

PHENOTYPIC SELECTION ON *DALECHAMPIA* BLOSSOMS: HONEST SIGNALING AFFECTS POLLINATION SUCCESS

W. SCOTT ARMBRUSTER,^{1,2,3,4} LIV ANTONSEN,² AND CHRISTOPHE PÉLABON²

¹*School of Biological Sciences, University of Portsmouth, Portsmouth PO1 2DY UK*

²*Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway*

³*Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775-7000 USA*

Abstract. Pollinators may choose which flowers to visit by direct assessment of rewards or by indirect assessment of “honest” advertisements or other traits correlated with the quantity or quality of reward. We wished to know whether selection generated by pollinators acted directly or indirectly on floral rewards, on advertisement traits, and/or traits affecting pollinator efficiency (their fit with flowers) in *Dalechampia* vines (Euphorbiaceae) and whether the advertisement (bract size) was correlated honestly with reward amount (measured by resin-gland area). In Gabon we studied bee visitation and pollen arrival rates to blossoms of *D. ipomoeifolia* Benth. and found that, despite the apparent visibility of the resin reward (and its volume), the strongest bee-mediated natural selection acted directly on bract size rather than gland area. Blossoms with larger bracts were visited more often by the only pollinators, female *Heriades* nr. *spiniscutis* (Apoidea: Megachilidae), and these blossoms received more pollen on their stigmas. Blossoms with larger resin glands were also visited more often and received more pollen overall, but this effect disappeared when bract size (which was phenotypically correlated with gland size) was controlled for statistically. These observational data were confirmed by experimental reduction of bract size, which significantly decreased pollen arrival rates. Thus, the bees appear to rely on the “honest” correlation between advertisement and reward in choosing the best blossoms to visit, and this behavior generates direct selection for larger bracts and indirect selection for larger resin glands. Bees visiting blossoms with larger separation between the gland and stigmas contacted the stigmas less frequently, and such blossoms received less pollen on their stigmas. Because gland area, bract size, and gland–stigma separation are positively correlated phenotypically, response to selection for larger bracts may be limited in this population by conflicting selection against large gland–stigma separation.

Key words: *Dalechampia ipomoeifolia*; evolution; floral advertisement; genetic correlation; *Heriades*; honest signaling; indirect selection; phenotypic selection; pollination.

INTRODUCTION

Pollinators choose among flowers by direct perception and assessment of reward quality and quantity, by assessment of traits correlated with rewards, and by their past experience of the means, variances, and covariances of reward quality, quantity, and related floral traits (e.g., Heinrich and Raven 1972, Real 1981, Ott et al. 1985, Harder and Real 1987, Cartar 1991, Shafir et al. 1999, 2003). In turn, these choices generate many of the selective pressures influencing the evolution of floral traits. However, the interplay between pollinator choice, the natural selection it generates, and the nature, amount, and scale of floral variation remain poorly understood (Fenster et al. 2004).

Phenotypic selection analysis (Lande and Arnold 1983) has been used a number of times to investigate the selective pressures that pollinators impose on flowers (e.g., Campbell 1989, Galen 1989, Campbell et al.

1991, Stanton et al. 1991, Melendez-Ackerman and Campbell 1998, Caruso 2002, Totland 2004). Despite a well-developed theoretical background (Lande and Arnold 1983, Phillips and Arnold 1989), there have been fewer attempts to understand how response to selection may be influenced by phenotypic and genetic correlations among floral traits. Nevertheless, interest in selection on correlated traits has grown sharply in the past decade (e.g., Dorn and Mitchell-Olds 1991, Campbell et al. 1994, 1996, Meagher 1994, Stanton and Young 1994, Andersson 1996, 1997, O’Connell and Johnston 1998, Worley and Barrett 2000, Cariveau et al. 2004, Caruso 2004).

Reward quantities often vary dramatically within and among closely related plant species, even though reward type may remain very similar (Fenster et al. 2004). When rewards are perceived directly, pollinators may choose among flowers by assessing the presence and amount of reward (e.g., Thorp et al. 1975, Armbruster and Webster 1982, Armbruster and Herzig 1984, Cresswell and Robertson 1994, Dobson et al. 1996, Dobson and Bergstrom 2000). When rewards

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⁴ E-mail: scott.armbruster@port.ac.uk

cannot be directly perceived by pollinators, however, floral visitors must select flowers on the basis of traits that are correlated with reward amounts (e.g., Cresswell and Galen 1991). The commonness of flowers with hidden rewards and the prevalence of costly advertisements (e.g., Galen et al. 1999, Galen 2000) in plants with readily perceived rewards (e.g., most pollen-reward flowers) suggest that advertisements are usually important in floral choice, even when rewards are not hidden (see Chittka and Thomson 2001). The “honesty” of the reward–advertisement correlation may influence the evolutionary dynamics of the system (Schaefer et al. 2004) much as does honest signaling in animal mate choice and sexual selection (see Grafen 1990, Johnstone 1995, Maynard-Smith and Harper 2003). Lack of a correlation (e.g., Cresswell and Robertson 1994) or even “dishonest” advertisement may lead to the evolution of food deception and mimicry pollination systems (e.g., Dafni 1984, Ackerman 1986, Roy and Widmer 1999, Smithson and Gigord 2002, Thakar et al. 2003) and may increase selection for pollinators to assess rewards directly (see Cresswell and Robertson 1994, Dobson et al. 1996, Dobson and Bergstrom 2000). A strong reward–advertisement correlation (“honest” signaling) allows evolutionary and/or behavioral shifts towards choosing flowers on the basis of easily perceived advertisements (Cresswell and Galen 1991).

