DR. ANTON PAUW (Orcid ID : 0000-0002-4012-648X)

The Least Effective Pollinator Principle: specialized morphology despite generalized ecology.

Anton Pauw[a], Andrea A. Cocucci[b] and Alicia N. Sérsic[b]

[a]Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

[b] Instituto Multidisciplinario de Biología Vegetal (UNC-CONICET), Casilla de Correo 495, X5000JJC Cordoba, Argentina.

Corresponding author: Anton Pauw (apauw@sun.ac.za)

Short title: The Least Effective Pollinator Principle

Summary

- The large body of work on the adaptation of plants to pollinators is still somewhat incomplete because most studies focus on one-to-one interactions. How will adaptation proceed in a multi-pollinator environment? According to Stebbins' Most Effective Pollinator Principle, "the characteristics of the flower will be molded by those pollinators that visit it most frequently and effectively".

- To test this hypothesis, we studied the pollination biology of *Pelargonium incrassatum* (Geraniaceae) in the Namaqualand Region of Southern Africa. The species has a long floral tube and we expected its most important pollinator to have a long proboscis.

- Contrary to expectations the most important pollinator was a short proboscid fly (a new species of *Prosoeca*), while *Prosoeca peringueyi*, which had a proboscis that matched the floral tube length, was a rare visitor. Consistent with the high degree of trait mismatching, we did not detect selection on tube length at most sites.

- The paradox of mismatching traits can be resolved by considering the strength of the trade-off involved. Adaptation to the rare species can apparently occur without incurring the cost of reduced pollination by the abundant species. Generally, species may often evolve specialized morphology if they do not incur the cost of ecological specialization.

Introduction

The process whereby floral tube length evolves in response to pollinator proboscis length has been revealed in exquisite detail. Firstly, it has been shown that there is heritable variation in floral tube length within populations (Campbell 1996; Anderson *et al.* 2016) and, secondly, that this variation is often correlated with reproductive success (Nilsson 1988; Maad 2000; Alexandersson & Johnson 2002; Little *et al.* 2005; Benitez-Vieyra *et al.* 2006; Pauw *et al.* 2009; Muchhala & Thomson 2009; Nattero *et al.* 2010; Sletvold & Ågren 2010; Moré *et al.* 2012; Paudel *et al.* 2016; Soteras *et al.* 2020). Thirdly, we know that tube length changes over time in response to selection (Whittall & Hodges 2007; Anderson *et al.* 2014; Abrahamczyk & Renner 2015; Serrano-Serrano *et al.* 2017; Pauw *et al.* 2017), resulting in divergent populations, new species and an overall correlation between floral tube length and pollinator proboscis length (Nattero & Cocucci 2007; Anderson & Johnson 2008; Pauw *et al.* 2009; Boberg *et al.* 2014; Newman *et al.* 2014; Anderson *et al.* 2014; Paudel *et al.* 2016). Results are consistent across a wide taxonomic range that includes Orchidaceae, Iridaceae, Zingiberaceae, Campanulaceae, Polemoniaceae, Solanaceae, Fabaceae, Ranunculaceae, Geraniaceae and Gesneriaceae among others.

Although very detailed, the picture painted by these studies is still somewhat unrealistic because most assume that plants are under selection by a single pollinator species, whereas we know that plant species are usually linked to several pollinator species in the interaction network (Sazatornil *et al.* 2016). What is the expectation when a plant population is visited by several pollinator species with different morphologies? According to Stebbins' Most Effective Pollinator Principle, "the characteristics of the flower will be molded by those pollinators that visit it most frequently and effectively (Stebbins 1970)". The prediction is that, in a multi-pollinator environment, floral tube length should match the proboscis length of the most important pollinator, i.e. the pollinator that contributes most to plant fitness either by having a high per visit effectiveness (Spears 1983), a high visitation rate, or both (Waser *et al.* 1996).

Stebbins' Principle has been the dominant paradigm in understanding floral adaptation in a multi-pollinator environment (Ollerton 1996; Armbruster *et al.* 2000), and was formulated into a model of floral adaptation by Waser et al. (1996). Central to the principle is the assumption of strong trade-offs. The traits of a plant are expected to come to match those of the potentially most

rewarding pollinator because any adaptation to a potentially less rewarding pollinator is expected to decrease fitness gains from the potentially more rewarding pollinator. Indeed, several studies show that floral morphologies with high fitness in the presence of one type of pollinator perform poorly when interacting with other types of flower visitors, suggesting the existence of trade-offs (Muchhala 2007). Adaptation to birds, for example, may be traded off against adaptation to insects, which are often observed to visit bird-adapted flowers without contacting the anthers or stigmas because of a poor fit with floral morphology (Pauw 2019).

