

**NATURAL SELECTION ON INFLORESCENCE COLOR
POLYMORPHISMS IN WILD *PROTEA* POPULATIONS: THE
ROLE OF POLLINATORS, SEED PREDATORS, AND INTERTRAIT
CORRELATIONS¹**

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- *Premise of the study:* The processes maintaining flower color polymorphisms have long been of evolutionary interest. Mechanistic explanations include selection through pollinators, antagonists, local environments, drift, and pleiotropic effects. We examined the maintenance of inflorescence color polymorphisms in the genus *Protea* (Proteaceae) of South Africa, in which ~40% of species contain different color morphs.
- *Methods:* We studied 10 populations of four bird-pollinated *Protea* species and compared adult performance, floral and leaf morphology, vegetative pigmentation, germination, and seedling survival between co-occurring pink and white morphs. We also tested for differences in pollination success and pre-dispersal seed predation.
- *Key results:* White morphs produced seeds 10% heavier and 3.5 times more likely to germinate, which all else being equal, should fuel positive selection on white. In one studied population per species, however, white morphs were more susceptible to seed predation by endophagous larvae. Pollinators had no morph-specific effects on female fecundity, as measured by amount or probability of seed set. Differences in stem color indicated that white morphs produced smaller quantities of pigment and associated compounds throughout, possibly explaining their higher seed palatability.
- *Conclusions:* Our findings suggest a mechanism for some white protea polymorphisms: deleterious pleiotropic effects on pink morphs are occasionally offset by reduced losses to seed-eating larvae. Because trends were repeated across species, we suggest that similar processes may also occur in other proteas, placing a new emphasis on seed predators for influencing some of South Africa's amazing floral diversity.

Key words: anthocyanin; Cape Floristic Region; South Africa; color polymorphism; flower color; natural selection; pre-dispersal seed predation; Proteaceae; white protea.

Color polymorphisms within plant species are a striking component of many natural landscapes, inspiring decades of research on the ecological and evolutionary processes maintaining them (e.g., Wright, 1943; Mogford, 1978; Fineblum and Rausher, 1997; Schemske and Bierzychudek, 2007). The persistent co-occurrence of different color morphs has been attributed to a variety of evolutionary processes, ranging from direct or indirect selection mediated by pollinators, herbivores, or environmental effects, to random genetic drift. In a well-known example, Wright (1943, 1978) speculated that the persistent flower color polymorphism in *Linanthus parryae* was largely

due to genetic drift, in support of his shifting balance theory of evolution; however, subsequent work has shown that color differences are likely maintained by strong selection along environmental gradients (Epling et al., 1960; Schemske and Bierzychudek, 2001, 2007).

The adaptive maintenance of flower color polymorphisms is most frequently attributed to pollinators, in part because pollinators tend to promote assortative mating—i.e., they preferentially visit certain phenotypes (Kay, 1978; Waser and Price, 1981; Stanton, 1987; Jones and Reithel, 2001). When preferences for flower color vary among individuals, pollinator guilds, or through time, assortative mating can maintain distinct color morphs side by side in a population as well as in allopatry (Chittka et al., 2001; Geegar and Laverly, 2001; Jones, 2001; Fenster et al., 2004). When preferences are constant, however, additional selection pressures may be involved, including non-pollinating visitors or deleterious pleiotropisms of alleles affecting flower color. In fact, almost no studies show that pollinators are the sole evolutionary force maintaining flower color polymorphisms (Rausher, 2008).

Plant consumers and pathogens have increasingly been recognized as important agents of selection on flower color polymorphisms, usually through indirect selection on pleiotropically related traits (Linhart, 1991; Strauss and Whittall, 2007). The idea that herbivores respond to flower color and the chemicals associated with pigmentation is not new. As early as 1876, Darwin suggested that certain floral pigments were distasteful to insects, based on observations that blue morphs had fewer nectar-robbing holes than white morphs in *Aconitum napellus*

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(p. 428). Since then, numerous studies have shown that selection through enemies and pleiotropic effects can contribute to the maintenance of polymorphisms (Simms and Bucher, 1996; Irwin et al., 2003; Frey, 2004; Irwin and Strauss, 2005). Other evolutionary mechanisms for flower color polymorphisms exist and likely interact with those mentioned, though they have fewer supporting examples. These additional mechanisms include negative frequency-dependent selection (Gigord et al., 2001), variable selection resulting from heterogeneous environments (Schemske and Bierzychudek, 2001, 2007), heterozygote advantage, and as mentioned, neutral mutation and drift (Wright, 1943; Falconer, 1989).

Flower color polymorphisms are common worldwide (Kay, 1978; Rafinski, 1979; Waser and Price, 1981; Warren and Mackenzie, 2001). In fact, they are a conspicuous component of one of the world's most diverse and endemic floras, in the Cape region of South Africa. In and around the Kogelberg Biosphere Reserve (~5000 km² adjacent to Cape Town area), nearly a quarter of the 500 most common angiosperms are polymorphic for flower color (excluding grasses and grass-like species; data extracted from Bean and Johns, 2005). The most common color polymorphism is pink to white, occurring in at least 70 species in this small region. Within the iconic genus *Protea* [Proteaceae], ~40% of all species exhibit variation in their showy involucral bracts, ranging from green or cream to increasingly bright pink or crimson (data from Rebelo, 2001). Similarly, flower color polymorphisms are documented for ~38% of species in the genus *Erica* [Ericaceae] in the southwestern Cape (Rebelo and Siegfried, 1985). Other polymorphic Cape taxa include geraniums, irises, amaryllids, asters, beetle daisies, and orchids (Johnson, 1994; Bean and Johns, 2005; Ellis and Johnson, 2009).

Flower color polymorphisms are also frequent in other floras and clades. In the British flora, Warren and Mackenzie (2001) estimated that 20% of species are polymorphic for flower color (217 of 1032 species). For the Polemoniaceae, the frequency is ~40% (Schemske and Bierzychudek, 2007). In these estimates as in those from South Africa, however, it is often unclear whether morphs are sympatric or allopatric, and some observations may be of transient mutant individuals rather than persistent polymorphisms. Despite inexact counts, many of the distinct color morphs in the Cape Flora are well recognized, widespread, persistent, and even horticulturally important (Vogts, 1982; Rebelo, 2001; Bean and Johns, 2005; Coetzee and Littlejohn, 2007). Even so, almost no attempts have been made to explain why polymorphisms are common in certain Cape lineages (but see Rebelo and Siegfried, 1985) or how they are maintained evolutionarily.

In this paper, we explore mechanisms associated with maintenance of one of the most conspicuous flower color polymorphisms in the Cape Floristic Region, that in the genus *Protea*. We focus on a monophyletic clade of six species, known as the white proteas (*Protea* sect. *Exsertae*; Valente et al., 2010). Using four polymorphic species, we seek to understand the role of intertrait correlations (i.e., pleiotropic effects), pollinators, and seed predators in the maintenance of both pink and white color morphs within populations. To this end, we measured plant traits, seed production, and seed predation rates in 10 populations, and we compared offspring in a common garden. With these data, we addressed the following questions:

(1) Does inflorescence color covary with pigmentation in adult stems or leaves, or with pigmentation in leaves of offspring? Does it covary with any functional leaf or floral

traits, with adult performance, seed characteristics, or seedling survival?