Previous studies have suggested that pollen-arrival rates are determined by the product of the rate of pollinator attraction (total visitation rate) and the pollinator efficiency (per-visit contact with anthers and stigmas or pollen pickup and deposition; e.g., Armbruster 1988; see reviews in Armbruster et al. 2000, Fenster et al. 2004), although this has rarely been measured empirically (but see Campbell et al. 1994, Engel and Irwin 2003). In this study, therefore, we used *Dalechampia* vines (Euphorbiaceae) to examine the direct and indirect effects of natural variation in reward quantity (resin-gland area), advertisement (bract size), and pollinator–flower fit (gland–stigma distance) on pollinator visitation rate, frequency of pollinator contact with stigmas, and the female component of allogamous-pollination success (i.e., number of pollen grains from other blossoms arriving on stigmas; note this includes geitonogamous [within-plant] and xenogamous [between-plant] pollen movement). We tested the results of our study of the effects of natural phenotypic variation by analyzing the effect of experimentally induced variation in bract size on pollen arrival rates.

The evolutionary response to selection on floral traits may be affected by conflicting selection on and genetic correlations between floral traits. Pollinators may often choose to visit and hence select for larger flowers and larger amounts of reward. But there may also be conflicting selective pressure on flower size driven by pollinator fit and pollination efficiency. For example, when small bees are the main pollinators of a *Dalechampia*

species, there may be directional selection for a decrease in the distance between the stigma, where pollen needs to be deposited, and the resin gland, where bees obtain the resource they are seeking (see Armbruster 1990). Thus, concurrent selection for an increase in bract size and reward amount that is driven by pollinator choice may be restricted or promoted by positive genetic correlations between gland–stigma distance, bract size, and reward amount (Lande and Arnold 1983, Hansen et al. 2003a, Armbruster et al. 2004). In other situations, such as when very large bees are common, there may be selection for increases in the size of all blossom traits. Thus, in some cases genetic correlations between floral traits may limit response to directional selection, and in others they may promote response to selection (Armbruster 1990, 1991, Armbruster and Schwaegerle 1996, Schluter 1996, Hansen et al. 2003a).

The goals of this study were to assess the interplay between reward amount, advertisement size, visitation, and pollen arrival rates on stigmas and between floral shape and pollination efficiency in one population of *Dalechampia ipomoeifolia* in Africa. Specifically, we wished to determine whether: (1) floral advertisements (large involucre bracts) constitute an honest signal indicating the amount of resin reward present in a blossom; (2) the amount of resin reward and/or size of the involucre bracts influence the choice of blossoms by resin-collecting bees and rate of pollinator visitation; (3) the rate of pollen arrival on the stigma is positively influenced by increasing amounts of resin and/or size of the involucre bracts; (4) increasing distance between the stigmas and resin gland negatively influences pollination efficiency (the frequency with which bees contact stigmas), and hence the rate of pollen arrival; (5) the gland–stigma distance positively covaries with other blossom size traits (e.g., the size of the resin gland and involucre bracts), hence acting in combination with (4) potentially to constrain response to selection on blossom size by bees.

MATERIALS AND METHODS

Study system

Dalechampia (Euphorbiaceae) is a tropical vine bearing self-compatible, unisexual flowers clustered into open, bisexual, blossom inflorescences (pseudanthia), which, in most species, secrete resin from specialized glands to attract pollinators (Webster and Webster 1972, Armbruster 1984, 1993). The resin is collected by several species of megachilid and apid bees for use in nest construction. Previous work on *Dalechampia* has led to the conclusion that the amount of resin (as measured by the size of the resin gland) offered by each blossom determines the type and size of bees that will visit inflorescences, at least at the level of variation among species of plants and species of bees (Armbruster 1984, 1988, Armbruster and Herzig

1984). This follows logically from the expectations of pollination energetics (Heinrich and Raven 1972), wherein it is inferred that large floral visitors cannot energetically afford to visit flowers offering small amounts of reward, whereas smaller visitors, with their lower payload and energy costs, can (Armbruster 1984, 1988, 1991). Plants are also under selection to offer the minimal amount of reward that will still attract pollinators. Excess reward not only wastes energy and nutrients, it may also reduce the number of flowers visited (Heinrich and Raven 1972). Indeed, *Dalechampia* pollinators usually visit numerous blossoms on multiple plants in a single foraging bout, suggesting this balance has been struck.

