

# Direct selection at the blossom level on floral reward by pollinators in a natural population of *Dalechampia schottii*: full-disclosure honesty?

Geir H. Bolstad<sup>1</sup>, W. Scott Armbruster<sup>2,3,4</sup>, Christophe Pélabon<sup>1</sup>, Rocío Pérez-Barrales<sup>2,5</sup> and Thomas F. Hansen<sup>6</sup>

<sup>1</sup>Department of Biology, Centre for Conservation Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway; <sup>2</sup>School of Biological Sciences, University of Portsmouth, Portsmouth PO1 2DY, UK; <sup>3</sup>Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway; <sup>4</sup>Institute of Arctic Biology, University of Alaska, Fairbanks, AK 99775, USA; <sup>5</sup>Plant Biology and Ecology Department, University of Seville, 41080, Seville, Spain; <sup>6</sup>Centre for Ecological and Evolutionary Synthesis, Department of Biology, University of Oslo, PO Box 1066, N-0316 Oslo, Norway

## Summary

Author for correspondence:

Geir H. Bolstad

Tel: +47 92 03 76 65

Email: geir.bolstad@bio.ntnu.no

Received: 14 June 2010

Accepted: 14 July 2010

*New Phytologist* (2010) **188**: 370–384

doi: 10.1111/j.1469-8137.2010.03429.x

**Key words:** *Dalechampia schottii*, fitness function, euglossine bees, multivariate selection, natural selection, pollination, selection gradient.

- Both floral rewards and advertisements can be important in the attraction of pollinators, but few studies have separated the individual contributions of rewards and advertisements to fitness.
- Here, we investigated selection by pollinators on individual blossoms in *Dalechampia schottii*. This Neotropical vine, endemic to the Yucatán Peninsula, rewards bees by secreting fully visible, deep-blue resin from a gland subtended by two conspicuous petaloid bracts that may play the role of advertisement.
- We used contextual analysis to build a fitness function for four morphological traits of individual blossoms: the amount of the reward as measured by gland area; the size of the advertisement trait as measured by bract length; the flower-pollinator fit as measured by the shortest distance between reward and stigma; and the potential for self-pollination as measured by the shortest distance between anthers and stigma.
- Larger gland area and increased potential for self-pollination directly increased the seed production of individual blossoms. However, bract size or flower-pollinator fit did not influence the number of seeds produced by blossoms. Therefore, in this *Dalechampia* species, pollinators seem to select directly on the reward of individual blossoms but not on the advertising bracts.

## Introduction

Pollinators usually visit flowers to obtain rewards, but if the reward is hidden the pollinators have to rely on ‘honest’ advertisements that are correlated with the reward to make good foraging decisions (e.g. Cresswell & Galen, 1991; Fenster *et al.*, 2006). Under these conditions, pollinator-mediated selection on rewards is indirect through the phenotypic correlation between the reward and the signal (Lande, 1979). The strength of the correlation determines the honesty of the signal (i.e. the accuracy of the information provided to the pollinators). Plants have evolved very different levels of honesty (reviewed in Schaefer *et al.*,

2004), from deceptive, nonrewarding orchids (Johnson, 2000) to flowers providing information about the amount of reward present by changing corolla colour after they have been pollinated and are generally unrewarding (Casper & La Pine, 1984; Weiss, 1991, 1995). The most honest advertisement is, however, the direct and conspicuous display of the reward itself. This gives the reward a dual function as both signal and reward (Hansen *et al.*, 2007; Raguso, 2008). We refer to this as ‘full-disclosure honesty’, defining ‘signal’ broadly as a phenotypic character that provides information to other organisms. Full-disclosure honesty bears similarity to signals that are impossible to fake, termed ‘indices’ in the animal literature, in contrast to the signals

that are honest because they are costly (Maynard Smith & Harper, 2003).

Full-disclosure honesty has evolved in several species, creating the potential for direct selection on the reward. The most obvious example is scent-rewarding plants (Dressler, 1968; Williams & Dodson, 1972; Ackerman, 1983). Other examples include flowers in which bees can detect the presence of nectar by visual inspection (Goulson *et al.*, 2001) or smell (Howell & Alarcón, 2007). Some plants have even evolved coloured (Hansen *et al.*, 2006, 2007) or fluorescent nectar (Thorp *et al.*, 1975), which appears to make detection and assessment easier for pollinators. Similarly, pollen-collecting pollinators can assess the amount of pollen by visual inspection (Cresswell & Robertson, 1994; Lunau, 2000; Goulson *et al.*, 2001), and several plants have scented pollen, allowing bees to assess the amount of pollen by olfaction (Dobson *et al.*, 1999; Dobson & Bergström, 2000). Plants have also evolved coloured floral oils (Buchmann, 1987), which may facilitate detection by pollinators. Furthermore, direct selection for reward production has been demonstrated by experimentally varying the amount of nectar (Mitchell & Waser, 1992; Mitchell, 1993; Hodges, 1995). Despite of all this, selection for reward has usually been assumed to be indirect, and surprisingly few studies have focused on disentangling selection on signal from selection on reward (but see Cresswell & Galen, 1991; Golding *et al.*, 1999; Schemske & Bradshaw, 1999; Armbruster *et al.*, 2005; Makino & Sakai, 2007), and we know of only four studies that have estimated selection gradients jointly for signal and reward (Mitchell *et al.*, 1998; Caruso, 2000, 2001; Castro *et al.*, 2009).

Most species of *Dalechampia* reward their pollinators with terpenoid resins secreted from a fully visible gland subtended by two showy petaloid bracts. In two studies, on *Dalechampia ipomoifolia* in Gabon (Armbruster *et al.*, 2005), and on *D. scandens* in Mexico (R. Pérez-Barrales *et al.*, unpublished), the visitation rate of pollinating bees was not influenced by the size of the resin-secreting gland (hereafter called the 'resin gland'), but was influenced by the size of the involucre bracts. Notably, these species of *Dalechampia* have nearly transparent resins, which may be hard for the bee to see even though they are not hidden by floral structures. Other species of *Dalechampia*, such as *Dalechampia schottii*, secrete brightly coloured resins, which may be more conspicuous to pollinators (Armbruster, 1996, 2002).

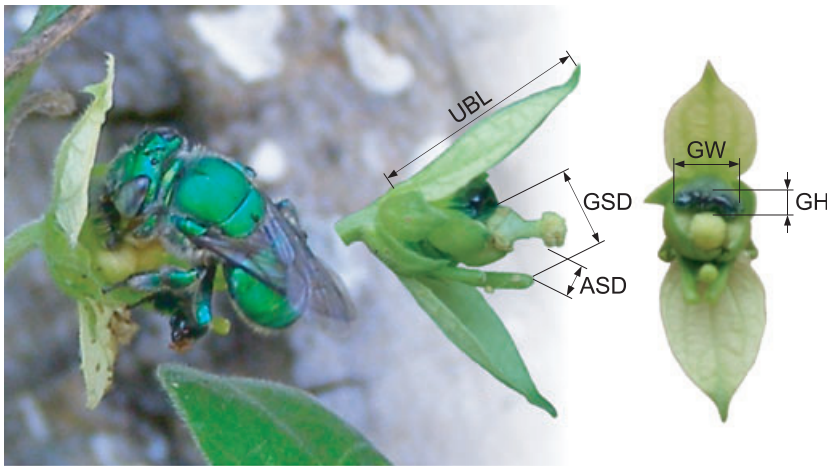
In this study, we investigated how floral traits of a *Dalechampia* species secreting coloured resin (as seen by humans) affect the reproductive output of blossoms, as mediated by pollinator choice. More specifically, we wanted to see whether pollinators selected blossoms directly on the size of the resin gland, instead of indirectly through selection on correlated structures, such as involucre bract size, as

observed in studies of *Dalechampia* species with transparent resins (Armbruster *et al.*, 2005; R. Pérez-Barrales *et al.*, unpublished). We tested this expectation by conducting phenotypic selection analysis at the level of blossoms in *D. schottii*. This species, endemic to the Yucatán Peninsula, has small blossoms secreting a deep-blue resin, and like virtually all species of *Dalechampia*, it is partially protogynous (with a female phase preceding a bisexual phase). We studied the effects of blossom morphology on components of blossom seed set with three separate analyses. The first was a contextual analysis exploring the effect of resin-gland size and bract size on pollinator visitation at two levels, among patches of blossoms, and among blossoms within patches. The second analysis explored the effect of visitation and flower-pollinator fit on pollen load at the end of the female phase ('cross-pollen'; technically allogamous – from other blossoms on the same and other plants). In this analysis, we included the distance between the resin gland and the stigma, because this affects the likelihood that a floral visitor contacts the stigma when collecting resin ('flower-pollinator fit'; Armbruster, 1988; Armbruster *et al.*, 2005). The third analysis explored the effect of visitation, flower-pollinator fit, and a proxy for ability to self-pollinate (the distance between the anthers and the stigma) on pollen arrival on the first day in the bisexual phase (cross- plus self-pollen). We then combined the parameter estimates from these three analyses with a previously established empirical relationship between pollen load and seed set to build a function that describes the effect of morphological variation on the seed set of individual blossoms, through pollinator visitation and pollen arrival. Construction of this function was based on generalized mixed-effects models, but was inspired by path analysis and structural-equation modelling, and shares several of their advantages. Most importantly, these approaches take causal relationships between variables into account, and allow the causal relationships to be estimated and tested (see Kingsolver & Schemske, 1991; Mitchell, 1992; Scheiner *et al.*, 2000). Moreover, the generalized mixed-effects framework allows for complex error structures among observations and is not restricted to normally distributed residuals as traditional path analysis and structural-equation models are.

