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Pollinator-mediated selection on flower-tube length in a hawkmoth-pollinated *Gladiolus* (Iridaceae)

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Darwin's mechanistic model whereby selection favours plants with flower tubes that exceed the tongue length of the primary pollinator, was tested using unmanipulated plants of the hawkmoth-pollinated South African iris, *Gladiolus longicollis*. The study population was characterized by exceptionally large phenotypic variation in flower-tube length (range 56–129 mm). Directional selection on tube length was revealed by a significant positive relationship between this trait and both fruit and seed set. Selection was attributed to the effect of tube length on pollen receipt, as supplemental hand pollinations showed fruit and seed set in the population to be pollen limited. Indirect selection on tube length may also occur through the correlation of this trait with inflorescence height, although direct selection on the latter trait was significant only for seed set. The main pollinators at the study site were individuals of the large hawkmoth *Agrius convolvuli* that had tongue lengths of 85–135 mm. Other hawkmoths had tongues that were much too short to reach the nectar in *G. longicollis* flowers and seldom carried pollen of *G. longicollis*. Flowers with tubes shorter than the tongues of *A. convolvuli* are apparently not effectively pollinated because stigmas do not contact the moth's head effectively. This study demonstrates that selection may occur among plants with natural phenotypic variation in flower-tube length, and supports Darwin's model of pollinator-mediated selection.

Keywords: *Agrius convolvuli*; flower tube; flower morphology; *Gladiolus longicollis*; hawkmoth pollination; selection

1. INTRODUCTION

A traditional view of flower shape is that it reflects differences in foraging preferences and morphology of pollinators (Faegri & van der Pijl 1966; Johnson & Steiner 2000). Flower shape affects plant fitness through both attraction of pollinators to the floral display (cf. Herrera 1989), and efficiency of pollination due to the mechanical 'fit' between plant and pollinator (cf. Nilsson 1988; Campbell *et al.* 1996). Examples of morphological traits that influence the functional fit between pollinator and plant include width of the corolla tube (Campbell *et al.* 1996), column length in orchids (R. Alexandersson and J. Ågren, unpublished data) and length of the flower tube or spur (Nilsson 1988; Johnson & Steiner 1997).

Many plant species have a narrow flower tube (or spur) that usually contains nectar as a reward for flower visitors. Darwin (1862) reasoned that the flower tube should be longer than the pollinator's tongue to facilitate removal and deposition of pollen. To maximize nectar uptake, the pollinator must insert its tongue as far as possible into the flower and, if the tube is longer than the length of the tongue, the pollinator's head will be pressed against the anthers or the stigma (figure 1), resulting in pollen removal and deposition. We expect to find directional pollinator-mediated selection for longer flower tubes up to a limit where opposing selection pressures will stabilize selection. Opposing selection pressures could be limitation of resources for flower production or pollinator avoidance of flowers with inaccessible rewards (cf. Creswell 1998; Galen 1999).

Experimental manipulations of tube or spur length have shown a positive relationship between this trait and reproductive success (Nilsson 1988; Johnson & Steiner 1997). Nilsson (1988) experimentally shortened the spurs in the two hawkmoth-pollinated orchids *Platanthera bifolia* and *P. chlorantha*, resulting in a decrease in both removal and deposition of pollinia. Johnson & Steiner (1997) shortened spur length in the orchid *Disa draconis* and found a significant decrease in fruit production, but no difference in removal of pollinia. One of the weaknesses of tube-shortening experiments is that they do not reveal the consequences for plants of having tubes that are longer than the population average, and are thus ambiguous with respect to directional versus stabilizing or disruptive selection. However, it has proved difficult to document selection acting on tube length in natural, unmanipulated plant populations. Herrera (1993), for example, found no correlation between spur length and seed production in a five-year study of the hawkmoth-pollinated *Viola cazorlensis*. Nilsson (1988) documented a positive relationship between spur length and fruit set in a population of *P. bifolia*, when pooling data from 15 years. More recently, Maad (2000) found directional selection for longer spurs in the same species during one of the three years of her