Floral resin is openly presented and indeed often brightly colored and presumably visible to approaching bees, but it is unclear whether bees choose *Dalechampia* blossoms on the basis of the amount of reward or some advertisement trait(s) correlated with resin quantity. The size of the visiting bees affects the probability of pollen being transferred to the stigmas, with only large bees expected to be effective pollinators of species with large blossoms (and hence large distances between the resin gland and sexual parts [Armbruster 1988, 1990]). It is unclear, however, whether the correlated evolution of various reward and advertisement traits seen at the level of populations and species (e.g., amount of resin, area of resin gland, size of petaloid bracts, gland–stigma distance, and overall blossom size [Armbruster 1988, 1990, 1991, Hansen et al. 2003a, Armbruster et al. 2004]) is the result of correlational selection (Endler 1986, Armbruster and Schwaegerle 1996) or a combination of direct and indirect selection manifested through genetic correlations (Lande and Arnold 1983, Armbruster 1991, 2002, Hansen et al. 2003a, Armbruster et al. 2004).

We studied a population of *Dalechampia ipomoeifolia* Benth. in a large opening in a lightly disturbed, lowland, evergreen forest (~490 m elevation), ~20 km east of Lastoursville, Gabon, from December 1996 to March 1997. The functionally bisexual blossoms comprise three pistillate flowers, ~12–16 staminate flowers, a resin gland, and two large involucre bracts. The blossoms are protogynous and open for pollination for ~10 d. During the first 2–3 d the bracts are open and the stigmas are receptive, but male flowers are still closed (“female phase”). During the subsequent week the pistillate flowers remain receptive and 1–4 male flowers open daily (“bisexual phase”); each male flower sheds pollen for 1–2 d and then drops off.

Pollinator visitation

We recorded insect visits to 60 blossoms discretely labeled by numbering the nearest leaf with black ink (to minimize effect on visitor behavior), on seven clumps of plants (blocks), with 3–19 blossoms (median = 6) observed per block. Each clump in this and the other phenotypic-selection experiments contained one

to several plants and was treated as a block, so that variation among sites and among genetic individuals (plants) could be estimated and controlled statistically. Each labeled blossom was observed for set periods of time between 0.25 and 3.8 h/d (median = 2 h), depending on weather and time available, throughout the study period. We estimated visitation rate from these data (number of visits per hour). We measured a series of morphological traits for each blossom, including the length of the lower bract (“bract length”), the length and width of the resin-bearing surface of the cluster of bractlets forming the resin gland (multiplied to obtain “gland area,” which correlates tightly with the standing crop of resin [Armbruster 1984]), and the minimum distance separating the gland and the stigma (gland–stigma distance or “GSD”).

We examined the effect of GSD on the rate of stigma contact by floral visitors. We recorded whether the visitor touched the stigma on each observed visit to a target (measured) blossom. In order to increase the range of GSD sizes in female blossoms, we also created “artificial” females by removing the open staminate flowers (which might otherwise interfere with bee contact with the stigmas) from bisexual blossoms (GSD increases with age, and bisexual blossoms generally have larger GSDs). This manipulation increased the mean GSD and standard deviation from 2.32 ± 0.48 mm for the unmanipulated blossoms to 2.59 ± 0.69 mm in the full data set.

Effect of natural blossom variation on pollen arrival

Eighty-four unopened blossoms on 11 clumps of plants (blocks) in the same population of *D. ipomoeifolia* were selected haphazardly and discretely labeled by marking leaves, as above. As the bracts opened on each blossom, we counted, using a 10× hand lens, the pollen grains on the stigmas on each day of female phase (before anthers were open). The same blossom traits as in the visitation study were measured on each blossom on each day. By making measurements on all days of the female phase we were able to select the counts and measurements from the final day of the female phase for further analysis, under the assumption that the opening of the first male flower is a good temporal/developmental landmark. We chose the last day of the female phase as the critical observation point, because it captures the maximum amount of pollinator-transported pollen arriving from other blossoms (functionally allogamy; i.e., xenogamy plus geitonogamy) prior to the possibility of “self” (within-blossom) pollination, without the need for emasculation. The number of pollen grains on the stigma is thus a measure of the female component of success in allogamous (inter-blossom) pollination and presumably fitness.

Effect of bract manipulation on pollen arrival

Seventy-three pairs of unopened, nearly adjacent blossoms on the same shoot were chosen from 29

plants. We chose one pair of blossoms on small plants, but up to three pairs on large plants. On the first blossom, prior to its opening, we cut the extreme edge of both upper and lower involucre bracts with a pair of small scissors ("cut long" = control). On the second blossom we cut both involucre bracts so that the bract area was reduced to less than one-third of the original ("cut short"). Pollen loads were counted as described above and also at 3 d after the manipulation. Therefore, one measure (pollen load after 3 d) was made at a constant time interval since the manipulation, while the other measure (pollen load at end of female stage) was made at a constant ontogenetic stage. We report below the results of analyses of both measurements.

Statistical analysis

The analysis of the pollinator data required assessment of heterogeneity and sequential pooling to achieve useful sample sizes. The initial data comprised the visitation rates for each blossom, organized by plant, block (= clump of plants), time of day (period), and date (day). As a result, the data have a repeated-measures structure (multiple observations per blossom, on different days), with morphological variation introduced by blossom growth between days with repeated observations. Thus, the levels of variation in blossom measurement are: (1) blossom size with age (tagged blossoms measured on different days) and (2) blossom nested within block.