(2) Do plants with pink inflorescences differ from those with white inflorescences in the level of pollinator limitation on seed set, as measured by the proportion of seed heads that set seeds or the total number of seeds produced?

(3) Do plants with pink inflorescences differ from those with white inflorescences in rates of seed predation, as measured by the number of seed heads attacked or proportion of seeds consumed per seed head?

MATERIALS AND METHODS

Study species—We investigated the maintenance of color polymorphism in white proteas species *Protea aurea* (Burm.f.) Rourke subsp. *aurea*, *P. lacticolor* Salisb., *P. punctata* Meisn., and *P. subvestita* N.E.Br. The white proteas are endemic to the Cape Floristic Region of South Africa, except for *P. subvestita*, which extends farther east and north in southern Africa (Rebelo, 2001). All studied species are large, upright shrubs that occur on semiarid mountain slopes up to 2300 m a.s.l. (Rebelo, 2001). They have nonoverlapping distributions that are partially determined by climate gradients such as rainfall, temperature, and elevation (Latimer et al., 2009). Most *Protea* species, including all white proteas, produce broad leaves that are sclerophyllous, long-lived, and low in nutrients (Coetzee et al., 1997; Rebelo, 2001).

The reproductive biology and pollinators of white proteas are relatively unstudied. Individuals take at least 3 years to reach reproductive maturity and then flower annually between January and June, with significant overlap in flowering among species (Rebelo, 2001). Like all other *Proteas*, white protea inflorescences consist of many florets, each with a single ovule and reduced perianth, aggregated on a woody receptacle, and surrounded by showy bracts (Rebelo, 2001). The colors of the bracts, perianths, and styles can vary from white to crimson pink. In some *Protea* species, hybrids, and other Proteaceae, bract and perianth color variation are known to have a genetic component (Vogts, 1982; Bickford and Sedgley, 1994; Coetzee and Littlejohn, 2007), and for many species of Australian Proteaceae, the pigments responsible are anthocyanins (Gascoigne et al., 1948; Bickford and Sedgley, 1994), as is also likely for *Proteas*. *Protea* florets are protandrous, with pollen attached to the sides of a modified style upon opening and maximum stigma receptivity occurring 2+ days later (Collins and Rebelo, 1987; Van der Walt and Littlejohn, 1996). Some *Protea* species are self-compatible (e.g., *P. roupelliae*, Hargreaves et al., 2004; *P. repens* and *P. nerifolia*, Coetzee and Littlejohn, 2007), but data on three white protea species indicate that self-pollen does not produce viable seed in this group (Horn, 1962).

The main pollinators of white proteas are almost certainly sugarbirds and sunbirds, as for other closely related *Protea* species (Vogts, 1982; Rebelo, 2001; Hargreaves et al., 2004). In the Cape Floristic Region, avian visitors to white proteas include cape sugarbirds, orange-breasted sunbirds, malachite sunbirds, and greater and lesser double-collared sunbirds (Rebelo et al., 1984; de Swardt, 2008; J. Carlson, personal observation). For *Protea subvestita*, visits are made by Gurney's sugarbird, and Malachite and other sunbirds (de Swardt, 2008; J. Carlson, personal observation). Beetles and other insects also frequent inflorescences, but are likely ineffective or less effective pollinators of white proteas, as has been shown for some closely related *Protea* species (Wright et al., 1991; Wright, 1994a; Hargreaves et al., 2004). Bird-pollinated *Proteas* generally require at least one visit for substantial seed set, indicating there is little to no autonomous selfing in the group (Horn, 1962; Coetzee and Giliomee, 1985; Hargreaves et al., 2004).

Seed set in white proteas tends to be low, as is characteristic of the genus and much of the family (Collins and Rebelo, 1987). The average ratio of fertile seeds to florets is 9.2% for the genus *Protea* (Collins and Rebelo, 1987). Unsuccessful florets produce empty, brittle achenes, whereas successful florets produce plump, firm, seed-containing achenes, which we henceforth call seeds. White proteas retain seeds on the plant in serotinous infructescences, henceforth seed heads, usually until the whole plant is killed by fire (occurring historically every ~12 to 30 years; van Wilgen, 1982). Thus, current total lifetime seed production depends on stand age or time since last fire, and can be counted directly on plants. Once *Protea* seeds are released, the vast majority germinate within 2 months into the next rainy season (May–July in Western Cape; Bond, 1985; Rebelo and Rourke, 1986). Ungerminated seeds may experience heavy post-dispersal predation, and there appears to be little to no long-term underground storage in these species (Bond, 1984, 1985).

Protea seed heads harbor a diversity of seed-eating insects (Wright and Samways, 1999; Giliomee, 2003). Endophagous insects have been extensively surveyed for *P. repens* and a few other *Protea* species (Coetzee and Giliomee, 1987a, b; Wright and Samways, 1999; Roets et al., 2006). Among white proteas, known pre-dispersal seed predators include beetle larvae of *Genuchus hottentotus* (Scarabaeidae), *Sphenoptera* spp. (Buprestidae), and *Euderus* spp. (Curculionidae), and stem-boring lepidopteran larvae of the genera *Argyroloce* (Olethreutidae), *Tinea* (Tineidae), and *Syanthodon* (Sesiidae; Coetzee and Giliomee, 1987b; Wright and Samways, 1999). Infestation by most known seed predators of white proteas usually occurs after seeds are partially or entirely developed, i.e., 2+ months postfertilization (Coetzee and Giliomee, 1987a, b; J. Carlson, personal observation). Larvae can consume large portions of a population's potential seed output, e.g., up to 84% in 2 years for *P. repens* (Coetzee and Giliomee, 1987b), but the rates of seed predation are unknown for most *Protea* species.

Sampling design—Adult performance and vegetative traits—We chose study populations after a field survey of all six white protea species in 2008. During the survey, we visited roughly five populations per species, which consisted of our four focal species, plus *P. mundii* and *P. venusta*. We visually assessed populations for relative frequencies of plants with white vs. pink inflorescences, based predominantly on bract color, but also on perianth and style-tip color. The five *P. venusta* populations were deemed monomorphic and were therefore excluded; all observed individuals had bicolored bracts with pink tips and white bases. Of the 25 remaining populations, we found only four more that were monomorphic for inflorescence color, each with exclusively white morphs. Just over half of the polymorphic populations, i.e., 11 of 21, were dominated by white-flowered individuals (i.e., over two-thirds of the population had white inflorescences), seven had roughly equal frequencies of pink and white morphs, and only three populations were pink dominant (represented by only *P. punctata*). *Protea mundii* had almost no pink morphs in four of five populations, excluding it from further study. From the remaining polymorphic populations, 10 were chosen for study, which we list in Table 1.