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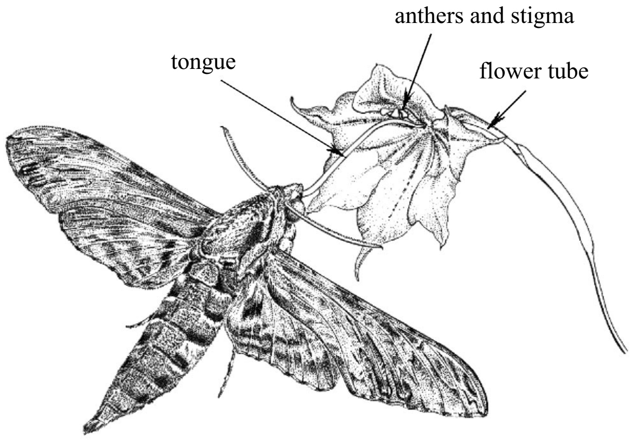

Figure 1. The hawkmoth, *Agrius convolvuli*, probing a flower of *Gladiolus longicollis*. Flower tube, anthers, stigma and the tongue of the hawkmoth are indicated.

study. One factor that might make it difficult to detect selection in fitness-correlated traits, such as spur length, is that such traits are often characterized by low phenotypic variation (Creswell 1998; R. Alexandersson and J. Ågren, unpublished data). Therefore, it can be difficult to detect a relationship between floral spur or tube length and reproductive success in natural populations, even when a functional relationship actually exists.

The iris *Gladiolus longicollis* Baker shows tremendous morphological variation in flower-tube length among populations in southern Africa (Goldblatt & Manning 1998; R. Alexandersson and S. D. Johnson, unpublished data). Generally, populations in the northern part of the distribution consist of long-tubed plants (tubes up to 130 mm), while populations situated in the south consist mainly of short-tubed plants (tubes as short as 35 mm). We studied a large population in the zone where the distributions of the long- and the short-tubed morphs coincide. In this area, which might represent a hybrid zone between pollination ecotypes, we expected to find greater phenotypic variation in tube length than in other parts of the species' distribution.

The aims of this study were to determine: (i) whether a positive correlation exists between tube length and reproductive success in *G. longicollis*; and (ii) whether such a correlation, if it does exist, can be explained by Darwin's hypothesis that plants with flower tubes that exceed the length of the pollinator's tongue will be favoured by natural selection (Darwin 1862).

2. MATERIAL AND METHODS

(a) *Study species*

Gladiolus longicollis (Iridaceae) is a self-incompatible (F. Field and S. D. Johnson, unpublished data), perennial herb, with creamy yellow or white flowers possessing a narrow flower tube 35–130 mm in length that contains nectar at the base (figure 1; Goldblatt & Manning 1998). It grows in low open grassland, and fire in the preceding winter is crucial for the stimulation of flowering. It is widespread in southern Africa, and occurs from the southern Cape up to the Northern Province and Swaziland (Goldblatt & Manning 1998). In the past, the species has been divided into two subspecies: short-tubed subspecies *longicollis* and long-tubed subspecies *platypetalus*, but current taxonomic opinion is that the floral variation is clinal and not easily partitioned into intraspecific categories (Goldblatt & Manning 1998).

Flowering in *G. longicollis* occurs in spring to early summer, and plants normally produce a single flower, although up to seven flowers can occur on an inflorescence (R. Alexandersson, personal observation). The flower is closed during the daytime and opens gradually from about 17.00 on sunny days, or earlier during cloudy conditions. The flower produces a strong sweet fragrance during the evening. There are few records of pollinators, but the long-tongued, nocturnal hawkmoth *Agrius convolvuli* (L.) (Sphingidae) has been documented as a flower visitor in long-tubed populations in Swaziland (Goldblatt & Manning 1998). Fruit maturation occurs three to four weeks after flower senescence.