After testing for, and rejecting, the existence of significant heterogeneity across periods, all periods from the same day were pooled. This yielded a mean hourly visitation rate per day (sum of all the observation periods for one day divided by the number of hours of observation) for each blossom. Although we had repeated observations of blossom visitation over up to three consecutive days, the design was too unbalanced for us to employ a mixed-effects model that took growth-induced changes in flower size into account. Therefore we used only the first day of observation for each blossom. As a consequence, the variation in size among blossoms was due to variation in both the ontogenetic stage of the blossom and the true ontogeny-independent size. This does not seem to be a serious problem, because variation in blossom size due to ontogenetic stage during the 2–3 d of the female stage is small compared to the total size variation among blossoms, and the ontogenetically induced variance only adds to the size variation whose functional significance we are examining.

We analyzed the effect of variation in gland area and bract length on visitation rate using a mixed-effects model (lme in S-plus; Venables and Ripley 2002) where the block (clump of plants) was entered as a random factor. The effect of gland–stigma distance (GSD) on the probability of stigma contact by bees was analyzed using a generalized linear model (GLM) with binomial error structure. We did not enter block as a variable in

this model, because unlike total visitation rate or pollen arrival, the probability of stigma contact is not likely to be influenced by block factors, and GSD varied as much within blocks (clump) as among. However, this treatment of the data reduced the full independence of the observations, and hypothesis testing and interpretation of marginal *P* values require additional caution.

We subjected the data on pollen arrival and visitation rate to path analysis (Wright 1921, Li 1975) because we were interested in the relationships among the morphological traits as well as their direct and indirect effects on pollen arrival and pollinator visitation. All predictor variables showed nearly normal distributions. The number of pollen grains on the stigma showed a normal distribution after square-root transformation. We first ran an ANCOVA between the pollen load and each predictor variable separately, with plant or clump of plants (block) as a categorical factor, to test whether the plant on which a blossom occurred had any effect on the relationship between the pollen load and the predictor variables. We did not find any significant interactions between the plant (or clump of plants) and the predictor variables (not shown) and concluded that the relationships between the pollen load and each predictor variable were similar among plants and could be analyzed jointly.

We then calculated the Pearson correlations between the different predictor variables (Table 1). To calculate path coefficients, we analyzed the multiple regression between the pollen arrival or visitation rate and the different predictor variables using a linear mixed-effects model (lme in S-plus) where each predictor variable was entered as a fixed effect and the block (clump) was entered as a random effect (because several blossoms per clump were measured [see Wright 1921, Li 1975, Shipley 2000]). Thus the inter-block (-clump) variation (effects of site and individual) has been effectively removed from the path analysis. All variables were standardized (mean = 0, SD = 1) before analysis.

The design of the manipulative experiment described above allowed comparison of stigmatic pollen loads in the two treatments with a paired *t* test. Variations in plant phenotype, local environment, and time are largely controlled in the paired comparisons. All analyses were performed in S-plus (Venables and Ripley 2002).

RESULTS

Effect of natural phenotypic variation on visitation rate

The only floral visits observed, excepting a few incidental landings on the bracts by flies, were by female *Heriades* nr. *spiniscutis* (Megachilidae), which collected resin, pollen, or sometimes both. These bees are ~6 mm long, roughly 2.5 times the mean GSD (Table 1). Visitation rates ranged from 0 to 3.2 visits per blossom per hour (median = 0.2). Although blossoms with large glands tended to receive more visits, the visitation

TABLE 1. Phenotypic correlations between predictor and response variables, with *P* values in parentheses (above diagonal) and means \pm SE for each variable given on the diagonal (*n* = 81).

Variable	Bract length	Gland area	Gland–stigma distance	Pollen arrival
Bract length	20.1 \pm 0.41 mm	0.36 (0.001)	0.04 (0.72)	0.42 (<0.001)
Gland area		2.90 \pm 0.08 mm ²	0.32 (0.003)	0.23 (0.03)
Gland–stigma distance			2.35 \pm 0.06 mm	–0.09 (0.42)
Pollen arrival				37.5 \pm 3.62

Notes: The study population of *Dalechampia ipomoeifolia* Benth. was in a large opening in a lightly disturbed, lowland, evergreen forest (~490 m elevation), about 20 km east of Lastoursville, Gabon. Pollen arrival was measured as the number of pollen grains on the stigma at the end of the female phase.

rate was affected primarily by the size of the bracts (Table 2, Figs. 1, 2). Because gland area is a good measure of the standing crop of resin (Armbruster 1984), the significant phenotypic correlation between bract length and gland area (*r* = 0.32; Table 1) indicates that the floral advertisement is honest: blossoms with larger bracts indeed usually offer more reward.

Effect of blossom shape on pollination efficiency

The probability of bees contacting the stigmas decreased with increasing GSD (Fig. 3). The coefficients of the GLM were: intercept (\pm SE) = 5.58 (\pm 0.71) and slope = –1.58 (\pm 0.24). The Akaike Information Criterion (AIC) of the model including GSD was 307.48, and the AIC of the model lacking GSD was 406.23; this large difference indicates a highly significant effect (Burnham and Anderson 2002). We observed no sign of overdispersion (dispersion parameter = 1.23). The inflection point was roughly where GSD was 3 mm or about half the length of the pollinating bee. The proportion of stigma contacts approached 0 as GSD approached the length of the bee (6 mm). Because GSD covaries positively with gland area and bract size, however, this negative effect on pollinator efficiency did not translate into a reduction in the rate of pollen arrival (Table 1) unless the effects of gland area and bract size were controlled statistically.

