


RESEARCH ARTICLE

Pollination syndrome accurately predicts pollination by tangle-veined flies (Nemestrinidae: *Prosoeca s.s.*) across multiple plant families

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Keywords

Adenandra; coevolution; convergent evolution; floral deception; Fynbos vegetation; long-proboscid flies; *Stuckenbergina africana*.

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ABSTRACT

- The idea that a syndrome of floral traits predicts pollination by a particular functional group of pollinators remains simultaneously controversial and widely used because it allows plants to be rapidly assigned to pollinators. To test the idea requires demonstrating that there is an association between floral traits and pollinator type.
- I conducted such a test in the Cape Floristic Region of South Africa, by studying the pollination of eight plant species from six families that flower in spring and have scentless, actinomorphic, upwards-facing flowers, with orbicular petals all held in the same plane. The petals are brilliant-white with red-purple nectar guides. The tubes are short and hold small volumes of concentrated nectar, except in the rewardless *Disa fasciata* (Orchidaceae). Pollinators were photographed and captured, pollen loads were analysed and pollination networks were constructed.
- Consistent with the pollination syndrome hypothesis, the species with the defined syndrome shared a small group of pollinators. The most frequent pollinators belonged to a clade of four tangle-veined fly species with relatively short proboscises (Nemestrinidae: *Prosoeca s.s.*), while functionally similar Bombyliidae and Tabanidae played minor roles. Among the four *Prosoeca* species, only *Prosoeca westermanni* has been described, a result that highlights our ignorance about pollinators.
- The demonstration of an association between the syndrome of traits and pollination by this group of flies explains the repeated evolution of the syndrome across multiple plant families, and allows prediction of pollinators in additional species. More generally, the result validates the idea that the traits of organisms determine their ecology.

INTRODUCTION

The idea that traits predict ecology is a cornerstone of biology (McGill *et al.* 2006; Vellend 2016). Species that share traits, and hence ecological function and niche, are often grouped into a guild or functional group (Simberloff & Dayan 1991; Blondel 2003). Examples include pioneer plants, whose small seeds and high growth rates allow them to seize disturbed areas (Tilman 1994), and megaherbivores, whose large body mass results in predictable changes in vegetation (Owen-Smith 1988). Species in the same guild need not be related, but may be if traits are evolutionarily conserved (Webb *et al.* 2002).

In pollination biology, the idea that traits predict a niche is distilled in the pollination syndromes concept, according to which a characteristic combination of co-occurring floral traits is associated with the utilization of a specific group of pollinators (Delpino 1873; Vogel 1954; Faegri & van der Pijl 1979; Fenster *et al.* 2004; Dellinger 2020). For example, in the South African flora, 200 plant species with nectar tubes measuring 30–90 mm, nectar volumes ranging from 1.1 to 5.0 μl , nectar sugar concentrations of 20–30% and no scent, are pollinated by long-proboscid flies in the Nemestrinidae and Tabanidae (Goldblatt & Manning 2000).

The pollination syndrome concept is logically extended into predictions of pollinator type on the basis of floral features, and this allows large numbers of plant species to be rapidly assigned to pollinators. In ecological studies, the aim may be to combine information about pollinator use with plant community composition data to test for the existence of pollinator mediated competition or facilitation in the assembly of communities (Pellissier *et al.* 2010; Heystek & Pauw 2014; Pauw 2018). In evolutionary studies, pollination mode (inferred from floral syndromes) is typically plotted onto a phylogeny. The aim may be to test whether higher diversification rates are associated with a particular mode of pollination (Serrano-Serrano *et al.* 2017), or whether switches in pollination mode drive plant adaptation and speciation (Waterman *et al.* 2011).

Despite its apparent utility, the use of pollination syndromes to assign species to pollinators is open to criticism because there have been too few attempts at testing the predictive power of floral traits (Ollerton *et al.* 2009; Dellinger 2020). The challenge is to find “detectable associations between plant phenotypes and pollinators” (Waser *et al.* 1996). Some studies have found this association (Hargreaves *et al.* 2004; Wilson *et al.* 2004; Pauw 2006; Geerts & Pauw 2009; Marten-Rodriguez *et al.* 2009; Danieli-Silva *et al.* 2012; Rosas-Guerrero *et al.* 2014; Johnson & Wester 2017; Lagomarsino *et al.* 2017;

Bilbao *et al.* 2021; Rose & Sytsma 2021), whereas in other cases, floral traits and pollinators were not associated, because each plant species was found to be pollinated by many kinds of animal (Waser *et al.* 1996; Ollerton *et al.* 2009; Hilpman & Busch 2021; Amorim *et al.* 2022), or primarily by animals that do not match the prediction (Huang & Fenster 2007; Li & Huang 2009; Pauw *et al.* 2020).

Here I contribute to this debate by testing whether a group of very distantly related plant species with a characteristic combination of floral traits share a mode of pollination. The plants grow in Fynbos vegetation in the mountains of the South-Western Cape, South Africa, and include Rutaceae, Ericaceae, Geraniaceae, Lobeliaceae, Iridaceae and Orchidaceae. The previously unrecognized syndrome of traits which they share includes (i) mid-September to early October flowering and flowers that are, (ii) large (20–60 mm), (iii) scentless, (iv) actinomorphic and (v) upwards-facing, (vi) with petals all held in the same plane. The petals are (vii) orbicular and (viii) brilliant-white, with (ix) small, red-purple nectar guides that lead into a (x) short (2–20 mm) nectar tube. The co-occurrence of these traits across multiple species suggested that they constitute a pollination syndrome that results from adaptation to a particular set of pollinators.

First, I confirmed that the focal plant species share these and additional traits using detailed measurements. Then I tested the prediction that the plant species are associated with a particular group of pollinators by observing them at 11 field sites over 6 years and by analysing pollen sampled from the bodies of captured pollinators. Plants that share pollinators often reduce heterospecific pollen transfer by placing pollen on different parts of the pollinator (Steiner & Whitehead 1988; Manning & Goldblatt 1996; Pauw 2006; Huang & Shi 2013). To assess whether the plant species partition the pollinator's body among them, I separately sampled pollen from different parts of the body.

MATERIAL AND METHODS

Floral traits

At 11 sites (Appendix S1), I studied all species that had at least seven of the ten floral traits listed above. To test whether these proposed guild members also conform in more detailed traits, I measured the size of the display (maximum diameter across petal tips) and floral tube length to the nearest 0.1 mm using a ruler. Nectar volume was measured with graduated 5- μ l microcapillary tubes, and sugar concentration (in sucrose equivalents) was measured in the field with handheld refractometers (Eclipse 0–50% and 45–80%; Bellingham & Stanley, UK). The standing crop of nectar was measured, *i.e.* the nectar volume in flowers that were open to natural pollinator visitation. Measurements were made early in the morning, before pollinators became active. Only fresh flowers were used and rainy days, when nectar may be diluted, were avoided. For colour measurements, flowers on long stems were stood in water and transferred to the lab in a cooler box. Flower colour was measured on the day of collection using an USB2000 fibre optic spectrophotometer with a deuterium–tungsten halogen lamp. The predominant petal colour was measured, and the nectar guides were avoided. Specular reflectance was reduced by mounting the fibre optic probe at 45° to the corolla, 1 mm from the surface. A white standard (WS-1-SS) was used to

re-calibrate between each measurement, and SpectraSuite software was used for data capture (Ocean Insights, Rostock, Germany).