In contrast to these ideas, Aigner (2001) suggested that there may be situations where the trade-offs in adapting to one versus the other pollinator is weak or absent. If there is no trade-off, a trait may evolve to enhance pollination by an unimportant pollinator because the change in the trait does not reduce fitness gains from the more important pollinator species and only adds fitness. If there is a weak trade-off, a trait may still evolve to enhance pollination by an unimportant pollinator because the cost of a slight reduction in pollination by the important pollinator can easily be compensated for by fitness gains from occasional visits by the unimportant pollinator (Fig. 1). Without strong trade-offs, the expected outcome is that plant traits may be poorly matched with the traits of their most important pollinators, i.e. those that are most frequent and/or effective. Despite the theoretical appeal of Aigner's (2001) idea, relatively few examples of adaptation to unimportant pollinators have been documented and the general expectation for a match between plant and pollinator morphology remains (Mayfield *et al.* 2001; Aigner 2004, 2006). The continued reliance on syndromes of floral traits to predict the most important pollinator, is a case in point (Pauw 2006; Rosas-Guerrero *et al.* 2014; Serrano-Serrano *et al.* 2017).

With Stebbins' and Aigner's ideas in mind, we studied variation in the length of the floral tube of *Pelargonium incrassatum* in the Namaqualand region of southern Africa. We asked:

1) Which is the most important pollinator of *Pelargonium incrassatum*? To answer this question, we quantified pollinator abundance, and the pollen loads that they carried, across ten sites. As an additional measure of the relative importance of each pollinator species to pollination, we correlated pollen deposition on stigmas with the abundance of the different pollinator species using multiple regression. Based on a study by Manning and Goldblatt (1996), we expected the long-proboscid fly, *Prosoeca peringueyi* (Nemestrinidae), would

be the most frequently captured pollinator, and that variation in its abundance would explain most of the variation in pollen receipt across populations.

2) How does floral tube length in *Pelargonium incrassatum* match up with the proboscis length of its pollinators across populations? To answer this question, we overlaid the frequency distributions of floral tube lengths on frequency distributions of proboscis length per site. We expected that the median floral tube length should coincide with the median proboscis length of the most important pollinator at each site.

1) Is phenotypic selection on tube length still ongoing? To answer this question, we correlated pollen receipt with tube length variation within each population. Based on the studies cited above, we expected to observe ongoing directional selection for longer tubes if some individuals had tubes shorter that the proboscises of the most important pollinators in the community.

Materials and Methods

Study species and sites

Pelargonium incrassatum (Andrews) Sims (Geraniaceae: Section Hoarea) has a 380 km long range that follows the granite escarpment between the west coast lowlands and the high-lying interior of southern Africa in the Namaqualand Region. It is a perennial geophyte, with deciduous pinnate leaves and an umbel-like cluster of ~15 scentless, magenta flowers on a simple peduncle, ~30 cm tall. The stamens are exerted, and the anthers drop off before the stigma unfurls such that self-pollen is not deposited on the stigma (Fig. 2). Stigmas unfurl in the morning and are receptive for about one day. We examined 383 flowers and found only one flower with partial overlap in the male and female phase (an anther was still present while the stigma was already unfurled). The nectar is held in a ~35 mm deep rigid hypanthium. *P. incrassatum* flowers in spring along with a group of other long-tubed, scentless, magenta-flowered species that have been described as the '*Prosoeca peringueyi* pollination guild' because preliminary observations indicated that they share this long-proboscid Nemestinid fly as their pollinator (Manning & Goldblatt 1996).

During the peak flowering period in September 2011 we located ten study sites that spanned the range of *Pelargonium incrassatum*. Variation in proboscis length at these sites was placed in the context of our larger data set of proboscis length variation in *Prosoeca peringueyi*, which included additional sites at which *P. incrassatum* did not occur (Table S1).

Determination of the most important pollinator

To determine which is the most frequent pollinator of *Pelargonium incrassatum*, we conducted 120 hrs of pollinator observations, capturing all visitors to *P. incrassatum*. Important pollinators should make frequent visits, or they should transfer large amounts of conspecific pollen per visit (Stebbins 1970; Waser *et al.* 1996; Mayfield *et al.* 2001). Pollen grains on pollinators were identified under a stereomicroscope by comparison with a reference pollen collection from the site and pollen loads were quantified.