In each of the 10 populations, we sampled 10–40 plants along transects through the approximate population center (mean $N = 28$ plants). We attempted to sample plants alternating by color along transects, but sample sizes were occasionally limited by plant abundance and pink morph frequency. In most populations, we observed but did not include intermediate phenotypes, e.g., plants with very light pink bracts or white bracts with pink perianths. From each plant, we collected the following data: total height, number of branching events, number of seed heads, and the area, length, width, and mass of a representative leaf. We used the leaf data to calculate leaf length to width ratio and specific leaf area (SLA; leaf area divided by leaf mass). In one population per species, that is, populations RP, PO, SP, and SA, we also recorded two indices of vegetative tissue pigmentation. First, we recorded stem color from a representative young shoot, categorized as without red, up to half red, and mostly red. Second, we recorded the amount of red pigment around the edges of fully expanded leaves on that shoot, categorized as without red, or with some red. None of the plants measured in population SA had any leaf edge pigmentation, so we excluded it from this analysis. In a subset of plants from each of the 10 populations (mean $N = 21$ plants), we collected 2–5 fresh inflorescences per plant and measured total inflorescence length, style length, and diameter of the inflorescence base.

Seed production and seed predation—We measured seed production and seed predation rates in the same subset of plants used for inflorescence dimension. From each plant, we collected 10 seedheads that were between 2 and 5 years old, and we dried them until opening (~48 h at 50°C). We checked for any signs of larval damage or infestation, including frass, chewed interior of the woody receptacle, damaged seeds, or actual larva presence. Whenever possible, we collected voucher larvae for identification. On five seedheads per plant, we counted floret receptacles—each corresponding with a single floret—and plump, intact seeds. We also measured mean seed mass per seed head on each plant, excluding damaged seed heads to prevent any confounding effects of infestation (i.e., minor, undetected damage to individual seeds).

Germination and seedling survival—We germinated wild-collected seeds from one population per species (RP, PO, SP, and SA) in a greenhouse at Kirstenbosch Botanical Garden in May 2008. Fifteen plump, undamaged seeds per maternal plant were sown in pots of sandy soil, and seeds were watered three times a week for 9 weeks, by which time most viable seeds should have germinated (Rebello and Rourke, 1986). For each maternal plant, we recorded the mean number of days until germination and the proportion of seeds

germinated after the 9-week trial. In July 2008, we transplanted seedlings into each of two experimental gardens, one on Jonaskop mountain (944 m a.s.l.; mean annual rainfall ~350 mm), and one at Kirstenbosch Botanical Gardens (175 m a.s.l.; mean annual rainfall ~1130 mm). At 6 months, we recorded the redness of a representative new leaf (approximate percentage of leaf surface colored red). We used only young, similarly aged leaves to quantify leaf redness, because many older seedling leaves had lost red pigmentation. We did not record the stem redness because nearly all stems were red. One year after seeds germinated, we recorded seedling survival. Population SA was excluded from analysis of garden data due to low germination.

Statistical analyses—Intraplant correlates of inflorescence color—We compared morphology, performance, seed characteristics, and offspring success of pink vs. white morphs using a series of linear and generalized linear mixed models. Fixed effects were inflorescence color, species, population nested in species, and the population \times color interaction. We included populations and species as fixed effects because we were peripherally interested in whether they differed in focal traits and principally interested in whether differences between color morphs varied among populations, perhaps in relation to characteristics of the site (e.g., elevation or dominant morph color). The random effects of plant nested in population were included where appropriate. All analyses were performed using Proc MIXED or Proc GLIMMIX in the program SAS v. 9.1.3 (SAS Institute, Cary, North Carolina, USA). Significance tests were performed using Kenward–Roger approximations for degrees of freedom (SAS Institute, 2005).

We compared pink and white morphs while accounting for intertrait correlations using MANOVAs on three sets of response variables. These were fresh inflorescence dimensions (style length and inflorescence length and diameter), leaf morphology (SLA, leaf area, and length to width ratio), and adult performance (seed heads per plant, height, and mean growth rate between semiannual branching events). The following response variables were log transformed to improve normality of residuals: SLA, leaf area, seed heads per plant, height, and growth rate. The MANOVA on adult performance included the random effect of stand age class to account for among-population differences in lifetime opportunity for growth and reproduction. Age classes were young (6–8 yr), intermediate (9–17), and old (18–25; see also Table 1). For any MANOVAs that were significant, we performed protected ANOVAs on individual response variables. We expected correlations among a few other variables, but differences in sample sizes and underlying data set distributions precluded the use of MANOVAs. Hence, we tested each separately but also tested the strength of correlations. We included population and species as fixed effects in correlation tests where appropriate.

We used individual ANOVAs to compare pink and white morphs for four continuous and four categorical variables. Continuous responses were florets per seed head, mean seed mass per seed head (uninfested seed heads only), mean number of days till germination, and seedling leaf color. Of these, the first required a log transformation to meet assumptions of normality. Categorical variables were stem color, leaf edge color, probability of germination, and probability of seedling survival. Species effects were excluded from these four analyses, because only one population per species was measured (also true for the latter two continuous variables). To analyze the three classes of stem color, we used a cumulative logit model, which is appropriate for ordered categorical responses of three or more categories (SAS Institute, 2005). Leaf edge color, probability of germination, and seedling survival were modeled as binary responses. For both germination models, source plant was a random effect. Whenever we detected a significant color by population interaction, we used linear contrasts to determine how morphs differed within populations. For ANOVAs on seedling leaf pigmentation and seedling survival, we tested both common gardens simultaneously, with garden and garden by color interaction as additional fixed effects.

Seed set: Pollination and predation—We tested for differential responses of plant visitors to morph colors using separate generalized linear mixed models for each of three responses. The first and second models aimed to detect strong binary responses of pollinators and seed predators, respectively. In the first model, we tested whether pollinator limitation on seed set differed between pink and white morphs using the probability of setting seed in uninfested seed heads. We did so under the assumption that morphs disfavored by pollinators are more likely to be pollinator limited, i.e., less likely to be visited once per inflorescence, and thereby fail to set seed more often than preferred morphs. To allow for the possibility of some autogamy, we tested cut-off values to 3% seed set (autogamous seed set in *P. roupelliae*; Hargreaves et al., 2004), but results did not differ between the chosen (0 vs. ≥ 1) and alternative pollinator limitation models. We reran the same model on the all-seed head data set with infestation

TABLE 1. White protea species and populations sampled for this study. All populations were in the Western Cape province of South Africa, except for SA and RN, which were in Kwa-Zulu Natal. Veld age class was determined from the time since the most recent fire to the sampling dates, based on fire data for the Western Cape from Cape Nature. For SA and RN, veld age was estimated on-site. Young is 6–8 yr, intermediate is 9–17, and old 18–35. The dominant morph color was also estimated on-site, typically over multiple visits. In this category, the pink (P) or white (W) morph color is dominant if it comprises >2/3 of the population. W≈P means neither color dominates.