Pollinator observations

To test whether the plant species that share the defined floral syndrome share pollinators, I photographed and captured all pollinator species visiting the focal flowers. Pollinator observations were made on 30 calm, sunny days distributed over 6 years (September to October 2016–2021; Appendix S2). Potential pollinators were captured with an insect net and killed with potassium cyanide in a clean killing jar (BioQuip Products, Compton, CA, USA). The head and body length, wing length and proboscis length was measured to the nearest 0.1 mm using a ruler.

Pollen analysis

In the hope of detecting additional links in the network of observed interactions, I sampled pollen from the bodies of captured pollinators. The analysis was restricted to 49 individuals belonging to the seven most abundant pollinator species. Pollen was sampled using 3 × 3 × 2 mm blocks of fuchsin gel held in forceps (Beattie 1971). The gel was dabbed all over the body part being sampled, which removed about half of the pollen. I melted the gel onto slides and compared the sampled pollen under a compound microscope with a reference collection from the focal plant species. *Erica fastigiata* pollen was observed to be aggregated into tetrads, so the number of tetrads was counted and multiplied by four. In *Disa fasciata* (Orchidaceae) the pollen is aggregated into two, sectile pollinaria, each with its own sticky viscidium. The mean number of pollen grains per flower in the Orchidoideae has been estimated at 101237 (N = 8 species) (Staedler *et al.* 2018). This number was halved to obtain an estimate of the number of pollen grains per pollinarium in *D. fasciata*. To test whether the plant species use mutually exclusive parts of the pollinator, pollen was sampled separately from six body regions (dorsal abdomen, dorsal thorax, frons, proboscis, ventral thorax, ventral abdomen).

Data presentation

The data are presented as pollination networks, in which links represent either the number of visits observed, the average number of pollen grains transported per individual insect, or the number of sites at which either direct observation or pollen data indicate that the interaction had occurred. The latter network combines information from the former two datasets and gives an indication of how ubiquitous the interaction is. Networks were drawn using the function 'plotweb' in the 'bipartite' package in R statistical software (R Core Team 2016). Network connectance was calculated as the number of observed links divided by the number of possible link, which is the number of animal species multiplied by the number of plant species.

RESULTS

Guild composition and distribution

At the 11 study sites (Appendices S1, S3), I found eight plant species that conform to the proposed pollination syndrome

(Table 1). The members of the proposed pollination guild were found to occur in the well-watered, Fynbos-clad, coastal mountains of the South Western and Southern Cape, in particular the Cape Peninsula, the Boland, Hottentots Holland, Rivier-sonderend, Kleinrivier and Langeberg Mountains, where rainfall exceeds 500 mm-year⁻¹. The distribution range is bound by the Doring river, 200 km north of Cape Town, and by the Gourits river, 330 km east of Cape Town. Within this region the proposed guild is absent from the drier, coastal lowlands and does not occur in the arid interior.

Guild richness ranged from three to six plant species per study site, and composition varied with post-fire successional stage. Flowering in *Geissorhiza ovata* and *D. fasciata* peaked in the first years after a fire, but by year 5, these small geophytes were overshadowed, and the shrubs, *Adenandra villosa* and *E. fastigiata*, were in peak flower. Guild composition was also geographically structured: *E. fastigiata* dominated at Jonkershoek and Kogelberg, but was absent from the Cape Peninsula, where *Ad. villosa* dominated.

Floral traits

Detailed observation showed that although the flowers of the proposed guild members were overall similar, there were some interesting differences (Table 1). Only five of the eight species had vertically oriented flowers. Flower size generally ranged from 22 to 43 mm, but the flowers of *E. fastigiata* were unusually small. The small size of the flowers was, however, compensated for by clustering them in fours at the ends of the branches. Floral tube length for most species fell in the range 8–20 mm. The outliers were *Ad. villosa* and *Aristea spiralis*. In *Ar. spiralis*, there was no tube, and the nectar formed exposed droplets at the bases of the long filaments. In *Ad. villosa*, there is a hypanthium of only 2.1 mm, which is extended for another 5.0 mm by a funnel formed from the connivent stamens and staminodes, each tipped with a large, viscous gland (Appendix S4). Nectar volumes were small, but variable (0.3–6.5 µl), and concentrations were high, but variable (12.5–67.0 g·100 g⁻¹). The exception was *D. fasciata* which contained no nectar, or miniscule amounts of fluid (mean = 0.018 µl, N = 5; Table 1).

Flower colour measurements showed that all flowers had consistently high reflectance throughout the human visible

spectrum, as might be expected from white flowers (Fig. 1). Reflectance continued below 400 nm, *i.e.* into the UV range, typically dropping off sharply before 350 nm. *Ar. spiralis* had a small peak at 300 nm and *E. fastigiata* had a pronounced peak at 270 nm, outside the detection ability of most insects.

Pollinator observations

Visitation rates were generally very low. I observed 354 visits by 11 insect species to the flowers of the eight focal plant species (Fig. 2a). Four species of large-bodied, short-proboscid *Prosoeca* (Diptera: Nemestrinidae) contributed 297 of the visits (Fig. 3). Body length (including head) varied from 13.9–18.0 mm and proboscis length ranged from 3.1 to 7.0 mm (Table 2). The large, black *Prosoeca westermanni* was the most widespread, occurring at five of the 11 sites (Fig. 3a,b). The remaining three *Prosoeca* species had small ranges within the distribution of *Pr. westermanni*. Three of the *Prosoeca* species have not been described and are therefore given preliminary names, which are placed in italics to avoid invalidation of a more detailed description. Representative collection accession numbers are listed in Appendix S5. Recent phylogenetic work showed that these three species, along with *Pr. westermanni*, constitute a small, deeply diverged clade of unusually short-proboscid species that is sister to the remaining 60 *Prosoeca* species (Theron *et al.* 2022). Since *Pr. westermanni* is the type species for the genus, the clade is referred to as the *Prosoeca sensu stricto* clade.