As an additional measure of pollinator importance, we collected one stigma from each of 60 plants just before sunset, at the end of the female phase. We mounted the stigmas on microscope slides in fuchsin gel and counted conspecific as well as heterospecific pollen grains (Beattie 1971). While the former indicates effective pollination, the latter indicates the activity of poor pollinators, because interspecific pollen transfer results in the loss of pollen and the contamination of stigmas (Morales & Traveset 2008). To separate the contribution made by each pollinator species to the observed stigmatic pollen loads, we correlated variation in the mean number of conspecific pollen grains per stigma with variation in the abundance of the different visitor species across populations. In a multiple regression analysis, variation in the abundance of an important pollinator is expected to explain a relatively large percentage of the variance in pollen receipt among populations. To estimate pollinator abundance, all three authors simultaneously patrolled each site from 8 AM to 12 PM on a warm, windless day, capturing all visitors to *Pelargonium incrassatum*. Stigmas were collected in the late afternoon of the same day. Although our pollinator observations amounted to a total of only 12 h per site, observations did span the period of peak pollen receipt by the focal stigmas.

Trait matching

To test the hypothesis that floral tube length should match the length of the proboscis of its most important pollinator, we measured the proboscis length of all pollinators that were captured at each of the ten sites. At each site we also measured the floral tube length of one flower on 60 haphazardly selected *Pelargonium incrassatum* plants and overlaid the frequency distribution of floral tube lengths on a frequency distribution of proboscis lengths.

We measured floral tube length from its entrance, at the point where the sepals split, up to its end, which is marked by a small bump on the abaxial surface of the hypanthium close to its proximal end. We measured proboscis length at rest in freshy killed specimens by straightening the proboscis along the edge of a ruler. The proboscis can be extended slightly by pulling on it, and this extension may be used during feeding, so we also measured extended length in a subset of flies. Because it is unclear how far flies extend their proboscis during feeding, we conducted analyses using both fully extended and non-extended lengths.

Phenotypic selection on tube length

To test whether phenotypic selection on tube length was potentially occurring via differential pollen receipt, we collected the stigmas of the measured flowers and recorded conspecific pollen receipt as above. Selection analyses were performed for each site separately, using floral tube length as the predictor variable and conspecific pollen receipt (a measure of female fitness) as response variable. Regression models were of the form: pollen $= \beta*$tube $+ \gamma*$tube$^2 + c$. A significant, positive linear model coefficient, β, would be consistent with directional selection for longer tubes, a negative β would suggest directional selection for shorter tubes, a significant positive quadratic coefficient, γ, would be consistent with stabilizing selection, and a significant negative γ would be consistent with disruptive selection that favours either long or short tubes (Lande & Arnold 1983; Brodie *et al.* 1995). Number of flowers per inflorescence was initially used as an additional term in this model, since variation in pollen receipt per flower could also be accounted for by variation in display size but was finally excluded because it did not greatly alter the conclusions. Analyses were conducted on 60 flowers per site, giving us 20 observations per model parameter. Tube length and display were standardized to a mean of zero and a standard deviation of one separately by population and pollen receipt was divided by the population mean

in order to calculate standardized selection coefficients that can be compared with other studies. Because the fitness measure are counts, the statistical significance of regressions was obtained through quasi-generalized linear models (GLM), with Poisson error distributions and log link function using R (R Core Team 2016). Quadratic selection coefficients were quantified as twice the partial regression coefficients extracted from these models (Stinchcombe *et al.* 2008). Linear selection gradients were estimated from models that included linear terms only, whereas quadratic selection gradients were estimated from models that included both linear and quadratic terms (Lande & Arnold 1983). To depict the relationships between tube length and pollen receipt we calculated cubic splines using the *mgcv* package (R Core Team 2016; Wood 2017). Smoothing parameters were obtained by minimizing the generalized cross-validation scores, and Bayesian standard errors were obtained according to Wood (2017).

Results

Relative pollinator importance

Surprisingly, the most frequent visitor captured on *Pelargonium incrassatum* was not *Prosoeca peringueyi* Lichtwardt (1920), but rather an undescribed *Prosoeca* species that had a considerably shorter proboscis and co-occurred with *Prosoeca peringueyi* at some sites (Table S2; Fig. S1 & S2). Here we name this species *Prosoeca 'namaquensis'*, for ease of reference, and use quotation marks to not invalidate formal description. In total we captured 64 *P. 'namaquensis'* and 11 *P. peringueyi* individuals at the ten sites. *P. 'namaquensis'* was the only visitor at seven sites and co-occurred with *P. peringueyi* at one site. At the remaining two sites only *P. peringueyi* was collected. There was no obvious difference in the temporal pattern of activity of the two fly species throughout the course of a day. All specimens have been lodged with the South African Museum, Cape Town (Table S3).

