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# Geographical covariation and local convergence of flower depth in a guild of fly-pollinated plants

Bruce Anderson<sup>1,2</sup> and Steven D. Johnson<sup>1</sup>

<sup>1</sup>School of Biological and Conservation Sciences, University of KwaZulu-Natal, P. Bag X01, Scottsville, Pietermaritzburg 3209, South Africa; <sup>2</sup>Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa

## Summary

Author for correspondence:

Bruce Anderson

Tel: +27 21 8083586

Email: [banderso.bruce@gmail.com](mailto:banderso.bruce@gmail.com)

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**Key words:** adaptation, divergent evolution, coevolution, convergent evolution, geographic mosaic of coevolution, maladaptation, mimicry, pollination syndrome.

- Plant adaptations to pollinators are usually studied at the species level, but are expected to occur at the local population level and be reflected in fine-scale patterns of floral variation.
- Here, we examined whether a guild of *c.* 20 South African plant species pollinated by the long proboscis fly *Prosoeca ganglbaueri* (Nemestrinidae) exhibits fine-scale patterns of geographical covariation and convergent evolution at a local scale.
- Fly proboscis length is highly variable among sites (20–50 mm). Plant adaptation results in floral depths of plants within the guild being closely matched with the proboscis length of their fly pollinator across numerous sites. This results in patterns of divergence among allopatric populations and convergence among species within a site.
- The most likely evolutionary processes driving these patterns include coevolution between the fly and plants with consistent and abundant rewards, as well as one-sided evolution in rare and nonrewarding species that do not influence the coevolutionary process. Pollinator-mediated selection on spur length was confirmed for a nonrewarding orchid species in the guild by a reciprocal translocation experiment. Thus, rarer and nonrewarding species in the guild are forced to keep pace with the coevolutionary race between common rewarding flowers and flies.

## Introduction

Natural selection is seldom more apparent than when totally unrelated organisms have very similar traits resulting from similar selective pressures acting on them. This pattern of convergent evolution is most striking when organisms use nonhomologous parts to arrive at the same solution to a common problem. Some of the most striking examples of convergent evolution occur in guilds of unrelated plants that are adapted to a common pollinator or functional group of pollinators (Fenster *et al.*, 2004). These ‘floral syndromes’ are most evident when pollination systems are specialized (Johnson & Steiner, 2000). Typically, plants show convergence in both advertising traits (e.g. colour) and morphological traits that accommodate the pollinator’s body, and thereby result in effective pollen transfer (Fenster *et al.*, 2004). An example of the latter is that flowers pollinated exclusively by long-tongued insects often have correspondingly long nectar spurs or corolla tubes (e.g. Nilsson, 1988; Johnson & Steiner, 1997; Whittall & Hodges, 2007; Anderson & Johnson, 2008; Pauw *et al.*, 2009).

Studies of floral syndromes have traditionally been limited to general descriptions of similarities among flowers pollinated by a particular pollinator or group of pollinators (Brown & Kodric-Brown, 1979; Johnson & Bond, 1994; Goldblatt & Manning, 2000). Although some recent studies have gone beyond this by using multivariate analyses to show similarities and convergence between taxa pollinated by the same pollinator (e.g. Wilson *et al.*, 2004; Ollerton *et al.*, 2003), few have considered fine-scale patterns of convergence at the population level. Most population-level studies of floral adaptation in plants have been carried out in the context of pollination ecotypes, the formation of which may be very important in the process of plant speciation (Coyne & Orr, 2004). One of the earliest was Grant & Grant’s (1965) study of pollination ecotypes in *Gilia leptantha*, which seems to have undergone pollinator shifts between bees and bee-flies, as reflected in floral variation across its range. A number of studies have subsequently demonstrated firm connections between population-level variation in floral traits and shifts between different pollinators (e.g. Johnson, 1997; Johnson & Steiner, 1997; Nattero & Cocucci, 2007). In other

cases, ecotypes may reflect adaptations to, or coevolution with, a single geographically variable pollinator species (Anderson & Johnson, 2008; Pauw *et al.*, 2009) or even the outcome of interactions with other plant species (Johnson, 1994; Hansen *et al.*, 2000).