The members of the *Prosoeca s.s.* clade were usually detected by the buzzing sound of their flight. They spent the vast majority of their time either patrolling small territories (males) or searching for egg-laying sites (females), and occasionally made brief foraging bouts. In contrast with related *Moegistorhynchus longirostris* (Nemestrinidae) (Pauw *et al.* 2009), the males' territories usually did not contain nectar plants. Also unlike *M. longirostris*, the members of the *Prosoeca s.s.* clade did not hover while feeding, but always landed on the flower. The elongated hind legs often found purchase underneath the petals and drew the body and proboscis into the flower (Fig. 3). In cases where the nectar tube is longer than the proboscis, the flies were presumably accessing that portion of the nectar that wells up inside the tube.

Table 1. Floral traits of the plants that conform to the *Prosoeca westermanni* pollination syndrome shown as mean ± SD (N), where N is the number of plants.

plant family	plant species	flower orientation	diameter of display (mm)	nectar tube length (mm)	nectar volume (µl)	conc. (g 100 g ⁻¹)
Ericaceae	<i>Erica fastigiata</i> L. var. <i>fastigiata</i>	Vertical	6.5 ± 0.5 (3)	8.6 ± 1.2 (9)	1.0 ± 1.3 (10)	26.7 ± 22.7 (5)
Geraniaceae	<i>Pelargonium longicaule</i> Jacq. var. <i>longicaule</i>	Horizontal	41.2 ± 2.3 (3)	18.6 ± 2.3 (21)	2.1 ± 2.3 (14)	15.0 ± 10.1 (5)
Iridaceae	<i>Aristea spiralis</i> (L.f.) Ker Gawl.	Horizontal	59.0 ± 1.4 (2)	0 ± 0 (3)	6.5 ± 0.7 (2)	12.5 ± 0 (2)
Iridaceae	<i>Geissorhiza ovata</i> (Burm.f.) Asch. & Graebn. LC	Vertical	28.3 ± 3.3 (6)	17.2 ± 2.7 (17)	0.7 ± 1.0 (8)	33.0 ± 7.0 (3)
Iridaceae	<i>Gladiolus debilis</i> Sims	Horizontal	42.8 ± 3.3 (5)	16.3 ± 1.8 (12)	3.2 ± 2.0 (6)	43.6 ± 17.6 (5)
Lobeliaceae	<i>Cyphia volubilis</i> (Burm.f.) Willd. var. <i>volubilis</i>	Vertical	23.1 ± 2.1 (4)	9.4 ± 1.2 (8)	0.3 ± 0.5 (4)	20.0 (1)
Orchidaceae	<i>Disa fasciata</i> Lindl.	Vertical	22.0 ± 1.4 (4)	8.6 ± 0.4 (5)	0.0 ± 0 (5)	NA
Rutaceae	<i>Adenandra villosa</i> (Berg.) Licht. ex Roem. & Schult.	Vertical	24.7 ± 2.3 (9)	2.1 ± 0.4 (6)	0.4 ± 0.8 (39)	67.0 ± 4.1 (8)

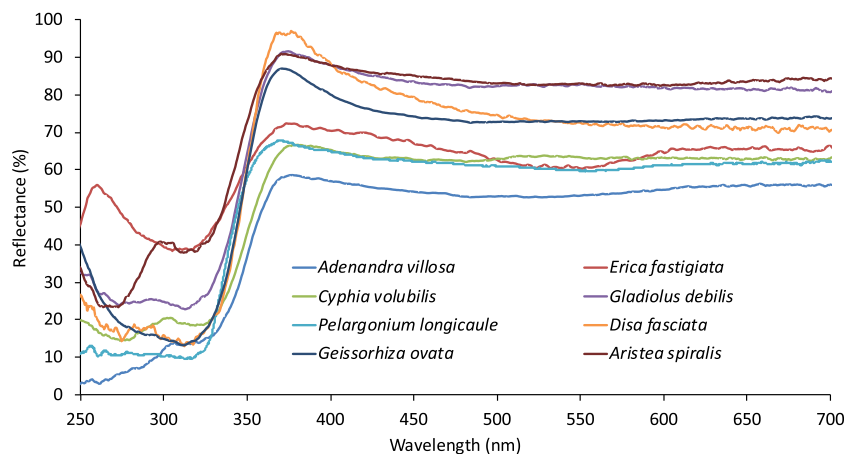


Fig. 1. Spectral reflectance for confirmed members of the *Prosoeca westermanni* pollination guild.

The proboscises of the flies in the *Prosoeca s.s.* clade were found to be short and stout relative to those of the well-studied members of the genus *Prosoeca*, in which proboscis elongation is achieved mainly by the remarkable elongation of the labium, which can be more than twice as long as the labrum (Table 2) (Karolyi *et al.* 2012). In the *Prosoeca s.s.* clade, the labium is only marginally longer than the labrum. The four members of the *Prosoeca s.s.* clade were further characterized by having fleshy, spreading labella that form a conspicuous bulbous end to the proboscis, whereas the labella of long-proboscid Nemestrinidae are typically slender, able to diverge only slightly and do not form a conspicuous thickening at the tip of the proboscis (Appendix S6). The exposed inner surfaces of the labella were traversed by a series of channels (pseudotracheae) along which the nectar presumably moves, and these channels are deep and conspicuous in the *Prosoeca s.s.* clade.

Among the less frequently observed pollinators (Fig. 2a), the largest number of visits was contributed by *Stuckenbergina africana* Oldroyd (Tabanidae: Pangoniinae: Pagoniini). It occurred at one site where it visited *Ge. ovata* and the rewardless *D. fasciata*, often switching between these two species (Fig. 4). The next most frequent of the occasional visitors was the butterfly, *Papilio demodocus*, which visited *Ge. ovata* at one site, where it grew in close proximity to the larval host plant, *Notobubon* (Apiaceae). Next in order of importance was the fly *Australoechus* sp. (Bombyliidae), which visited *Ge. ovata* and was the only visitor observed on *Cyphia volubilis* (Appendix S7). These less frequent pollinators are not closely related to the *Prosoeca s.s.* clade, but nevertheless have similar functional traits, including a slender, medium length proboscis and spring activity.

Pollen analysis

Pollen identification on the bodies of captured insects showed that they transported large loads of pollen from the guild members (Appendix S5). The pooled samples from an individual fly, on average, contained 427 ± 503 pollen grains (excluding *Disa* pollinaria) belonging to 2.27 ± 0.97 guild members (mean \pm SD). Many interactions that were not directly observed were revealed (Fig. 2b), and when these interactions are added to those that were directly observed, the connectance

of the network increased from 0.20 to 0.40 (Fig. 5). For example, *Pr. westermanni* was observed on four guild members, but carried pollen of six, and in the case of *Pr. Alticola*, the number of guild members known to be visited increased from one to six. In many cases, flies carried pollen from species that were not present within 200 m of the site of capture, indicating long-distance pollen transport.