Species	Population	Location	Dates visited (month/year)	Coordinates (S, E)	Elev. (m)	Ann. rainfall (mm)	Veld age class	Dominant morph color
<i>Protea aurea</i>	MP	Marloth Park, near Koloniesbos	3/08, 6/08, 4/09	–33.9963, 20.45651	239	882	young	W
	PV	Protea Valley, Marloth Park	8/08, 4/09	–33.9502, 20.37055	1062	439	intermediate	W≈P
	RP	Robinsons Pass, Outeniqua Reserve	3/08, 5/09	–33.9087, 22.02348	544	723	old	W≈P
<i>Protea lacticolor</i>	PK	Pofaddersnek, Hottentots Holland Res.	2/08, 4/09	–34.0188, 19.08011	836	1260	intermediate	W≈P
	PO	Purgatory outspan, Hottentots Holland Res.	3/08, 4/09	–33.9676, 19.14433	435	926	intermediate	W
<i>Protea punctata</i>	GB	Gydoberg vicinity, near Ceres	2/08, 5/09	–33.254, 19.48446	1363	394	old	W≈P
	JK	Jonaskop, near Villiersdorp	3/09, 3/09	–33.9695, 19.5017	1396	323	young	W
	SP	Swartberg Pass, Swartberg Reserve	3/08, 3/09	–33.3626, 22.06647	1208	932	intermediate	P
<i>Protea subvestita</i>	SA	Sani Pass, Southern Drakensberg	4/08, 3/09	–29.6093, 29.36219	1851	1063	intermediate	W≈P
	RN	Royal Natal Park, Northern Drakensberg	4/08, 3/09	–28.6852, 28.91599	1833	969	young	W

status as a covariate, to determine whether patterns could be detected in infested seed heads as well. In the second model, we tested whether seed predators preferentially infested seed heads of white vs. pink morphs, using the probability of infestation. This analysis used a full data set including both infested and uninfested seed heads. For both models, we included plant nested in population as a random effect, and as for the intraplant analyses, fixed effects were population nested in species, inflorescence color, their interaction, and species.

Our third analysis aimed to detect more subtle effects of enemies and pollinators. The response variable was percentage seed set, or the fraction of florets setting intact, fertile seeds. The model was similar to the seed set model above except that the response variable had a binomial distribution rather than a Bernoulli distribution, and it had the additional predictors of infestation status (yes/no) and its two- and three-way interactions. We focused primarily on these interactions to determine whether seed predators consumed more seeds of one morph, regardless of infestation probability. If the rate of seed consumption differs between morphs, the more palatable morph should have fewer seeds in infested seed heads relative to uninfested seed heads from the same or a similar plant. We assessed significance of this effect using preplanned comparisons of differences in least squared means (seed set in uninfested minus infested seed heads) between pink and white morphs within populations, and the associated odds ratios and 95 or 99.9% confidence intervals.

In the event that white proteas experienced pollinator limitation on female fecundity beyond the first seed, we sought additional evidence for pollinator preferences using the same model as above. If one color morph consistently receives more visits, it may set a higher proportion of seeds in uninfested seed heads. We used tests of simple effects to compare seed set in uninfested seed heads of pink vs. white morphs. These results must be interpreted with caution, however, because they assume that morphs are equivalent in resource status as seed set increases. The analysis using probability of setting seed should be less confounded, as it assumes resource base equivalence only up to the first seed. We excluded from associated analyses all populations with extreme rates of infestation or failure to set seed (≤ 5 or $>95\%$ of seed heads), because in all cases, data for at least one color morph was based on ≤ 2 seed heads. These populations were JK for all three analyses, PK for the first and RN for the second and third.

RESULTS

Intraplant correlates of inflorescence color—Although there were significant trait differences among populations and

species, pink and white morphs were indistinguishable in all adult characteristics measured, except those related to pigmentation (Table 2). Inflorescence color was not related to any of the following characters: inflorescence dimensions, florets per seed head, leaf morphology, leaf edge color, adult growth, height, or seed head production. Adult plants with pink inflorescences were, however, seven times more likely to produce higher quantities of red pigment in nonwoody stem tissue than plants with white inflorescences (odds ratio, pink : white = 6.9; 95% CI = 1.7–29).

We detected significant differences in seed mass and viability between pink and white morphs. White morphs produced slightly but significantly heavier seeds than pink morphs (in the absence of insect damage), and their seeds were more likely to germinate in a subset of sampled populations (Table 2, Fig. 1A, B). The odds of germination were 18 times greater for white vs. pink morphs of *P. subvestita* SA, and roughly three and four times greater for white vs. pink of *P. punctata* SP and *P. lacticolor* PO, respectively (overall odds ratio for pink : white = 0.3; 95% CI = 0.1–0.9; Fig. 1A). We detected a significant population effect on seed mass but not germination probability, and for neither was the color by population interaction significant (Table 2, Fig. 1B). Seed mass also differed among species, based on post hoc comparisons with Tukey–Kramer adjustments. *Protea subvestita* had the lightest seeds at 10.9 ± 0.3 (53) mg [raw mean \pm SE (*N*)], followed by *P. punctata* at 16.1 ± 0.3 (158) mg, and finally *P. aurea* and *P. lacticolor*, each with similarly heavy seeds at 29.9 ± 0.6 (113) and 29.4 ± 0.9 (42) mg, respectively.

Offspring of pink vs. white morphs did not differ in survival probability or leaf pigmentation (Table 2). Seedlings had fairly high mortality in both gardens, although losses in the Kirstenbosch garden were significantly greater. There were also no detectable differences between morphs in the amount of red pigment on seedling leaves, although sample sizes were limited by mortality during the preceding 6 months.

Jonaskop seedlings had redder leaves in general, and there was a marginally significant effect of source population on leaf pigmentation (Table 2).

Some traits of seeds and of inflorescences could not be tested with MANOVAs but were nevertheless intercorrelated. Plants with broader inflorescence bases produced more florets per inflorescence ($F_{1,192} = 14.7, P = 0.0002$), but there were no detectable relationships between seed mass and either trait ($P > 0.1$ for both). The probability of germination increased with seed mass, and this was not dependent on the source population (seed mass: $F_{1,14} = 6.03, P = 0.028$, pop: $F_{3,14} = 0.87, P = 0.47$, seed mass \times pop: $F_{3,14} = 1.17, P = 0.35$). Neither seed mass nor germination probability was detectably associated with days to germination, and again population and interaction effects were nonsignificant ($P > 0.15$ for all effects). On adult plants, we detected no relationship between the degree of leaf pigmentation and pigmentation of stems ($P > 0.35$).

Seed set: Pollination—All studied white protea populations showed some evidence of pollinator limitation, i.e., at least one nonaborted, uninfested seed head failed to set any seed (Table 3). Not all populations and species were limited to the same degree, however (population: $F_{1,128} = 4.14, P = 0.004$; species: $F_{3,134} = 10.6, P < 0.0001$). *Protea subvestita* RN was by far the most pollinator limited with only 18% of measured seed heads containing seed, whereas in populations *P. punctata* JK and *P. laticolor* PK, we encountered only one uninfested, empty seed head apiece. Pollinator limitation could be unambiguously detected only in uninfested seed heads, because in infested seed heads, larvae occasionally consumed all seeds per seed head. Infested seed heads were four times more likely to be empty than uninfested seed heads, based on the all-seed head data set (infestation status: $F_{1,544} = 27.9, P < 0.0001$; 95% CI on odds ratio: 2–7).

Pink and white morphs did not differ significantly in the probability of setting seed in uninfested seed heads, suggesting pollinator limitation was similar for both morphs (color: $F_{1,150} = 0.28, P = 0.60$). The apparent absence of strong pollinator preferences was consistent across populations (interaction: $F_{7,127} = 0.20, P = 0.99$). Including infested seed heads in the data set did not change these results. All main and interaction effects that involved morph color were nonsignificant in the all-seed head data set ($P > 0.48$ for all color-related effects).