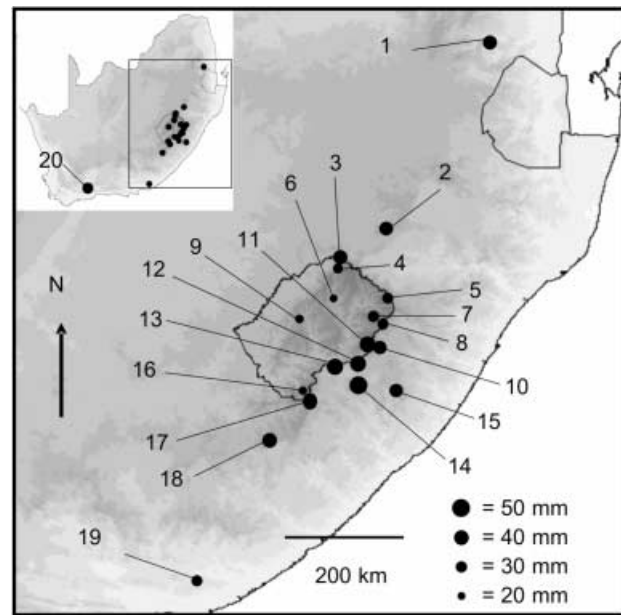
In this study, we take a more in-depth approach to convergence than most past studies by examining patterns of trait matching at the site level instead of the species level. We study geographical covariation and local convergence in a guild of plants pollinated by the long-tongued nemestrinid fly *Prosoeca ganglbaueri*, which functions as the near-exclusive pollinator of *c.* 20 plant species in southern Africa (Goldblatt & Manning, 2000). Flowers in this guild tend to be white-pink in colour, possess long corolla tubes, and have no obvious scent. In a previous study, the tongue length of *P. ganglbaueri* was shown to covary with the corolla length of the most common nectar plant (*Zaluzianskya microsiphon*) in a manner consistent with coevolution (Anderson & Johnson, 2008). However, rarer and non-rewarding members of the guild are unlikely to influence the evolution of tube length in flies. It has been suggested that the corolla tubes of these rare/nonrewarding plants may nevertheless track the evolving tongue lengths of their pollinators to ensure efficient pollen transfer (e.g. Johnson & Steiner, 1997). This is a process of unilateral evolution, unlike coevolution which is a reciprocal process of co-adaptation between organisms (*sensu* Janzen, 1980).

Convergence of floral traits among species has seldom been demonstrated at the local scale. Because of the enormous variation in the proboscis length of *P. ganglbaueri*, we predicted that members of the guild of plants pollinated by this fly would show fine-scale geographical covariation with the fly's proboscis length and that convergent evolution would be evident among plant species at the local site level. We also tested whether spur length of a nonrewarding orchid in the guild is under pollinator-mediated selection.

## Materials and Methods

### Measurements of morphological traits

*Prosoeca ganglbaueri* Lichtwardt flies and flowers from 20 study sites over an area of *c.* 253 000 km<sup>2</sup> in South Africa (Fig. 1) were examined during the months of January–March 2004 and 2005. At each site, the functional proboscis lengths of 3–60 flies and the floral tubes of 20–89 plants per species in the pollination guild were measured (using methods outlined in Anderson *et al.*, 2005), the only exception being *Disa nivea* H.P. Linder at the Bastervotepad site, where only three of these orchid plants were found and measured. In most plants pollinated by *P. ganglbaueri*, flowers have a long narrow corolla tube or spur into which the fly probes with its tongue for nectar. Depending on the plant species, pollen is placed on the head, chin, thorax or abdomen (dorsal or ventral) of the fly (see Goldblatt & Manning, 2000 for descriptions of pollen deposition sites in



**Fig. 1** A geographic mosaic for the guild of plants pollinated by the long-tongued fly *Prosoeca ganglbaueri*. Each circle represents a site from which we sampled both flies and plants, where the size of each circle is proportional to the length of the average fly proboscis in each population.