(b) *Study site*

The study was carried out during November and December 1999 on Mount Gilboa (29°19′ S, 30°17′ E), about 30 km north of Pietermaritzburg in KwaZulu–Natal, South Africa. The altitude at the study site is about 1700 m above sea level. The site could be characterized as low open grassland with rocky outcrops. The area was burnt during the winter of the same year in which the study was performed.

(c) *Floral traits and treatments*

To determine if there was a correlation between female reproductive success and flower-tube length, we marked 289 single-flowered plants (*ca.* 95% of plants in the population had a single flower) with a small piece of red tape around the stem and measured tube length to the nearest mm with digital calipers. Aware of the possibility of selection operating on a correlated character (e.g. Lande & Arnold 1983), we also measured inflorescence height to the nearest cm with a metre rule, as preliminary analyses at Mount Gilboa had indicated that this trait is correlated with tube length ($r = 0.31$; $p = 0.02$; $n = 60$). Other display traits, such as horizontal and vertical diameters of the open flower, were not measured in all plants, as preliminary analyses showed that they were not significantly correlated with tube length ($r = 0.07$ and 0.17, respectively; $n = 60$). At the end of the flowering season, mature fruits were picked and the number of seeds counted.

To determine if fruit and seed production were limited by pollen availability, 15 plants were randomly chosen for supplemental hand pollination. Pollen used for hand pollination was taken from a donor plant at least 5 m away from the recipient plant. Fruit and seed set in these plants were compared with randomly chosen unmanipulated control plants.

To find the minimum tongue length required by a pollinator to reach the nectar in the tube of a *G. longicollis* flower, 15 randomly chosen flowers (tube-length range: 84–108 mm) were picked between 16.30 and 18.00 h, which was shortly before the activity period of hawkmoths. The bracts covering the base of the tube (figure 1) were removed and the flowers were backlit with a flashlight so that the nectar level was visible through the wall of the flower tube. The distance from the constricted entrance of the flowers to the top level of the nectar was measured using digital calipers.

(d) *Hawkmoths*

To quantify the pollinator fauna, hawkmoths were caught using a light trap equipped with a 250 W mercury vapour lamp.

Moths were captured on four evenings between 27 November and 16 December from about 18.30 to 21.30 h. To determine whether captured hawkmoths carried pollen, the forehead, base of the antennae and tongue were scrubbed with a piece of sticky fuschin-gel (Beattie 1971). Each sample was placed on a slide, investigated under a microscope, and presence of *G. longicollis* pollen was recorded. The tongue length of captured hawkmoths was measured to the nearest mm using a ruler.

(e) *Statistics*

We used least-square regression for seed-set data and logistic regression for fruit-set data to assess the form and strength of selection. We calculated univariate (single-trait) regression coefficients (i.e. selection differentials) to estimate the total strength of selection acting on a trait (including selection acting indirectly through correlated traits). Multivariate (multiple-trait) regression coefficients (i.e. selection gradients) were calculated to quantify the strength and mode of selection acting on a trait independently of variation in the other characters included in the regression model. The regression coefficients estimate the linear directional selection coefficients (β) and nonlinear selection coefficients (γ) acting on the traits examined. We also included the interaction between traits to assess possible correlational selection (Lande & Arnold 1983; Maad 2000).

Logistic regression is most appropriate for fruit-set data as the response variable is binary (Hosmer & Lemeshow 1989), but logistic coefficients are not directly comparable with the selection differentials and gradients obtained from least-square regression. Therefore, logistic regression coefficients were transformed to approximate selection differentials and gradients, which are possible to compare straightforwardly with coefficients obtained from least-square regressions (Janzen & Stern 1998).

Relative fitness was regressed on standardized trait values in all analyses. Relative fitness was calculated by dividing an individual's absolute fitness by the population average. Trait values were standardized by subtracting the individual trait value from the population mean and dividing by the standard deviation.

The confidence intervals for regression coefficients were obtained from 5000 bootstrap iterations (Efron & Tibshirani 1991).

