The importance of flower visitors not predicted by floral syndromes

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Abstract

Flower visitors that do not fit a perceived floral syndrome are often over-looked and deemed unimportant. In *Tritoniopsis revoluta*, an irid with a very long corolla tube conforming to the long proboscid fly pollination syndrome, we determine visitation rates of bees and long proboscid flies, as well as ascertain whether bees could be important pollen vectors in this system. We confirmed the presence of extremely long proboscid *Prosoeca longipennis* flies in one *T. revoluta* population, but found that fly visitation was absent in most populations. Instead, we found that in the absence of flies, nectar wells up the corolla tubes and becomes accessible to *Amegilla* bees, which were the most frequent visitors to *T. revoluta* at most sites and carried pollen, suggesting they could effect pollination. The highest bee visitation rates were in *T. revoluta* populations with unusually short tubed plants, where nectar was more accessible than in plants with long corolla tubes. Short tubed populations with more bee activity had higher average seed set than long tubed populations, suggesting that bees might contribute significantly to fitness in this apparently long-proboscid fly pollinated plant. Although these results do not support the use of syndromes for predicting all of a flower's important pollinators, they do support the idea that floral morphology will be shaped by the most effective pollinators at the time.
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1. Introduction

Plant guilds with similar suites of floral traits or syndromes may evolve in order to attract and utilize specific functional groups of pollinators (Vogel, 1954; Faegri and Van der Pijl, 1979; Johnson and Steiner, 2000, 2003; Fenster et al., 2004). The strikingly similar plants within these guilds are often only distantly related (e.g. Goldblatt and Manning, 2000), suggesting independent and often convergent evolution of floral traits to match the traits of their common pollinators (see Anderson and Johnson, 2009; Pauw et al., 2009) — one of the most visual testimonies to natural selection.

Trait complementarity of flowers and their pollinators is widespread (e.g. Goldblatt and Manning, 2000; Anderson and Johnson 2008, 2009; Pauw et al., 2009) and has traditionally

been explained by the 'most effective pollinator principle' developed by Grant and Grant (1965), and Stebbins (1970). There has however been considerable debate about the degree to which the match between floral traits and pollinator traits are due to specialization in plant-pollination systems. In a controversial but influential paper, Waser et al. (1996) proposed that most plants show 'moderate to substantial' generalization in their (seemingly specialized) pollination system. However, Fenster et al. (2004) found widespread specialization when this same data set was analyzed after excluding non-pollinating visitors and after organizing pollinators into functional groups. Ollerton (1996) also argued for widespread generalization because many flowers that seem to show specialization in floral traits, are in fact visited by diverse assemblages of animals that could be *equally or more effective* pollinators. These studies have proposed that the evolution of floral traits in response to selection imposed by observed pollinators might involve more complex adaptive pathways than previously imagined, and have triggered research demonstrating that flowers might attract a broader spectrum of visitors than might be expected based on

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their pollination syndromes (Goldblatt et al., 2001; Gomez, 2002; Fenster et al., 2004; Gomez et al., 2007).

Aigner (2001) also points out that *overall* fitness of a plant is actually a function of all its pollinators, not just the most effective one. As a result, it may be possible to adapt to less effective pollinators in addition to a more effective one, as long as those adaptations increase the overall fitness of the plant. This allows for complex adaptive landscapes with fitness peaks corresponding to evolutionary outcomes spanning the continuum from generalization to specialization. However in reality, trade-offs may make it difficult to adapt to a less effective pollinator without compromising prior adaptations to a more effective pollinator (but see Aigner, 2004).

Many species from several families of Cape plants are pollinated exclusively by long tongued flies and consequently display similar traits, such as elongated tubular flowers, exerted unilateral stamens, a lack of odour, similar colouring and markings and a reward accessible only to long-tongued insects (Goldblatt and Manning, 2000). *Tritioniopsis revoluta* (Burm.f.) Goldblatt (Iridaceae) is an excellent example of a plant displaying characteristics of the long proboscid fly pollination syndrome (Fig. 1). This species underwent a name change from *T. apiculata* (F.Bolus) G.J Lewis after the plant drawings featured in Wijnands and Goldblatt (1992) and it occurs in the Langeberg, Swartberg and Potberg mountain ranges (Western

Cape, South Africa). In the Swartberg mountains, *T. revoluta* has tube lengths of 14–34 mm and Goldblatt and Manning (1999) observed the flowers being visited by the long proboscid fly *Prosoeca ganglbaueri*. In the Langeberg mountains the floral tubes attain much longer lengths of up to 84 mm and until now no pollinators have been observed visiting plants of *T. revoluta* in this area. Manning and Goldblatt (1995, 2005) did, however, capture specimens of *Prosoeca longipennis* visiting the pink, long tubed flowers of other guild members near Riversdale in the Langeberg, but these flies had proboscides only half the length (38–40 mm) of the corolla tubes of the Langeberg *T. revoluta* plants.