the guild). At the apex of the corolla, the flower 'flares' and often forms a broad chamber that accommodates the head or body of the fly. For each plant species, we measured the distance from the end of the tube to the point where the corolla flares, on the basis that it is this section that accommodates the length of the pollinator's proboscis. In orchids we measured the length of the spur, from its tip to the stigma. We also included the amaryllid genera *Brunsvigia* and *Nerine* in the analysis, both of which produce a very short tube. Instead of a tube matching the tongue length of the flies, these plants have long protruding anther filaments and styles which ensure placement of pollen on the abdomen of the insect while it probes the nectaries (Supporting Information Fig. S1). As the tube length of non-amarlyllid plants is hypothesized to match only the proboscis length of flies, we subtracted the total body length of the flies (averaged for each population) from the stigma–nectary distance (Fig. S1) of each *Brunsvigia* and *Nerine* population. Flowers of these genera are protandrous and were measured in the female phase, after the stigma had curved around into the position occupied by the stamens in Fig. S1 (supplementary material).

We measured and recorded all species that we observed to be visited only by *P. ganglbaueri* as well as those species previously recorded to be part of the *P. ganglbaueri* pollination guild (see Goldblatt & Manning, 2000). Observations and data collection took place over a time frame of between 1 and 5 d per site. The total number of days spent recording data was 72.

## Analyses of covariation and convergence

To determine whether tube lengths of guild members covary geographically with the proboscis length of *P. ganglbaueri*, we performed regression analyses based on mean population measurements for individual guild members, and grand means (means of means) for the entire guild. As a formal test of convergence, we tested whether the tube lengths of the three most widespread guild members (*Zaluzianskya microsiphon* (Kuntze) K. Schum., *D. nivea* and *Gladiolus oppositiflorus* J. D. Hook) converged on fly proboscis length at each site. Here we ran a general linear model on flower tube length with the average proboscis length as a continuous predictor and plant species as a categorical factor, as well as the interaction of these two terms to test for differences among species in the slope of the proboscis–flower tube length relationship. If the interaction term was not significant, we reran the analysis as an ANCOVA without the interaction term. The statistical package SPSS (release 16.0; SPSS Inc., Chicago, IL, USA) was used throughout. As a further test of fine-scale guild convergence among plant species, we used regression to explore the relationship between the flower tube length of the most common guild member (*Z. microsiphon*) and those of all the other guild members at each site.

## Is tube length adaptive?

To determine whether floral tube length is an adaptive trait, we performed a selection experiment using the nonrewarding guild member *D. nivea* (Orchidaceae), which is a mimic of the flowers of *Z. microsiphon* (Anderson *et al.*, 2005; Anderson & Johnson, 2006). To simulate a polymorphism for spur length, we performed reciprocal translocations between site 12 (Fig. 1), where plants have spurs measuring  $42.13 \pm 0.68$  (SE) mm, and site 10, where plants have spurs measuring  $24.27 \pm 0.69$  mm, thus creating mixtures of long- and short-tubed plants at each site. The two sites are 27 km apart. Plants in both populations were bagged in bud while their flowers were allowed to open. Once open, these virgin inflorescences were cut and placed in test tubes of water. Eighteen pairs of cut inflorescences (consisting of an individual from each site) were randomly placed in a *Z. microsiphon* population at site 10 (the Lodge site). Similarly, 19 pairs of inflorescences were placed in a *Z. microsiphon* population at site 12 (the Rama site). They were left in the field for 1 wk, during which the test tubes were topped up with water when necessary. Thereafter, each flower was examined for removal of pollinaria and deposition of pollen massulae. Female pollination success was taken as the proportion of flowers on each inflorescence that had been pollinated, while male pollination success was taken as the proportion of flowers with pollinaria removed. Proportion data were arcsine square-root transformed and after the residuals tested positively for normality we performed a general linear model with site and tube length ecotype and their interaction as categorical factors and floral

display size as a continuous predictor. If the efficiency of pollen transfer is related to spur length as predicted by Darwin (1862), then we expect longer-spurred ecotypes to always perform equally or better than shorter-spurred ecotypes in terms of pollen receipt and removal. Furthermore, selection favouring longer flower tubes should be stronger at site 12, where flies have a longer proboscis.