3. RESULTS

The mean tube length (± s.d.) of *G. longicollis* at the study site was 93 ± 9 mm (range: 56–129 mm, $n = 289$). Herbivores damaged about one-third of the marked plants, which decreased the tube-length range of plants used in the selection experiments to 72–115 mm ($n = 204$; figure 2*a*). The mean distance (± s.e.) from the entrance of the flower to the nectar was 83 ± 2 mm and the range was 67 to 97 mm ($n = 15$).

Seven species of hawkmoth were caught and four of these carried pollen (table 1). The only hawkmoth species with a tongue longer than 45 mm was *A. convolvuli* (table 1, figure 2*d*). This long-tongued *A. convolvuli* was the most commonly caught hawkmoth species and 80% of caught individuals ($n = 15$) carried *G. longicollis* pollen (table 1). The three individuals that did not carry pollen were characterized by exceptionally long tongues (120, 130 and 135 mm; figure 2*d*).

Supplemental hand pollination increased fruit and seed set significantly. All hand-pollinated plants (not eaten by herbivores) produced a fruit ($n = 10$), while only 67% of the open-pollinated plants ($n = 204$) produced a fruit (d.f. = 1; $\chi^2 = 4.8$; $p = 0.03$). Supplemental hand pollination increased mean seed set (± s.e.) per flower by two and a half times, from 34 ± 3 seeds per plant in open-pollinated plants to 85 ± 13 seeds per plant in hand-pollinated plants ($Z = 3.63$, $p = 0.0003$; Mann–Whitney *U*-test).

In general, female reproductive success varied significantly with the length of the flower tube. Overall, plants producing a fruit had longer tubes ($x \pm \text{s.e.} = 94.3 \pm 0.7$ mm, $n = 137$) than those that did not set fruit (89.6 ± 1.1 mm, $n = 67$) (d.f. = 202, $t = 3.6$, $p = 0.003$). Univariate selection analysis showed highly significant positive linear selection on tube length (table 2), both via fruit and seed set. The analysis also detected a positive relationship between inflorescence height and seed set.