We investigated the pollination biology of *T. revoluta* subpopulations in the Langeberg mountains of the Western Cape (South Africa). We asked whether dominant visitors matched guild predictions from floral syndromes. Specifically we wanted to test the prediction of Manning and Goldblatt (1995, 2005), that *T. revoluta* in the Langeberg is pollinated by *P. longipennis*, even though flies of this species captured previously elsewhere in the region have tongue lengths which are much shorter than most *T. revoluta* flowers recorded from the Langeberg. In addition we investigated the role of nectar accessibility in facilitating visitation by non-guild visitors to these apparently specialized flowers and infer contributions of non-guild visitors to pollination success in *T. revoluta* populations.

2. Material and methods

2.1. Study species

Tritoniopsis revoluta (Burm.f.) G.J. Lewis (Iridaceae: Crocoideae) is a seasonal geophyte of moderate size, typically 35–75 cm high and endemic to South Africa. The species grows on acidic oligotrophic Fynbos soils and has a deeply buried and swollen stem (a corm). Individuals annually produce a branched inflorescence with spirally arranged and horizontally orientated flowers which develop acropetally. The flowers are zygomorphic with a prominent dorsal tepal and are strongly bilabiate with unilateral, arcuate stamens and style that are well exerted from the floral tube. The lower three tepals are marked with streaks of dark red, assumed to be nectar guides (Manning and Goldblatt, 2005; Fig. 1). The flowers are unscented to the human nose. Flowering occurs mainly in autumn, between March and May and is synchronized in a population (Manning and Goldblatt, 2005). The floral tube is funnel shaped and elongated, typically 30–70 mm long and pale pink (Manning and Goldblatt, 2005). Nectar is secreted by the septal nectaries and can accumulate in the floral tube (Rudall et al., 2003; Manning and Goldblatt, 2005). *T. revoluta* is protandrous and herkogamous (Goldblatt et al., 1998; Goldblatt and Manning, 1999; Manning and Goldblatt, 2005) which means that autonomous and facilitated autogamy are not likely. Furthermore, preliminary results from breeding system experiments (Ros et al., unpublished data) also suggest that *T. revoluta* is only partially self compatible. Thus in the absence of pollinators, protandry, herkogamy and very limited self compatibility should ensure that seed set is extremely low.
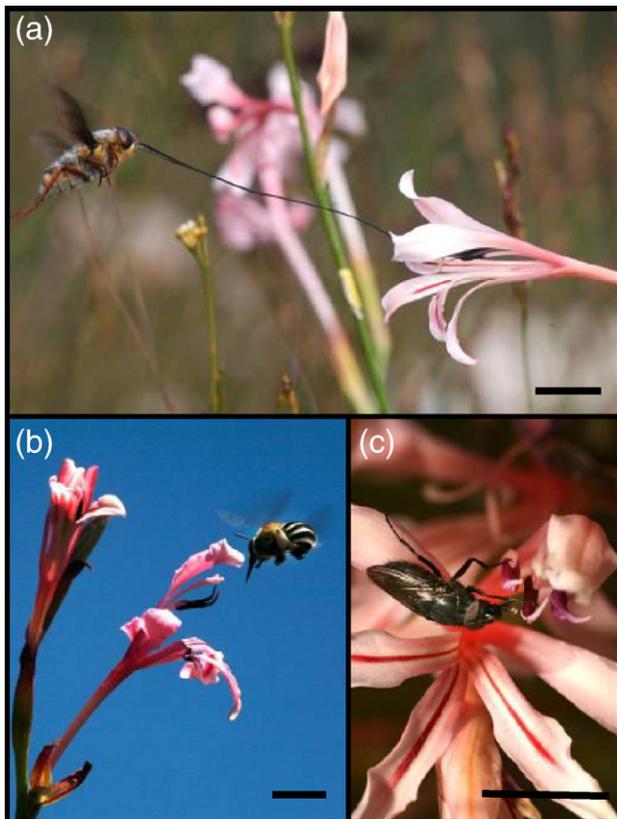

Fig. 1. Visitors to *Tritoniopsis revoluta*, (a) *Prosoeca longipennis* (photo Caitlin von Witt), (b) *Amegilla fallax*, (c) *Cosmina fuscipennis*. Scale bars represent 1 cm in each case.