## Materials and Methods

### Study system

We studied a population of *Dalechampia schottii* Greenman (Euphorbiaceae) in a lightly disturbed subperennial tropical forest in the botanical garden 'Dr Alfredo Barrera Marin' (20°51'11"N, 86°53'43"W) 1 km south of Puerto Morelos, in north-eastern Quintana Roo, Mexico, in September and October 2007. *Dalechampia schottii* is endemic to the Yucatán Peninsula. As illustrated in Fig. 1, the



**Fig. 1** *Dalechampia schottii* blossoms with pollinator (female *Euglossa* cf. *viridissima*) and with lines indicating the morphological measurements: upper bract length (UBL), gland–stigma distance (GSD), anther–stigma distance (ASD), gland width (GW), and gland height (GH). The blossom in the middle is in bisexual phase, and the blossom to the right is in female phase.

relatively small blossoms are functionally bisexual with three pistillate flowers situated below four staminate flowers. Above the staminate flowers is a resin gland composed of packed bractlets which secrete blue resin. Above and below the blossom are two 5–15-mm, pale-green to white involucral bracts. The blossoms are partially protogynous. During the first 2–4 d after they open, the stigmas are receptive but the male flowers remain closed ('female phase'); subsequently one to two male flowers open each day, while the stigmas remain receptive ('bisexual phase'). Resin starts to be produced in the beginning of the female phase.

The study population of *D. schottii* grows in sympatry with *Dalechampia scandens* L., which has much larger blossoms with transparent to whitish resin and two large pale-green to white bracts. *Dalechampia* resins are collected by several species of megachilid and apid bees for use in nest construction (Armbruster, 1984, 1985, 1988). At this field site, *D. schottii* was pollinated by large resin-collecting female *Euglossa* cf. *viridissima* Friese and small resin-collecting female *Hypanthidium* cf. *melanopterum* Cockerell. In the study period, however, we observed very few *Hypanthidium*, so most of the pollination was probably by *Euglossa*. This contrasts to the year previous to this study when *Hypanthidium* was abundant, and probably the main pollinator (R. Pérez-Barrales, pers. obs.). *Dalechampia schottii* has generally been described as a 'small-bee-pollinated' *Dalechampia* (Armbruster, 1993; Hansen *et al.*, 2000), and in other populations *Trigona* sp. and *Hypanthidium* cf. *melanopterum* have been reported as pollinators (Armbruster, 1985, 1988).

### Field observations

*Dalechampia schottii* blossoms grow in patches consisting of single plants or entangled clumps of plants. We examined up to 20 blossoms in all patches we could find in the local population, ending up with a total of 278 blossoms in 27 patches. These blossoms were scored for number of pollen

grains on the stigma and evidence of resin removal once a day during their female phase, and on the first day of their bisexual phase. Pollen counting was aided by a  $\times 10$  hand lens. Accurate counting of pollen grains was difficult when there was abundant pollen, and was complicated by the germination of older pollen grains, which reduced their visibility. Therefore, lower amounts of pollen ( $c. < 25$ ) were counted precisely as each pollen grain was easy to spot, but the accuracy of the counts probably decreased as the amount of pollen increased and each pollen grain became harder to separate out visually. On the first day of the bisexual phase, we measured a series of morphological traits on each blossom (see next paragraph). There was extensive floral herbivory by weevils and larvae of the butterflies *Dynamine* sp. and *Hamadryas* sp.: 61 out of the 278 observed blossoms were completely eaten and 39 were severely damaged. Therefore, morphological measurements were possible on only 178 blossoms from 25 patches, with complete morphological measurements on 137 blossoms from 25 patches. The number of blossoms measured per patch ranged from one (in three patches) to 20 (in three patches) with a median of six. We obtained a measure of blossom production by counting all the blossoms and developing fruits in each patch once during the study period. This is referred to as number of blossoms (or #blossoms).

### Morphological traits and components of blossom reproductive success

We measured the following morphological traits (Fig. 1).

- Upper bract length (UBL) or 'bract size'. We chose UBL as a measure of bract size because there were more missing data in the other bract size measures (upper bract width (UBW), lower bract length (LBL), and lower bract width (LBW)). However, the correlations between the different bract measurements were high (UBL with UBW,  $r = 0.73$ ; UBL with LBL,  $r = 0.60$ ; and UBL with LBW,  $r = 0.59$ ),

and using the other measures did not alter the conclusions of the study.

- The height and width of the resin-bearing surface of the cluster of bractlets forming the resin gland (multiplied to obtain 'gland area' (GA)), which correlates tightly with the standing crop of resin in several species of *Dalechampia* (*Dalechampia dioscoreifolia*,  $r = 0.81$ ; *Dalechampia heteromorpha*,  $r = 0.78$ ; *D. scandens*,  $r = 0.88$ ; and *Dalechampia osana*,  $r = 0.89$ ; Armbruster, 1984).
- The minimum distance separating the gland from the middle stigma ('gland–stigma distance' (GSD)). This is a well-established measure of flower–pollinator fit in *Dalechampia* (Armbruster, 1988, 1990; Armbruster *et al.*, 2009).
- The minimum distance separating the anthers of the central staminate flower from the middle stigma ('anther–stigma distance' (ASD)). Anther–stigma distance correlates with the ability of blossoms to self-pollinate (Armbruster, 1988).

Means and correlations of the traits are given in Table 1. Repeatabilities, the ratios of among-blossom variance to total variance (Lessells & Boag, 1987), of the different measures estimated from two measurements taken on the same day on 21 blossoms were: UBL, 0.94; GA, 0.91; GSD, 0.98; ASD, 0.99.

We measured several components of blossom reproductive success.

- The probability,  $V$ , of a blossom being visited during 1 d (one day). This was estimated from indirect evidence: resin removal or pollen arrival during the female phase.
- The number of pollen grains,  $P_F$ , that had arrived on the three stigmatic surfaces by the end of the female phase, given at least one visit during the female phase. We used the highest count of pollen grains, obtained in the female phase, on each stigmatic surface to avoid underestimating the amount of pollen.
- The number of pollen grains,  $P$ , on the stigmatic surfaces on the first day of the bisexual phase.
- The number of pollen grains,  $P_B$ , arriving on the first day of the bisexual phase. This was estimated as the difference between the number of pollen grains on a stigmatic surface on the first day of the bisexual phase and the number of

pollen grains on the same stigmatic surface on the last day of the female phase (negative values were set to zero), summed over all three stigmatic surfaces.

### Fitness model

Male reproductive success is difficult to estimate in this system, and we focused our study on the female reproductive success of individual blossoms. Our goal was to study the direct selection on blossoms that results from pollinator behaviour. Selection at the blossom level should not be confused with selection at other levels of organization, for example the plant level. Pollinator-mediated selection on average blossom traits on the plant may not be fully explained by selection at the blossom level if pollinators can distinguish between plants within a patch, and this creates a pollination pattern that cannot be explained by within-patch differences in blossom phenotype (i.e. if pollinators choose among blossoms within a plant differently from among blossoms on different plants within a patch). However, the blossom-level selection probably captures most of the selection at the plant level as bees are unlikely to distinguish between plants in a patch except by differences in blossom phenotype. The measures of reproductive success at the blossom level are only valid measures of individual fitness and individual selection if there are differences in mean floral characteristics (i.e. if individuals differ in the distribution of these characters among flowers on a plant). For this selection to induce an evolutionary response there also needs to be heritable variation in these differences. However, for convenience, we refer to our measurements as 'fitness' and 'selection' and refer to the estimated relationships between fitness and characters as 'selection gradients'. Note also that this study does not account for other types of selection such as herbivory, selection on the male function, selection during other phases of development, and indirect selection resulting from correlation among traits, all of which will contribute to the total selection acting on the traits.

To build our model of blossom seed set, we combined our field observations of pollen arrival with an empirically

**Table 1** Phenotypic correlations among morphological characters of *Dalechampia schottii* with 95% confidence interval above the diagonal. Character means with standard deviation are shown on the diagonal, and covariances below the diagonal. The number of blossoms used to obtain the estimates is given by  $n$

Variable	UBL	GA	GSD	ASD
UBL	8.63 (1.47) mm ( $n = 159$ )	0.47 (0.34, 0.58) ( $n = 159$ )	0.30 (0.14, 0.44) ( $n = 146$ )	0.16 (–0.01, 0.32) ( $n = 137$ )
GA	1.25 mm <sup>3</sup>	4.44 (1.86) mm <sup>2</sup> ( $n = 178$ )	0.46 (0.33, 0.58) ( $n = 163$ )	0.38 (0.24, 0.51) ( $n = 154$ )
GSD	0.55 mm <sup>2</sup>	1.05 mm <sup>3</sup>	3.99 (1.27) mm ( $n = 163$ )	0.75 (0.67, 0.81) ( $n = 154$ )
ASD	0.30 mm <sup>2</sup>	0.88 mm <sup>3</sup>	1.21 mm <sup>2</sup>	1.97 (1.29) mm ( $n = 154$ )

The characters are: upper bract length (UBL), gland area (GA), gland–stigma distance (GSD), and anther–stigma distance (ASD).

established relationship linking pollen arrival to seed set. This relationship corresponds to an increasing function where  $P$ , the number of pollen grains, maps into  $S$ , the number of seeds set with an asymptotic value of nine seeds. The general function is described as:

$$S = 9 \frac{aP}{1 + aP}, \quad \text{Eqn 1}$$

where  $a$  describes the increase in the number of seeds with an increase in pollen load. In *D. scandens*,  $a$  was estimated as 0.0850 (R. Pérez-Barrales *et al.*, unpublished). This value of  $a$  maps 1 pollen grain into 0.71 seeds, 10 pollen grains into 4.1 seeds, and 50 pollen grains into 7.3 seeds, and explained 24.2% of the variation in seed set in the study on *D. scandens*. Although this relationship might be different for *D. schottii*, the similarity between the two species (same number and arrangement of pistillate flowers; same type of pollination system) suggests that this relationship should be close enough to be useful for comparing seed production under different pollination scenarios.