## Results

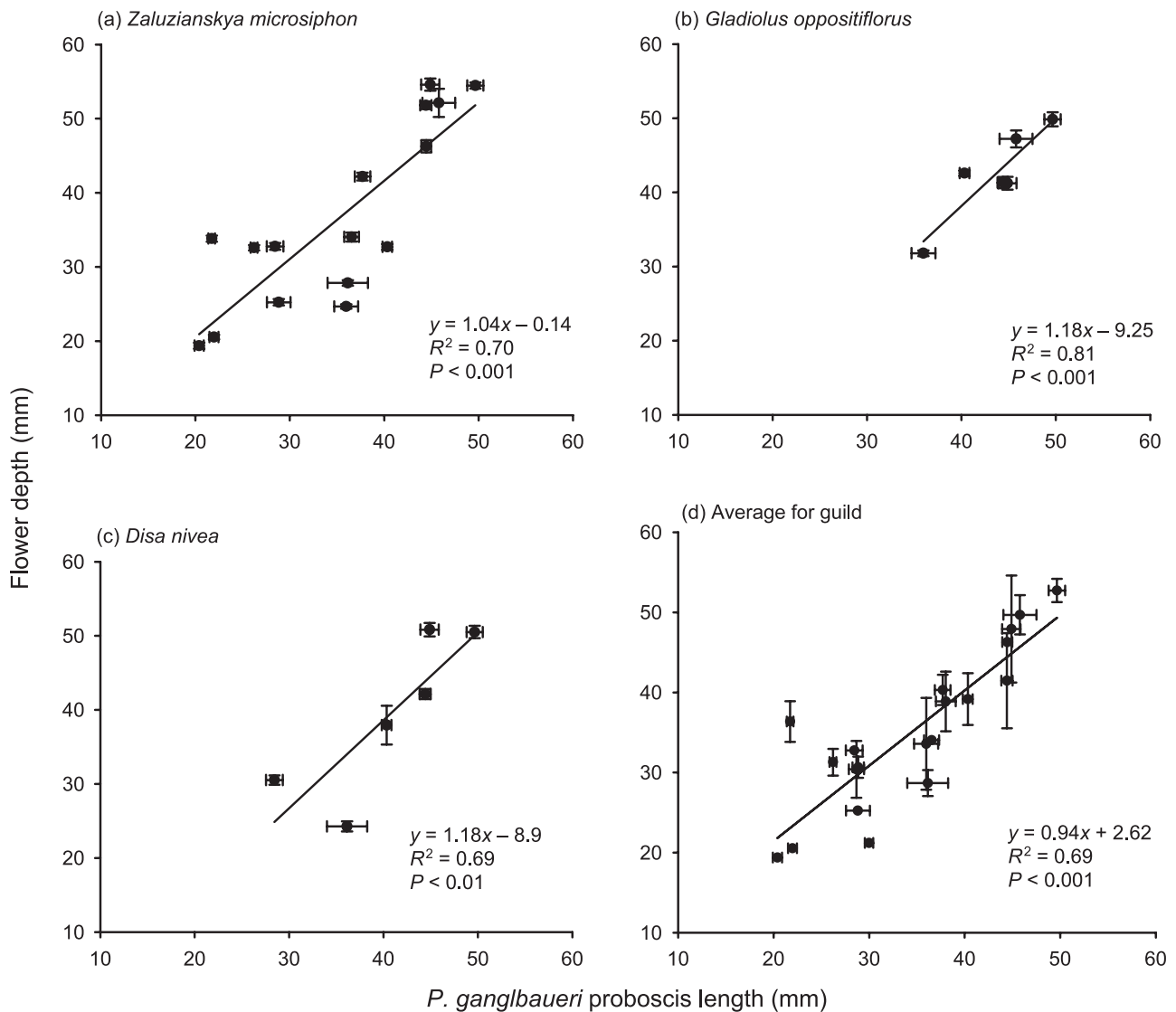
### Covariation and convergence

The corolla tube lengths of all three widely distributed plant species, *Z. microsiphon*, *G. oppositiflorus* and *D. nivea*, were strongly correlated with the proboscis length of *P. ganglbaueri* ( $R^2 > 0.68$ ,  $P < 0.02$  for all; see Fig. 2). In addition, the grand mean for the flower tube length of all guild members at each site correlated strongly with the proboscis lengths of the flies ( $R^2 = 0.73$ ,  $P = 0.001$ ; Fig. 2). The slopes of the proboscis–tube length regressions for all three species tested were not significantly different ( $F_{2,22} = 0.042$ ,  $P = 0.9590$ ). In a model that excluded this nonsignificant interaction term, fly proboscis length was a significant predictor of tube length across all three plant species ( $F_{1,24} = 54.173$ ,  $P < 0.0001$ ) and the intercepts for the proboscis–tube length regressions did not differ significantly among species ( $F_{1,24} = 0.252$ ,  $P = 0.6200$ ; Table S2). Moreover, the average tube length for the guild (excluding *Z. microsiphon*) at each site was positively correlated with the tube length of *Z. microsiphon* ( $R^2 = 0.59$ ,  $P < 0.006$ ). Guild members within a site also clustered closely to the tube lengths of *Zaluzianskya* (Fig. 3). It is also evident from this scatter plot that for wide-ranging species there is considerable divergence in tube length between populations, and all of the species with more than one population showed significant divergence in tube length ( $F > 21$ ,  $P < 0.001$ ; Table S1).

## Is tube length adaptive?

For pollen deposition, a measure of the female component of pollination success, the long-spurred ecotype significantly outperformed the short-spurred ecotype at both the Rama and Lodge sites (Fig. 4). The average proboscis length of fly pollinators was longer than the average spur length of the short-spurred ecotype at both sites (Fig. 4). Both *D. nivea* spur length ecotype and site were significant predictor variables for pollen deposition ( $F_{1,67} = 7.93$ ,  $P = 0.006$  and  $F_{1,67} = 33.6$ ,  $P < 0.0001$ , respectively; Table S3). However, the interaction between these two terms was not significant ( $F_{1,67} = 0.15$ ,  $P = 0.6889$ ), implying that the long-tubed ecotype had the same relative advantage over the short-spurred ecotype at both sites.