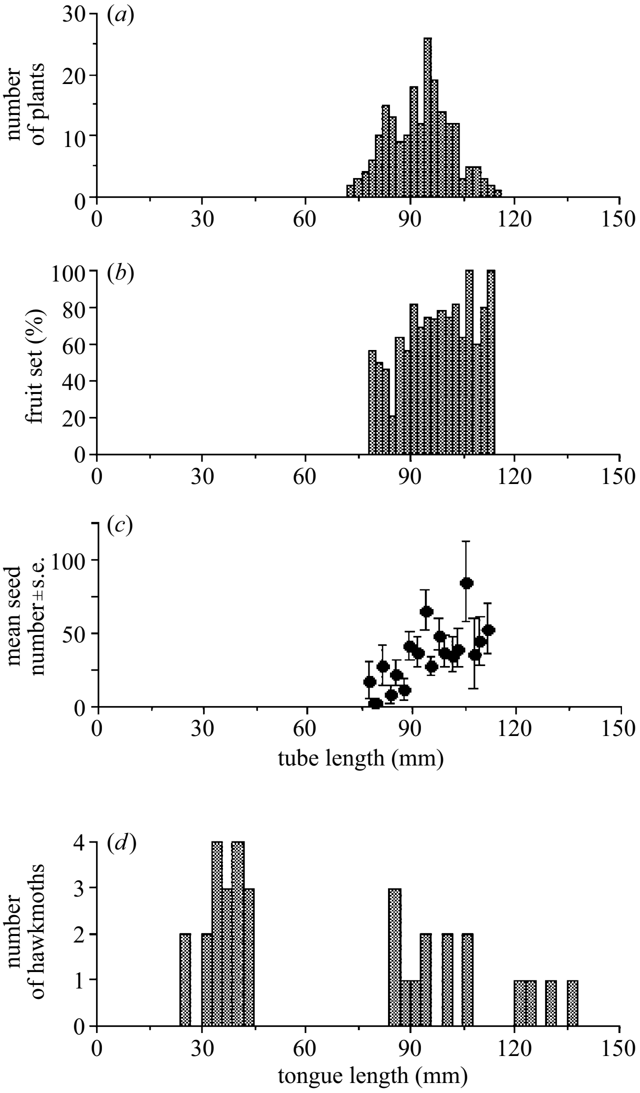

Figure 2. (*a*) The distribution of flower-tube length in the study population of *Gladiolus longicollis*. (*b*) The relationship between flower-tube length and fruit set. All bars show an interval of 2 mm in tube length, except the first and last bar, showing the interval less than 78 mm and equal to or more than 110 mm, respectively. (*c*) The relationship between flower-tube length and mean seed set (± s.e.) per flower. Interval of each point, see (*b*). (*d*) The tongue-length distribution for hawkmoths caught at Mount Gilboa (see table 2 for details).

Table 1. Tongue length and presence of *Gladiolus longicollis* pollen on hawkmoths caught during four evenings using a light trap at Mount Gilboa, KwaZulu–Natal, South Africa.

species	mean tongue length ± s.d. (mm)	range (mm)	hawkmoths carrying *Gladiolus* pollen (%)	*n*
Agrius convolvuli	102.9 ± 6.7	85.0–35.0	80	15
Basiotha schenki	39.5 ± 2.9	35.04–2.0	33	6
Hippotion celerio	36.8 ± 1.7[a]	32.0–42.0[a]	50	8
Nephele sp.	37.0 ± 3.0	34.0–40.0	0	3
Theretra cajus	27.0 ± 2.6	25.0–30.0	33	3
Theretra capensis	35.0	—	0	1

[a] $n = 5$.

The multivariate analysis revealed strong directional selection on tube length (fruit and seed set) and inflorescence height (seed set). This analysis also detected significant, but weaker effects of stabilizing selection on inflorescence height (negative γ) and interacting effects of flower-tube length and inflorescence height.

4. DISCUSSION

This study provides several lines of evidence for directional selection on flower-tube length in a natural plant population. The mean tube length of plants producing a fruit was significantly longer than in plants not producing a fruit. Indeed, the probability of producing a fruit increased from roughly 50% to almost 100% over the range of flower-tube length (figure 2*b*) and tube length showed a significant linear correlation with both fruit and seed set in the selection analyses (table 2). Morphometric data showed that flowers with longer tubes enjoy a higher probability that a visiting hawkmoth would have a tongue that is shorter than the flower tube (figure 2*a*,*d*), thus lending support for Darwin's (1862) mechanistic model of selection.

It is unlikely that the strong selection on tube length is mediated solely via correlated traits. On the one hand, in the present study, flower size (measured as horizontal and vertical diameter) was not correlated with tube length. Inflorescence height, on the other hand, correlated significantly with tube length and had a weak indirect effect on fruit production (table 2). The positive relationship between seed set and inflorescence height may reflect a display effect (e.g. Peakall & Handel 1993). Nevertheless, tube length overwhelms the effect of inflorescence height in its direct effect on seed set. Other traits that may be correlated with tube length that were not measured in this study include ovule number and nectar production. Ovule number is unlikely to explain the variance in reproductive success among plants, as supplemental hand pollinations showed clearly that seed set was pollen limited. It was not possible to measure nectar production for each plant without destructive sampling (see figure 2). Other studies have shown high coefficients of variation for nectar production, most proabably reflecting environmental vagaries (reviewed in Creswell 1998).

Hawkmoths with tongues that are excessively long compared with the length of the flower tube will not pick up pollen efficiently. An interesting observation was that the three moths that did not carry pollen had very long tongues. Two of these three individuals of *A. convolvuli* had tongues that measured 130 and 135 mm, respectively, which is longer than any flower tube measured in the population (figure 2*a*,*d*). The third had a tongue of 120 mm, which was just shorter than the longest measured flower tube (129 mm). This is in accordance with the model, as these moths could not press their foreheads against anthers during flower visits.