The flies carried pollen mainly on the thorax and the ventral abdomen with very small amounts on the head. We examined 63 *Prosoeca 'namaquensis'* and found that 55 carried *Pelargonium incrassatum* pollen grains ventrally on the thorax (avg. across all individuals = 143, max. = 1200 grains). Particularly large amounts of *P. incrassatum* pollen occurred in the depression between the coxae. *Hesperantha pauciflora* (Iridaceae) pollen co-occurred with that of *P. incrassatum* on the ventral surface of 8 of the 55 specimens (avg. = 25, max. = 400 grains), from where it might

potentially be transferred to *P. incrassatum*. The campanulate, actinomorphic flowers of *H. pauciflora*, contact all parts of the body with their long anthers and substantial amounts were also found on the dorsal surface of the thorax (avg. = 20, max. = 400). In contrast, precise, differential pollen placement ensured that there was limited opportunity for interspecific pollen transfer between *P. incrassatum* and other guild members. *Lapeirousia silenoides* (Iridaceae) pollen occurred on 13 individuals (avg. = 127, max. = 5000 grains) but was restricted to the dorsal surface of the thorax. Two specimens also carried *Babiana curviscapa* (Iridaceae) pollen on the dorsal thorax.

We examined 11 *Prosoeca peringueyi* and found that they all carried *Pelargonium incrassatum* pollen grains ventrally on the thorax (avg. across all individuals = 2213, max. = 5000 grains). *Lapeirousia silenoides* pollen occurred on the frons or dorsal thorax of 6 individuals (avg. = 63, max. = 200 grains).

The composition of pollen loads on stigmas is consistent with these observations. At site four, where *Hesperantha pauciflora* co-occurred with *Pelargonium incrassatum*, 24 out of 58 sampled stigmas contained *H. pauciflora* pollen (avg. = 2.7, max. = 29 grains). No stigmas contained *Lapeirousia silenoides* pollen despite co-occurrence at seven sites, indicating effective partitioning of the pollinator between these species.

The quantity of conspecific pollen received by stigmas varied among populations from a mean (median) of 5.7 (1) to 41.8 (33.5) and was strongly related to the abundance of *Prosoeca.* The abundance of *Prosoeca 'namaquensis'* explained 73% of the variance in pollen receipt and the abundance of *Prosoeca peringueyi* contributed an additional 1% of uniquely explained variance (Table 1; Fig. 3).

In conclusion, *Prosoeca peringueyi* was nowhere common and was entirely absent from seven of ten sites where *Prosoeca 'namaquensis'* was the only pollinator observed. Correlations between pollinator abundance and pollen receipt suggested that *P. 'namaquensis'* was responsible for the bulk of pollen transfer to focal flowers. Although *P. 'namaquensis'* did carry smaller pollen loads, this deficiency was apparently more than compensated for by substantially higher visitation rates, making *P. 'namaquensis'* the most important pollinator overall.

Trait matching

Contrary to our expectations, floral tube length in *Pelargonium incrassatum* did not match the proboscis length of the most important pollinator. Within Namaqualand, there was a bimodal distribution of proboscis length variation with the first mode corresponding to the short proboscid *Prosoeca 'namaquensis'* and the second mode to the long-proboscid *Prosoeca peringueyi*, with no overlap. Floral tube length of most *P. incrassatum* (median = 35 mm; N = 595) fell above the range of proboscis length variation in *P. 'namaquensis'* (range = 13.5-23.9 mm; N = 68), but coincided with the proboscis length of *P. peringueyi* (Fig. 4). Floral tube length in *P. incrassatum* varied little among sites, but was slightly and significantly longer at site two, where only *P. peringueyi* was observed (F_9 = 18.89, p < 0.001, Fig. 5, Table S4).

We reach a similar conclusion when we repeat the analysis using extended proboscis length instead of relaxed proboscis length. Extended proboscis length could be calculated for all specimens using the following straight-line relationship that was calculated from the subset of flies for which both measurements were available (Table S3). For proboscis length in *Prosoeca peringueyi*, extended = 1.084*relaxed + 4.245 (Adjusted R^2 = 0.574, P = 0.001, df = 12). For *Prosoeca 'namaquensis'*, extended = 0.896*relaxed + 6.216 (Adjusted R^2 = 0.5249, P = 0.0004, df = 16).

Selection on floral tube length via pollen receipt

Pollen receipt was significantly related to floral tube length at three sites (Table 2 & S4, Fig. S3). At site three a significant linear selection gradient is consistent with negative directional selection. At site four significant linear and quadratic selection gradients indicated the joint effect of positive directional selection and disruptive selection, respectively. Finally, at site five a significant quadratic gradient indicated stabilizing selection. Results were not appreciably different when we excluded the number of flowers per inflorescence from the model.

Discussion

In contradiction of Stebbins's Most Effective Pollinator Principle, floral tube length of *Pelargonium incrassatum* did not match the proboscis length of the most important pollinator, a relatively short-proboscid fly, but instead matched the proboscis length of a less important long-proboscid pollinator that was absent at most sites (Fig. 4 & 5).