For pollinaria removal, a measure of the male component of pollination success, the long-spurred ecotype also significantly outperformed the short-spurred ecotype at both sites (Fig. 4).



**Fig. 2** Relationships between the flower depth of various guild members and the proboscis length of *Prosoeca ganglbaueri* across the study sites. Symbols are means ( $\pm$  SE). (a) *Zaluzianskya microsiphon* (from Anderson & Johnson, 2008), (b) *Gladiolus oppositiflorus* and (c) *Disa nivea*. (d) The entire guild of plants pollinated by *P. ganglbaueri* (see Supporting Information Table S1).

Spur length was a significant predictor variable for pollinaria removal ( $F_{1,67} = 18.76$ ,  $P < 0.0001$ ; Supporting Information Table S3) and the interaction between spur length and site was significant ( $F_{1,67} = 5.94$ ,  $P = 0.0017$ ), reflecting stronger selection for long tubes through the male component of pollination success at the Rama site, where flies had the longest proboscides and the long-spurred ecotype is native (Fig. 4).

## Discussion

### Trait matching

In this study, we found that fine-scale trait matching between pollinators and plants has led to patterns of strong phenotypic convergence between different plant species from the same site

and phenotypic divergence between plants from different sites (Figs 2, 3). Trait matching of the fly and floral traits may be brought about by either coevolution or unilateral evolution, reiterating the need to show reciprocal selection in order to demonstrate coevolution (see Janzen, 1980). Some species in this guild, such as *Z. microsiphon*, appear to have coevolved with *P. ganglbaueri* (Anderson & Johnson, 2008). *Zaluzianskya microsiphon* is the most common member of the guild and pollen load analysis shows that at the majority of sites it is the species most relied on for nectar by *P. ganglbaueri* (Johnson & Anderson, 2008). Other species that are rare or nonrewarding (e.g. *D. nivea*) would not have influenced the evolution of the fly proboscis length (and thus would not have coevolved with the fly), but they exhibit the same pattern of correlation with the fly proboscis length (Figs 2, 3). The evolution of tube length