The proportion of florets to produce viable seeds averaged 9% for uninfested seed heads of both color morphs, again revealing no differences between morphs (color: $F_{1,100} = 0.0, P = 0.96$, pop \times color: $F_{15,102} = 1.47, P = 0.13$). There were slight differences among populations and species (Table 3; pop: $F_{1,103} = 2.6, P = 0.02$; species: $F_{3,99} = 2.9, P = 0.04$), but individual species differences could not be identified in post hoc tests.

Seed set: Predation—Larval infestation rates of white proteas were generally high, with over one third of seed heads infested in most populations (Fig. 2A). The only two populations in which larvae were rare were *P. subvestita* RN and *P. punctata* JK, with 0.5% and 5% infestation rates respectively. The majority of seed predators identified from white protea seed heads were lepidopteran larvae in the genus *Synanthedon* (Sesiidae). We also encountered some beetle larvae in the genus *Sphenoptera* (Buprestidae). Most larvae could not be kept or reared to adulthood, making it likely that some additional species in seed heads went unrecognized.

Pink and white morphs were equally likely to contain larvae, both within and across populations (Fig. 2A; color: $F_{1,107} = 0.12, P = 0.73$; pop \times color $F_{7,107} = 0.67, P = 0.69$), but there were significant differences in infestation probability among species (species: $F_{3,107} = 13.1, P < 0.0001$; population: $F_{4,107} = 2.18, P = 0.08$). *Protea punctata* and *P. subvestita* had significantly lower infestation rates than did *P. aurea* and *P. laticolor* (Tukey–Kramer adjusted $P < 0.0008$). Excluded populations JK and RN also conformed to these species-level differences.

Once entering the seed head, larvae inflicted significantly less damage in pink vs. white inflorescences (Fig. 2B, color: $F_{1,100} = 0.40, P = 0.53$; infestation \times color: $F_{1,645} = 10.8, P = 0.0011$). Uninfested seed heads of pink morphs had three times the seed set of those infested by larvae, whereas uninfested seed heads of white morphs had five times the seed set (95% CI on odds ratios: pink = 2.5–4.1; white = 4.4–6.4). Only half of the studied populations showed significant differences in susceptibility, however (infestation \times color \times pop: $F_{7,645} = 4.4, P < 0.0001$). The greatest morph-specific differences were observed in populations *P. punctata* SP and *P. subvestita* SA, in which white inflorescences lost three to four times more of their potential seed set than did pink inflorescences (Fig. 2B; $P < 0.0001$ for both comparisons). This equated to at least one additional percentage point of seed set lost to larvae. Populations *P. aurea* RP and *P. laticolor* PK lost approximately 1.5 times more from white vs. pink morphs ($P < 0.05$ for both). For populations MP, PV, PO, and GB, there were no significant differences in amount of seeds lost to predation (Fig. 2).

DISCUSSION

Our results reveal a plausible mechanism by which inflorescence color polymorphisms are maintained in at least some white protea populations. First, white inflorescences are favored over pink through indirect selection on seed mass and germinability, due to either pleiotropic effects or genetic linkage. Second, positive selection on higher quality seeds in white inflorescences is occasionally countered by negative selection on the same morphs, due to increased losses to predispersal seed predators. Third, these polymorphisms are apparently maintained without the involvement of pollinators, at least in terms of pollinator effects on female fecundity. Many recent articles suggest that pollinators are not the sole biotic agents of selection on flower color polymorphisms (Strauss and Whittall, 2007; Rausher, 2008), yet only a few studies provide strong evidence that enemies are involved (e.g., Irwin et al., 2003; Frey, 2004). This study demonstrates that white morphs occasionally suffer greater losses to larvae across four species and that selection through enemies is heterogeneous among populations. It also reveals trait correlations between inflorescence color, stem color, and seed quality that are generally consistent across populations and species.

Consequences of pigment production for plants—*Whole plant pigmentation*—Pink and white morphs of studied proteas did not differ in most morphological aspects; however, the correlation between stem and inflorescence color may hint at important physiological differences between morphs. In many plant species, vegetative pigmentation increases with floral pigmentation, and the same pigment compounds are often present in both tissues (Schoen et al., 1984; Strauss and Whittall, 2007).

TABLE 2. Trait values and statistical comparisons of pink and white color morphs for adult traits, seed traits, seedling traits, adult performance and seedling survival in four species of white proteas in South Africa. Means, standard errors (SE), and percentages are on raw data. For sample sizes, *N* is the total number of plants measured, except for germination analyses, where *N* is the total number of seeds. The following variables were log transformed: florets per seedhead, specific leaf area (SLA), leaf area, seedheads, growth rate, and height. Plant nested in population was a random effect in the first three analyses and in germination analyses. In all models, species effects were significant ($P < 0.0001$) and interactions were nonsignificant ($P > 0.15$); those *P*-values are not reported below. NS = $P > 0.12$.

Plant traits	Inflorescence color raw mean \pm SE (<i>N</i>) or raw % (<i>N</i>) for ^a			Inflorescence color			Population		
	Pink	White	Pops.	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Inflorescence dimensions (mm) ^b				3, 2182	0.61	NS	18, 2182	8.95	<0.0001
Total length	68.9 \pm 1.4 (104)	67.8 \pm 1.3 (109)	10						
Style length	80.0 \pm 1.3 (104)	79.0 \pm 1.3 (107)	10						
Base diameter	25.3 \pm 0.1 (104)	25.5 \pm 0.1 (109)	10						
Florets per seed head	117 \pm 2.5 (104)	116 \pm 2.2 (109)	10	1, 194	0.18	NS	6, 194	4.90	<0.0001
Mean seed mass (mg)	20.1 \pm 0.66 (94)	22.0 \pm 0.67 (99)	10	1, 123	5.24	0.02	6, 120	8.31	<0.0001
Adult performance ^b				3, 899	0.89	NS	27, 899	31.9	<0.0001
Seed heads	43.9 \pm 3.1 (137)	41.6 \pm 2.9 (136)	10						
Growth rate (m/yr)	0.24 \pm 0.01 (137)	0.24 \pm 0.01 (136)	10						
Height (m)	2.3 \pm 0.06 (137)	2.3 \pm 0.06 (136)	10						
Leaf morphology ^b				3, 288	1.14	NS	27, 288	16.2	<0.0001
SLA (cm ² /g)	43.8 \pm 1.0 (116)	45.4 \pm 1.0 (117)	10						
Leaf area (cm ²)	15.9 \pm 0.5 (116)	16.9 \pm 0.5 (117)	10						
Length: width	2.9 \pm 0.07 (116)	3.0 \pm 0.06 (117)	10						
Leaf color (% of plants with) ^a				1, 46	1.24	NS	2, 46	1.64	NS
No red on leaf edge	46.4% (28)	54.2% (24)	3						
Up to half red leaf edge	53.6% (28)	45.8% (24)	3						
Stem color (% of plants with) ^a	—	—	—	1, 55	7.34	0.009	3, 55	8.01	0.0002
No red on stem	30.3% (33)	67.9% (28)	4						
Up to half red	33.3% (33)	25.0% (28)	4						
Mostly red	36.4% (33)	7.1% (28)	4						
Days to germination	36.9 \pm 1.9 (72)	35.0 \pm 1.3 (107)	4	1, 18	0.85	NS	3, 18	0.44	NS
Germination rate (%) ^a	34.2% (212)	52.3% (191)	4	1, 21	4.94	0.04	3, 20	2.15	NS
Seedling survival (% alive) ^a				1, 84	0.02	NS	2, 84	2.97	0.06
Kirstenbosch	27.8% (18)	20.7% (29)	3						
Jonaskop	52.9% (17)	67.9% (28)	3						
Seedling leaf color (prop. red) ^c				1, 46	0.40	NS	2, 46	2.97	0.06
Kirstenbosch	0.38 \pm 7.7 (10)	0.41 \pm 6.2 (14)	3						
Jonaskop	0.60 \pm 9.1 (11)	0.70 \pm 7.3 (19)	3						

^a Response variables were categorical (see Materials and Methods for details). For stem color, one population had no white morphs with red on the stems, precluding a test of two-way interactions.