We can think of at least three possible explanations for the unexpected results. Firstly, the result can be interpreted as an example of maladaptation. A poor fit between plants and their observed pollinators may result if the pollinator fauna varies over time such that the plants are adapted to a fauna other than the one observed. Sampling in additional years will provide information on short-term fluctuations in the pollinator fauna, however we currently lack methods for reconstructing long-term patterns of variation. It is possible that the long-proboscid *Prosoeca peringueyi* was the most frequent pollinator in former times and that the long tubes of *Pelargonium incrassatum* are anachronisms. We see only a snapshot in evolutionary time.

Another possibility is that, despite being an infrequent visitor, *Prosoeca peringueyi* was the most important visitor because it carried larger and purer pollen loads. If this were the case, the abundance of *P. peringueyi* would explain most of the variance in pollen deposition, but our results clearly show that variation in the abundance of *P. peringueyi* is a poor predictor of variation in pollen deposition (Fig. 3, Table 1).

Finally, it is possible that the long tubes of *Pelargonium incrassatum* are in fact the optimal solution to the observed pollination environment. The implication is that Stebbins' Most Effective Pollinator Principle is overly simplistic and that the assumption of strong trade-offs in adaptation to one or the other pollinator needs to be qualified. Aigner's (2001) refinement of Stebbins' (1970) model does just that by allowing the strength of the trade-off to vary. The model clearly shows that, when the trade-off is weak, plants may adapt to the least important pollinator (Fig. 1). Thus, the optimum tube length may be substantially longer than the proboscis length of the most frequent pollinator if longer-tubed flowers benefit from improved pollen transfer efficiency by infrequent, long-tongued pollinators without sacrificing fitness gains from more frequent, shorter-proboscid species. High pollination success in the presence of *Prosoeca 'namaquensis'* despite trait mismatching suggest that this short-proboscid pollinator is effective over a wide range of floral phenotypes, i.e. there is a weak trade-off in adapting to a less important pollinator.

Adaptation without trade-offs may be a widespread but under-reported phenomenon (Aigner 2004; Sahli & Conner 2011). In many plant species, nectar wells up inside the floral tube within reach of short-proboscid pollinators, making it possible for flowers to play this double game. In *Tritoniopsis revoluta* (Anderson *et al.* 2014) and *Habenaria* (Moré *et al.* 2012), for example, the most frequent visitors have short proboscises, but floral tube length matches that of very rare long-proboscid species. More generally, a population may adapt to a relatively unimportant resource if the specialized phenotype required to access it does not impede exploitation of more abundant resources (Robinson & Wilson 1998). Counterintuitively, phenotypic specialization may, under these circumstances, broaden the range of resource use rather than forcing ecological specialization.

The lack of ongoing selection at most sites (Table 2, Fig. S3) is consistent with the observation of trait mismatching (Fig. 5). The range of tube length in *Pelargonium incrassatum* falls above the range in proboscis length of *Prosoeca 'namaquensis'*, with the result that all flowers, regardless of their tube length, will make maximum contact with the pollinator during an interaction (Pauw *et al.* 2009; Moré *et al.* 2012). Generally, a very poor match between trait and environment precludes selection because all individuals will fail or succeed in the task at hand (Toju & Sota 2006; Hanifin *et al.* 2008). While all *P. incrassatum* may be equally successfully pollinated by *P. 'namaquensis'*, only longer-tubed individuals will benefit from full pollination by *Prosoeca peringueyi,* but, given the scarcity of the fly, this selective advantage was too slight for us to detect. Of course, pollen receipt is only the first of a long series of steps that ultimately leads, via seed production, to mature individuals in the next generation. It seems likely that any correlation between female fitness and tube length will weaken through these several stages.

The conclusions of this study are based on correlative data. It would be very interesting to follow this up with an experimental study. Our data on pollinator abundance suggest that the short proboscid *Prosoeca 'namaquensis'* is a frequent visitor of the long-tubed *Pelargonium incrassatum*, and the positive correlation between *P. 'namaquensis'* abundance and the abundance of conspecific pollen on stigmas, coupled with information on the magnitude and composition of pollen loads on the bodies of flies, suggests that they are also effective pollinators. Thus, correlational data suggest that *P. 'namaquensis'* fulfils the two criteria of an important pollinator,

namely, frequent and effective visits. A more experimental approach would be to quantify pollen deposition on stigmas after a single visit to a previously unvisited flower (Spears 1983), and additionally to quantify the number of visits per flower per hour by each pollinator species in natural populations. The use of seed set, in addition to pollen receipt, as a measure of female fitness will be a further improvement, as will comparisons with seed set in pollen supplemented flowers.