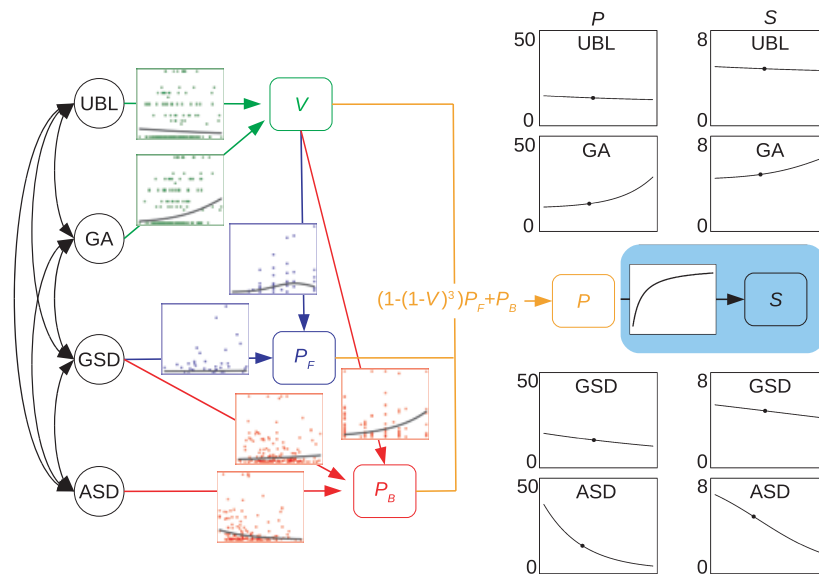
To understand the effects of the different floral traits on seed set, we modelled the total number,  $P$ , of pollen grains that arrived at the three stigmatic surfaces during the first day of the bisexual phase as a function of three variables: the probability,  $V$ , of a blossom being visited during 1 d, the number of pollen grains,  $P_F$ , arriving in the female phase

given at least one visit, and the number of pollen grains,  $P_B$ , arriving on the first day of the bisexual phase. These were combined as

$$P = (1 - (1 - V)^3)P_F + P_B, \quad \text{Eqn 2}$$

where  $1 - (1 - V)^3$  gives the probability of being visited during 3 d (i.e. the median duration of the female phase in this population), and  $(1 - (1 - V)^3)P_F$  gives the expected amount of pollen arriving during the female phase. In the rest of the bisexual phase, not included in this study, the blossom continues to self-pollinate and the arrival of additional cross-pollen is probably less important, because the male flowers are positioned above the female flowers and will form a platform that may hinder contact between the stigma and the pollinator. Although we did not include the total amount of self-pollen, the pollen arrival on the first day in the bisexual phase gives us a relative difference in the ability of the blossoms to self-pollinate. Fig. 2 provides a graphical representation of the model.

The probability,  $V$ , of a blossom being visited during 1 d can be influenced by the characteristics of each single blossom, but also by the floral display of the patch to which each blossom belongs. Several studies have showed the importance of floral display in the foraging decision of pollinators (Harder & Barrett, 1995; Harder *et al.*, 2001; Harder & Johnson, 2005). We therefore allowed  $V$  to be



**Fig. 2** Graphical representation of the fitness model (Eqns 1, 2, 4, 5 and 6). Double-headed arrows indicate correlations, single-headed arrows indicate direct effects, and the shape and signs of the effects at the population mean are indicated by the small graph on each arrow.  $V$  is the probability of a blossom being visited during 1 d,  $P_F$  is the amount of pollen deposited on the stigma in the female phase given a visit,  $P_B$  is the amount of pollen deposited on the stigma on the first day in the male phase, and  $P$  is the total amount of pollen deposited on the stigma in both phases. In the blue box the relationship between the total amount of pollen and the number of seeds,  $S$ , is indicated. The graphs marked UBL (upper bract length), GA (gland area), GSD (gland-stigma distance), and ASD (anther-stigma distance) show the predicted effect of each trait on the total amount of pollen (column  $P$ ) and the predicted effect of each trait on the number of seeds (column  $S$ ) over the range of each trait when all other traits are at the population mean. The dot marks the trait mean in these graphs.

influenced by the size of the bract, the size of the resin gland, and the number of blossoms in a patch. To analyse this relationship we used contextual analysis (Heisler & Damuth, 1987), which allowed us to investigate the effects on the probability of being visited during 1 d both among and within groups of blossoms (patches) in the same model. The data have four levels; each observation ( $i$ ) was taken on a particular day ( $j$ ) on a particular blossom ( $k$ ), each blossom being nested within a patch ( $l$ ). We made only one observation per blossom on each day, and hence  $V$  is given by the probability of observing a visit at observation  $i$ . We used a mixed-effects model with logit link and binomially distributed errors to analyse the data. The log odds for a blossom of being visited during 1 d is given by:

$$\log\left(\frac{V_i}{1 - V_i}\right) = \alpha_{1j[i]} + \alpha_{2k[i]},$$

$$\alpha_{1j} = \varepsilon_j^{\alpha_1},$$

$$\alpha_{2k} = \beta_{0l[k]} + \beta_1(\text{UBL}_k - \overline{\text{UBL}}) + \beta_2(\text{GA}_k - \overline{\text{GA}}) + \varepsilon_k^{\alpha_2},$$

$$\beta_{0l} = \gamma_0 + \gamma_1(\overline{\text{UBL}}_l - \overline{\text{UBL}}) + \gamma_2(\overline{\text{GA}}_l - \overline{\text{GA}}) + \gamma_3(\#\text{blossoms}_l - \overline{\#\text{blossoms}}) + \varepsilon_l^{\beta_0}, \quad \text{Eqn 3}$$

for observations  $i = 1, \dots, 484$ , days  $j = 1, \dots, 38$ , blossoms  $k = 1, \dots, 159$ , and patches  $l = 1, \dots, 25$ . The subscript  $j$  or  $k$  with  $i$  in brackets denotes the corresponding subscript  $j$  or  $k$ , respectively, for observation  $i$ . The subscript  $l$  with  $k$  in brackets denotes the corresponding subscript  $l$  for blossom  $k$ . One bar denotes the patch mean (for UBL and GA) and two bars denote the grand mean of the trait.  $\alpha_{1j}$  ( $= \varepsilon_j^{\alpha_1}$ ) is a random effect that gives the deviation for a day  $j$  from the grand mean log odds of being visited during 1 d,  $\alpha_{2k}$  is the predicted log odds of blossom  $k$  being visited during 1 d,  $\beta_{0l}$  is the predicted log odds of being visited for patch  $l$ ,  $\beta_1$  is the effect of upper bract length within patch,  $\beta_2$  is the effect of gland area within patch,  $\varepsilon_k^{\alpha_2}$  is a random effect that represents the deviation of the predicted value of blossom  $k$  from the rest of the model at the blossom level,  $\gamma_0$  is the grand mean log odds of being visited during 1 d,  $\gamma_1$  is the effect of upper bract length among patches after the within-patch effect ( $\beta_1$ ) has been removed (the effect of upper bract length among patches is  $\gamma_1 + \beta_1$ ),  $\gamma_2$  is the effect of gland area among patches after the within-patch effect ( $\beta_2$ ) has been removed,  $\gamma_3$  is the effect of the number of blossoms in a patch, and  $\varepsilon_l^{\beta_0}$  is a random effect that represents the deviation of the predicted value for patch  $l$  from the rest of the model at the patch level. The random effects/error terms ( $\varepsilon$ ) at each level are assumed to be normally distributed with a mean of zero. In the fitness model we are only interested in the within-patch effects of the morphological traits, while controlling for the among-patch effects. Hence, the probability of being visited during 1 d,  $V$ , is given by

$$V = \frac{e^{\gamma_0 + \beta_1 \text{UBL} + \beta_2 \text{GA}}}{1 + e^{\gamma_0 + \beta_1 \text{UBL} + \beta_2 \text{GA}}}, \quad \text{Eqn 4}$$

where  $\gamma_0$ ,  $\beta_1$  and  $\beta_2$  are estimated by Eqn 3, and GA and UBL are centred on their grand means.

The pollen arrival in the female phase, given that the blossom has been visited,  $P_F$ , is potentially influenced by the amount of visitation and the fit between the pollinator and the pollen-receiving structures (i.e. the stigma). This fit is influenced by the distance between the reward and the stigma, estimated by GSD. We investigated these effects using a generalized linear model with a log link and quasipoisson-distributed errors to account for overdispersion. The probability of being visited during 1 d and the gland-stigma distance were entered as fixed effects. The estimates (intercept  $\alpha_1$ , and slopes  $\beta_{1.1}$ ,  $\beta_{1.2}$ ,  $\beta_{1.3}$  and  $\beta_{1.4}$ ) from this analysis were used to model pollen arrival in the female phase as

$$P_F = e^{\alpha_1 + \beta_{1.1} V + \beta_{1.2} V^2 + \beta_{1.3} \text{GSD} + \beta_{1.4} V \times \text{GSD}}, \quad \text{Eqn 5}$$

where GSD is centred on its grand mean.

Pollen arrival in the bisexual phase,  $P_B$ , is potentially influenced by the visit of pollinators and by autogamy. Autogamy may be influenced by visitation by pollinators or other biotic and abiotic factors that provoke the fall of pollen on the stigma, but also by the distance between anther and stigma (Armbruster, 1988; Armbruster *et al.*, 2009). We analysed the pollen arrival during the bisexual phase using a generalized mixed-effects model with a log link and quasipoisson-distributed errors to correct for overdispersion, with the probability of being visited during 1 d, the gland-stigma distance, and the anther-stigma distance as fixed effects, and patches as random effects to control for differences in disturbance between patches. These traits, together with visitation, can interact in a complicated way, and indeed, the best model included linear terms and their two- and three-way interactions. We modelled pollen arrival in the bisexual phase,  $P_B$ , from the estimates from this analysis as:

$$P_B = e^{\alpha_2 + \beta_{2.1} V + \beta_{2.2} \text{GSD} + \beta_{2.3} \text{ASD} + \beta_{2.4} V \times \text{GSD} + \beta_{2.5} V \times \text{ASD} + \beta_{2.6} \text{ASD} \times \text{GSD} + \beta_{2.7} V \times \text{ASD} \times \text{GSD}}, \quad \text{Eqn 6}$$

where GSD and ASD are centred on their grand means.