2.2. Study sites

We studied *T. revoluta* populations along the Gysmanshoek pass (S33°55′54.43″ E21°4′18.65″) in the Langeberg (Fig. 2) for a period of 1 week at the end of March 2008. We quickly realized that plants on the northern side of the pass had very long corolla tubes whereas plants on the southern side had much shorter corolla tubes. Short and long tubed plants were separated by a very narrow transition zone (<100 m) near the top of the pass. We thus divided the Gysmanshoek pass into two subpopulations: Gys long and Gys short. We also conducted observations in a population of very long tubed *T. revoluta* plants on the northern side of the Tradouws pass in the Langeberg (S33°56′48.78″ E20°41′59.22″). A population of short tubed (about 10 mm) *Tritoniopsis ramosa* plants on the southern side of the Tradouws pass (S33°59′9.38″ E20°42′57.11″), which Manning and Goldblatt (2005) record as being pollinated by *Amegilla* bees, served as a comparison between seed set of bee adapted plants visited by bees and putatively fly adapted plants visited by bees.. Observations were also made in a population of shorter tubed *T. revoluta* plants (20–38 mm) in De Hoop Nature Reserve near Potberg (S34°27′15.85″ E20°23′56.27″). This site was very overgrown with proteoid vegetation, making observation work difficult. Fynbos, dominated by *Erica* spp. and *Protea* spp., was the dominant vegetation type at all sites. All sites, except for the De Hoop and *T. ramosa* sites, had burnt within the last five years. The Tradouws and Gysmanshoek sites were located on fire breaks. *Tritoniopsis* plants were flowering abundantly at all sites, suggesting that flowering in this species is not confined to post-fire environments. Although other putatively long tongue fly pollinated plants occur in the area (Manning and Goldblatt, 2005), no other guild members were found at our sites.

2.3. Data collection

We observed and captured pollinators and measured floral traits at all five study sites. Three of the sites (Gys short, Gys long and *T. ramosa*) were more intensively sampled and were used to additionally test pollinator preferences and to assess pollination effectiveness.

2.4. Floral traits

2.4.1. Tube and nectar distance

For the youngest open flower on randomly sampled inflorescences we measured the length of the floral tube and the nectar column to the nearest mm. Tube length was measured as the distance between the top of the ovary and the corolla tube opening (petal split). Nectar welling in the tube was measured after removing the bracts covering the base of the tube. The flowers were backlit using sunlight or a flash light to see the nectar level. The nectar distance, defined as the distance between corolla opening and top nectar level, was calculated as the difference between tube length and nectar level. Nectar levels were measured on unbagged, open-pollinated plants collected between 1000 h and 1400 h. To control for the possibility that nectar production (or depletion) changes with flower age we recorded the stage (male or female) of each flower. Flowers were categorized as female when the style was unfolded and recurved, exposing the stigmatic surfaces and when the anthers were dehisced and without pollen. Male phase flowers had an un-split style and anthers that clearly contained pollen.

2.4.2. Nectar concentration

After removing the bracts and the ovary, the nectar was withdrawn from the base of the floral tube using 5 µL and 10 µL
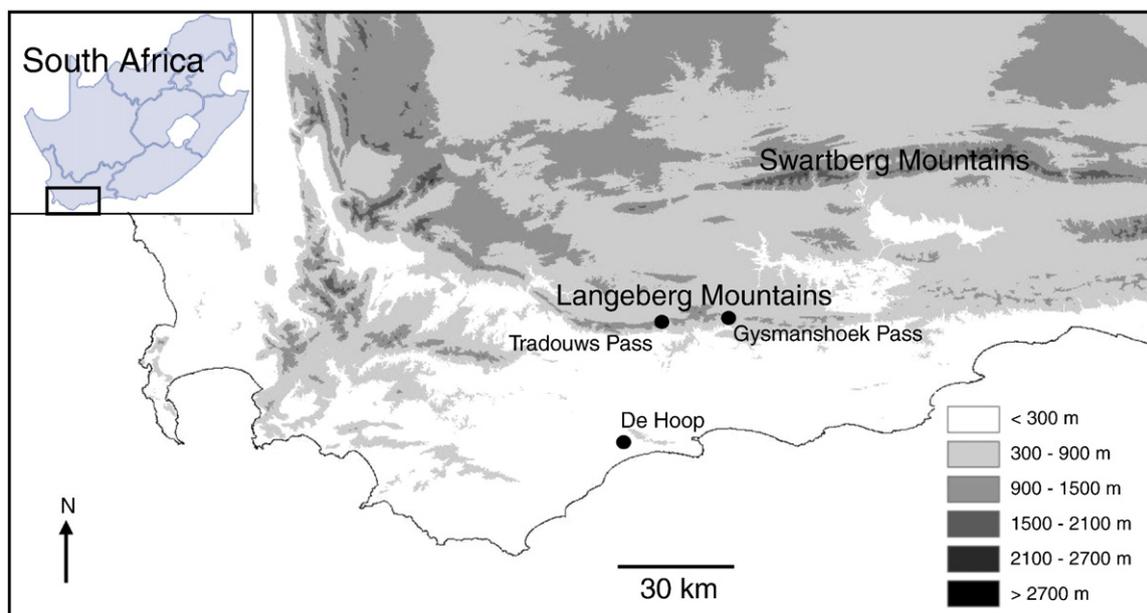