The pollinators carried relatively little pollen from species outside the guild. This ‘foreign’ pollen comprised on average 10.2% of the pollen load per individual. Large amounts of foreign pollen were detected on *Moegistorhynchus “pictus”* (Appendix S7) and *S. africana* (Fig. 4a). The former species carried 950.3 ± 999.9 pollen grains of *Gladiolus carneus* and the latter carried 103.0 ± 75.3 pollen grains of *Gerbera* sp. (mean \pm SD; $N = 3$ for both species). When these species are excluded and only the four members of the *Prosoeca s.s.* clade are considered, the percentage of foreign pollen transported drops to 4.3%.

Pollen placement

Analysis of pollen distribution on the bodies of the pollinators shows precise placement, but a fair amount of overlap among species (Fig. 6): peak pollen abundance for *Ge. ovata* occurred on the frons region; for *Gladiolus debilis* on the dorsal thorax; and for *Pelargonium longicaule* and *Ar. spiralis* on the ventral abdomen. Pollen of *D. fasciata*, *E. fastigiata* and *Ad. villosa* co-occurred on the proboscis, but interspecific pollen transfer between *Disa* and the other two species will be limited because they flower during different post-fire successional stages. *Erica* and *Adenandra* pollen, however, often occurred on the same proboscis. *Adenandra* pollen usually occurred as aggregated masses that were precisely glued to the base of proboscis by the viscous secretion from the glands on the tips of the stamens and staminodes (Appendix S4). Although relatively little *Geissorhiza* pollen occurred on the smooth proboscis, it was interesting to see large amounts of *Geissorhiza* pollen adhered to the *Disa* pollinaria, suggesting a potential benefit provided by the *Disa* to its model. The effectiveness of pollen delivery was very high in *D. fasciata*. I examined all flowers on 41 *D. fasciata* plants (70 flowers) and found that $62.6 \pm 41.7\%$ (mean \pm SD) of flowers had pollen massulae on their stigmas (Fig. 4c).

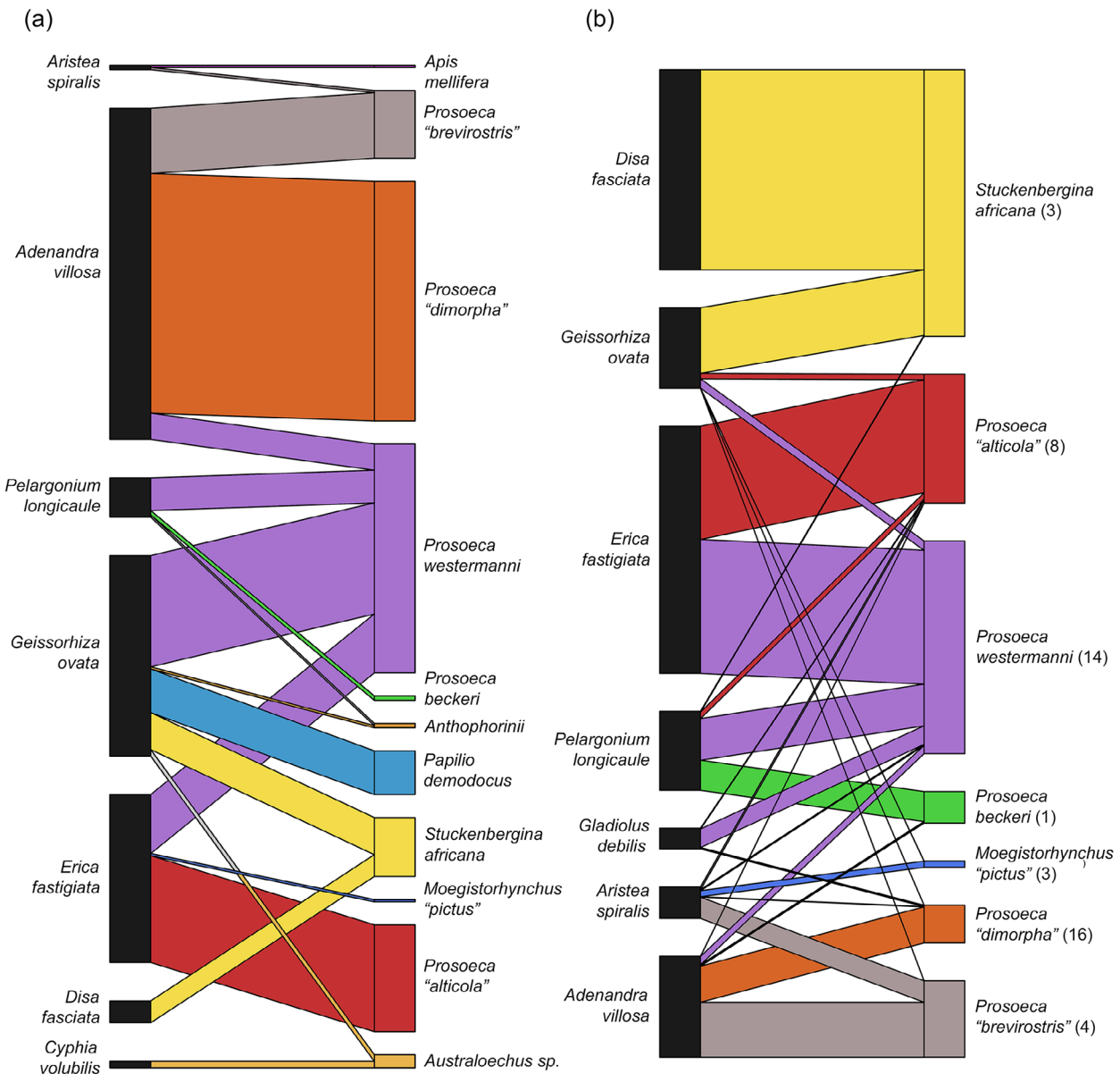


Fig. 2. Bipartite networks of interaction between the plants that conform to the *Prosoeca westermanni* pollination syndrome and their pollinators. (a) Network of 354 observed visits, with line thickness varying from 1 to 110 visits. (b) Pollen transport network, where line thickness is the average number of pollen grains carried per sampled insect. The thinnest lines represent 0.125 pollen grains, and the thickest is 666 grains. The number of individuals sampled is in brackets. The link from *Disa fasciata* is shown at 1% of its actual width because its pollen is aggregated into pollinaria that contain thousands of pollen grains.

DISCUSSION

The results presented here are consistent with the prediction of the pollination syndrome concept that a group of eight plant species, which share a characteristic set of floral traits, share a functional group of pollinators. This finding is contrary to the expectation of generalization in pollination systems and a consequent lack of an association between plant phenotype and pollinators (Waser *et al.* 1996). More broadly, the results validate the idea that the traits of organisms are one of the key determinants of their ecology (McGill *et al.* 2006).

