

Pollen loads and pollen diversity on bodies of *Eulonchus tristis* (Diptera: Acroceridae): implications for pollination and flower visitation

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Abstract—Acroceridae is a family of spider-parasitic flies that often visit flowers as adults, although little is known about their possible role as pollinators. *Eulonchus tristis* Loew visiting flowers of *Brodiaea elegans* Hoover (Liliaceae s.l.) and *Iris douglasiana* Herbert (Iridaceae) were collected in California. Individuals carried large pollen loads, although visitors to *B. elegans* carried significantly more pollen grains than visitors to *I. douglasiana*. Visitors to *B. elegans* also carried a higher percentage of focal-plant pollen (91%) than visitors to *I. douglasiana* (38%). There was no difference in the diversity of pollen species (approximately nine) carried by visitors to either plant species. For visitors to *B. elegans*, no difference was seen in the amount or diversity of pollen carried with respect to the sex of the visitor. The behaviour potentially resulting in the acquisition of these pollen loads is discussed. These results show that *E. tristis* has the potential to be an important pollinator for these plant species, particularly *B. elegans*.

Résumé—Les Acroceridae sont des mouches parasitoïdes d'araignées dont les adultes visitent souvent les fleurs, bien qu'on connaisse mal leur rôle potentiel comme pollinisateurs. Nous avons récolté en Californie des individus d'*Eulonchus tristis* Loew en train de visiter des fleurs de *Brodiaea elegans* Hoover (Liliaceae s.l.) et d'*Iris douglasiana* Herbert (Iridaceae) en Californie. Ces insectes portaient d'importantes charges de pollen, bien que ceux qui visitaient *B. elegans* transportaient significativement plus de grains de pollen que ceux qui visitaient *I. douglasiana*. Les individus qui visitaient *B. elegans* transportaient aussi un pourcentage plus élevé de pollen de cette plante ciblé (91%) que ceux qui visitaient *I. douglasiana* (38%). Il n'y avait pas de différence de diversité dans les espèces de pollen (environ neuf) transportées par les visiteurs de chacune des plantes deux plantes. Chez les insectes qui visitaient *B. elegans*, ni la quantité ni la diversité du pollen ne variaient en fonction du sexe. Les comportements possiblement reliés à ces charges polliniques y sont aussi discutés. Nos résultats montrent donc qu'*E. tristis* peut potentiellement être un important pollinisateur des ces espèces végétales, particulièrement de *B. elegans*.

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Introduction

The role of Diptera (true flies) in pollination remains seldom studied, especially considering their prevalence as flower visitors (Kearns and Inouye 1994; Larson *et al.* 2001; Borkent and Harder 2007). One group of Diptera that shows an obvious propensity for flower feeding are those species with long (at least one-half of

body length), tubelike mouthparts, hovering flight, and a hair-covered body. The majority of dipteran species with this combination of characters belong to the families Nemestrinidae, Bombyliidae, and Acroceridae. Although some study has been made on the pollinating abilities of nemestrinids (Goldblatt *et al.* 1995; Goldblatt and Manning 1999, 2000; Devoto and Medan 2006) and bombyliids (Deyrup 1988;

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Grimaldi 1988; Johnson and Midgley 1997; Johnson and Dafni 1998; Souza-Silva *et al.* 2001), acrocerids have received little attention (Borkent and Schlinger 2008). This is partly due to the apparent rarity of many acrocerid species, although this cannot be the sole reason, as some species are locally abundant (Cole 1919; Schlinger 1960; Luz 2004). Their presence may have been disregarded because of the perception that they are unimportant as pollinators or components of the pollinator fauna in a region.

The amount of pollen that an insect carries and the diversity of species in that pollen load can provide useful, though indirect, information on the pollinating ability of that insect (Beattie 1971; Davis 1997; Kwak and Velterop 1997; Wiesenborn 2003). This is particularly true in species whose flower-visiting behaviours are unpredictable or difficult to follow. To provide effective pollination to a plant species, a pollinator must remain relatively constant to a given plant species and carry enough pollen in a location suitable for deposition on the stigma of a subsequent flower. If an insect grooms pollen away from the location where it was deposited, that pollen is no longer available to subsequent flowers/stigmas of that species. If the insect does not groom substantially, then layers of pollen from multiple flowers and (or) species may form on the insect. The diversity within a pollen load allows the constancy of an individual insect to a given plant species to be measured (Beattie 1971; Kwak and Velterop 1997; Wiesenborn 2003). If the insect is long-lived and grooms relatively infrequently, this diversity may also give a history of focal plants chosen by the insect. Little is known about the amount of pollen that flies can carry and the diversity of pollen loads. Studies that have looked at dipteran pollen loads have concentrated principally on Syrphidae (Beattie 1971; Yeboah Gyan and Woodell 1987; Kwak and Velterop 1997), although some data are available on pollen loads carried by the three dipteran families that contain long-proboscis pollinators.