Effect of natural phenotypic variation in size and shape on pollen arrival

There were on average 37.5 \pm 3.62 grains of allogamous pollen on the stigmatic surface at the end of the female phase (i.e., pollen from other blossoms) (range = 0–140 grains). This large range was in part the result of a tendency for the amount of allogamous pollen on the stigma to increase with the length of the lower bract (Fig. 4; see Fig. 5 for significance testing). The relationships among floral morphological variables and between floral morphology and pollen arrival are presented in Table 1 and Fig. 5. Bract length appears to be the most important factor directly affecting pollen arrival. Gland–stigma distance had a marginally significant (*P* = 0.12) negative direct effect on pollen arrival (as GSD increased pollen number tended to decrease). The gland area had no detectable effect on pollen arrival (Fig. 5). Thus, while pollen arrival was significantly correlated with gland area (*r* = 0.23; Table 1), this was largely due to the effect of bract length on pollen load and the correlation between gland area and bract length. If this correlation is the result of the influence of a general growth or size factor on both bract and gland size (the most likely alternative), then the pollen–gland correlation is largely a spurious relationship (sensu Wright 1921; see Bollen 1989:50–51). This would also be the case if bract size influences gland

TABLE 2. Analysis of the relationships between bract length, gland area, and visitation rate.

Model	AIC	Δ AIC	<i>K</i>	<i>w_i</i>
Bract length \times gland area + bract length + gland area	138.57	11.10	5	0.002
Bract length + gland area	130.91	3.44	4	0.101
Gland area	128.55	1.08	3	0.330
Bract length	127.47	0	3	0.566

Notes: We performed a multiple linear regression on visitation rate with both bract length and gland area as predictor variables. We used a mixed-effects model (lme in S-plus) where the block (plant or clump of plants) was entered as random factor. We selected the best model using the Akaike Information Criterion (AIC). *K* is the number of estimable parameters; *w_i* is the Akaike weight of evidence in favor of model *i*. See Burnham and Anderson (2002) for further details on the calculation and meaning of these parameters. The final model is in boldface type. Note that despite a rather low evidence ratio for the model (bract length) vs. the model (gland area), the SE of the parameters were much lower in the former than in the latter model. Coefficients (\pm SE) of the best model are intercept = –0.25 \pm 0.37, bract length = 0.052 \pm 0.018; coefficients of the gland-area model are intercept = –0.10 \pm 0.56; gland area = 0.26 \pm 0.18.

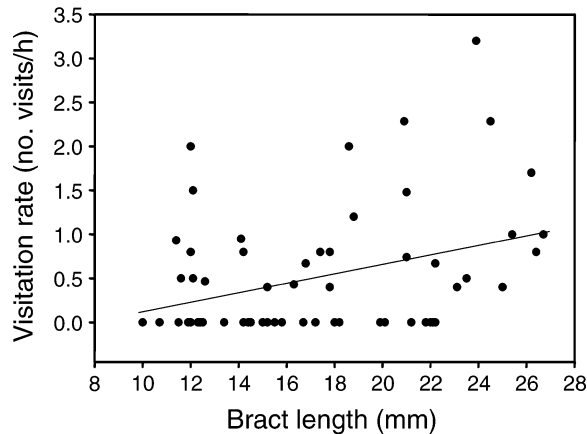


FIG. 1. Effect of natural phenotypic variation in the length of the lower involucral bract on the number of visits by *Heriades* sp. (Megachilidae) observed per unit time. The coefficients (\pm SE) for the regression line are: intercept = -0.38 ± 0.34 , slope = 0.053 ± 0.019 ; $R^2 = 0.12$. See Fig. 2 for significance testing.

area. Alternatively, if gland area influences bract size (which seems very unlikely), an indirect relationship between gland area and pollen arrival would generate much of the correlation. The correlated effect involving bract length was $0.14 (= 0.36 \times 0.39)$, whereas the direct effect of gland area on pollen arrival was 0.12 (Fig. 5).

Manipulation of bract length

The stigmatic pollen load at day 3 was significantly lower in blossoms with bracts cut short than in blossoms with bracts cut "long" (Fig. 6; mean difference = 14.84 pollen grains; 95% CI, 6.97 – 22.70 ; paired t test, $t = 3.77$, $df = 61$, $P < 0.001$). Similarly, at the end of the female phase, the stigmatic pollen load in cut-short blossoms was less than in the cut-long blossom (mean \pm SE, cut-short = 39.3 ± 4.39 ; control = 52.3 ± 4.15 , mean difference = 12.95 , 95% CI, 2.31 – 23.58 ; paired t test, $t = 2.43$, $df = 60$, $P = 0.018$).