Fig. 2. Map of the study area. Circles represent approximate positions of the study sites used. *Tritoniopsis revoluta* also occurs in the Swartberg Mountains.

capillary tubes. The percentage of sucrose equivalents in fresh nectar was measured with a Bellingham and Stanley hand-held refractometer (0–50%). This technique is suitable for Iridaceae, where the potential for cell sap to dilute the nectar has been shown to be insignificant (Manning and Goldblatt, 2005). Sample sizes for all data collection can be found on the relevant figures.

2.5. Floral visitors

2.5.1. Observations

At each of the sites eight observers walked around for about 60 min and noted every animal visiting the pink *Tritoniopsis* flowers. Attempts were made to catch all flower visitors for pollen analysis. Observations were made between 0900 h–1300 h. No observations were made during the evening, thus although un-likely, we cannot exclude the possibility of hawkmoth visitation.

2.5.2. Pollen analysis

Captured insects were killed in separate vials, labelled and brought back to the University of Stellenbosch for pollen analysis. As the anthers of *T. revoluta* are arcuate, we expected to find *T. revoluta* pollen only on the dorsal part of the thorax of the pollinators. However, in addition to swabbing the dorsal side of the thorax, we also swabbed the frons, the base of the proboscis, the base of the antennae, the legs, the abdomen and the ventral side of the thorax of the insects with a piece of sticky fuschin-gel (Beattie, 1971). Each piece of gel was then liquefied by heating, placed on a glass slide and examined under a microscope. *T. revoluta* pollen on the insects was identified by comparison with a reference slide.

2.5.3. Tongue length

Tongue (glossa) lengths were measured after relaxing the pollinators in a humid jar for 2 days, which allows the tissue to soften and the tongue to be pulled out.

2.6. Pollinator preference

To investigate which animals visit *T. revoluta* and whether these potential pollinators show preference for flowers with either short or long tubes, we conducted choice experiments between 900 h and 1300 h on two consecutive days in the long and short-tubed populations at Gysmanshoek. We observed pollinator visitation to pairs of adjacent long and short-tubed inflorescences.

On both days four observers (two in Gys long and two in Gys short) each watched 5 inflorescence pairs arranged 0.5 m apart. Observers noted pollinator choices on each pair and whether contact was made with the reproductive parts of the flowers. Insects that were observed probing the floral tube or brushing the anthers or stigmas were captured when they left the array.

2.7. Seed set

Seed capsules of open-pollinated *T. revoluta* plants were collected at the Gys long, Gys short, and *T. ramosa* sites on 16 and 17 May 2008 in order to estimate differences in pollination success, particularly between the putatively bee-adapted *T. ramosa* and fly-adapted *T. revoluta* populations. High herbivory at Tradouws eliminated all inflorescences and we were unable to get seed set samples from there. At each site we picked the bottom three capsules of 20 randomly chosen plants, leaving the rest of the capsules untouched. Sixty capsules were collected per site. The capsules were preserved in labelled envelopes, brought back to the University of Stellenbosch and stored in a dry place. The numbers of viable seeds were counted as soon as possible to prevent fungal growth in the capsules. We used average number of viable seeds in the three capsules of each plant as a measure of seed set.

2.8. Data analysis

Kruskal–Wallis rank sum tests, in combination with post hoc Wilcoxon rank sum tests with Bonferroni p value correction, were used to test for differences in tube length, nectar distance and seed set between populations because these data were not normally distributed (tested with the Shapiro–Wilk normality test) and because of heteroscedasticity (tested with the Bartlett test of homogeneity of variances). One way ANOVA was used to test for nectar concentration differences between populations. Two-sample t-tests that do not assume equal variance (Welch t-test) were used to test for differences in nectar distance and sucrose equivalents between male and female phase flowers, and for differences in sucrose equivalents between tube length morphs. Standard regression analyses were used to explore the relationship between tube length and nectar distance across sites as well as within each population. Resampling (permutation, 10^6 iterations) was used to test for significance of regressions because this method is less sensitive to heteroscedasticity and non-normality of residuals than other 'standard' linear model analyses. Pollinator preference for short or long-tubed flowers was tested using

Table 1
Mean tube length, nectar distance and nectar concentration for each study site.