The main pollinators of the eight study species were a small group of large flies with proboscises measuring 3–7 mm and a

period of activity spanning mid-September to early October (Figs 3–5). The four most frequent pollinators were the sole members of the *Prosoeca s.s.* clade, a small, well-defined clade of tangle-veined flies (Nemestrinidae), of which *Pr. westermanni* is the only described species. Within an interaction network, a guild may be named for an important hub species that its members collectively exploit. Hence, the plants may be said to belong to the *Pr. westermanni* pollination guild and the pollinators to the *Ad. villosa* flower visitor guild.

The results of this study show how asymmetrical our knowledge of plant–pollinator interactions often is. The plant species in the system were all named more than 150 years ago, whereas four of the important tangle-veined fly species (Nemestrinidae)



Fig. 3. Interactions between members of the *Prosoeca* s.s. clade (Nemestrinidae) and their nectar plants: (a) *Prosoeca westermanni* pollinates *Geissorhiza ovata* (Iridaceae) in burnt vegetation at Houwhoek. The orange pollen of *G. ovata* is on the frons while ivory pollen of *Gladiolus debilis* is on the thorax. (b) A female *Pr. westermanni* pollinates *Pelargonium longicaule* at Eerste Waterval, Jonkershoek; the stigma contacts the ventral abdomen. (c) A female *Prosoeca* “*dimorpha*” pollinates *Adenandra villosa* at Steenberg Peak, Cape Peninsula. (d) A male *Prosoeca* “*alticola*” pollinates *Erica fastigiata* at Pic-sans-Nom, Jonkershoek. The sizes of the flies are in Table 2.

Table 2. Traits of the most important flies in the *Adenandra villosa* flower visitor guild shown in mm as mean \pm SD (N). Hottentots Holland includes Houwhoek, Kogelberg and Jonkershoek.

fly species	proboscis	wing	head & body
<i>Prosoeca</i> “ <i>alticola</i> ”	5.5 \pm 0.3 (14)	14.4 \pm 1.1 (14)	14.2 \pm 1.1 (14)
<i>Prosoeca</i> “ <i>brevirostris</i> ”	3.1 \pm 0.3 (10)	15.9 \pm 1.3 (10)	14.4 \pm 1.9 (10)
<i>Prosoeca</i> “ <i>dimorpha</i> ”	4.3 \pm 0.4 (17)	13.9 \pm 0.9 (17)	13.9 \pm 1.0 (17)
<i>Prosoeca westermanni</i> (Cape Peninsula form)	4.8 \pm 0.2 (3)	16.8 \pm 0.9 (3)	15.9 \pm 0.8 (3)
<i>Prosoeca westermanni</i> (Hottentots Holland form)	7.0 \pm 0.8 (17)	18.4 \pm 1.1 (15)	18.0 \pm 1.5 (17)
<i>Stuckenbergina africana</i>	6.3 \pm 0.9 (3)	11.0 \pm 0.3 (4)	12.2 \pm 0.5 (4)

have not been collected before, although they are large insects and occur within sight of Cape Town. In addition, the anomalous genus *Stuckenbergina*, in the horsefly family (Tabanidae; Fig. 4), was previously known from only three specimens. It is the only southern Africa representative of the tribe Pangoniini (Oldroyd 1962).

Pollen analysis added additional links to the network of observed interactions (Fig. 2a versus b). Pollen from different guild members often co-occurred on the body of an individual pollinator. However, opportunities for interspecific pollen transfer were somewhat reduced by differential pollen placement (Fig. 6). Flies carried remarkably little pollen from other

plant genera outside the guild, suggesting that not only the plants, but also the flies were specialized.

Pollen could confidently be assigned to genera, but not to species. However, usually only one species per genus was in flower at a site. The genus *Erica* was an exception in this respect. There were often several species in flower at a site, but I did not see visits to any species other than *E. fastigiata*. *Erica* pollen was only found on pollinators at sites where this species was present. The result suggests that the other *Erica* species lack the necessary floral traits for attracting and interacting with the flies in the *Ad. villosa* flower visitor guild. It is remarkable to observe such differential specialization within one genus.

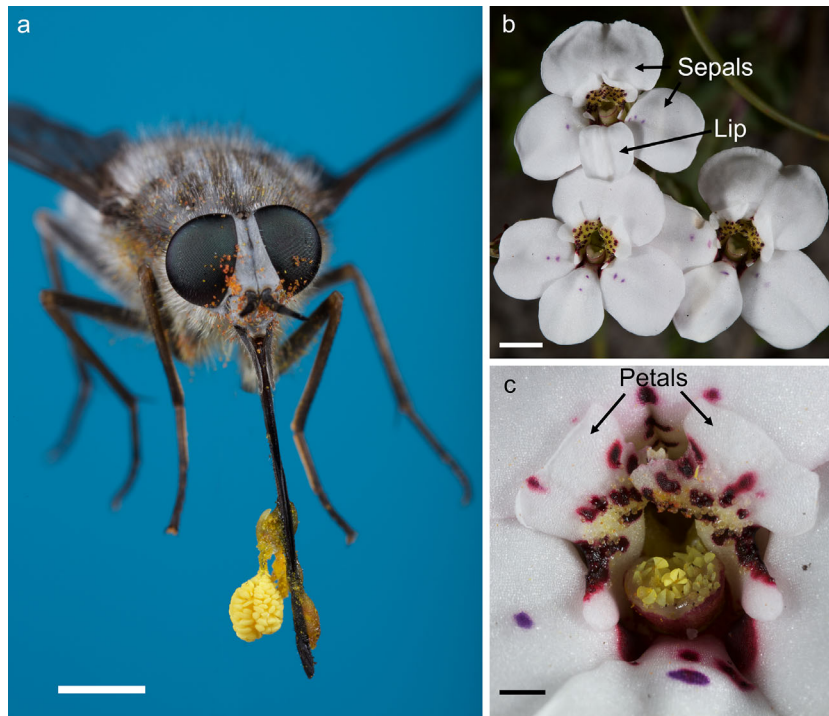


Fig. 4. Pollination of the rewardless *Disa fasciata* (Orchidaceae). (a) A female horsefly *Stuckenbergina africana* (Tabanidae), with a pollinarium and several viscidia of *D. fasciata* attached to its proboscis. Orange pollen grains of *Geissorhiza ovata* adhere to the frons and eyes. Scale bar = 2 mm. (b) *D. fasciata* is unusual in *Disa* in having a lip that resembles the sepals, and in this way actinomorphy is achieved. Scale bar = 5 mm. (c) Pollen massulae on the stigma flanked by the lateral petals (Voorberg, Kogelberg). Scale bar = 1 mm.