To aid interpretation, we obtained linear ( $\beta$ ) and quadratic selection gradients by multiple regression on the predicted values (transformed to relative fitness). We first included only the linear terms to estimate  $\beta$ . We then included linear, quadratic and pairwise interaction terms to estimate quadratic selection gradients (Lande & Arnold, 1983). Note that the quadratic terms in the regression were multiplied by 2 in order to obtain the diagonal of quadratic selection-gradient

matrix. We also provide mean-standardized selection gradients, as they can be interpreted as percent of selection on fitness (for which  $\beta = 1$ ) and therefore used to estimate the strength of selection (Hansen *et al.*, 2003b; Hereford *et al.*, 2004). Mean-standardized selection gradients are given in percentages. Selection gradients are estimated for both the predicted amount of pollen and the predicted seed set. Seed set is most probably a better measurement of reproductive success than the amount of pollen, but it does not take into account the effect of pollen competition on seed quality. Pollen competition has been shown to influence seed quality in *D. scandens* (Armbruster & Rogers, 2004).

We investigated the curvature of the fitness function using canonical analysis (Phillips & Arnold, 1989). In a canonical analysis, the axes of the quadratic selection matrix are rotated so that the cross-product terms are eliminated. The new axes are linear combinations of the traits given by the eigenvectors of the quadratic selection matrix and the new quadratic selection gradients are the eigenvalues of this matrix. Hence, the new quadratic selection gradients are fewer and orthogonal. This method has been recently used to study natural selection on *Silene virginica* (Reynolds *et al.*, 2010).

There are several benefits of using such a fitness model instead of estimating selection gradients directly from an observed fitness component, as is normally done in selection studies. First, the fitness model estimates functional relationships, which provide insight into the selection process. Secondly, by taking the functional relationships into account it filters away many environmental correlations and some of the noise that may obscure the relationship between the traits and fitness, and it has a higher probability of avoiding problems of unmeasured correlated traits affecting fitness. Lastly, it can incorporate known biological constraints such as the relationship between the amount of pollen and the number of seeds. The downside of this approach is that the use of link functions without explicit biological bases may affect the estimation of quadratic selection gradients, because the link functions affect the curvature. It is therefore important to consider quadratic terms and interactions in the model.

### Statistical analyses

We performed model selection by using AICc scores for the model with binomial errors and QAICc scores for the models with quasipoisson error distribution (Burnham & Anderson, 2002). We evaluated quadratic and interaction terms in addition to the linear terms. The only term that was kept in a model even when it did not improve the AICc or QAICc scores was the linear term for upper bract length in the visitation model (Eqn 3).

The analysis of pollen arrival in the female phase given that the blossom had been visited was performed on 36 blossoms in 11 patches after the exclusion of two outliers that had a strong leverage. The analysis of pollen arrival in

the bisexual phase was performed on 149 observations (blossoms) in 24 patches.

We estimated the 95% confidence intervals for the selection gradients using parametric bootstrapping (Davison & Hinkley, 1997), re-sampling 1000 times the probability,  $V$ , of being visited from binomial distributions, and the pollen arrival in the female phase given visit,  $P_F$ , and the pollen arrival in the bisexual phase,  $P_B$ , from negative binomial distributions. The parameters in Eqns 4, 5 and 6 were re-estimated from each of these runs, by exchanging the old variables for the new (obtained from the parametric distributions), both for the response and for the fixed effects. This gave us 1000 different fitness surface estimates, which we used to calculate confidence intervals.

To estimate the proportion of the variance in blossom traits explained among patches and among blossoms within patch, we performed variance-component analyses with patches as random effects. All analyses were performed in R 2.11.1 (R Development Core Team 2010) using the packages MASS (Venables & Ripley 2002) and lme4 (Bates & Maechler 2010).

## Results

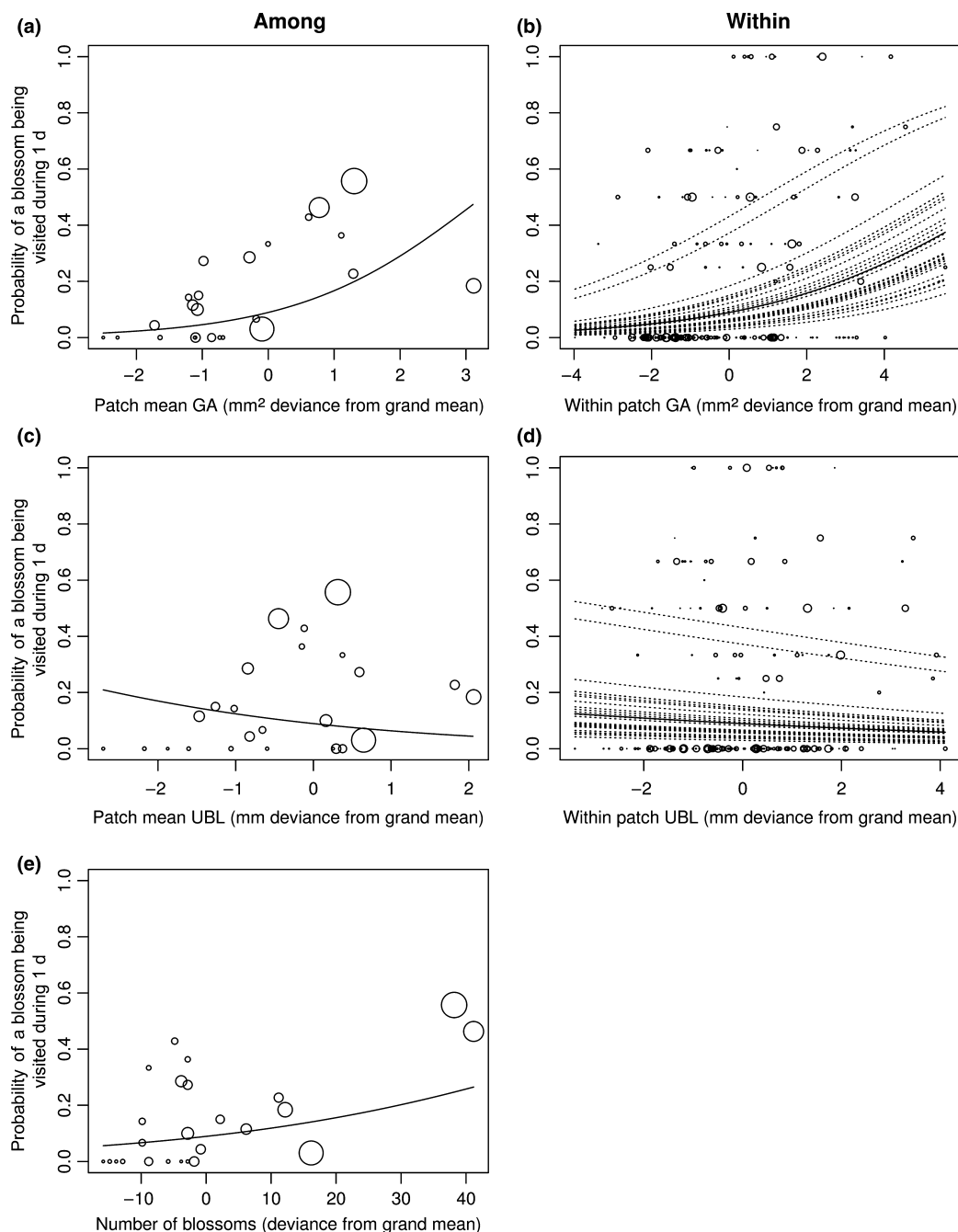
### Patterns of variation

The traits varied more among blossoms within patches than among patches. For bract size, differences among patches explained 36.2% of the variance, while 63.8% was explained by the blossom differences within patches. The pattern was similar for gland area (36.1 vs 63.9%), gland-stigma distance (30.8 vs 69.2%) and anther-stigma distance (20.9 vs 79.1%). The variation among patches may be attributable to both genetic and environmental differences. The variation among blossoms within patches can, in addition to genetic and environmental differences, result from developmental noise in blossoms within a plant.

### Bee visitation

Although we did not detect much influence of bract size on the rate of bee visitation, blossoms with larger resin glands received more visits (Fig. 3, Table 2). Our model predicts that an increase of the gland area by one standard deviation (1.86 mm<sup>2</sup>; 42%) from the population mean (4.44 mm<sup>2</sup>) would nearly double the daily probability of a blossom receiving a visit, from 8.9 to 15.2%. Over 3 d, the median duration of the female phase, the same increase in the gland area would increase the probability of a visit from 24.5 to 39.1%.