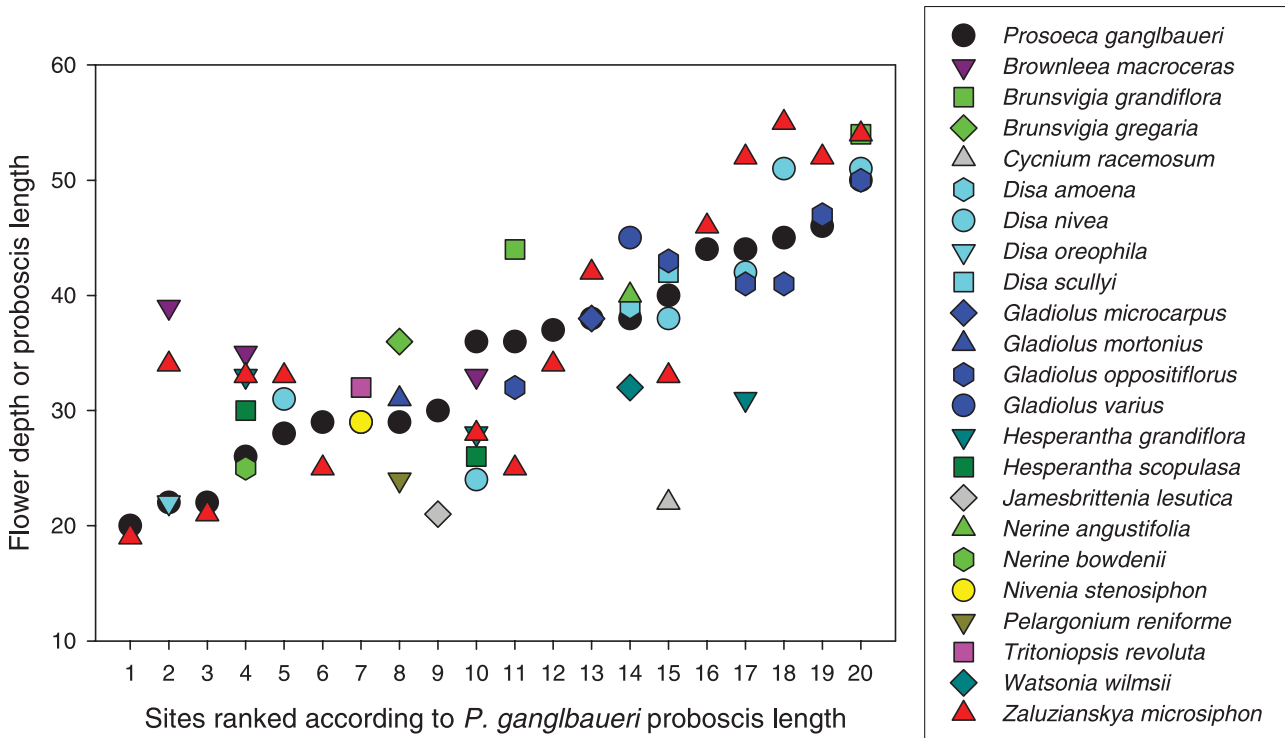


Fig. 3 Study sites ranked in order of *Prosoeca ganglbaueri* proboscis length, showing variation in plant reproductive traits within and among sites for each plant species.

