rather than explicitly among species, yet two lines of evidence suggest that this better represents divergent selection in the white protea clade. First, most white protea taxa can survive in fairly broad, often overlapping, environmental conditions within the CFR (this study, Latimer 2006; Latimer et al. 2009), and as a result, trait divergence along environmental clines is as likely within as across species. Second, recent work by Prunier and Holsinger (2010) shows that there is as much neutral genetic diversity between conspecific white protea populations as there is between species, and that there is little gene flow between conspecific populations. Taken together, these results suggest that after speciation occurred, spatially separated white protea populations evolved independently (and often adaptively) due to low dispersal and geographic isolation (see also Latimer et al. 2009). Future work comparing molecular to trait diversity should help clarify the relative importance of adaptive and neutral processes in the white protea radiation (i.e., Schluter 2000). Our results show that currently, adaptive processes are driving differentiation in SLA, leaf area, and growth among white protea populations, making this study the first to link ecologically based divergent selection to phenotypic diversity within a CFR radiation.

ACKNOWLEDGMENTS

We are grateful to A.G. Rebelo and G.F. Midgley for their advice, generosity, and logistical support during project development and data collection. We also thank L. Nurrish and M. McQuillan for providing space and resources for our Kirstenbosch common garden, D.M. DeWitt for amazing greenhouse support and practical advice, and the du Plessis family for granting access to Jonaskop through G.F. Midgley. Additional South African support came from B. Mantlana, C. Musil, and others at SANBI at Kirstenbosch. We thank A.M. Latimer and A.M. Wilson for help with climate data in GIS, and G. J. Anderson and C. S. Jones for sharing equipment and laboratory techniques. C.S. Jones also provided helpful editorial comments, as did two anonymous reviewers and the handling editor. A.M. Gawel, C.A. Adams, B. Tomb, and M. El Harduchi provided excellent assistance in the field and/or laboratory. Data were collected under Cape Nature permits AAA005-00093-0028 and AAA005-00125-0028, permit No. CRO.23/08CR from the Chief Directorate of Environmental Affairs of the Eastern Cape Province, Ezemvelo KZN Wildlife permit 1789/2008, and unnumbered Eastern Cape permits from the Parks Board and the Department of Water Affairs and Forestry; We thank the above agencies, the many reserve managers, and the private landowners for granting access to our wild study populations. This research was funded by NSF grant DEB-0716622 awarded to Kent Holsinger.

LITERATURE CITED

- Ackerly, D. D., S. A. Dudley, S. E. Sultan, J. Schmitt, J. S. Coleman, C. R. Linder, D. R. Sandquist, M. A. Geber, A. S. Evans, T. E. Dawson, et al. 2000. The evolution of plant ecophysiological traits: recent advances and future directions. *BioScience* 50:979–995.
- Agrawal, A. A., A. C. Erwin, and S. C. Cook. 2008. Natural selection on and predicted responses of ecophysiological traits of swamp milkweed (*Asclepias incarnata*). *J. Ecol.* 96:536–542.