DISCUSSION AND CONCLUSIONS

Dalechampia ipomoeifolia blossoms with larger bracts were visited more frequently by pollinating bees. Surprisingly, gland size, which reliably indicates the quantity of resin reward present, had no detectable effect on visitation rate. Bees thus appeared to rely on the honest signal manifested through the phenotypic correlation between bract size and gland area. Higher rates of visitation suggest increasing pollen export (male fitness) with bract size, as well as higher female fitness.

Blossoms with larger bracts achieved higher female fitness as measured by arrival of allogamous pollen on the stigmas. Thus, the increase in visitation results in an increase in the rate of pollen arrival on the stigmas. This relationship is often assumed in pollination studies

but very rarely documented. The same relationship has also been demonstrated recently in *Ipomopsis aggregata* (Polemoniaceae; Engel and Irwin 2003).

Although the resin–gland area accurately predicts the standing crop of resin (Armbruster 1984), which is what the bees seek, gland area did not appear to influence visitation or pollen arrival directly. The significant phenotypic correlation between gland area and bract size, the basis of the honest signal, was probably mostly the result of a general size factor (such as growth rate or duration) affecting both gland area and bract size and thus visitation rate via the latter. The correlation between gland area and pollen arrival would thus be primarily the result of a "spurious effect" in the path analytical sense (see Bollen 1989:50–51), and flower choice by bees thus generates indirect, not direct, selection for larger glands (see Scheiner et al. 2000). The two phenotypic selection studies (visitation and pollen arrival) and the manipulative experiment all yielded results strongly supporting this surprising conclusion that bract size rather than gland area was the main trait influencing pollination success.

Variation in the separation between the site of reward deployment (e.g., resin gland) and the stigmas is expected to affect the pollinator efficiency (amount of pollen deposited per visit), although this has been hard to document in the field (e.g., Engel and Irwin [2003] expected but failed to detect this effect). Our study is one of only a few to show an apparent relationship between floral morphology and pollinator efficiency (see also Campbell et al. 1994). As the gland–stigma distance (GSD) increased, the proportion of visits in which visitors contacted the stigmas dropped significantly, and the rate of pollen arrival on the stigmas also dropped (after the effect of bract size on total visitation had been removed statistically).

The results of the phenotypic-selection study and experimental trimming of bracts both point to the adaptive role of the involucral bracts in attracting pollinators to flowers. This confirms previous speculation

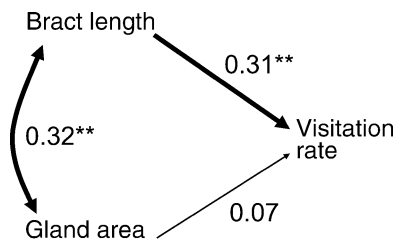


FIG. 2. Path diagram showing estimated direct effects of reward (gland area) and advertisement (bract length) traits on the rate of visitation by *Heriades* sp. (Megachilidae). The double-headed arrow indicates an unanalyzed correlation between morphological traits. Arrow thickness indicates relative importance of the independent variable's effect on the dependent variable. The analysis was conducted using a mixed-effects model including block as a random factor, and the diagram thus represents the relationship after the block effect is removed. $**P < 0.01$.

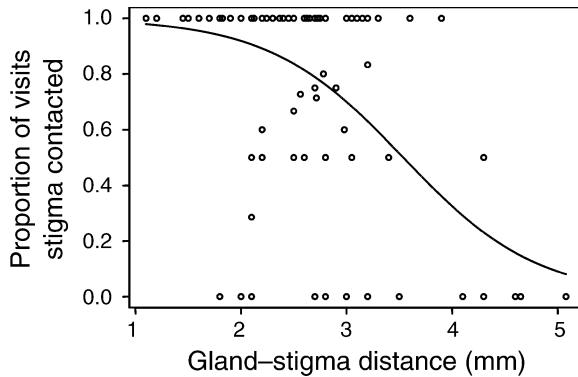


FIG. 3. Variation in the likelihood of visiting bees contacting the stigmas in relation to variation in the distance between the resin gland and the stigmas (GSD).

(Armbruster 1997) explaining the origin of large involucre bracts in this group as an adaptation to attract pollinators. Interestingly, large bracts also play an important role in defense of flowers against nocturnal florivores in those species in which the bracts close at night (Armbruster 1997).

Selection for large bracts may also have played a role in the divergence of populations and species of *Dalechampia*. Although the population studied here was visited by only one species of resin-collecting bee, and these bees showed only limited size variation, larger species of resin-collecting bees are found in other parts of Africa (e.g., *Pachyanthidium* spp.; Steiner and Whitehead 1991, Armbruster and Steiner 1992, Armbruster et al. 1993) and vastly larger resin-collecting bees are found in the neotropics (e.g., *Euglossa*, *Eulaema*; Armbruster 1988). Larger bees may select for larger bracts (and ultimately glands), leading to the evolution of larger blossoms in those populations (Armbruster 1985, Hansen et al. 2000) and species (Armbruster 1988, 1991, 1993) pollinated primarily by larger bees. Smaller bee pollinators may drive divergence in the opposite direction in other populations and species.