Bees also appeared to ignore bract size when selecting between different patches, as they preferentially visited patches with many blossoms and large average gland sizes (Fig. 3, Table 2). Among patches, an increase in mean



**Fig. 3** Effect of gland area (a and b), upper bract length (c and d), and number of blossoms (e) of *Dalechampia schottii* on the probability of a blossom being visited during 1 d, both among patches (a, c, and e) and among blossoms within patches (b and d). Circles differ in size relative to the number of observations. Parameter estimates are from Table 1; in (a) the slope is given by  $\beta_1 + \gamma_1$ , in (c) by  $\beta_2 + \gamma_2$ , and in (e) by  $\gamma_3$ . In (b) and (d), dotted lines give the effect within each patch, given by the estimates for  $\beta_{0l}$  and  $\beta_1$  for (b) and  $\beta_{0l}$  and  $\beta_2$  for (d), where the subscript  $l$  is the patch. The solid lines in (b) and (d) give the mean effects ( $\beta_1$  and  $\beta_2$  with  $\gamma_0$  as the intercept). See Eqn 3 for explanations of the symbols.

gland area by 1 standard deviation ( $1.86 \text{ mm}^2$ ; 42%) from the population mean ( $4.44 \text{ mm}^2$ ) increased the probability of a random blossom in a patch receiving a visit in 1 d from 8.9 to 27.1%. During 3 d this probability increased from 24.5 to 61.2%. Therefore, gland area seemed to have a stronger effect among patches than among blossoms

within patches, although this difference was not statistically significant (Table 2). In comparison, an increase in the number of blossoms by one standard deviation (16.2 blossoms; 79.5%) above the population mean (20.5 blossoms) would increase the probability of a random blossom in a patch being visited during 1 d from 8.9 to 14.9%.



Parameter	Estimate	±SE		%σ <sup>2</sup>
Intercept ( $\gamma_0$ )	-2.320	± 0.294	log odds	
UBL within ( $\beta_1$ )	-0.110	± 0.167	log odds mm <sup>-1</sup>	1.9
UBL among – UBL within ( $\gamma_1$ )	-0.257	± 0.371	log odds mm <sup>-1</sup>	6.3
GA within ( $\beta_2$ )	0.325	± 0.127	log odds mm <sup>-2</sup>	26.4
GA among – GA within ( $\gamma_2$ )	0.389	± 0.331	log odds mm <sup>-2</sup>	17.6
#blossoms ( $\gamma_3$ )	0.030	± 0.020	log odds per #blossoms	15.1

The percent variance explained (%σ<sup>2</sup>) by the log odds of being visited during 1 d is given for each of the fixed factors; the rest of the variance is explained by the three residual terms: day ( $\epsilon_i^{\alpha_1} = 6.3\%$ ), blossom ( $\epsilon_k^{\alpha_2} = 16.0\%$ ), and patch ( $\epsilon_l^{\beta_0} = 10.4\%$ ).

Over a period of three days this probability would increase from 24.5 to 36.6%. Note that the different traits were all positively correlated among patches (Pearson correlations with 95% confidence intervals for 25 patch means: number of blossoms with gland area,  $r = 0.56$  (0.21, 0.78); number of blossoms with bract size,  $r = 0.42$  (0.03, 0.71); gland area with bract size,  $r = 0.61$  (0.29, 0.81)).

### Pollen arrival in the female phase

Blossoms with a high probability of being visited during 1 d had more pollen deposited on their stigma in the female phase than blossoms with a lower probability (blossoms that did not receive any visits were excluded from this analysis; Table 3). When the probability of visitation was low, blossoms with small gland-stigma distance had more pollen deposited on their stigma than blossoms with large gland-stigma distance, while the opposite pattern was found at a high probability of visitation (Table 3). The inflection point was at a visitation probability of 12.5%, which means that the gland-stigma distance had only a slight negative effect at the population mean ( $V = 8.9\%$ ).

### Pollen arrival in the bisexual phase

Gland-stigma distance, anther-stigma distance, the probability of being visited and the different interactions between these three factors all affected the amount of pollen deposited on the stigma during the first day of the bisexual phase

**Table 3** Effect of the probability of being visited in 1 d ( $V$ ) and gland–stigma distance (GSD) on pollen arrival in the female phase of *Dalechampia schottii* given a visit (Eqn 5)

Parameter	Estimate	± SE	
Intercept ( $\alpha_1$ )	-2.436	± 1.692	log $P$
$V$ ( $\beta_{1,1}$ )	12.003	± 4.493	log $P V^{-1}$
$V^2$ ( $\beta_{1,2}$ )	-7.942	± 3.113	log $P V^{-2}$
GSD ( $\beta_{1,3}$ )	-0.119	± 0.513	log $P \text{ mm}^{-1}$
$V \times \text{GSD}$ ( $\beta_{1,4}$ )	0.956	± 0.718	log $P V^{-1} \text{ mm}^{-1}$

$P$ , the number of pollen grains;  $V$ , the probability of being visited in 1 d.

**Table 2** Effect of upper bract length (UBL) and gland area (GA) within and among patches, and number of blossoms of *Dalechampia schottii* (#blossoms) among patches on the probability of a blossom being visited during 1 d (Eqn 3)

(Table 4). The general pattern was that the probability of being visited in 1 d had a positive effect, anther-stigma distance had a negative effect, and gland-stigma distance had both positive and negative effects. The median pollen arrival in the bisexual phase for blossoms without any recorded visits was six, while for blossoms with recorded visits the median was 18.

### Fitness function

We estimated selection gradients on the traits of individual blossoms using the estimates of amount of pollen and seed set from our fitness model, which included an empirically established relationship between amount of pollen and seed set. We observed directional selection for larger glands, smaller bracts, shorter gland-stigma distance, and shorter anther-stigma distance (Fig. 2, Table 5). The directions of selection on bract size and gland-stigma distance were uncertain, however, as their 95% confidence intervals largely overlapped zero (Table 5). We observed moderate to strong selection on all traits, from 6 to 71% of the strength of selection on fitness when pollen arrival was used as fitness currency, and from 13 to 34% when seed set was used as fitness currency. Importantly, all selection gradients, except for on upper bract length, were weaker when we used the

**Table 4** Effect of the probability of being visited in 1 d ( $V$ ) and morphological traits (gland–stigma distance (GSD) and anther–stigma distance (ASD)) on pollen arrival in the bisexual phase of *Dalechampia schottii* (Eqn 6)

Parameter	Estimate	± SE	
Intercept ( $\alpha_2$ )	2.408	± 5.701	log $P$
$V$ ( $\beta_{2,1}$ )	2.363	± 1.889	log $P V^{-1}$
ASD ( $\beta_{2,2}$ )	-0.536	± 0.820	log $P \text{ mm}^{-1}$
GSD ( $\beta_{2,3}$ )	-0.132	± 0.745	log $P \text{ mm}^{-1}$
$V \times \text{ASD}$ ( $\beta_{2,4}$ )	0.338	± 1.828	log $P V^{-1} \text{ mm}^{-1}$
$V \times \text{GSD}$ ( $\beta_{2,5}$ )	0.363	± 2.065	log $P V^{-1} \text{ mm}^{-1}$
ASD $\times$ GSD ( $\beta_{2,6}$ )	0.233	± 0.377	log $P \text{ mm}^{-2}$
$V \times \text{ASD} \times \text{GSD}$ ( $\beta_{2,7}$ )	-0.459	± 0.813	log $P V^{-1} \text{ mm}^{-2}$

$P$ , the number of pollen grains;  $V$ , the probability of being visited in 1 d.

**Table 5** Directional selection gradients with 95% confidence interval at the blossom level using the amount of pollen or seeds as absolute fitness

Trait	Pollen			Seeds		
	$\beta$	$\beta_\sigma$	$\beta_\mu$ in %	$\beta$	$\beta_\sigma$	$\beta_\mu$ in %
UBL (mm)	-0.0067 (-0.0887, 0.0483) mm <sup>-1</sup>	-0.0098 (-0.1307, 0.0712)	-5.75 (-76.52, 41.70)	-0.0096 (-0.0429, 0.0158) mm <sup>-1</sup>	-0.0142 (-0.0632, 0.0232)	-8.30 (-37.03, 13.60)
GA (mm <sup>2</sup> )	0.0572 (0.0008, 0.2240) mm <sup>-2</sup>	0.1065 (0.0015, 0.4174)	25.39 (0.35, 99.51)	0.0329 (0.0069, 0.0861) mm <sup>-2</sup>	0.0612 (0.0128, 0.1604)	14.60 (3.06, 38.25)
GSD (mm)	-0.1507 (-0.6848, 0.4133) mm <sup>-1</sup>	-0.1907 (-0.8665, 0.5229)	-60.13 (-273.20, 164.87)	-0.0272 (-0.3288, 0.2158) mm <sup>-1</sup>	-0.0344 (-0.4160, 0.2731)	-10.84 (-131.16, 86.11)
ASD (mm)	-0.3619 (-0.7674, 0.1409) mm <sup>-1</sup>	-0.4656 (-0.9873, 0.1812)	-71.37 (-151.33, 27.78)	-0.1716 (-0.4354, 0.0462) mm <sup>-1</sup>	-0.2208 (-0.5601, 0.0595)	-33.84 (-85.85, 9.12)

$\beta_\sigma$  is variance standardized, and  $\beta_\mu$  is mean standardized.

UBL, upper bract length; GA, gland area; GSD, gland–stigma distance; ASD, anther–stigma distance.

number of seeds as the fitness currency than when we used the amount of pollen (Table 5). The uncertainty in the strength of selection estimates was large, however, and for all traits but gland area, the 95% confidence interval included zero (Table 5). For gland area, the 95% confidence interval ranged from 3 to 38% of the strength of selection on fitness when we used seed set as fitness currency. For anther-stigma distance the 95% confidence interval ranged from 0 to 85% of selection on fitness when we used seed set as fitness currency.

We observed quadratic selection only for anther-stigma distance. The curvature of the selection was positive and statistically significant with pollen as fitness currency and almost significant with seed set as fitness currency (Table 6). The canonical analysis further showed that there was a saddle point close to the population mean for two of the eigenvectors of the quadratic selection matrix. These two eigenvectors were mainly influenced by gland-stigma distance and anther-stigma distance (Fig. 4). However, the quadratic selection gradients (eigenvalues) in this analysis also had 95% confidence intervals that substantially overlapped zero. Along the two other eigenvectors, mainly influenced by gland area and upper bract length, the fitness surface was predicted to be almost linear in the neighbourhood of the population (Fig. 4).