The discovery that the plant species in the *Prosoeca peringueyi* pollination guild are used by two very closely related, sympatric fly species begs many questions regarding the ecology and evolution of *Prosoeca.* The coexistence of these fly species may be mediated by small-scale partitioning of larval resources, or by differences in the use of floral resources (Maglianesi *et al.* 2014). *Prosoeca 'namaquensis'* may be able to visit short-tubed species, such as *Hesperantha pauciflora*, more rapidly than *P. peringueyi*, but *P. peringueyi* will be able to access nectar levels beyond the reach of *P. 'namaquensis'* in long-tubed species (Zhang *et al.* 2013). Indeed, the broader pattern of proboscis length variation in *P. peringueyi* is suggestive of the evolution of resource partitioning by character displacement (Diamond *et al.* 1989). In the Cape Floral Region, where *P. peringueyi* occurs alone, it has a shorter proboscis than in Namaqualand, where it co-occurs with *P. namaquensis* (Fig. S2).

The observation of high variance in pollen receipt among populations of *P. incrassatum* and a strong positive correlation with *Prosoeca 'namaquensis'* abundance highlights the importance of this little-known pollinator in plant reproduction (Fig. 3). In three populations where *Prosoeca* were rare, *Pelargonium incrassatum* flowers received a median of one pollen grain per stigma despite having five ovules, a result suggestive of pollen limitation of plant reproduction. To conserve plants, and the interactions on which they depend, we clearly need more information on the lifecycle of *Prosoeca* and the factors that limit their abundance.

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Author contributions

AP, A.A.C and A.N.S conducted the fieldwork and analyzed the results. AP wrote the manuscript with input from A.A.C and A.N.S.

ORCID

Anton Pauw https://orcid.org/0000-0002-4012-648X

Andrea Arístides Cocucci https://orcid.org/0000-0002-3464-3309

Alicia N. Sérsic https://orcid.org/0000-0003-4718-6861

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Supporting Information

Table S1. Location of study sites.

Table S2. Traits used to distinguish between the two *Prosoeca* species.

Table S3. Proboscis length measurements.

Table S4. Conspecific pollen receipt, floral tube length and number of flowers per inflorescence in *Pelargonium incrassatum.*

Fig. S1 A comparison of Prosoeca *'namaquensis'* and *Prosoeca peringueyi*.

Fig. S2. Broader geographical context of tongue length variation in *Prosoeca peringueyi*.

Fig. S3. Cubic spline regressions of pollen receipt on standardized floral tube length in *Pelargonium incrassatum* populations.

Table 1. Multiple linear regression estimates for the relationship between mean pollen receipt in *Pelargonium incrassatum* and abundance of two *Prosoeca* fly species across 10 populations. Multiple R^2 = 0.7368, Adjusted R^2 = 0.6615, p = 0.0093, df = 7 (Fig. 2).

	Estimated slope	Std. Error	t- value	P	Sum of Squares (% variance explained)
P. peringueyi	5.5883	1.8337	3.0480	0.0187	10.58 (1%)
P. 'namaquensis'	2.4268	0.5518	4.3980	0.0032	812.74 (73%)

Table 2. There is little evidence for ongoing selection acting on floral tube length via female fitness. Linear (β) and quadratic (γ) selection gradients were estimated from the relationship between conspecific pollen receipt (a fitness proxy) and phenotypic traits: (1) display size and (2) floral tube length (Fig. S4). Standard errors are in brackets. * P < 0.05, ** P < 0.01, *** P < 0.001.

| Site	β1	β2	γ1	γ2
1	-0.13 (0.16)	0.28 (0.16)	0.26 (0.18)	0.10 (0.25)
2	0.01 (0.16)	-0.14 (0.16)	-0.04 (0.16)	0.47 (0.21)
3	-0.06 (0.12)	-0.27 (0.13)*	-0.25 (0.14)	-0.01 (0.16)
4	0.25 (0.08)***	0.33 (0.08)***	0.26 (0.08)	0.46 (0.14)***
5	0.11 (0.14)	-0.02 (0.14)	-0.05 (0.13)	-0.65 (0.23)**
6	-0.11 (0.32)	0.31 (0.32)	0.31 (0.34)	-0.37 (0.42)
7	0.59 (0.24)*	0.10 (0.24)	0.16 (0.27)	0.36 (0.25)
8	-0.40 (0.29)	0.04 (0.29)	0.23 (0.34)	0.60 (0.49)
9	0.05 (0.15)	-0.09 (0.15)	-0.13 (0.16)	-0.33 (0.21)
10	-0.18 (0.18)	0.11 (0.18)	0.10 (0.18)	-0.32 (0.30)

Figure captions

Fig. 1. A model for the evolution of floral phenotype in an environment with two pollinator species. The lower two curves represent the individual contributions of two pollinator types, 1 and 2, to plant fitness. The upper curve is the net fitness function (offset slightly upward for clarity) assuming that the effects of the individual pollinators are purely additive. Each numbered arrow below the x-axis indicates the phenotype that is optimally adapted to that pollinator by itself. The solid triangles above the x-axis indicate the optimal phenotype in the two-pollinator environment. The graph show that selection may drive adaptation to an infrequent pollinator (2) if in so doing more fitness is gained than sacrificed. For this to occur, the frequent pollinator (1) needs to be effective over a wide range of phenotypes, i.e. there needs to be a weak trade-off. Redrawn from Aigner (2001).