The directional-selection gradients on flower-tube length ($\beta = 0.121$ and 0.357, for seed set and fruit set, respectively; table 2) are in the upper range of directional-selection gradients reported in other studies. Hoekstra *et al.* (2001) recently reviewed selection gradients from 63 studies and found that the median directional-selection gradient for reproductive selection of β is 0.180, and a mean value of β is 0.250 (including plants, vertebrates and invertebrates). Thus pollinator-mediated selection can be a rather strong selection pressure during certain years. If long-tubed individuals are that much more successful than short-tubed plants, why do not all plants possess long tubes? At least three factors may maintain the relatively wide range in flower-tube length. First, the study population is situated in the zone between long- and short-tubed populations, so that gene flow from these populations could maintain high phenotypic variance. Second, occasional visits by short-tongued hawkmoths could contribute to the maintenance of short-tubed plants in the populations. During the year of this study, the long-tongued *A. convolvuli* was the most commonly observed hawkmoth (table 1), but in other years short-tongued hawkmoths may be more frequent. A few individual short-tongued hawkmoths carried *G. longicollis* pollen, but as their maximum tongue length was only 42 mm (table 1) and the minimum tongue length required to reach the nectar was 67 mm, they are not able to obtain nectar from *G. longicollis*. Short-tongued hawkmoths probably stop visiting the plants after a few unrewarding visits, as documented in bumble-bees visiting non-rewarding plants (Heinrich 1979) and artificial non-rewarding flowers (Smithson & Macnair 1997). Visits by short-tongued hawkmoths to flowers of *G. longicollis* have been observed in the study population (S. D. Johnson, personal observation). Third, herbivory can favour smaller plants and flowers (Herrera 2000). However, at least during this year, the incidence of being eaten by herbivores did not vary significantly with the length of the flower tube in *G. longicollis* (logistic regression coefficient = −0.002, $\chi^2 = 0.01$, $p = 0.9$, $n = 289$).

Table 2. Univariate and multivariate selection on fruit set (analysed by logistic regression) and seed set (by multiple regression) in a population of *Gladiolus longicollis*. Univariate directional (β) and non-directional (γ) selection differentials (approximate selection differentials for fruit set; see § 2) include both direct effect of traits and indirect effects of correlated traits, while multivariate directional (β) and non-directional (γ) selection gradients (approximate selection gradients for fruit set) only include direct effects of traits on female reproductive success ($n = 204$). Confidence intervals (CI) were evaluated by bootstrapping.

		selection differentials (univariate selection)				selection gradients (multivariate selection)			
		fruit set		seed set		fruit set		seed set	
trait	form of selection	β or γ[a]	95% CI	β or γ[a]	95% CI	β or γ[a]	95% CI	β or γ[a]	95% CI
tube length	directional	0.122***	0.052–0.192	0.447***	0.224–0.658	0.121**	0.047–0.195	0.357**	0.114–0.582
tube length	non-directional	−0.014	−0.078–0.050	−0.095	−0.282–0.105	−0.040	−0.106–0.026	−0.184	−0.441–0.052
inflorescence height	directional	0.042	−0.024–0.108	0.393***	0.169–0.614	0.006	−0.064–0.076	0.275*	0.050–0.497
inflorescence height	non-directional	−0.041	−0.095–0.013	−0.099	−0.295–0.065	−0.064*	−0.124–(−0.004)	−0.148	−0.318−0.024
inflorescence height × tube length	correlational	—	—	—	—	0.096*	0.010–0.182	0.207	−0.060−0.456

[a] * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

In conclusion, this study shows evidence for Darwin's (1862) mechanism of pollinator-mediated selection on flower-tube length in a plant population that exhibits specialization for pollination by a long-tongued hawkmoth species. Selection imposed by pollinator morphology in different geographical regions may explain the tremendous diversification in flower-tube length in *G. longicollis* and other plant species in southern Africa (cf. Johnson & Steiner 1997). Studies over a larger geographical area are being planned to test whether the expected correlation between pollinator and plant morphology in populations of *G. longicollis* does indeed exist.

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