- Arntz, A. M., and L. F. Delph. 2001. Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. *Oecologia* 127:455–467.
- Ashton, P. M. S., and G. P. Berlyn. 1994. A comparison of leaf physiology and anatomy of *Quercus* (section *Erythrobalanus*-Fagaceae) species in different light environments. *Am. J. Bot.* 81:589–597.
- Benowicz, A., R. D. Guy, and Y. A. El-Kassaby. 2000. Geographic pattern of genetic variation in photosynthetic capacity and growth in two hardwood species from British Columbia. *Oecologia* 123:168–174.
- Bond, W. J. 1984. Fire survival of Cape Proteaceae: influence of fire season and seed predators. *Vegetatio* 56:65–74.
- Byars, S. G., W. Papst, and A. A. Hoffmann. 2007. Local adaptation and cogradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradient. *Evolution* 61:2925–2941.
- Carlson, J. E., and K. E. Holsinger. 2010. Natural selection on inflorescence color polymorphisms in wild *Protea* populations: the role of pollinators, seed predators and inter-trait correlations. *Am. J. Bot.* 97:934–944.
- Clausen, J., D. D. Keck and W. M. Hiesey. 1940. Experimental studies on the nature of species. I. Effects of varied environments on western North America plants. Carnegie Inst. Washington. Publ. 520:1–452.
- Clay, K., and J. A. Quinn. 1978. Density of stomata and their responses to a moisture gradient in *Danthonia sericea* populations from dry and wet habitats. *Bull. Torrey Bot. Club* 105:45–49.
- Comes, H. P., A. Tribsch, and C. Bittkau. 2008. Plant speciation in continental island floras as exemplified by *Nigella* in the Aegean Archipelago. *Proc. R. Soc. Lond. B* 363:3083–3096.
- Cowling, R. M., D. M. Richardson, and P. J. Mustart. 1997. Fynbos. Pp. 99–130 in R. M. Cowling, D. M. Richardson, and S. M. Pierce, eds. *Vegetation of Southern Africa*. Cambridge Univ. Press, Cambridge.
- Cunningham, S. A., B. Summerhayes, and M. Westoby. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecology* 69:569–588.
- Dai, A., K. E. Trenberth, and T. R. Karl. 1999. Effects of clouds, soil moisture, precipitation, and water vapor on diurnal temperature range. *J. Clim.* 12:2451–2473.
- Dobzhansky, T. 1947. Adaptive changes induced by natural selection in wild populations of *Drosophila*. *Evolution* 1:1–16.
- Dudley, S. A. 1996. Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102.
- Dunlap, J. M., and R. F. Stettler. 2001. Variation in leaf epidermal and stomatal traits of *Populus trichocarpa* from two transects across the Washington Cascades. *Can. J. Bot.* 79:528.
- Ellis, A. G., and A. E. Weis. 2006. Coexistence and differentiation of ‘flowering stones’: the role of local adaptation to soil microenvironment. *J. Ecol.* 94:322–335.
- Ellis, A. G., A. E. Weis, and B. S. Gaut. 2006. Evolutionary radiation of “stone plants” in the genus *Argyroderna* (Aizoaceae): unraveling the effects of landscape, habitat, and flowering time. *Evolution* 60:39–55.
- Endler, J. A. 1986. *Natural selection in the wild*. Princeton Univ. Press, Princeton, NJ.
- Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. 1. Clinal patterns of selection along an environmental gradient in the great plains. *Evolution* 58:1446–1458.
- Fonseca, C. R., J. M. Overton, B. Collins, and M. Westoby. 2000. Shifts in trait-combinations along rainfall and Phosphorus gradients. *J. Ecol.* 88:964–977.
- 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.
- Galston, A. W., P. J. Davies, and R. L. Satter. 1980. *The life of the green plant*. Prentice Hall, NJ.
- Geber, M. A., and L. R. Griffen. 2003. Inheritance and selection on plant functional traits. *Int. J. Plant Sci.* 164(Suppl. 3):S21–S42.
- Gelman, A., and D. B. Rubin. 1995. Avoiding model selection in Bayesian social research. Discussion of “Bayesian model selection in social research”. *Sociol. Methodol.* 25:165–173.
- Gelman, A., J. B. Carlin, H. S. Stern, and D. B. Rubin. 2004. *Bayesian data analysis*, 2nd ed. Chapman and Hall, Boca Raton, FL. pp. 696.
- Givnish, T. J. 1987. Comparative studies of leaf form—assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytol.* 106:131–160.
- Goldblatt, P., and J. C. Manning. 2000. Cape plants: a conspectus of the vascular plants of the Cape Region of South Africa. *Strelitzia* 7. National Botanical Institute of South Africa, Cape Town.
- Goldblatt, P., V. Savolainen, O. Porteous, I. 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. *Mol. Phylogenet. Evol.* 25:341–360.
- Grime, J. P. 1977. Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111:1169–1194.
- Gurevitch, J. 1992. Differences in photosynthetic rate in populations of *Achillea lanulosa* from two altitudes. *Funct. Ecol.* 6:568–574.
- Hallik, L., U. Niinemets, and I. J. Wright. 2009. Are species shade and drought tolerance reflected in leaf-level structural and functional differentiation in Northern Hemisphere temperate woody flora? *New Phytol.* 184:257–274.
- Hodges, S. 1997. A rapid adaptive radiation via a key innovation in *Aquilegia*. Pp. 391–405 in T. Givnish and K. Sytsma, eds. *Molecular evolution and adaptive radiations*. Cambridge Univ. Press, Cambridge.
- Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. *Evolution* 52:1564–1571.
- Jonas, C. S., and M. A. Geber. 1999. Variation among populations of *Clarkia unguiculata* (Onagraceae) along altitudinal and latitudinal gradients. *Am. J. Bot.* 86:333–343.
- Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Lett.* 7:1225–1241.
- 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 Phytol.* 160:337–347.
- Lamont, B. B., P. K. Groom, and R. M. Cowling. 2002. High leaf mass per area of related species assemblages may reflect low rainfall and Carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. *Funct. Ecol.* 16:403–412.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Latimer, A. M. 2006. Environmental and geographical controls on species distributions: a case study on proteas of the Cape Floristic Region. Pp. 259. Department of Ecology and Environmental Biology. Univ. of Connecticut, Storrs, CT.
- Latimer, A. M., S. Wu, A. E. Gelfand, and J. A. Silander. 2006. Building statistical models to analyze species distributions. *Ecol. Appl.* 16:33–50.
- Latimer, A. M., J. A. Silander, A. G. Rebelo, and G. Midgley. 2009. Experimental biogeography: the role of environmental gradients in high geographic diversity in Cape Proteaceae. *Oecologia* 160:151–162.