Fig. 2. Herkogamy in *Pelargonium incrassatum*. In the lower flower, the anthers are present and are dehiscing pollen, the style is short and hidden inside the flower, and the style branches are closed. In the top flower, the anthers have dropped off, the style has elongated, and the style branches have opened to reveal a receptive, papillose stigma. Male and female phases are concurrent in the same inflorescence (Photo: A. Pauw).

Fig. 3. Pollen receipt by stigmas of *Pelargonium incrassatum* is related to variation in the abundance of two *Prosoeca* fly species across 10 populations.

Fig. 4. Trait matching and mismatching between the flowers of *Pelargonium incrassatum* and two fly species, *Prosoeca 'namaquensis'* (top) and *P. peringueyi* (bottom). The histogram of fly proboscis length (grey bars) has two peaks, the first corresponding to the abundant, short-proboscid *P. 'namaquensis'* and the second to the rare *P. peringueyi,* the long proboscis of which matches the floral tube length of *P. incrassatum* (pink bars). *P. incrassatum* is represented by a random sample of the same size as the *Prosoeca* sample ($N = 79$).
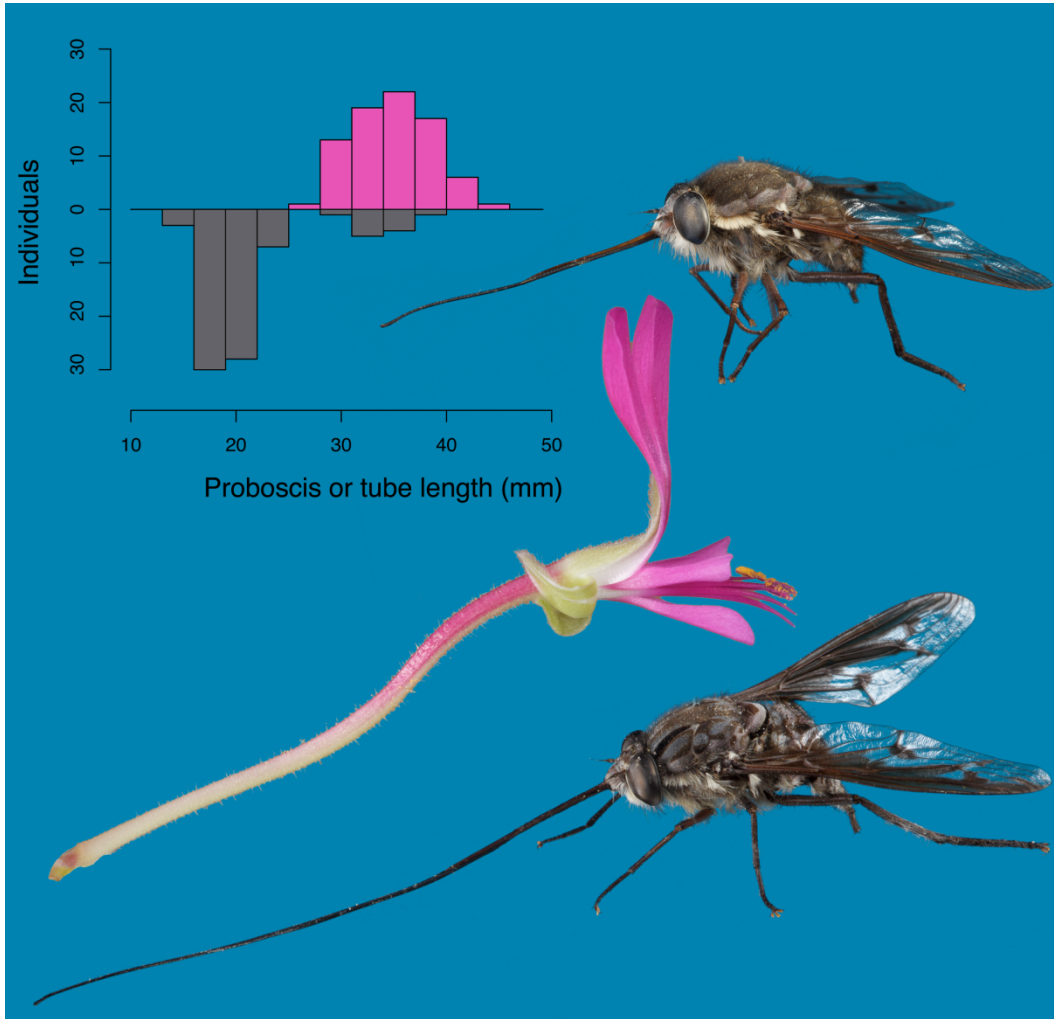
Fig. 5. Variation in the degree of trait matching across ten populations of *Pelargonium incrassatum* pollinated by *Prosoeca* flies in Namaqualand. The location of the study sites is provided in Table S1.