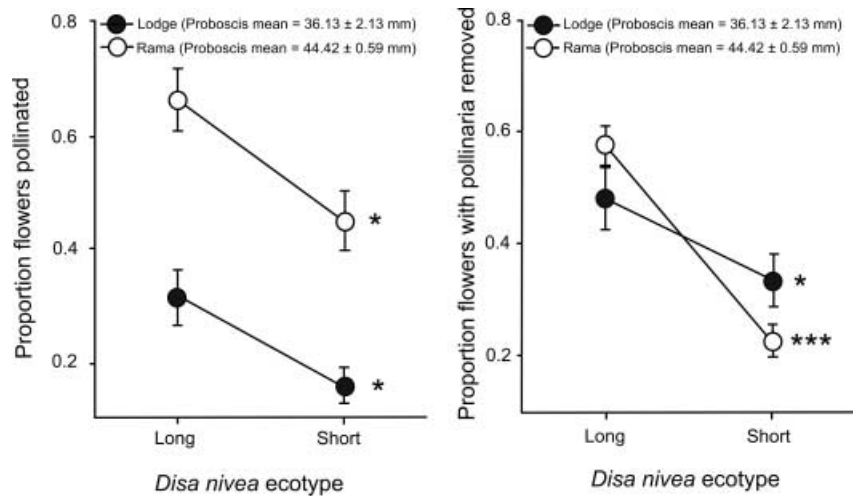


Fig. 4 Female and male pollination success of long- and short-spurred *Disa nivea* plants at two of the study sites. Short-spurred plants are native to the Lodge site where flies have medium-length proboscides whereas long-spurred plants are native to the Rama site where flies have long proboscides. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

in the rare or rewardless species is probably a consequence of unilateral tracking of the coevolutionary process between the pollinator and common rewarding plants. As these rare and rewardless plants have a specialized pollination system, they have kept pace with the evolutionary race because having floral tubes that match fly tongues facilitates pollen transfer efficiency (Nilsson, 1988; Johnson & Steiner, 1997; Pauw *et al.*, 2009). The *P. ganglbaueri* guild in the Drakensberg mountains (this study) and the *Moegistorhynchus longirostris* one in the Cape documented by Johnson & Steiner (1997), Manning & Goldblatt (1997)

and Pauw *et al.* (2009) exemplify how the coevolutionary process can have indirect effects by driving the divergence of species that are not strictly part of the process.

### Is tube length adaptive?

Our results add to the growing number of studies showing the adaptive significance of flower tube length and strong selective pressures acting on this trait in plants with specialized pollination systems (Nilsson, 1988; Johnson & Steiner, 1997; Alexandersson

& Johnson, 2002; Anderson & Johnson, 2008; Pauw *et al.*, 2009).

Anderson & Johnson (2008) showed by means of correlation analyses and phenotypic selection experiments that tube length in the common rewarding species *Z. microsiphon* is adaptive and probably arose through the process of coevolution with its pollinator. Here we showed that selection on spur length through the male component of pollination success in the rewardless (and thus unilaterally evolved) orchid *D. nivea* was stronger at a site with relatively long-tongued flies than it was at a site with relatively short-tongued flies (Fig. 4). This phenotype–environment interaction helps to explain how spur length could diverge among sites. Selection through the female component of pollination success also always favoured long spurs, but, unlike selection through male fitness, did not differ between sites (Fig. 4). This means that divergence in spur length between sites is more likely to have been driven by selection through male than through female fitness. Nevertheless, our results suggest that long-spurred mutants would be favoured through both components of fitness at the Lodge site where plants currently have relatively short spurs. The apparent maladaptation of the native short-spurred plants at this site could have resulted from range shifts where longer-tongued flies recently invaded and plants have not had time to adapt. Maladaptations are expected and are to some extent diagnostic of systems that are driven by coevolution, and it is thought that they represent areas where future bouts of coevolution may take place (Thompson *et al.*, 2002). The causes of mismatches in adapted traits are discussed in Thompson *et al.* (2002) and reflect the geographic mosaic structure of coevolved processes including asymmetries in gene flow, differences in community structure, time lags and the antagonistic nature of arms races.

### Convergence

Patterns of phenotypic convergence among the three most widespread species in this system (Fig. 3) have probably arisen because they are adapted to the same pollinator in each site. This was evident from the lack of significant differences in the slopes and intercepts of the proboscis length–tube length relationships for three common members of the guild (Fig. 2a–c, Table S2), the significant relationship between the mean fly proboscis length and grand means for the flower tube lengths of all guild members across the study sites (Fig. 2d), and the significant relationship between the mean tube length of *Z. microsiphon* and those of other guild members at each site (see Results). Despite strong convergence among guild members, there were nevertheless some outliers (see Fig. 3) that may correspond to plants with multiple pollinators that impose additional selection on tube length. For example, we suspect that *Cycnium racemosum* is also pollinated by short-tongued tabanid flies as it has been observed to be visited by these flies at other sites (Johnson, 2000) and that *Brownleea macroceras* may be

pollinated by another unidentified pollinator because its strong fragrance and blue colour are exceptions to the general *P. ganglbaueri* floral syndrome. These two species with mismatched flowers may be in the process of incorporation into the guild after range expansion into areas without their usual pollinators or where their usual pollinators are not as effective as *P. ganglbaueri* for pollination (cf Stebbins, 1970).