The demonstrated association between plant phenotype and pollinators allows prediction of the pollinators of species that occur outside of the study area. Based on their floral traits, *Erica ampullacea* Curtis, *Erica vallis-gratae* Guthrie & Bolus, and some forms of *Erica walkeria* Andrews are predicted to be members of the guild. In the genus *Adenandra* (Rutaceae), it seems likely that at least *Ad. uniflora* (L.) Willd., *Ad. brachyphylla* Schltdl., *Ad. viscida* Eckl. & Zeyh., *Ad. marginata* (L.f.) Roem. & Schult., *Ad. obtusata* Sond., *Ad. mundiifolia* Eckl. & Zeyh., *Ad. coriacea* Licht. ex Roem. & Schult. and *Ad. acuta* Schltr. belong to the guild, but knowledge of their nectar properties will be required for a more confident prediction. *Cyphia subtubulata* E.Wimm. (Lobeliaceae) and *Geissorhiza schinzii* (Baker) Goldblatt (Iridaceae) are additional candidates for inclusion, although corolla tubes in the latter species are long (mean \pm SD = 24.8 \pm 3.2 mm, N = 20).

Prosoeca westermanni has previously been reported to visit *Adenandra* (Pauw & Johnson 1999), *Gl. debilis* (Goldblatt *et al.* 2001), *Geissorhiza schinzii* (Goldblatt *et al.* 2009) and *D. fasciata* (Liltved & Johnson 2012). These records are brief, no dates or locations are provided and no specimens were captured. Nevertheless, these anecdotes provide valuable, independent confirmation of the observations reported here.

Earlier anatomical studies of the members of the pollination guild provide additional insight into the nature of the interactions. Marloth (1923) described the viscid glands on the tips of the stamens and staminodes in *Adenandra* (Rutaceae; Appendix S4), found that the secretions contained no sugar, and correctly inferred that the function was to glue pollen to the mouthparts of the pollinators. He saw two

kinds of long-proboscid flies, tentatively identified as *Pangonia* (syn. *Philoliche*: Tabanidae), but was surprised at how low the visitation rates were. Marloth (1932) also made observations of *C. volubilis*, and recorded visits by small hawkmoths without giving further details. The accompanying illustration shows a moth visiting a form of *C. volubilis* with zygomorphic, horizontally oriented flowers, not the form studied here (Appendix S7). Goldblatt & Manning (1997) describe nectar secretion in *Ar. spiralis* from perigonal nectaries at the base of the tepals and record seeing visits by *Philoliche rostrata*, and later by *Philoliche gulosa* (Goldblatt & Manning 2000), but provide no details. At my study sites, *Ar. spiralis* had finished flowering before these long-proboscid flies were on the wing.

Bolus (1918) was the first to recognize the similarity in floral traits between the rewardless orchid *D. fasciata* and the shrub *Ad. villosa* (Rutaceae), and this similarity is often referred to (e.g. Liltved & Johnson 2012) (Figs 4, 5). At my study sites, these two species did not co-flower because they peaked at different post-fire successional stages, but post-fire resprouting is more common in other *Adenandra* populations, and this may allow them to co-flower with *D. fasciata*. If the traits of the *Disa* are indeed modelled on those of a specific nectariferous species, then, at my study site, the model seems likely to be *Ge. ovata*. Indeed, three of the four flies captured on *D. fasciata* carried copious pollen of *G. ovata*. The importance of mimicry in the evolution of pollination syndromes, generally, remains an open question. The answer hinges on the extent to which the flies' floral preferences are fixed *versus* learned from a prior, rewarding experience (Combs & Pauw 2009).