^b Results of MANOVAs on correlated response variables. ANOVAs on single responses were also nonsignificant.

^c The effect of garden was significant ($P < 0.0001$), but the interaction of inflorescence color and garden was not. Population SA was excluded from both gardens because almost no pink morph seedlings survived to be planted.

As such, inflorescence color may be just one manifestation of higher quantities of pigments—most likely anthocyanins—and their byproducts throughout the entire plant (Warren and Mackenzie, 2001; Armbruster, 2002). Below, we discuss the physiological functions of anthocyanins and some possible reasons for tissue-specific expression.

Anthocyanins in floral and vegetative tissues are associated with a broad range of effects, ranging from pollinator attraction to plant protection (Koes et al., 1994). In vegetative tissues, they function predominately as light screens to prevent photo-inhibition (Steyn et al., 2002), but they are also associated directly or pleiotropically with other benefits (see below). Because blocking light may reduce photosynthesis, anthocyanin expression is often early and transient in essential light-capturing tissues like leaves, but is more permanent in floral tissues and stems (Steyn et al., 2002). Correspondingly, we observed extensive but fading pigmentation on leaves of seedlings, almost none on mature leaves of adults, and strong pigmentation on seedling and adult stems. Furthermore, leaves produced in the higher elevation garden had 20–30% more of their surfaces pig-

mented than those in the lower elevation garden. Together, these observations suggest that leaf pigmentation in white proteas is tightly controlled and responsive to external stresses, and that morph-specific differences in whole-plant pigmentation still likely exist.

Plants that produce more pigment overall may have a greater capacity to respond to and endure stressful conditions. The production of anthocyanin and associated compounds often increases with cold, drought, UV radiation, and attack by enemies (Koes et al., 1994; Warren and Mackenzie, 2001; Steyn et al., 2002). In some cases, these correlations result from a shared biochemical pathway between pigment and protective or defensive compounds—e.g., the flavonoid pathway for anthocyanin also produces compounds with known antiherbivore functions, such as polyphenols or tannins (Fineblum and Rausher, 1997; Rausher, 2008). If the flavonoid pathway is blocked by inactivated genes, as may be the case for pigment-less morphs (Coberly and Rausher, 2003), plants may not only produce white flowers, but also may lack useful associated compounds through pleiotropic effects.

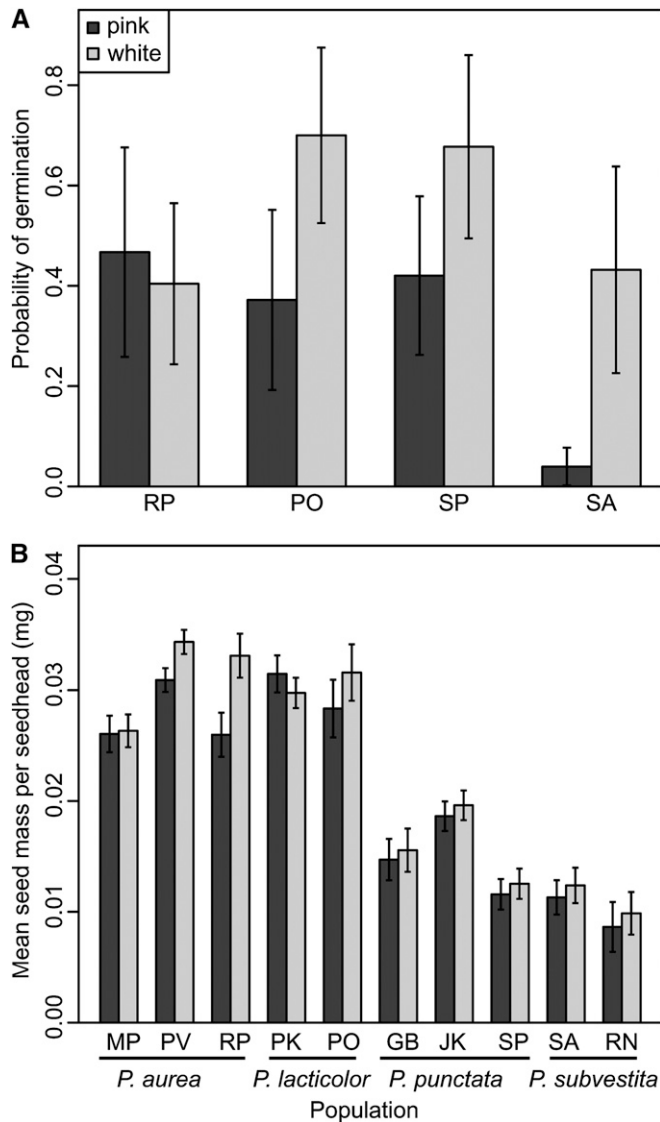


Fig. 1. (A) Probability of germination and (B) mean seed mass in uninfested seed heads for white proteas with pink vs. white inflorescences. White morphs had heavier seeds that were more likely to germinate in most tested populations, and seed mass varied among populations (see Results for statistical tests). Means are least squares \pm 1 SE.

An association between flower color and survival or performance under environmental stressors has been documented in many polymorphic taxa (Burdon et al., 1983; Schemske and Bierzychudek, 2001; Warren and Mackenzie, 2001; Coberly and Rausher, 2003). Morph color was not associated with the survival of 1-year-old white protea seedlings in either of our common gardens, but our ongoing study may reveal differences as seedlings mature and reproduce. Across all 25 white protea populations, there was a weak tendency for pink morphs to be most abundant at colder, higher altitude sites and white morphs to dominate at milder, lower sites (J. Carlson, unpublished data). We did not detect any association between dominant morph color and rainfall or drought stress, however. In either case, broader sampling and experimentation are needed to clarify potential trends. In ours, as for most other studies, it remains unclear whether anthocyanins and associated compounds are

themselves the targets of selection or whether they are indirectly favored through pleiotropic effects.