The frequency of visitation to a patch influences the directional selection gradients. Using the amount of pollen as fitness currency, an increase in the probability of being visited in a patch strengthens selection on gland area and bract size, and weakens selection on gland-stigma distance and anther-stigma distance (Fig. 5). This is not unexpected because, as the amount of cross-pollen went up with an increasing visitation rate during the female phase, the importance of the self-pollen deposited during the bisexual phase greatly decreased. When we used seed set as fitness currency in the model, an increasing visitation rate for a patch weakened the selection on gland-stigma distance and anther-stigma distance, but only slightly influenced selection on gland area and bract area (Fig. 5).

## Discussion

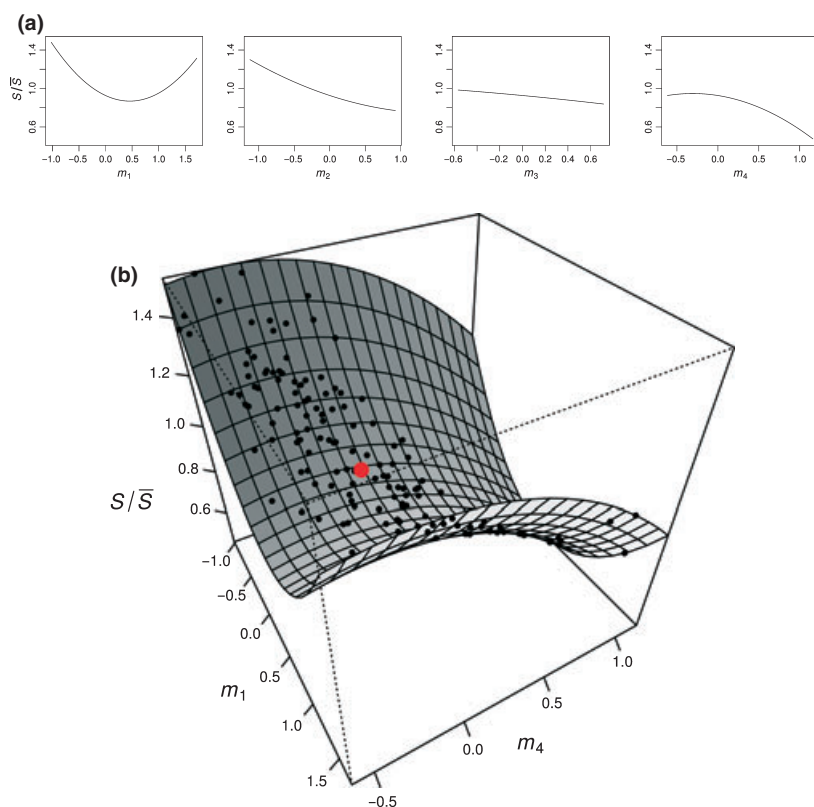
In order to study effects of floral signalling and reward on the reproductive success of individual blossoms in *D. schottii*, we built a fitness function based on functional relationships between traits and measures of reproductive success in individual blossoms. This fitness function revealed that bees chose among blossoms on the basis of the amount of reward offered, as measured by gland area. The variation in visitation rate created variation in the amount of pollen deposited on the stigma and eventually increased reproductive output for blossoms with larger gland size. In contrast, we could not find evidence that bract size influenced the reproductive output of blossoms. It thus appears

**Table 6** Mean-standardized\* quadratic selection matrix at the *Dalechampia schottii* blossom level

	Estimates				2.5% quantile				97.5% quantile			
	UBL	GA	GSD	ASD	UBL	GA	GSD	ASD	UBL	GA	GSD	ASD
UBL	-43.43	-48.68	14.06	10.98	-157.89	-274.28	-269.33	-110.80	154.26	79.72	172.56	180.32
GA		43.59	44.99	-38.08		-19.17	-53.29	-190.84		285.65	378.10	17.30
GSD			68.64	116.85			-136.81	-218.73			940.95	339.72
ASD				86.13				4.33				278.24
UBL	-5.82	-4.53	-13.12	5.63	-68.13	-54.35	-70.67	-15.11	73.48	20.87	31.53	42.03
GA		15.35	8.33	0.20		-3.46	-39.57	-32.09		63.18	66.28	17.81
GSD			15.10	46.03			-12.19	-53.93			127.90	137.48
ASD				0.11				-1.49				57.38

The upper four rows have pollen as absolute fitness, and the lower four rows have seeds as absolute fitness. Estimates are given as percentages (i.e.  $\times 100$ ).

\*The mean standardization is performed by multiplying each element in the quadratic selection matrix by the two corresponding trait means. UBL, upper bract length; GA, gland area; GSD, gland–stigma distance; ASD, anther–stigma distance.

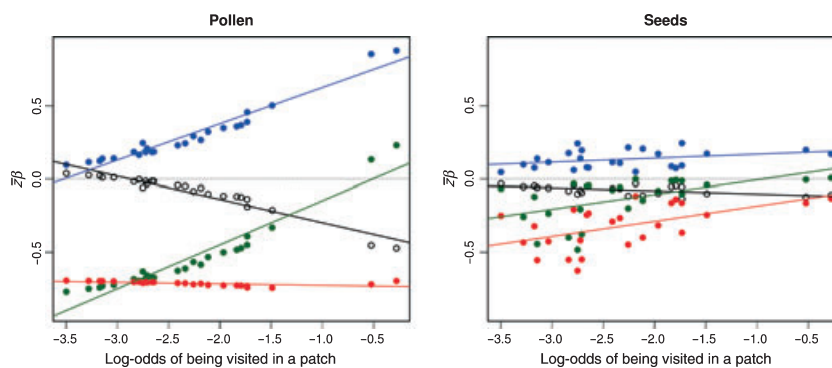


**Fig. 4** Predicted relative number of seeds ( $S/\bar{S}$ ) of *Dalechampia schottii* of the compound traits  $m_1$ ,  $m_2$ ,  $m_3$  and  $m_4$  from the canonical analysis. (a) The separate effects of each compound trait (the nonfocal traits equal zero); (b) the combined effect of  $m_1$  and  $m_4$ . In (b), the small black dots are individual blossom values, and the large red dot is the population mean. The canonical analysis was performed on the mean-standardized quadratic selection matrix (Table 6). Eigenvectors ( $m$ ), slopes ( $\theta$ ) along the new axes and eigenvalues ( $\lambda$ ),  $\theta$  and  $\lambda$  are given in percentages with 95% confidence interval:  $m_1 = (-0.118, 0.171, 0.761, 0.614)$ ,  $\theta_1 = -25.55 (-102.81, 29.96)$ ,  $\lambda_1 = 56.14 (-39.36, 186.39)$ ,  $m_2 = (0.224, -0.944, 0.057, 0.235)$ ,  $\theta_2 = -24.23 (-53.48, -10.27)$ ,  $\lambda_2 = 15.87 (-17.96, 99.27)$ ,  $m_3 = (0.914, 0.275, -0.134, 0.266)$ ,  $\theta_3 = -11.11 (-42.02, 21.61)$ ,  $\lambda_3 = -3.62 (-45.64, 48.29)$ ,  $m_4 = (-0.316, 0.063, -0.632, 0.705)$ ,  $\theta_4 = -13.46 (-104.54, 86.99)$ , and  $\lambda_4 = -43.65 (-117.69, 80.41)$ . The 95% confidence intervals are estimated from linear regressions using our bootstrap replicates of relative fitness as independent variables and composite traits as dependent variables.

that, in *D. schottii*, the resin gland plays a dual role as both signal and reward. Hence, pollinating bees may rely on full-disclosure honesty (i.e. the size of the resin gland) when making their foraging decisions, and do not have to depend on imperfect phenotypic correlations between amount of reward and size or intensity of advertising traits.

In contrast to *D. schottii*, which produces a deep-blue resin, many species of *Dalechampia* produce nearly transparent

(clear or whitish) resins (Armbruster, 1996, 2002). In two of these species, the pollinators base their foraging decisions on the size of the involucre bracts, which is phenotypically and genetically correlated with the size of the resin gland (Armbruster *et al.*, 2005; R. Pérez-Barrales *et al.*, unpublished; genetic correlations were 0.56 and 0.58 for upper and lower bract lengths; see Hansen *et al.*, 2003a). Other species of *Dalechampia* produce resins of



**Fig. 5** Relationship between pollinator environment in each patch and mean-scaled linear selection gradients (estimated at the blossom level). Pollinator environment is the mean log odds of being visited in a patch, given by  $\beta_{0i}$  in Eqn 3. Blue dots show selection gradients for gland area (GA), open circles for upper bract length (UBL), green dots for gland–stigma distance (GSD), and red dots for anther–stigma distance (ASD), and the dashed line is at  $\bar{z}\beta = 0$ . Linear models shown in the graph (intercept  $\pm$  SE; slope  $\pm$  SE), with pollen as absolute fitness: UBL ( $-0.456 \pm 0.0235$ ,  $-0.1588 \pm 0.0096$ ), GA ( $0.8739 \pm 0.0324$ ,  $0.2482 \pm 0.0132$ ), GSD ( $0.153 \pm 0.0465$ ,  $0.3006 \pm 0.019$ ), and ASD ( $-0.7359 \pm 0.0078$ ,  $-0.0102 \pm 0.0032$ ). With seeds as absolute fitness: UBL ( $-0.1232 \pm 0.0166$ ,  $-0.0212 \pm 0.0068$ ), GA ( $0.1962 \pm 0.037$ ,  $0.0269 \pm 0.0151$ ), GSD ( $0.0949 \pm 0.0814$ ,  $0.1003 \pm 0.0333$ ), and ASD ( $-0.0816 \pm 0.081$ ,  $0.1029 \pm 0.0331$ ).

different colours, from yellow to orange, maroon, or green (the resin of *D. schottii* is unique in being deep blue; Armbruster, 1996, 2002). Interestingly, the most colourful resins seem to be produced by the species related to *D. schottii* such as *D. dioscoreifolia*, which produces a maroon resin. Our results suggest that bees can take advantage of this shift in colour to adopt more reliable cues during foraging, but whether this shift in colour is an adaptation to promote full-disclosure honesty remains an open question. An alternative interpretation is that resin colour is not important, and instead different selection pressures in different *Dalechampia* species reflect differences in the behaviour or composition of pollinators. More species of *Dalechampia* would need to be studied to distinguish between alternative possibilities.