Fitness, $w(x)$

Floral phenotype, x

1   2
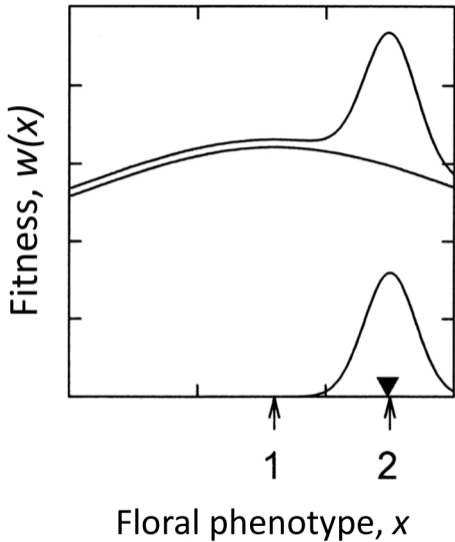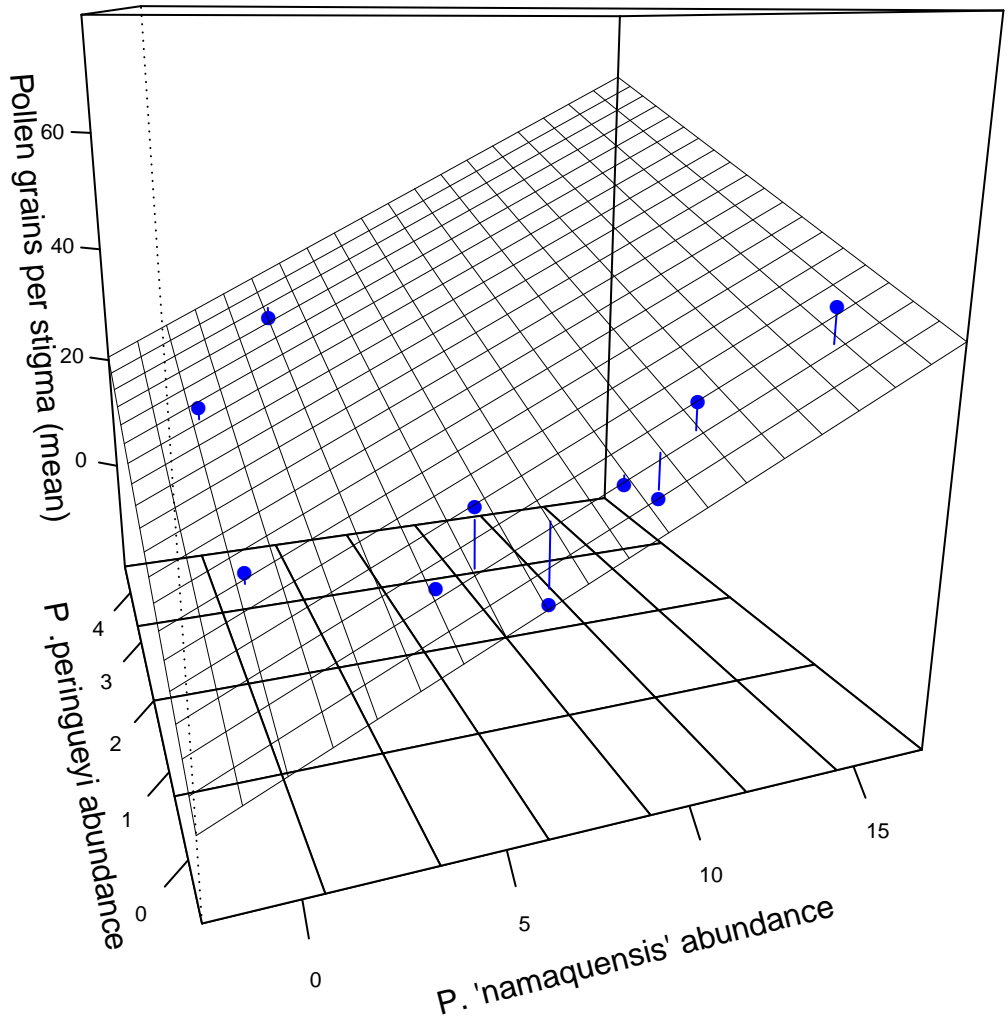
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Individuals (black = flies; white = plants)

Proboscis or floral tube length (mm)