Potential confounding effects

It has been recently pointed out that studies of phenotypic selection risk misinterpreting a correlation between fitness and phenotype as evidence for selection when the correlation actually reflects environmental covariance between fitness and phenotype (Rausher 1992, Scheiner 2002, Stinchcombe et al. 2002, Kruuk et al. 2003). This is the result of the environment (e.g., soil nutrition for a plant) having direct effects both on phenotype (e.g., flower size) and fitness (e.g., seed production). While this problem is probably common, especially when the measured component of fitness is offspring production, we think it is minimal here for several reasons. First, we employed path analysis, which may reduce the problem statistically (Scheiner 2002). Second, we measured visitation and pollen ar-

ival, components of fitness not strongly linked to nutrient or energy status. Third, all study plants were observed in fairly uniform conditions, especially with respect to light budget.

We can think of only one environmental variable that could affect both blossom size (via plant growth) and rates of pollinator visitation and pollen arrival. If plant growth is limited by available photosynthetically active radiation (PAR) and pollinator visitation is thermally constrained, then shady environments could have smaller blossoms and lower visitation rates than sunnier environments. This is because the total thermal environment (the “operative environmental temperature”; T_E) is influenced by radiation load as well as air temperature and wind (Bakken 1992). Bee activity is known to be sensitive to T_E (Bishop and Armbruster 1999), even in the tropics (Armbruster and Berg 1994), which means that a reduction in radiation load could affect both pollinator activity and photosynthesis (hence investment in blossoms). However, we do not think it likely that this environmental correlation affected our analysis, because all our study plants were in a nearly uniformly warm, open site, with ample PAR and high T_E during the observation periods. Furthermore, the manipulative experiment rigorously controlled environment in its paired design, largely precluding such environmental effects. That the results from this experiment were completely consistent with the phenotypic selection analysis confirms the validity of the latter.

Bee foraging decisions

It was somewhat surprising that bract size (an advertisement) rather than gland area (a component of reward quantity) influenced bees’ floral choices, given that the glands are openly visible when the blossoms

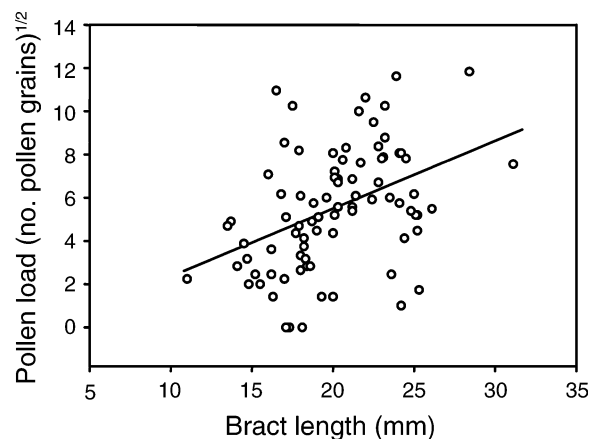


FIG. 4. Effect of natural variation in bract length on the amount of pollen found on the stigmas at the end of the female phase (i.e., pollen transported to stigmas by pollinators). The coefficients (\pm SE) for the regression line are: intercept = -0.88 ± 1.55 , slope = 0.31 ± 0.07 ; $R^2 = 0.18$. See Fig. 5 for significance testing.

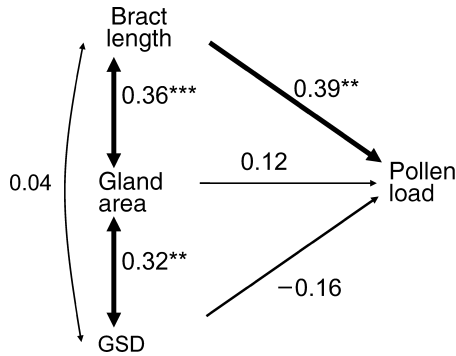


FIG. 5. Path diagram showing estimated direct effects of reward (gland area), advertisement (bract length), and pollinator-fit (gland–stigma distance [GSD]) traits on the arrival rate of pollen carried by pollinators (pollen load). The double-headed arrows indicate unanalyzed correlations between morphological traits. Arrow thickness indicates relative importance of independent variable's effect on the dependent variable. The analysis was conducted using a mixed-effects model including block as a random factor, and the diagram thus represents the relationship after the block effect is removed. ** $P < 0.01$; *** $P < 0.001$.

are receptive to visitation. Although we expected bract size might be important in long-distance signaling, we expected gland area to affect the final choice of blossoms by bees. Instead, it appears that bees' choice of more rewarding blossoms was based primarily on bract size and, ultimately, the phenotypic correlation between gland area and bract size. Bees visiting blossoms with large bracts do usually get more reward (from larger glands, on average) than bees visiting blossoms with small bracts. Although the advertisement is honest (bract length and gland area are significantly correlated; $r = 0.36$), this result is surprising because only a small portion of the variance in gland area and reward amount (~13%) is related to bract size. It is thus curious that the bees do not seem to choose blossoms based directly on the size of the gland and amount of resin visible. This may be explained by the fact that the bracts are larger and serve as better long-distance attractants, and bees are better able to detect differences in bract size. Another possibility is that, because *D. ipomoeifolia* resin is clear (in human vision), bees may not readily gauge the gland size and amount of resin as they approach the blossoms (Spaethe et al. 2001). Clear resin could be the product of selection against blossoms revealing "too much" information to bees, when this results in excessive discrimination and reduced rates of pollination. Many species of *Dalechampia* do, however, secrete brightly colored resin (Armbruster 1996, 2002). It would be interesting to see if bees choose blossoms of these species based directly on the amount of brightly colored reward present rather than, or in addition to, the size of the bracts.