Seed mass and germination—Plants with white inflorescences produced slightly heavier seeds than those with pink inflorescences in nine of the 10 populations. Furthermore, seeds from white morphs were 3.5 times more likely to germinate on average, possibly driven by the positive correlation between seed mass and germination probability. These differences have clear reproductive implications. First, all else being equal, pink morphs should contribute fewer offspring to the next generation due to their lower probability of germination. Second, lighter seeds of pink morphs may have a competitive disadvantage, given the well-supported relationships between seed mass and seedling size, competitive ability, and survival through hazards (reviewed in Westoby et al., 2002). Existing data on white proteas and other *Protea* species support these predictions, although our limited survival data do not. Smaller seeds make smaller seedlings across many *Protea* species (Stock et al., 1990; J. Carlson, unpublished data; R. Prunier, K. Holsinger, and J. Carlson, University of Connecticut, unpublished manuscript), and based on a larger data set than that used here, smaller white protea seedlings have higher mortality during their first year of life (J. Carlson, K. Holsinger, and R. Prunier, University of Connecticut, unpublished manuscript).

The association between flower color and seed quality is probably due to pleiotropic effects in white proteas, as has been suggested for other polymorphic taxa. It is true, however, that the production of anthocyanins or other flavonoids can be costly (Steyn et al., 2002; Warren and Mackenzie, 2001), and pink morphs may have fewer total resources with which to provision seeds. Costs of anthocyanin production are not apparent in other polymorphic taxa, however, given that pigmented morphs often have higher output than white morphs (but see Gomez, 2000). For example, relative to white morphs, pink morphs of *Phlox drummondii* have higher survivorship, flower production, and seed set (Levin and Brack, 1995), and blue morphs of *Hydrophyllum appendiculatum* have higher inflorescence and seed production, but similar seed mass (Wolfe, 1993).

In sum, our findings indicate that in most populations, natural selection favors plants with white inflorescences, albeit weakly, through small differences in seed mass and germinability. That pink morphs remain present and sometimes even dominate suggests that additional selection pressures may be acting on inflorescence color, either related to heterogeneous environmental conditions, as mentioned, and/or biotic interactions.

Consequences of pigment production for pollinators—Failure to set seed reduced total output in all four white protea species, but losses were equal between morphs; that is, pollinators did not discriminate in ways detectable through seed set. In all but three populations, at least 20% of inflorescence failed to set seed, independent of color. Pollinator limitation is the most likely cause of empty seed heads in both morphs, relative to other possible mechanisms, such as insufficient resources or seed predation (Collins and Rebelo, 1987). Predispersal seed predation can reduce percent seed set to zero in white proteas (present study) and is also significant in other species (Coetzee and Gilimee, 1987b). Because we accounted for damaged seed heads in the analyses, predation cannot explain the differences in seed output that we observe. Resource limitation is also an unlikely cause of empty seed heads or low percentage seed set, based on various

TABLE 3. Probability of seed set and percentage seed set in 10 white protea populations in South Africa. Data are based on uninfested seed heads only to control for effects of seed predation. Population means are raw \pm 1 SE. Pink and white morphs did not differ for probability of seed set or percentage seed set, and hence only population averages are provided. Populations JK and PK were excluded from analysis of percentage of seedheads to set seed because reliable estimates could not be obtained for both color morphs. The same was true for JK and RN in analysis of percentage seed set (each had \leq 5% infestation).

Species	Population	Percentage of seed heads to set seed (\geq 1 fertile seed)	Percentage seed set in seed heads (number of fertile seeds/floret receptacles)	N (uninfested seed heads)
<i>Protea aurea</i>	MP	77.1 \pm 6.1	11.4 \pm 1.4	48
	PV	90.0 \pm 5.6	14.3 \pm 1.5	30
	RP	61.5 \pm 9.7	5.1 \pm 1.1	26
<i>Protea laticolor</i>	PK	97.0 \pm 3.0*	11.5 \pm 1.1	33
	PO	66.7 \pm 12.6	4.8 \pm 1.7	15
<i>Protea punctata</i>	GB	77.4 \pm 7.6	6.8 \pm 1.2	31
	JK	98.7 \pm 1.3*	14.6 \pm 0.9*	79
	SP	77.8 \pm 4.9	5.8 \pm 0.7	72
<i>Protea subvestita</i>	SA	54.5 \pm 6.2	11.0 \pm 1.6	66
	RN	18.3 \pm 3.8	2.6 \pm 0.7*	104

* Excluded from associated analysis (see Materials and Methods)

lines of inferential and experimental evidence (Rebelo and Rourke, 1986; Mustart and Cowling, 1992). For example, controlled hand pollination on *Protea repens* and *P. eximia* indicate that seed set is limited by stages preceding pollen tube formation (Van der Walt and Littlejohn, 1996). Furthermore, *P. roupelliae* inflorescences that received just one natural visit set 1–11% of florets, whereas those that were open-pollinated set a median of 30%, a likely result of more visits (Hargreaves et al., 2004). Although some studies suggest pollen can limit *Protea* seed set, hand pollinations and pollen supplementation experiments provide equivocal results (Collins and Rebelo, 1987; Hargreaves et al., 2004; Coetzee and Littlejohn, 2007). The proximate causes of low seed set have received attention in some *Protea* taxa; however, explanations for its chronic occurrence throughout the genus and family require more experimental investigation.

Our estimates of pollinator limitation and seed set suggest that pollinators are not centrally involved in maintaining the color polymorphism in white proteas. This differs from the many studies that identify pollinators as important selection pressures favoring color morphs differentially across space, time, or pollinating species (Mogford, 1978; Stanton, 1987; Johnson, 1994; Gomez, 2000; Gigord et al., 2001; Jones and Reithel, 2001; Irwin and Strauss, 2005; Eckhart et al., 2006). At this stage, it is unclear whether pollinators should even be expected to respond to *Protea* color polymorphisms, especially in the absence of data on *Protea* nectar production. Sugarbirds and sunbirds prefer rewards of greater energetic value in some situations (Gill and Wolf, 1977; Downs and Perrin, 1996; Jackson et al., 1998), but it is unknown, and perhaps unlikely, that they otherwise have innate color preferences (Rebelo and Siegfried, 1985).

Our evidence against pollinator-mediated selection is based on equivalent seed set, yet paternal output of each morph remains unmeasured. Sexual selection theory predicts that pollinator-mediated selection on secondary floral traits is more likely to act through male function (reviewed in Willson, 1994; Skogsmyr and Lankinen, 2002), yet we lack estimates of male contribution in white proteas. Even if visit rates are similar, the timing or duration of visits may differ between morphs, which could affect male more than female output (e.g., Thomson, 1986; Jones and Reithel, 2001). Upcoming studies in white proteas will address whether pollinators preferentially visit and thereby potentially disperse more pollen of one color morph over the other.

Consequences of pigment production for plant antagonists—Our study provides compelling data that predispersal seed predation pressures are weaker on pink vs. white color morphs of white proteas. White morphs lost between 1.5 and 4 times more seed set than pink morphs in half of studied populations, whereas in the remainder, there were no morph-specific differences. It is perhaps unsurprising that pigmented flowers had less damage, given the interrelatedness of pigment, stress tolerance, and defense compounds discussed earlier. Even so, the morph-specific effects of seed predators in this study were subtle: we only observed differences in the potential output (i.e., uninfested minus infested), but not in the realized output of each morph. If fitness costs are indirect or similarly subtle in other plant species, enemy-mediated selection on color may be difficult to detect in general.