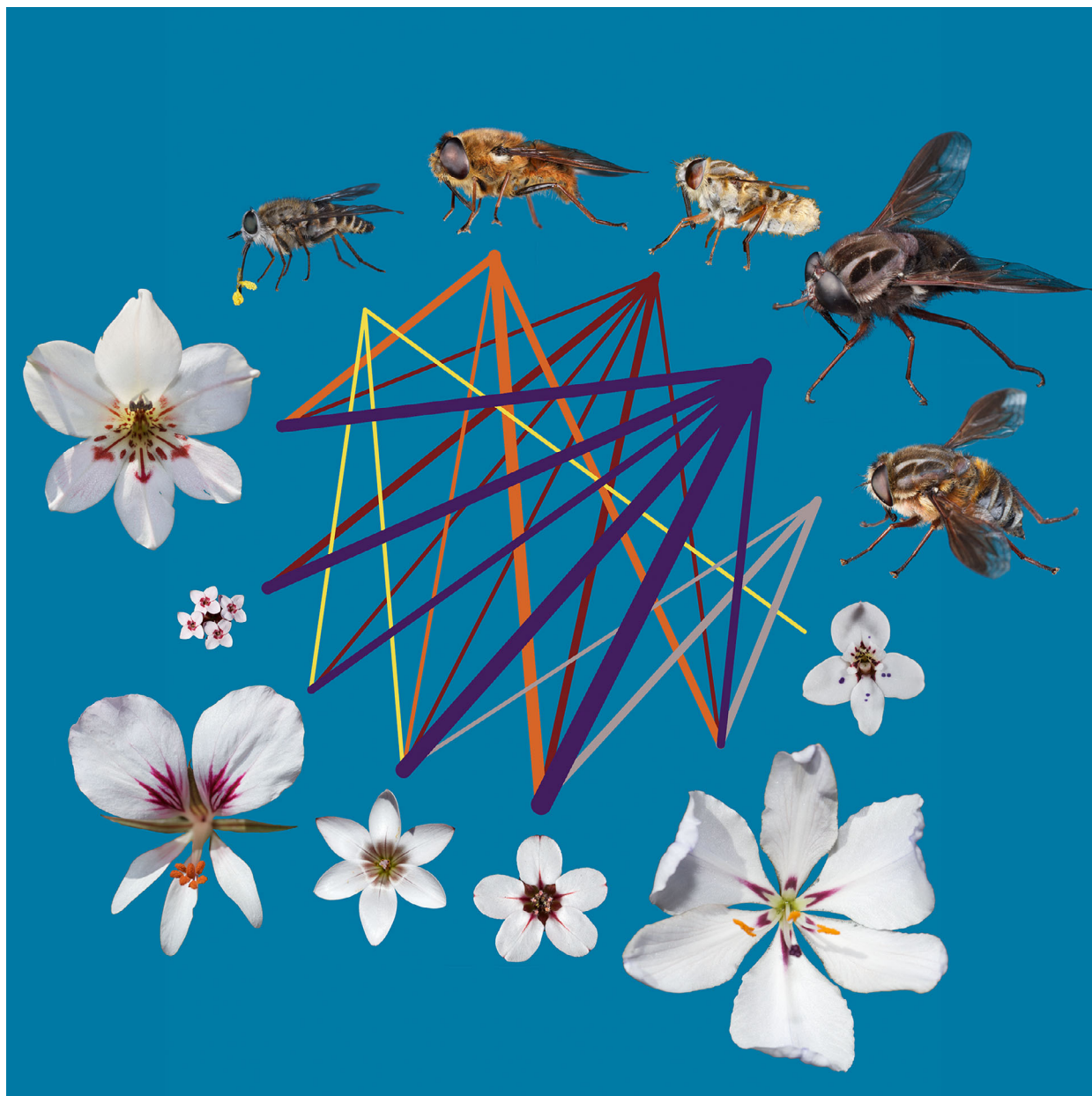


Fig. 5. The network of interactions between plants that conform to the *Prosoeca* s.s. pollination syndrome and their most important pollinators. Connecting lines represent the number of sites at which an interaction was observed. The thinnest lines represent interaction observed at one site; the thickest, five sites. Clockwise, the flies are: *Stuckenbergina africana*, *Prosoeca* "dimorpha", *Prosoeca* "alticola", *Prosoeca westermanni*, *Prosoeca* "brevirostris". The flowers: *Disa fasciata* (Orchidaceae), *Aristea spiralis* (Iridaceae), *Adenandra villosa* (Rutaceae), *Geissorhiza ovata* (Iridaceae), *Pelargonium longicaule* (Geraniaceae), *Erica fastigiata* (Ericaceae), *Gladiolus debilis* (Iridaceae). Pollinators for which 20 or fewer visits were recorded are excluded from the figure. When the image measures 29.7 cm across, the flowers are life size and the flies are 1.5 × life size.

It is somewhat surprising that the members of the *Pr. westermanni* pollination guild are specialized for pollination by such a narrow functional group of pollinators. One might think that spring-flowering, shallow, white, actinomorphic flowers should attract functionally diverse insects. What prevents bees, on the one hand, and longer proboscis flies, on the other hand, from visiting these flowers?

Within the study area, long-proboscid Nemestrinidae and Tabanidae (proboscis >15 mm) are well-known and important pollinators (Manning & Goldblatt 1997; Pauw *et al.* 2009). The

plant species that they pollinate have been grouped into the *Moegistorhynchus longirostris* pollination guild, and the syndrome of traits that identifies them includes floral tube lengths of 30–90 mm. The principal pollinators are *M. longirostris*, *Ph. rostrata*, *Ph. gulosa* and *Prosoeca nitidula*. The latter three species share the montane habitat with the *Pr. westermanni* pollination guild. Anatomy does not prevent the long-proboscid flies from also visiting shorter flowers, but the critical distinction is that these long-proboscid species are on the wing later in the season, emerging in early November, just after

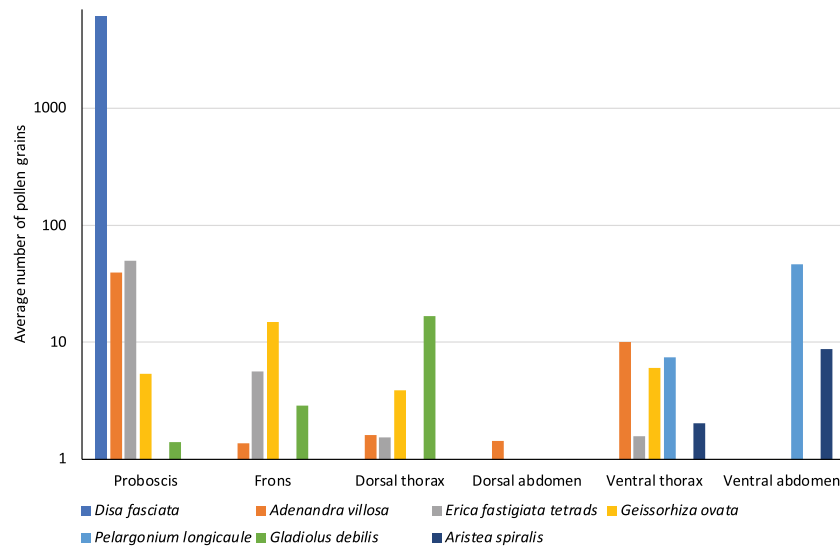


Fig. 6. Pollen distribution on the bodies of sampled flies. Standard deviations are not displayed because they are often larger than the means, as can be seen from the raw data (Appendix S5). The scale is logarithmic and values <1 are not shown.

the last members of the *Pr. westermanni* pollination guild have wilted and set seed. Several other short-proboscid flies that appear functionally similar to the members of the *Ad. villosa* flower visitor guild, especially the abundant *Philoliche lateralis* Fabricius and *Philoliche angulata* Fabricius (Tabanidae), are likewise excluded by their delayed phenology.

In addition to phenology, morphological trait matching can bring about specialization in plant–pollinator interactions. Members of the *Pr. westermanni* pollination guild typically have smaller volumes of nectar with higher concentrations than plant species that are pollinated by long-proboscid flies, and this may further restrict the pollinator fauna. The most abundant nectar plants (*Ad. villosa*, *E. fastigiata* and *Ge. ovata*) have volumes ranging from 0.4 to 1.0 μl and concentrations ranging from 33–67 $\text{g}\cdot 100\text{ g}^{-1}$ (Table 1), whereas long-proboscid fly-pollinated species have quantities mostly ranging from 1.1 to 5.0 μl and concentrations ranging from 20–30 $\text{g}\cdot 100\text{ g}^{-1}$ (Goldblatt & Manning 2000). The shorter, stouter proboscises of the members of the *Prosoeca s.s.* clade likely allow them to efficiently extract these small volumes of concentrated nectar. According to the Hagen–Poiseuille equation for laminar fluid flow through a pipe, the rate of flow is inversely proportional to both the length of the pipe and the viscosity of the fluid (Pivnick & McNeil 1985). Thus, the decrease in flow rate resulting from the higher viscosity of concentrated nectar can be offset by having a shorter proboscis.

The enlargement of the labella in the members of the *Prosoeca s.s.* clade relative to the long-proboscid flies will further enhance the extraction of the thin film of nectar that is offered by *Ad. villosa* and *E. fastigiata*. The labella spread open to form a disc that makes contact with the nectar film (Appendix S6). The underside of the disc is traversed by a series of channels (pseudo-tracheae), which presumably help to sponge up the nectar. The pseudo-tracheal system might also distribute saliva onto the surface when feeding on concentrated nectar (Krenn *et al.* 2005, 2021). The sponging mode of feeding, as performed by a short proboscis, represents the ancestral method of fluid uptake in Diptera (Krenn *et al.* 2021). In contrast, the long-

proboscid *Prosoeca* species, which extract larger volumes of more dilute nectar from narrow floral tubes, keep their elongated labella closed when feeding (Appendix S6) (Karolyi *et al.* 2012).

Flower colour can strongly influence the pollinator fauna. Pale flowers with reddish nectar guides are not unique to the *Pr. westermanni* pollination guild, but also occur in other Nemestrinidae and Tabanidae pollination guilds, suggesting the existence of a broader syndrome (Goldblatt & Manning 2000). However, the brilliance of the flowers, which can appear a solid porcelain white or paper white, is unusual outside the *Pr. westermanni* pollination guild. A recent study found that *Erica* species in the long-proboscid fly pollination syndrome tend to be UV reflecting, and that result is confirmed here for the members of the *Pr. westermanni* pollination guild (Fig. 1) (McCarren *et al.* 2021).