As a note of caution, the proportional measurement error (not estimated) was probably larger for gland area than bract length, because the gland is smaller and

somewhat harder to measure with calipers than are the bracts. This may have led to underestimation of the importance of gland area in influencing the choice of blossoms by bees, but would not have affected the importance of bract size. In a set of similar measurements on a closely related species, *D. scandens* (Armbruster and Baldwin 1998, Armbruster 2002), the measurement error variance was estimated as 1.05% of the mean for bract length and 2.14% for gland area. This difference of 1.09% is very small compared to the coefficient of total variance in gland area, which was 92.1% (Hansen et al. 2003b). Thus, we do not think that such a small difference in measurement error could have obscured a large effect of gland area on bee choice, but the possibility needs to be evaluated.

Evolutionary implications

The phenotypic correlation between bract length and gland area/resin quantity has a strong genetic basis in *D. scandens* (Hansen et al. 2003a), a species, as noted above, closely related to *D. ipomoeifolia*. The genetic correlation between gland area and lower bract length was 0.58, and the genetic correlation between gland area and lower bract width was 0.78 (Hansen et al. 2003a). Phenotypic correlations between bract size and gland area are seen also in all the other species in the genus that have been examined (Armbruster 1988, 1991). These observations, taken together, suggest that the honest phenotypic relationship between bract advertisement and reward may have a genetic basis and hence be an obligate signal of floral reward, at least during short-term evolution.

It appears that there may be a direct negative effect of increasing gland–stigma distance (GSD) on polli-

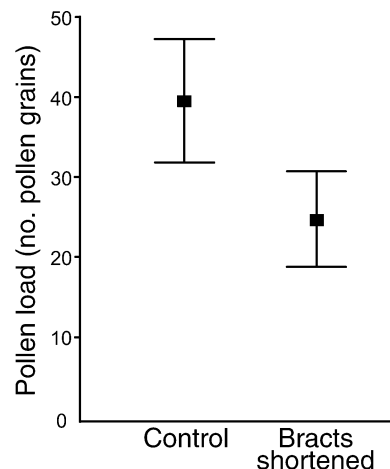


FIG. 6. Effect of cutting involucral bracts short on the amount of pollen on the stigmas on the three days after the treatment. The control had the bracts only slightly trimmed. Means and standard errors are shown. Note that they include a block effect. The paired-comparison analysis showed the difference to be significant (paired t test; $t = 3.77$, $df = 61$, $P < 0.001$).

nator efficiency in contacting stigmas and depositing pollen on stigmas (see also Cresswell 2000). This is consistent with observations of the pollination of various species of *Dalechampia* in which the probability of bees of a given length touching the stigma decreased as the distance between the stigma and the resin gland increased (Armbruster 1988, 1990). Note that the direct effect of GSD on pollen arrival has the opposite sign as the effect of variations in gland area and bract size. However, the phenotypic correlations between GSD, gland area, and bract size are positive. Although the observed correlations between GSD and gland area and between GSD and bract size were small (only the former was significant), they may represent stronger genetic correlations, as was observed for GSD \times gland area in the related *D. scandens* (phenotypic correlation = 0.50, genetic correlation = 0.75; Hansen et al. 2003b).

These observations raise the possibility that response to pollinator-mediated up-selection for honestly showy advertisements (larger bracts and more reward) is constrained by the negative fitness effect of the correlated increase in gland–stigma distance. Thus, despite apparent directional selection, current bract size in *D. ipomoeifolia* might actually be optimal because the up-selection generated by pollinators on an honest advertisement is balanced by stabilizing or down-selection on overall blossom size generated by the stigmatic fit with the relatively small pollinators. Additional selection against larger bracts and more resin may be generated by declining benefits in relation to the increased cost of carbon and nutrients (Armbruster 1990). Indeed, excessive resin production may even cause a drop in pollen arrival and dispersal as small bees visit fewer blossoms to achieve their payload (Heinrich and Raven 1972).

One longstanding question in evolutionary biology concerns the degree to which selection and/or genetic correlations measured in populations can explain the patterns of species divergence (Schluter 1996, Gould 2002). For example, which better explains the distribution of congeneric species in phenotypic space, the trajectory of multivariate selection or the trajectory of genetic correlations? Or do neither explain species divergence very well? The first possibility suggests that selection is largely responsible for species divergence, the second that genetic correlations constrain species divergence to a particular trajectory, and the third that macroevolution is not an extrapolation of microevolutionary adaptation or constraint. The data collected here, when integrated with published data for the same traits from multiple species (Armbruster 1988, 1990), suggest that both genetic correlations and the shape of the underlying adaptive surface influence the macroevolutionary patterns of floral diversification in *Dalechampia* (see also Armbruster 1991, 1996, Armbruster et al. 2004, Hansen et al. 2000, 2003a, b).

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