Unpigmented morphs of polymorphic species are demonstrated to be more attractive to enemies in only a few other studies. White and yellow morphs were preferred by slugs and caterpillars over anthocyanin-producing pink and bronze morphs of *Raphanus sativus*, although thrips and aphids had no preference (Irwin et al., 2003). A study on commercial *Petunia* flowers showed that two lepidopteran larvae species preferred uncolored tissue of multicolored flowers (Johnson et al., 2008). In both studies, most herbivores performed worse when fed on the anthocyanin-containing tissue, and for *Raphanus sativus*, this was linked to higher production of induced indole glucosinolates in pigmented morphs (Strauss et al., 2004). In contrast, research on *Ipomea purpurea* revealed either no evidence of increased susceptibility to enemies for white morphs (Fineblum and Rausher, 1997) or evidence that white morphs were less susceptible than pigmented morphs (Simms and Bucher, 1996). Similarly, Frey (2004) showed that slugs were less damaging to unpigmented morphs, although fungal pathogens sometimes countered these effects in *Claytonia virginica*. In many of these studies as in ours, the effects of enemies varied among sites or year, and thus, directional selection on preferred morphs was occasionally absent or reversed.

The absence of differential susceptibility in some populations suggests that seed predation pressures are not constant through space, which matches the heterogeneous environment (or variable selection) model for the maintenance of polymorphisms (Falconer, 1989). This model has some support from other color polymorphism studies as well (e.g., Schemske and

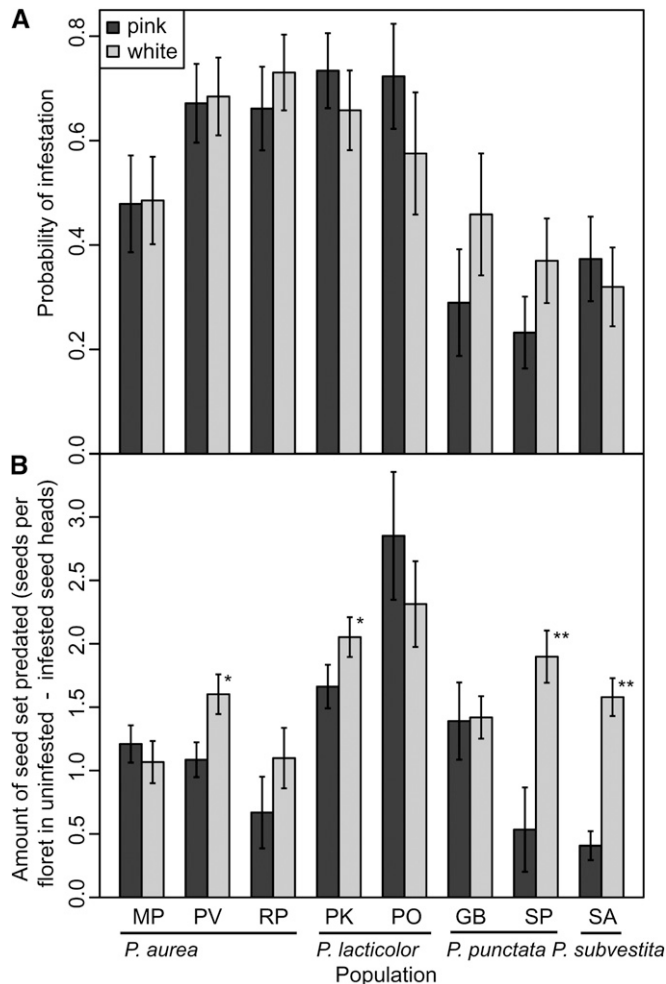


Fig. 2. Differences between white proteas with pink vs. white inflorescences in terms of (A) the probability of larval infestation and (B) the proportion of floret receptacles with fertile intact seed in uninfested seed heads minus those from infested seed heads. Color morphs did not differ in their rate of infestation, but in half of sampled populations, they differed in the amount of seeds lost to larvae (see Results for statistical tests). Means are least squares \pm 1 SE. * $P < 0.05$, ** $P < 0.001$.

Bierzychudek, 2001, 2007). In a study of fruit color polymorphisms in *Acacia ligulata*, Whitney and Stanton (2004) demonstrated that neither morph had highest reproductive output in all sites, that damage by heteropteran seed predators was also spatially variable, and that seed predators preferentially damaged yellow over red fruits in only 1 of 2 years. As in our study, Whitney and Stanton were unable to explain why selection mediated by enemies varied across sites and years. It has been suggested that plant genotype \times environment interactions can cause insect preferences to switch (i.e., the most palatable plants changes with local conditions; Cronin et al., 2001), and this may also be true for the white proteas. Next, we consider a related explanation for differential predator susceptibility among white proteas.

Endophagous insect communities in *Proteas* vary among sites and through time, likely in response to characteristics of the site and the host species (Wright and Samways, 1999; Roets et al., 2006). Because these insects must recolonize *Protea* stands following fire, their population dynamics are strongly

linked to veld age as well as to the previous fire's extent and the isolation and size of the plant population. Furthermore, insect communities in *Protea* seed heads vary across sites in relation to climate (Wright and Samways, 1999). In our study, veld ages ranged from 7 to 25 yr, and altitude and other climate predictors varied greatly across sites (see Table 1; J. Carlson, K. Holsinger, and R. Prunier, University of Connecticut, unpublished manuscript), suggesting that many of our white protea populations likely housed distinct larval communities. If some larval communities did not include species that responded to pigment, this could explain why only half of the *Protea* populations had morph-specific seed predation. There are no obvious environmental similarities among populations in which we detected higher white morph susceptibility, in terms of elevation and annual rainfall (Table 1); however, inferences are limited by a relatively small population sample size. Further research on seed predator communities, their individual feeding preferences, and their postfire colonization rates across a wide range of environments would be of particular value in this regard. These data would also serve in efforts to maximize *Protea* seed bank productivity, which is of interest to horticulturalists, fire managers, and conservationists.

The variable selection model involving seed predation is relevant to all four white protea species and likely applies to other *Protea* species as well. Color polymorphisms are present in roughly half of the bird-pollinated *Proteas*, including most of the bearded proteas (e.g., *P. neriifolia*, *P. laurifolia*, *P. magnifica*, *P. lorifolia*), the king protea *P. cyanaroides*, and the widespread *P. repens* (Vogts, 1982; Rebelo, 2001). Furthermore, the larva taxa detected in white proteas are damaging to many of the same bird-pollinated *Protea* species (Coetzee and Giliomee, 1987a, b; Wright and Samways, 1999). Because our study revealed trends that were not specific to a single species or environment, we suggest that pre-dispersal seed predators may have similar effects on polymorphisms throughout the *Protea* genus or even more broadly in the family Proteaceae. South Africa's amazingly high plant endemism and diversity have been explained through spatial variation in abiotic factors, and less frequently, selection mediated by pollinators (Johnson, 1996). Our results indicate that seed predators may also contribute to the region's floral diversity, at least in terms of *Protea* inflorescence color and associated characters, as well as to the diversity in reproductive strategies (e.g., Wright, 1994b). On a global scale, these findings add to a growing body of evidence that enemies are a major, though often overlooked, force in floral evolution.

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