Study site	Average tube length (mm±SE)	Nectar distance (mm±SE)	Nectar concentration (mm±SE)	*Amegilla* with pollen (%)	Other bees with pollen (%)	*Cosmina* with pollen (%)	*Prosoeca* with pollen (%)
Gys short	30.5±0.9[b]	15.3±1.6[cd]	28.4±1.2	83 (12)	33 (3)	11 (9)	–
Gys long	55.8±1.7[c]	31.9±3.3[b]	30.8±0.5	100 (4)	100 (1)	–	–
Tradouws	69.6±2.0[d]	56.8±2.7[a]	28.8±1.0	–	100 (3)	–	100 (1)
T. ramosa	10.6±0.3[a]	7.9±0.3[d]	32.3±0.9	–	–	–	–
De Hoop	29.6±0.8[b]	20.6±1.8[bc]	29.4±1.7	67 (3)	–	–	–

Superscript letters indicate significant differences between sites from posthoc tests using pairwise Wilcoxon rank sum tests with Bonferroni p value correction. The percentage of captured insects that carried pollen are also shown. Numbers in parentheses indicate the total number of individuals analysed for pollen.

binomial tests. All analyses were performed using "R" statistical software (R Development Core Team, 2008).

3. Results

3.1. Tube length

Mean tube length, which differed significantly between sites (Kruskal–Wallis $\chi_4^2 = 106.7$, $p < 0.0001$), was largest at Tradouws and smallest at the *T. ramosa* site (Table 1). All populations except Gys short and De Hoop had significantly different tube lengths (Table 1). Within the three focal sites, the average tube length at the Gys long site was almost twice that of the Gys short site and about five times the length of the *T. ramosa* site (Fig. 3a).

3.2. Nectar

In most subpopulations nectar distance ranged from small (nectar welling nearly to the top of the corolla tube) to the length of the entire tube (no nectar). There were significant differences in the distance to nectar between sites (Kruskal–Wallis $\chi_4^2 = 77.19$, $p < 0.0001$). The mean distance to the nectar was largest at Tradouws and smallest in the *T. ramosa* population (Table 1). Male and female phase flowers had similar nectar levels ($t_{61} = 0.82$, $p = 0.41$). Within the three focal sites, the distance to the nectar in the Gys long site was double that of the Gys short site and three times that of the *T. ramosa* site (Fig. 3b).

Regression analysis revealed that flowers with longer tubes have a greater distance to the nectar ($p < 0.0001$, $R^2 = 0.69$, slope = 0.79, Fig. 4). Even *within* three of the four study sites (Gysmanshoek, Tradouws pass and *T. ramosa*), this relationship between tube length and distance to nectar held true with $R^2 > 0.27$ and $p < 0.02$ at all three sites.

The mean percentage of sucrose equivalents (Table 1) in the nectar over all sites was 29.7 ± 5.0%, which did not differ between subpopulations ($F_{(4,100)} = 1.05$, $p = 0.41$), tube length morphs ($t_{115} = -0.2983$, $p = 0.77$) or male and female phase flowers ($t_{115} = 1.1325$, $p = 0.26$).