In conclusion, it seems that trait matching between the members of the *Pr. westermanni* pollination guild and their pollinators has played an important role in determining the specificity of the interactions by restricting the pollinator fauna to a very narrow subset of the available insects. It seems likely that within the broader community, the *Pr. westermanni* pollination guild and its pollinators forms a distinct module of closely interacting species.

How are new species added to a guild to produce the species-rich guilds described here? Species may be added from outside by convergent adaptation, or by speciation within the guild. There is evidence for both processes. Convergence is particularly striking in the plant guild, which has unusually high phylogenetic diversity – the eight confirmed species come from six different families.

When the members of the plant guild are compared to their close relatives outside the guild, it becomes obvious that convergent adaptation towards a shared phenotype has occurred in guild members. For example, *Ge. ovata* has an unusually long perianth tube for Section Weihea (Goldblatt 1985); *Ar. spiralis* is the only member of its large genus that secretes nectar (Goldblatt & Manning 1997); *Gl. debilis* and *D. fasciata*

tend towards actinomorphy, whereas the genera to which they belong are strongly zygomorphic (Bytebier *et al.* 2007); the upwards-facing flowers of *E. fastigiata* are very unusual in the genus; and the large flowers of *Adenandra* are unusual in the Diosmeae (Manning & Goldblatt 2012).

Pollinator-driven adaptation may be observed in several stages of development. At one extreme is *D. fasciata*, which is so morphologically divergent that it has been placed in a monotypic section (Bytebier *et al.* 2007), and at the other extreme we observe subtle divergence among populations. Both *Pe. longicaule* and *Gl. debilis* have late-flowering populations that are apparently adapted to flies with longer proboscises, such as *Philoliche rostrata*. *Pe. longicaule* at Eerste Waterval is a typical member of the *Pr. westermanni* pollination guild (Fig. 3a), but at Red Hill it flowers after the period of activity of these flies, has pink flowers and longer tubes (31.1 ± 2.7 mm, $N = 8$) (Pauw *et al.* 2009). At Kogelberg both early- and late-flowering *Gl. debilis* apparently occur, the latter differing in being pink and longer-tubed.

In contrast with the plants, speciation within the guild is apparently prevalent among the flies in the *Ad. villosa* flower visitor guild. They are phylogenetically clustered, with the four principal species all restricted to a small clade in one genus. Since the clade members overlap in interaction partners, it seems unlikely that host plant switching has driven diversification in this clade. However, subtle, geographically structured differentiation in relation to host plant association does seem to have occurred. On the Cape Peninsula, where *Ad. villosa* dominates the plant guild, *Pr. westermanni* has a shorter proboscis than in the Hottentots Holland mountains, where the longer-tubed *E. fastigiata* dominates (Table 2). Likewise, the Cape Peninsula endemic *Prosoeca "brevirostris"* has a shorter proboscis than its 'mainland' sister species *Prosoeca "alticola"*, which visits mainly *E. fastigiata* (Table 1).

The *Prosoeca s.s.* clade members with their short proboscises, provide a useful counterpoint to the closely related long-proboscid flies, which have become model systems for the study of coevolution (Zhang *et al.* 2013; Week & Nuismer 2019). Selection experiments have shown that reciprocal selection drives an inevitable evolutionary race for elongation between proboscises and nectar tubes (Pauw *et al.* 2009; Paudel *et al.* 2016), but it remains unclear why there is so much variation in the endpoints of these races (Anderson & Johnson 2008; Pauw *et al.* 2009). Environmental variation, which influences the cost of trait elongation, is one possible explanation for geographic variation in the outcome of Red Queen races (Toju 2008; Pauw *et al.* 2009). In particular, it has been proposed that windy conditions may limit the efficiency of long proboscises in some areas, but the *Prosoeca s.s.* clade are active before the onset of windy conditions. An outstanding feature of the *Pr. westermanni* pollination guild is the importance of *Adenandra* (Rutaceae), which is not included in the long-proboscid fly-pollinated guilds. The lack of petal fusion in this genus may mean that mutants with deeper flowers never occur, so the genus does not enter into a race with its pollinators.

The *Pr. westermanni* pollination guild and its pollinators is a fascinating evolutionary object. From its study we stand to learn a great deal about how selection imposed by interacting species shape traits and how those traits, in turn, shape interactions. Provisionally, it is clear that there is an association

between the floral traits of the guild and their pollinators, as required by the pollination syndrome concept.

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AUTHOR CONTRIBUTIONS

All work was conducted by the author.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Location of study sites.

Appendix S2. Observation data for interactions between plants in the *Prosoeca westermanni* pollination guild and their pollinators.

Appendix S3. Three members of the *Prosoeca westermanni* pollination guild in their habitat: (a) *Erica fastigiata* (Ericaceae), Pic-sans-Nom, Jonkershoek; (b) *Adenandra villosa* (Rutaceae), Steenberg Peak, Cape Peninsula; (c) *Disa fasciata* (Orchidaceae), on the burnt slopes of Voorberg, Kogelberg.

Appendix S4. Pollination in *Adenandra villosa*. (a) Male phase flower with gland-tipped stamens and staminodes. (b) Female phase flower with receptive, green stigma. (c) A male *Prosoeca "brevirostris"* pollinates *Ad. villosa* subsp. *sonderi*. Orange pollen on the ventral abdomen is *Aristea spiralis*. (d) Aggregated pollen masses from *Adenandra* on the proboscis of *Prosoeca westermanni*. a–c Klaasjagersberg; d Eerste Waterval, Jonkershoek. Scale bars: (a) 0.84 mm; (b) 1.0 mm; (c) 4.43 mm; (d) 0.19 mm.

Appendix S5. Numbers of pollen grains collected from captured pollinators.

Appendix S6. Proboscis morphology of two *Prosoeca* species (a) *Prosoeca "alticola"* with short, broad labella and pronounced pseudo-tracheal channels suitable for sponging up the small volumes of concentrated nectar that are produced by some members of the *Prosoeca westermanni* pollination guild. (b) *Prosoeca beckeri* with long, narrow labella and weakly developed pseudo-tracheal channels suitable for sipping larger volumes of dilute nectar.

Appendix S7. Less frequent pollinators of plants in the *Prosoeca westermanni* pollination guild. (a) A female *Moegistorhynchus "pictus"* (Nemestrinidae) at Platberg (Kogelberg); head and body = 16 mm; proboscis = 18 mm; wing = 19 mm. (b) *Australoechus* sp. (Bombyliidae) pollinating *Cyphia volubilis* (Lobeliaceae) at Houwhoek; head and body = 9 mm; proboscis = 5.4 mm; wing = 9.3 mm.

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