Although the benefits of full-disclosure honesty are clear from the pollinator's perspective, they are less obvious from the perspective of the plant. Plants adopting full-disclosure honesty run the risk of lowered visitation rates when the reward is depleted, but may also benefit from it. For example, a high level of honesty may facilitate floral constancy of pollinators in environments where interspecific competition for pollination occurs (sensu Waser, 1983; see also Hansen *et al.*, 2007; Raguso, 2008). Note, however, that the floral resins of *Dalechampia* are very sticky, and all of the resin cannot be removed by bees (Armbruster, 1984). Consequently, very dark resins (e.g. deep blue or dark maroon) may have the appearance of being abundant even when the gland has been scraped and only a thin layer of resin remains. Because we did not manipulate the amount of resin offered by the blossoms, we were not able to estimate whether bees assess it further than preferring blossoms with large resin glands.

The contextual analysis revealed differences in bee visitation among patches vs among blossoms within patches.

Increasing gland area increased visitation by bees both among patches and among blossoms within patches, but the effect was twice as strong among patches. This may reflect how bees process resource information in foraging decisions. For example, bees may assess patch averages in deciding where to collect resin and secondarily make decisions about individual blossoms. Alternatively, this difference could be explained by environmental variation. Several authors have pointed out that environmental variation can create a correlation between fitness and traits which is misinterpreted as evidence for selection (Rausher, 1992; Scheiner *et al.*, 2002; Stinchcombe *et al.*, 2002; Winn, 2004). We can think of only one such environmental correlation potentially influencing our study: if plant growth is limited by photosynthetically active radiation and pollinator visitation is thermally constrained, making blossoms small and visitation rates low in shady environments (see Armbruster & Berg, 1994; Armbruster *et al.*, 2005). While this could be a problem for the effect of bee choice among patches, the effect within patches should be much weaker because blossoms within a patch had similar solar orientation, while the different patches differed in solar orientation. Other environmental correlates that affect both flower traits and seed set, such as soil nutrition (Rausher, 1992; Scheiner *et al.*, 2002; Winn, 2004), should not influence the results in this study because of our fitness model approach.

In addition to visiting patches with large mean gland area, bees visited patches with many blossoms over patches with few blossoms. Attraction of pollinators by floral display is commonly reported in other species (Harder & Barrett, 1995; Harder *et al.*, 2001; Harder & Johnson, 2005), including one *Dalechampia*, *Dalechampia parvifolia* (Armbruster & Mziray, 1987).

The effect of gland area on the reproductive output of blossoms suggests that there is direct selection on the mean

gland area of the plant. As mentioned, the strength of selection on the plant mean of the trait may not be the same as selection on the level of individual blossom if pollinator choice among blossoms does not fully explain pollinator choice among plants. For selection at the blossom level to have evolutionary consequences, there must be blossom variation also at the plant level (differences in plant means) and this variation must have a genetic basis. The former is supported in our study by the large differences in mean gland area among patches, and the latter is suggested by genetic studies of other *Dalechampia* species (Hansen et al., 2003b) and studies suggesting that floral traits generally exhibit significant additive genetic variance (Ashman & Majetic, 2006).

There is, however, additional evidence for direct selection on gland size in *D. schottii*, in that this species has larger glands relative to the bracts when compared with the two species where pollinator selection of blossoms acted primarily on bract size. In *D. schottii* the ratio of mean gland area to mean upper bract area (GA/UBA) was higher than for *D. scandens*, and the ratio of mean gland area to lower bract length (GA/LBL) for *D. schottii* was higher than for *D. ipomeifolia* (*D. schottii*: GA/UBA = 0.085, GA/LBL = 0.47 mm; *D. scandens*: GA/UBA = 0.053; R. Pérez-Barrales et al., unpublished; *D. ipomeifolia*: GA/LBL = 0.14 mm; Armbruster et al., 2005).

Using the variation in pollinator environment among patches, we found that the effect of self-pollination on blossom seed set was lower in patches with high visitation than in patches with low visitation. This fits the theory wherein selfing is always selected for in plants with delayed selfing (as in *Dalechampia*) because of reproductive assurance, but the strength of selection depends on pollinator environment (Morgan & Wilson, 2005).

The estimated strength of directional selection on blossoms traits in our study was moderate to strong (6–71% of the strength of selection on fitness). This falls within what is typically observed in phenotypic selection studies (Hereford et al., 2004), and we remind the reader that the reproductive output of individual blossoms is only a small part of total fitness. Selection was weaker when seed set was used as fitness currency compared with when pollen load was used. Seed set is probably the best fitness currency and gives a better estimate of the strength of selection, but it does not take into account the difference in quality of the seeds resulting from pollen competition. Pollen competition has been shown to affect seed quality in *D. scandens* (Armbruster & Rogers, 2004), and selection gradients obtained by using seed set as fitness currency may therefore underestimate selection.

A long-standing paradox in evolutionary biology is the lack of evolutionary response in the presence of directional selection and additive genetic variance (Frank & Slatkin, 1992; Kruuk et al., 2000; Merilä et al., 2001; Hansen &

Houle, 2004; Walsh & Blows, 2009). Although we observed blossom-level directional selection for increased gland size, several factors may hinder it from evolving even in the presence of additive genetic variation. First, a fitness trade-off between pollinator-mediated selection and other selective forces, such as negative selection by herbivores, could create stabilizing selection. Secondly, multivariate genetic constraints (Hansen & Houle, 2008; Walsh & Blows, 2009), caused for example by trade-off in allocation of resources, could create a singular G-matrix. In *D. scandens*, the evolvability of gland area was substantially reduced when conditioning on other traits (Hansen et al., 2003a). Lastly, fluctuating selection, which is commonly observed in natural populations (Siepielski et al., 2009), can create stabilizing selection on average. Our study population may be under fluctuating selection as a result of fluctuations in the abundance of its alternative pollinators. During our field season, the main pollinator was a large Euglossine bee, but this may be atypical, as *D. schottii* has been classified as a 'small-bee-pollinated' *Dalechampia* (Armbruster, 1993; Hansen et al., 2000), and in the year before our study *Hypanthidium* cf. *melanopterum* was abundant and probably the main pollinator.

In conclusion, this study together with the study on *D. ipomeifolia* and *D. scandens* illustrates the diversity of selective pressures which may shape phenotypic differentiation in closely related species.

## Acknowledgements

The authors thank C. Elizondo, manager of *Jardín Botánico* 'Dr Alfredo Barrera Marin' in Puerto Morelos, who kindly provided permission to conduct this study. V. Rico-Gray, J. Lopez-Portillo and Victor Vazques from Instituto de Ecología in Xalapa provided valuable logistic advice and support. We also thank two anonymous reviewers and the Editor for helpful comments on the manuscript. This research was supported by NSF grant DEB-0444157 through Florida State University to TFH and DEB-0444754 through the University of Alaska to WSA.

## References

- Ackerman JD. 1983. Specificity and mutual dependency of the orchid-euglossine bee interaction. *Biological Journal of the Linnean Society* **20**: 301–314.
- Armbruster WS. 1984. The role of resin in angiosperm pollination: ecological and chemical considerations. *American Journal of Botany* **71**: 1149–1160.
- Armbruster WS. 1985. Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). *Evolution* **39**: 733–752.
- Armbruster WS. 1988. Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. *Ecology* **69**: 1746–1761.