3.3. Floral visitors

3.3.1. Observations

Six different species from three functional groups were observed on *T. revoluta* flowers at the sites (Fig. 5). *Amegilla fallax* bees were ubiquitous and visited flowers from all populations, where they were seen probing for nectar and occasionally collecting pollen. Of the 21 A. fallax bees identified,
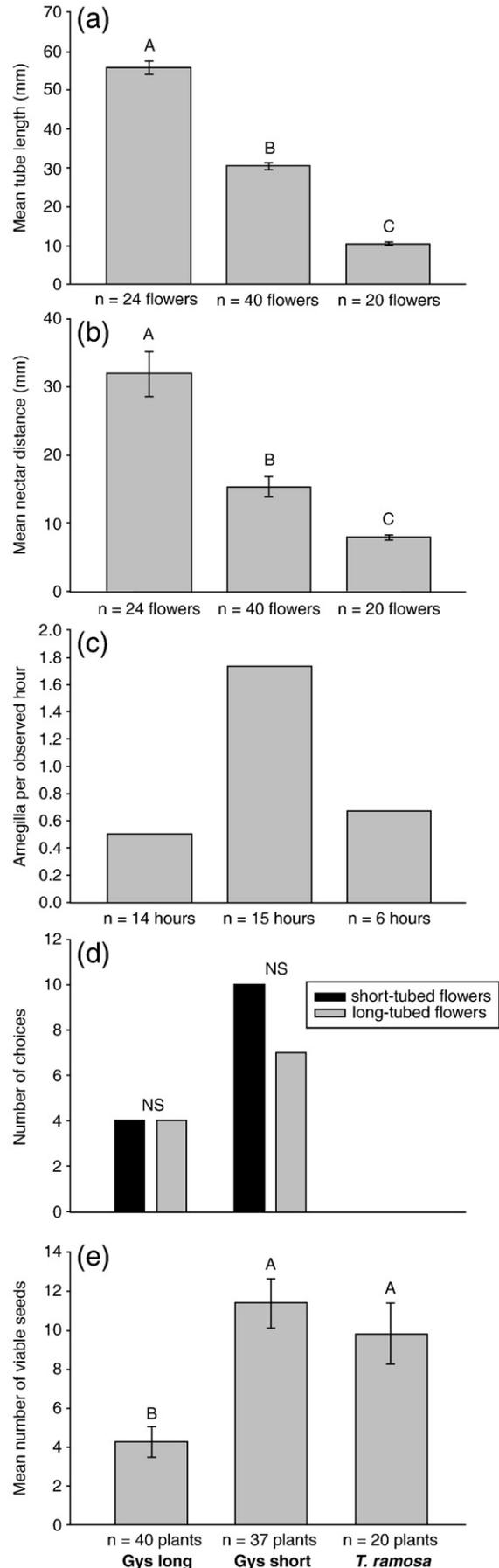

Fig. 3. Differences in the floral traits, foraging behaviour and resultant seed set in the three focal *Tritoniopsis* populations. The *T. ramosa* population served as a control for a bee adapted species. (a) Mean tube length (±SE), (b) mean distance from the corolla mouth to the nectar (±SE), (c) number of *Amegilla* bees observed visiting flowers per observation hour, (d) number of choices made by *Amegilla* bees for either long or short tubed plants at the Gys long site and the Gys short site, (e) mean number of viable seeds (±SE). Different letters indicate significant differences ($p < 0.05$) between the sites; NS = not significant. For each panel the sample size is given in parentheses below each column.
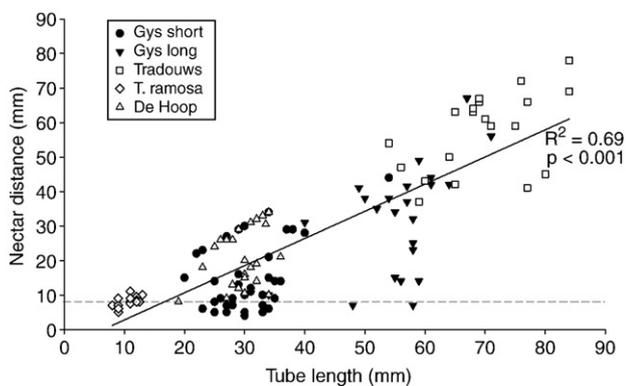
Fig. 4. The relationship between tube length and nectar distance for the five sites. The solid line is the regression line drawn through all the points on the figure and the dotted line is the maximal *Amegilla* bee tongue length measured in this study.

12 were female and nine were male. Carpenter bees, honey bees and an unidentified solitary bee also visited plants incidentally. One long-tongued fly was caught at Tradouws and identified as *P. longipennis* (Diptera: Nemestrinidae). Black calliphorid flies (*Cosmina fuscipennis*) were frequently observed moving slowly around flowers, apparently feeding on pollen grains at the sites on Gysmanshoek pass. At the three focal sites, *Amegilla fallax* bees were seen frequently visiting at Gys short and less frequently at Gys long and *T. ramosa* (Figs. 3c, 5).

3.3.2. Pollen analysis

Only one out of the nine captured calliphorid flies carried *T. revoluta* pollen (11%). Pollen grains of the study species were found on 18 of the 21 captured *Amegilla fallax* (86%), 5 of the 7 other bee species (71%) and on the long-tongued fly (100%) (Table 1).