- Latta, R. G., and C. McCain. 2009. Path analysis of natural selection via survival and fecundity across contrasting environments in *Avena barbata*. *J. Evol. Biol.* 22:2458–2469.
- Li, C., F. Berninger, J. Koskela, and E. Sonninen. 2000. Drought responses of *Eucalyptus microtheca* provenances depend on seasonality of rainfall in their place of origin. *Aust. J. Plant Physiol.* 27:231–238.
- Linder, H. P. 2003. The radiation of the Cape flora, southern Africa. *Biol. Rev.* 78:597–638.
- Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.* 27:237–277.
- Littell, R., G. Milliken, W. Stroup, R. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models. SAS Publishing, Cary, NC.
- Maron, J. L., S. C. Elmendorf, and M. Vila. 2007. Contrasting plant physiological adaptation to climate in the native and introduced range of *Hypericum perforatum*. *Evolution* 61:1912–1924.
- Martinez-Cabrera, H. I., C. S. Jones, S. Espino, and H. J. Schenk. 2009. Wood anatomy and wood density in shrubs: responses to varying aridity along transcontinental transects. *Am. J. Bot.* 96:1388–1398.
- Moeller, D. A., and M. A. Geber. 2005. Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution* 59:786–799.
- Niinemets, U. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82:453–469.
- Oleksyn, J., J. Modrzyński, M. G. Tjoelker, R. Zytowskiak, P. B. Reich, and P. Karolewski. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. *Funct. Ecol.* 12:573–590.
- Ordoñez, J. C., P. M. van Bodegom, J. P. M. Witte, I. J. Wright, P. B. Reich, and R. Aerts. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Glob. Ecol. Biogeogr.* 18:137–149.
- Pääkkönen, E., M. S. Günthardt-Goerg, and T. Holopainen. 1998. Responses of leaf processes in a sensitive birch (*Betula pendula* Roth) clone to ozone combined with drought. *Ann. Bot.* 82:49–59.
- Partridge, T. C. 1997. Evolution of landscapes. Pp. 1–20 in R. M. Cowling, D. M. Richardson, and S. M. Pierce, eds. *Vegetation of Southern Africa*. Cambridge Univ. Press, Cambridge.
- Preston, K. A., and D. D. Ackerly. 2003. Hydraulic architecture and the evolution of shoot allometry in contrasting climates. *Am. J. Bot.* 90:1502–1512.
- Prunier, R. E., and K. E. Holsinger. 2010. Was it an explosion? Using population genetics to explore the dynamics of a recent radiation within *Protea* (Proteaceae L.) *Mol. Ecol.* 19:3968–3980.
- Ramirez-Valiente, J. A., Z. Lorenzo, A. Soto, F. Valladares, L. Gil, and I. Aranda. 2009. Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance. *Mol. Ecol.* 18:3803–3815.
- Rauscher, M. D. 1992. The measurement of selection on quantitative traits—biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626.
- Rebello, A. G. 2001. *Proteas: a field guide to the proteas of southern Africa*. Fernwood Press, Vlaeberg, SA.
- . 2006. *Protea atlas project*. <http://protea.worldonline.co.za/default.htm>. Accessed 17 August 2010.
- Rebello, A. G., and J. P. Rourke. 1986. Seed germination and seed set in southern African Proteaceae: ecological determinants and horticultural problems. *Acta Hort.* 185:75–88.
- Rebello, A. G., W. R. Siegfried, and A. A. Crowe. 1984. Avian pollinators and the pollination syndromes of selected mountain fynbos. *S. Afr. J. Bot.* 3:285–296.
- Reich, P. B., I. J. Wright, J. Cavender-Bares, J. M. Craine, J. Oleksyn, M. Westoby, and M. B. Walters. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *Int. J. Plant Sci.* 164:S143–S164.
- Richardson, D. M., and F. J. Kruger. 1990. Water relations and photosynthetic characteristics of selected trees and shrubs of riparian and hillslope habitats in the south-western Cape Province, South Africa. *S. Afr. J. Bot.* 56:214–225.
- Schemske, D. W., and H. D. Bradshaw, Jr. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proc. Natl. Acad. Sci. USA* 96:11910–11915.
- Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford Univ. Press, Oxford.
- . 2009. Evidence for ecological speciation and its alternative. *Science* 323:737–741.
- Schulze, R. E. 1997. *South African atlas of agrohydrology and climatology*. Technical Report. Report TT82/96. Water Resource Commission, Pretoria, South Africa.
- . 2007. *South African atlas of climatology and agrohydrology*: WRC Report 1489/1/06. Water Research Commission, Pretoria, RSA.
- Spiegelhalter, D. J., G. B. Nicola, P. C. Bradley, and L. van der Angelika. 2002. Bayesian measures of model complexity and fit. *J. R. Stat. Soc. B Stat. Meth.* 64:583–639.
- Spigler, R. B., and S.-M. Chang. 2008. Effects of plant abundance on reproductive success in the biennial *Sabatia angularis* (Gentianaceae): spatial scale matters. *J. Ecol.* 96:323–333.
- Stanton, M. L., B. A. Roy, and D. A. Thiede. 2000. Evolution in stressful environments. I. Phenotypic variability, phenotypic selection, and response to selection in five distinct environmental stresses. *Evolution* 54:93–111.
- Stenström, A., I. J. Jónsdóttir, and M. Augner. 2002. Genetic and environmental effects on morphology in clonal sedges in the eurasian arctic. *Am. J. Bot.* 89:1410–1421.
- Stock, W. D., N. Allsopp, F. Van Der Heyden, and E. T. F. Witkowski. 1997. Plant form and function. Pp. 376–396 in R. M. Cowling, D. M. Richardson, and S. M. Pierce, eds. *Vegetation of Southern Africa*. Cambridge Univ. Press, Cambridge.
- Thorpe, R. S., J. T. Reardon, and A. Malhotra. 2005. Common garden and natural selection experiments support ecotypic differentiation in the Dominican Anole (*Anolis oculatus*). *Am. Nat.* 165:495–504.
- Thuiller, W., S. Lavorel, G. Midgley, S. Lavergne, and T. Rebelo. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 *Leucadendron* taxa. *Ecology* 85:1688–1699.
- Valente, L. M., G. Reeves, J. Schnitzler, I. P. Mason, M. F. Fay, T. G. Rebelo, M. W. Chase, and T. G. Barraclough. 2010. Diversification in the African genus *Protea* (Proteaceae) in the cape biodiversity hotspot and beyond: equal rates in different biomes. *Evolution* 64:745–760.
- Van Wilgen, B. W. 1982. Some effects of post-fire age on the above-ground plant biomass of fynbos (macchia) vegetation in South Africa. *J. Ecol.* 70:217–225.
- Verboom, G. A., H. P. Linder, and W. D. Stock. 2004. Testing the adaptive nature of radiation: growth form and life history divergence in the African grass genus *Ehrharta* (Poaceae: Ehrhartoideae). *Am. J. Bot.* 91:1364–1370.
- Woodward, F. I. 1986. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia* 70:580–586.
- Wright, I. J., and M. Westoby. 1999. Differences in seedling growth behaviour among species: trait correlations and shifts along nutrient compared to rainfall gradients. *J. Ecol.* 87:85–97.

- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.
- Wu, C. A., D. B. Lowry, L. I. Nutter, and J. H. Willis. 2010. Natural variation for drought-response traits in the *Mimulus guttatus* species complex. *Oecologia* 162:23–33.
- Yates, M. J., G. A. Verboom, A. G. Rebelo, and M. D. Cramer. 2010. Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. *Funct. Ecol.* 24:485–492.
- Yu, H., X. Chen, Y. Hong, Y. Wang, P. Xu, S. Ke, H. Liu, J. Zhu, D. J. Oliver, and C. Xiang. 2008. Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. *Plant Cell* 20:1134–1151.

Associate Editor: P. Tiffin

Supporting Information

The following supporting information is available for this article:

Figure S1. Drawings of significant correlations between environmental clines and traits of wild adults, seeds, and seedlings.

Table S1. Sampled species and populations, sample sizes for adults in the wild and common garden seedlings (J = Jonaskop garden, K = Kirstenbosch garden), locality name, latitude, longitude, rainfall, and elevation.

Table S2. Regression coefficients from multiple regressions on traits of plants measured in the wild and in the Kirstenbosch and Jonaskop common gardens.

Appendix S1. Description of the suite of GIS climate layers from the South African Atlas of Climatology and Agrohydrology (Schulze 2007) used to create the climate axes PPTCON, DRYPCA and COLDPCA.

Appendix S2. Details of the single hierarchical Bayesian model that we used to estimate local selection gradients and their relationships to the four environmental covariates.

Appendix S3. Methods and equations for calculating standardised selection gradients and differentials within each garden, including details on the method adapted from Janzen and Stern (1998) to convert logistic regression coefficients to selection gradients.

Supporting Information may be found in the online version of this article.

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