- Armbruster WS. 1990. Estimating and testing the shapes of adaptive surfaces: the morphology and pollination of *Dalechampia* blossoms. *American Naturalist* 135: 14–31.
- Armbruster WS. 1993. Evolution of plant pollination systems: hypotheses and tests with the neotropical vine *Dalechampia*. *Evolution* 47: 1480–1505.
- Armbruster WS. 1996. Cladistic analysis and revision of *Dalechampia* sections *Rhopalostylis* and *Brevicolumnae* (Euphorbiaceae). *Systematic Botany* 21: 209–235.
- Armbruster WS. 2002. Can indirect selection and genetic context contribute to trait diversification? A transition-probability study of blossom-colour evolution in two genera. *Journal of Evolutionary Biology* 15: 468–486.
- Armbruster WS, Antonsen L, Pélabon C. 2005. Phenotypic selection on *Dalechampia* blossoms: honest signaling affects pollination success. *Ecology* 86: 3323–3333.
- Armbruster WS, Berg EE. 1994. Thermal ecology of male euglossine bees in a tropical wet forest: fragrance foraging in relation to operative temperature. *Biotropica* 26: 50–60.
- Armbruster WS, Hansen TF, Pélabon C, Pérez-Barrales R, Maad J. 2009. The adaptive accuracy of flowers: measurement and microevolutionary patterns. *Annals of Botany* 103: 1529–1545.
- Armbruster WS, Mziray WR. 1987. Pollination and herbivore ecology of an African *Dalechampia* (Euphorbiaceae): comparisons with new-world species. *Biotropica* 19: 64–73.
- Armbruster WS, Rogers DG. 2004. Does pollen competition reduce the cost of inbreeding? *American Journal of Botany* 91: 1939–1943.
- Ashman T-L, Majetic CJ. 2006. Genetic constraints on floral evolution: a review and evaluation of patterns. *Heredity* 96: 343–352.
- Bates D, Maechler M. 2010. *lme4: Linear mixed-effects models using Eigen and Eigen++*. R package version 0.999375-34. <http://CRAN.R-project.org/package=lme4>
- Buchmann SL. 1987. The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics* 18: 343–369.
- Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. New York, NY, USA: Springer.
- Caruso CM. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54: 1546–1557.
- Caruso CM. 2001. Differential selection on floral traits of *Ipomopsis aggregata* growing in contrasting environments. *Oikos* 94: 295–302.
- Casper BB, La Pine TR. 1984. Changes in corolla color and other floral characteristics in *Cryptantha humilis* (Boraginaceae): cues to discourage pollinators? *Evolution* 38: 128–141.
- Castro S, Silveira P, Navarro L. 2009. Floral traits variation, legitimate pollination, and nectar robbing in *Polygala vayredae* (Polygalaceae). *Ecological Research* 24: 47–55.
- Cresswell JE, Galen C. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. *American Naturalist* 138: 1342–1353.
- Cresswell JE, Robertson AW. 1994. Discrimination by pollen-collecting bumblebees among differentially rewarding flowers of an alpine wild-flower, *Campanula rotundifolia* (Campanulaceae). *Oikos* 69: 304–308.
- Davison AC, Hinkley DV. 1997. *Bootstrap methods and their application*. New York, NY, USA: Cambridge University Press.
- Dobson HEM, Bergström G. 2000. The ecology and evolution of pollen odors. *Plant Systematics and Evolution* 222: 63–87.
- Dobson HEM, Danielson EM, van Wesep ID. 1999. Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb. (Rosaceae). *Plant Species Biology* 14: 153–166.
- Dressler RL. 1968. Pollination by euglossine bees. *Evolution* 22: 202–210.
- Fenster CB, Cheely G, Dudash MR, Reynolds RJ. 2006. Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *American Journal of Botany* 93: 1800–1807.
- Frank SA, Slatkin M. 1992. Fisher's fundamental theorem of natural selection. *Trends in Ecology and Evolution* 7: 92–95.
- Golding YC, Sullivan MS, Sutherland JP. 1999. Visits to manipulated flowers by *Episyrphus balteatus* (Diptera: Syrphidae): partitioning the signals of petals and anthers. *Journal of Insect Behavior* 12: 39–45.
- Goulson D, Chapman JW, Hughes WOH. 2001. Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *Journal of Insect Behavior* 14: 669–678.
- Hansen DM, Beer K, Müller CB. 2006. Mauritian coloured nectar no longer a mystery: a visual signal for lizard pollinators. *Biology Letters* 2: 165–168.
- Hansen DM, Olesen JM, Mione T, Johnson SD, Müller CB. 2007. Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait. *Biological Reviews* 82: 83–111.
- Hansen TF, Armbruster WS, Antonsen L. 2000. Comparative analysis of character displacement and spatial adaptations as illustrated by the evolution of *Dalechampia* blossoms. *American Naturalist* 156: S17–S34.
- Hansen TF, Armbruster WS, Carlson ML, Pélabon C. 2003a. Evolvability and genetic constraint in *Dalechampia* blossoms: genetic correlations and conditional evolvability. *Journal of Experimental Zoology* 296B: 23–39.
- Hansen TF, Houle D. 2004. Evolvability, stabilizing selection, and the problem of stasis. In: Pigliucci M, Preston KA, eds. *Phenotypic integration: studying the ecology and evolution of complex phenotypes*. Oxford, UK: Oxford University Press, 130–150.
- Hansen TF, Houle D. 2008. Measuring and comparing evolvability and constraint in multivariate characters. *Journal of Evolutionary Biology* 21: 1201–1219.
- Hansen TF, Pélabon C, Armbruster WS, Carlson ML. 2003b. Evolvability and genetic constraint in *Dalechampia* blossoms: components of variance and measures of evolvability. *Journal of Evolutionary Biology* 16: 754–766.
- Harder LD, Barrett SCH. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* 373: 512–515.
- Harder LD, Johnson SD. 2005. Adaptive plasticity of floral display size in animal-pollinated plants. *Proceedings of the Royal Society B-Biological Sciences* 272: 2651–2657.
- Harder LD, Williams NM, Jordan CY, Nelson WA. 2001. The effect of floral design and display on pollinator economics and pollen dispersal. In: Chittka L, Thomson JD, eds. *Cognitive ecology of pollination: animal behaviour and floral evolution*. Cambridge, UK: Cambridge University Press, 297–317.
- Heisler IL, Damuth J. 1987. A method for analyzing selection in hierarchically structured populations. *American Naturalist* 130: 582–602.
- Hereford J, Hansen TF, Houle D. 2004. Comparing strengths of directional selection: how strong is strong? *Evolution* 58: 2133–2143.
- Hodges SA. 1995. The influence of nectar production on hawkmoth behavior, self-pollination, and seed production in *Mirabilis multiflora* (Nyctaginaceae). *American Journal of Botany* 82: 197–204.
- Howell AD, Alarcón R. 2007. *Osmia* bees (Hymenoptera: Megachilidae) can detect nectar-rewarding flowers using olfactory cues. *Animal Behaviour* 74: 199–205.
- Johnson SD. 2000. Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behaviour. *Biological Journal of the Linnean Society* 71: 119–132.
- Kingsolver JG, Schemske DW. 1991. Path analyses of selection. *Trends in Ecology and Evolution* 6: 276–280.

- Kruuk LEB, Clutton-Brock TH, Slate J, Pemberton JM, Brotherstone S, Guinness FE. 2000. Heritability of fitness in a wild mammal population. *Proceedings of the National Academy of Sciences, USA* 97: 698–703.
- Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* 33: 402–416.
- Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.
- Lessells CM, Boag PT. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104: 116–121.
- Lunau K. 2000. The ecology and evolution of visual pollen signals. *Plant Systematics and Evolution* 222: 89–111.
- Makino TT, Sakai S. 2007. Experience changes pollinator responses to floral display size: from size-based to reward-based foraging. *Functional Ecology* 21: 854–863.
- Maynard Smith J, Harper D. 2003. *Animal signals*. New York, NY, USA: Oxford University Press.
- Merilä J, Sheldon BC, Kruuk LEB. 2001. Explaining stasis: microevolutionary studies in natural populations. *Genetica* 112: 199–222.
- Mitchell RJ. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modelling. *Functional Ecology* 6: 123–129.
- Mitchell RJ. 1993. Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field. *Evolution* 47: 25–35.
- Mitchell RJ, Shaw RG, Waser NM. 1998. Pollinator selection, quantitative genetics, and predicted evolutionary responses of floral traits in *Penstemon centranthifolius* (Scrophulariaceae). *International Journal of Plant Sciences* 159: 331–337.
- Mitchell RJ, Waser NM. 1992. Adaptive significance of *Ipomopsis aggregata* nectar production: pollination success of single flowers. *Ecology* 73: 633–638.
- Morgan MT, Wilson WG. 2005. Self-fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* 59: 1143–1148.
- Phillips PC, Arnold SJ. 1989. Visualizing multivariate selection. *Evolution* 43: 1209–1222.
- R Development Core Team. 2010. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. URL <http://www.R-project.org>.
- Raguso RA. 2008. Wake up and smell the roses: the ecology and evolution of floral scent. *Annual Review of Ecology Evolution and Systematics* 39: 549–569.
- Rausher MD. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46: 616–626.
- Reynolds RJ, Dudash MR, Fenster CB. 2010. Multiyear study of multivariate linear and nonlinear phenotypic selection on floral traits of hummingbird-pollinated *Silene virginica*. *Evolution* 64: 358–369.
- Schaefer HM, Schaefer V, Levey DJ. 2004. How plant-animal interactions signal new insights in communication. *Trends in Ecology and Evolution* 19: 577–584.
- Scheiner SM, Donohue K, Dorn LA, Mazer SJ, Wolfe LM. 2002. Reducing environmental bias when measuring natural selection. *Evolution* 56: 2156–2167.
- Scheiner SM, Mitchell RJ, Callahan HS. 2000. Using path analysis to measure natural selection. *Journal of Evolutionary Biology* 13: 423–433.
- Schemske DW, Bradshaw HD. 1999. Pollinator preference and the evolution of floral traits in monkey flowers (*Mimulus*). *Proceedings of the National Academy of Sciences, USA* 96: 11910–11915.
- Siepielski AM, DiBattista J, Carlson SM. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecology Letters* 12: 1261–1276.
- Stinchcombe JR, Rutter MT, Burdick DS, Tiffin P, Rausher MD, Mauricio R. 2002. Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *American Naturalist* 160: 511–523.
- Thorp RW, Briggs DL, Estes JR, Erickson EH. 1975. Nectar fluorescence under ultraviolet irradiation. *Science* 189: 476–478.
- Venables WN, Ripley BD. 2002. *Modern applied statistics with S, 4th edn*. Springer: New York.
- Walsh B, Blows MW. 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology Evolution and Systematics* 40: 41–59.
- Waser NM. 1983. Competition for pollination and floral character differences among sympatric species: a review of the evidence. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination ecology*. New York, NY, USA: Van Nostrand Reinhold, 277–293.
- Weiss MR. 1991. Floral color changes as cues for pollinators. *Nature* 354: 227–229.
- Weiss MR. 1995. Floral color change: a widespread functional convergence. *American Journal of Botany* 82: 167–185.
- Williams NH, Dodson CH. 1972. Selective attraction of male euglossine bees to orchid floral fragrances and its importance in long distance pollen flow. *Evolution* 26: 84–95.
- Winn AA. 2004. Natural selection, evolvability and bias due to environmental covariance in the field in an annual plant. *Journal of Evolutionary Biology* 17: 1073–1083.