3.3.3. Tongue lengths

The mean tongue length of the three carpenter bees was 4.7 ± 1.3 mm. The proboscis length of the long-tongued fly was 71.3 ± 0.1 mm, matching the average tube length of the flowers measured at Tradouws. The tongue lengths of *Amegilla fallax* bees ranged from 4.6 to 8.6 mm (mean 6.77 ± 1.33 mm, $n=7$).
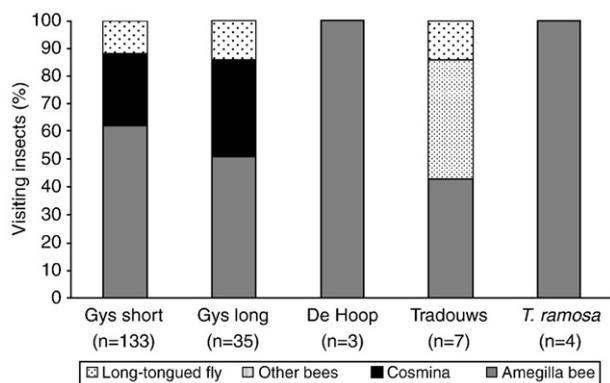

Fig. 5. The composition of *Tritoniopsis* flower visiting insects at each site as a percentages of the total number of observed visitors (in brackets).

3.3.4. Nectar accessibility to Amegilla bees

Thirty percent of measured flowers had nectar distances <8.6 mm, the maximum measured bee tongue length, and thus had nectar which was potentially accessible to *Amegilla fallax* bees. In all subpopulations, except Tradouws, some individual flowers had a nectar level high enough for it to be reached by these bees (Fig. 4, Tradouws — 0%, Gys long — 8%, De Hoop — 4%, Gys short — 31%, *T. ramosa* — 70%). Because nectar was measured in open pollinated flowers, either nectar depletion by pollinators (bees and flies) or insufficient nectar production may explain why 70% of measured flowers had nectar levels that were not accessible to bees.

3.4. Pollinator preference

Amegilla fallax did not show any preference for short tubed flowers at Gys short ($p=0.63$, probability$=0.59$) or at Gys long ($p=1$, probability$=0.5$) and visited the two morphs indiscriminately (Fig. 3d).

3.5. Seed set

Mean seed set per capsule was found to differ strongly between the subpopulations (Kruskal–Wallis $\chi_2^2=21.03$, $p<0.0001$, Fig. 3e). Plants at the Gys short site had similar seed set to the Anthophorid bee-adapted plants at the *T. ramosa* site. In contrast, the long tubed plants at the Gys long site set significantly fewer seeds ($p<0.01$).

4. Discussion

Our single observation of long-proboscid fly pollination in the Tradouws pass population supports the prediction based on floral syndromes, that *T. revoluta* in the Langeberg is indeed visited by *P. longipennis* (Manning and Goldblatt, 2005). In contrast, a similar study on *Pedicularis* in the Himalayas found that very long tubed plants had lost the ability to produce nectar and were pollinated only by pollen collecting bumblebees (Huang and Fenster, 2007). In this system, long proboscid pollinaters do not seem to be the selective agent for long corolla tubes (Huang and Fenster, 2007). In *T. revoluta* long tubed plants did produce nectar and in the population where the long proboscid fly was captured, this nectar was very depleted (Table 1) suggesting that visits from insects with long proboscides may be fairly common here. The fly had an extraordinarily long proboscis (71.3 mm) which matched the average tube length of the plants at this population very closely (69.5 mm), and was much longer than the tongues of individuals captured at Riversdale (38–40 mm) by Manning and Goldblatt (1995).

At most sites the most abundant pollinators were clearly not insects with tongue lengths that matched *T. revoluta* tube lengths. Long proboscid flies were conspicuously and consistently absent from Gysmanshoek and De Hoop during the observation period of this study. During many observation days at these sites in three additional years spanning the flowering time of *T. revoluta* we have never observed a long proboscid fly, suggesting that their absence is real and not a function of our

sampling approach. In their place, we found that *Amegilla fallax* bees with tongues shorter than the flower tubes were ubiquitous pollen-carrying visitors. Even at Tradouws pass bee visitors were more abundant than long proboscid flies. Calliphorid flies were also common visitors but they seldom moved between plants, and their very low pollen loads suggested that they were not important pollinators.