### Divergence

One of the most outstanding features of our study system is the dramatic variation in proboscis length of *P. ganglbaueri* (Fig. 1). This variation is not simply clinal, but is a patchwork mosaic of differing proboscis lengths across the distribution range (Fig. 1). Anderson & Johnson (2008) considered the possibility that differences in habitat microclimates may account for the mosaic of pollinator tongue lengths because it may be more difficult to evolve and maintain long tongues in harsh microclimates (e.g. the summit of the Drakensberg) than in more mild microclimates (e.g. the base of the Drakensberg). They found that altitude partly explains the proboscis length of *P. ganglbaueri*, but that flower tube length, regardless of altitude and other physical environmental predictors, was the strongest predictor of fly proboscis length.

Evidence suggests that the enormous site-by-site variation in this system arises from a coevolutionary process (Johnson & Anderson, 2008). Coevolutionary outcomes tend to occur at the population level and be geographically variable, which results in a mosaic of coevolutionary hotspots and coldspots (Thompson, 1994, 1999, 2005). These hotspots may be a result of different interacting communities at each site. In addition, gene flow patterns may affect coevolutionary outcomes by swamping local adaptations (Lenormand, 2002). However, gene flow could actually facilitate local adaptation by bringing about evolutionary novelties upon which natural selection can act (see Gandon *et al.*, 1996; Rieseberg & Carney, 1998; Rieseberg & Burke, 2001; Brockhurst *et al.*, 2003).

### Conclusion

The fine-grained patterns of floral variation documented in this study appear to have arisen from local adaptation to the long-proboscid fly *P. ganglbaueri*, which has been shown in earlier studies to be an effective pollinator of the plants in this guild (Goldblatt & Manning, 2000; Johnson *et al.*, 2002; Anderson *et al.*, 2005; Anderson & Johnson, 2008). We infer that two processes are at work: common members of the guild, such as *Z. microsiphon*, can influence the evolution of fly proboscis length because the availability of their nectar is critical for fly fitness (see Pauw *et al.*, 2009, for evidence of this in a different fly system) and would coevolve with the fly, while flower tube lengths of less common and nonrewarding members of the guild would evolve unilaterally to track the changing proboscis lengths of flies at the local scale (Fig. 2c). The outcome is a

floral syndrome that is not fixed at the species level, as assumed in previous studies of floral syndromes, but, instead, reflects a complex pattern of convergent evolution at the scale of local populations (Fig. 3).

In many ways, the patterns of morphological variation in this pollination guild system are similar to the extraordinary, fine-scale patterns of spatial colour variation found in butterfly Müllerian mimicry rings (e.g. Turner, 1976). While frequency-dependent selection is the main diversifying factor in butterfly mimicry systems, it appears to be coevolution in the *P. ganglbaueri* pollination system. However, the process of adverting evolution, whereby rarer species track the evolutionary changes in common species (Turner, 1977, 1984; Gilbert, 1983), appears to be a common feature of both of these systems and is deserving of far more attention in research on plant pollination systems.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Measurements of flowers in the guild.

**Table S1** The average tube lengths  $\pm$  SE ( $N$ ) of all guild members pollinated primarily by *Prosoeca ganglbaueri* and the average tongue lengths of *P. ganglbaueri* flies found in each population.

**Table S2** ANCOVA model testing the effects of population mean fly proboscis length (continuous variable) and plant species (grouping factor) on the population mean tube lengths of the three most widespread plant species in the guild of plants pollinated by *Prosoeca ganglbaueri*.

**Table S3** General linear models that test the significance of effects of *Disa nivea* spur length (grouping factor), flowers per inflorescence (continuous predictor), translocation site (grouping factor) and the interaction between site and spur length on the angular transformed proportion of flowers pollinated and the angular transformed proportion of flowers with pollinaria removed.

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