Although Manning and Goldblatt (2005) stated that the long tubes of *T. revoluta* would exclude bees from reaching nectar, we found that *Amegilla fallax* was able to access nectar in *T. revoluta* because it wells up the corolla in the absence of long proboscid flies. However, the nectar is more accessible in short tubed flowers than in long tubed flowers. This may explain why bees were commonly seen visiting flowers in the Gys short subpopulation (30.5 ± 0.9 mm) but were much less commonly seen visiting in the Gys long subpopulation (55.8 ± 1.7 mm). Interestingly bees were unable to distinguish between short and long flowers in choice tests. This means that the high visitation rates at Gys short may be due to foraging behaviour where bees learn that certain areas are better for foraging than others. Since Gys short and Gys long are separated by just a few hundred meters, bees foraging unsuccessfully on the flowers at Gys long would be expected to quickly move away until they find a richer nectar source (i.e. Gys short) or to change to another nectar source. This type of behaviour was observed in another study on long proboscid flies which quickly move away from areas with non rewarding plants but tend to stay in areas with rewarding plants, even if the two can not be distinguished on sight (Anderson and Johnson, 2006).

The importance of bees as pollinators in this system can be inferred by comparing the seed set between Gys long with its low bee visitation rate and Gys short with its high bee visitation rate. Gys short set as many seeds as bee adapted flowers at the *T. ramosa* (10.6 ± 0.3 viable seeds) site, and significantly more seeds than at Gys long. Since there were no other pollen carrying pollinators at these Gys sites this difference in seed set is likely based on bee abundance. Although the proximity of the Gys populations and unpublished data from hand pollination experiments (Ros et al., unpublished) render alternative explanations (like site specific differences in nutrients or the inherent ability of short-tubed plants to set more seeds) unlikely, further experimentation is required to directly establish the link between bee visitation and seed set in this species.

Bee pollination is the most common pollination system in the Iridaceae and is probably the ancestral system for the African irids (Goldblatt and Manning, 2006). In *Gladiolus*, *Lapeirousia* and *Sparaxis*, species pollinated by long-proboscid fly are nearly always most closely related to species pollinated by short-tongued bees of the Apidae family (Goldblatt and Manning, 1999, 2006). In fact, some *Tritoniopsis* species such as *T. toximontana* and *T. pulchella* have tube lengths which straddle the perceived adaptive peaks for both long proboscid fly and bee guilds. These so-called "bimodal systems" have been used to cast doubt on the existence of specialized pollination systems (Waser et al., 1996) although Manning and Goldblatt (2005) noted that bees are only able to operate in the absence of long proboscid flies, as flies are

better competitors for deep nectar. This makes these plants functionally specialized in time. Similarly, if long proboscid flies were abundant in any *T. revoluta* populations, bees would not be able to access the nectar and would not visit frequently (e.g. Tradouws). However, population densities of long-proboscis flies are often highly variable (Goldblatt and Manning, 1999) and so bees may be important in these bimodal systems for providing reproductive assurance (also see Anderson et al., 2003). The ease with which plants can shift from bee to long proboscid fly pollination (and vice versa) means that in any population, temporal fluctuations in pollinator composition may be driving corolla tube lengths towards either the short or the long end of the spectrum. In support of the 'most effective pollinator principle', it is likely that tube length evolution in many *Tritoniopsis* populations seldom reaches an equilibrium and is constantly being pulled one way or another by an abundance of bees or long proboscid flies.

Our results may also be interpreted in the context of Aigner's "total fitness" hypothesis. Bees may be the most effective pollinators most of the time, but overall fitness may be enhanced in some populations by the occasional presence of long proboscid flies. In these populations flowers could have long corollas despite the fact that bees may be the most effective pollinators most of the time. Evidence for this is that in all populations of *T. revoluta*, even where bees are clearly the most abundant pollinators, *Tritoniopsis* tube lengths were at least three times as long as the bee tongues, suggesting adaptation to long proboscid flies. Unlike studies which may support the "total fitness" hypothesis (e.g. Mayfield et al., 2001; Aigner, 2004), there is a clear trade-off in the *T. revoluta* system between adaptations to bees versus adaptations to flies: bees are less abundant visitors on plants with extremely long corollas than they are on plants with very short corollas. This is because long-tubed flowers effectively exclude most bee visitation by having less accessible nectar. As a result we tentatively reject Aigner's "total fitness" hypothesis as an explanation for long tubes in this system.

5. Conclusion

We conclude that although syndromes may provide clues about prospective pollinators, they are not always a fail-safe way of predicting all of a flowers' important visitors. Pollinators which do not fit the floral syndrome may still be important visitors that should not be ignored. It is likely that different functional groups of pollinators exert selective pressures on plants acting in different directions and as a result floral morphology may occasionally not match either functional group when pollinator abundances fluctuate in time and space. Since relative abundances of pollinators can change in space and time, the optimal trait characteristics of the plants can also change to match those pollinators, potentially giving rise to a geographic mosaic of plants with different tube lengths.

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