Some pollinating nemestrinids have received considerable recent study, owing to the pollination syndrome between them and a number of plant species in southern Africa (Goldblatt *et al.* 1995; Goldblatt and Manning 1999, 2000). These flies carry pollen loads sufficient to effect pollination in the plants they visit, and the plants deposit pollen in specific locations on the bodies of the flies to ensure pollen transfer to conspecifics even if

the flies visit many flowering species (Goldblatt and Manning 1999, 2000). Southern African nemestrinids have been recorded carrying up to seven pollen types on their bodies (Goldblatt *et al.* 1995). Members of the Australian nemestrinid genus *Austrogastromyia* Bequaert also carry heavy pollen loads and are highly constant to a given plant species (<5% foreign pollen; Williams and Adam 1998).

Bombyliids have been the subject of study in terms of the amount and diversity of pollen they carry. Grimaldi (1988) found that *Bombylius* L. species carried enough pollen from *Hedyotis caerulea* (L.) Hook. (Rubiaceae) to ensure effective pollination and that this pollen was carried primarily on the proboscis. An undetermined *Bombylius* species visiting *Plectranthus madagascariensis* (Pers.) Benth. (Lamiaceae) carried adequate pollen loads, of which 90% was from species of *Plectranthus* L'Hér (Potgieter *et al.* 1999). Various Bombyliidae observed visiting species of *Lapeirousia* Pourret (Iridaceae) were highly constant, carrying no more than two pollen species (Goldblatt *et al.* 1995). A number of bombyliid species in southern California visit the flowers of *Pholisma sonora* (Torr. ex A. Gray) Yatsk. (Lennoaceae) and carry small pollen loads (<50 grains) containing 50%–75% *P. sonora* pollen (Wiesenborn 2003).

Most data on pollen loads carried by the Acroceridae are restricted to the mention of pollen presence on acrocerid bodies (Schlenger 1960; Adler *et al.* 1997). However, Goldblatt *et al.* (1998) recorded that *Psilodera valida* (Wiedemann) in South Africa carried up to four pollen species (including pollen of the study plant). Two undetermined species of *Philopota* Wiedemann carry pollinia and are important pollinators of the Brazilian orchid *Rodriguezia bahiensis* Rchb. f. (Orchidaceae) (Carvalho and Machado 2006). No studies have been made of pollen carried by acrocerids in North America, although some genera (*e.g.*, *Eulonchus* Gerstaecker) are locally abundant on flowers (Cole 1919; Schlenger 1960) and exhibit behaviours that show constancy to particular plant species (Borkent and Schlenger 2008).

In this study we investigated the pollen loads on the bodies of *Eulonchus tristis* Loew (Diptera: Acroceridae). Based on the total number of pollen grains present and their location on the body we determined whether *E. tristis* visiting *Brodiaea elegans* Hoover (Liliaceae) and *Iris douglasiana* Herbert (Iridaceae) carried

pollen loads sufficient to effect pollination. We determined the diversity and percentage of focal-plant pollen present in these pollen loads to infer the constancy of *E. tristis* to these two plant species. Finally, we examined differences in pollen abundance and species diversity between the sexes of *E. tristis* visiting *B. elegans*.

Methods

Study site, species, and specimen collection

The observation and collection of *E. tristis* specimens took place at the Heath and Marjorie Angelo Coast Range Reserve (formerly the Northern California Coast Range Preserve) (10 km north of Branscomb, Mendocino County, California, United States of America, 39°44.5'N, 123°38'W). Insect collections were made on 18–21 May 1984, 30 May – 1 June 1986, and 5 and 20 May 1989. Pollen was sampled from flowering plants in the reserve from 10 to 21 May 2005.

Eulonchus tristis individuals from the study site were metallic green, blue, or purple in colour and 7–12 mm in total length, with females tending to be larger than males (scutum length 3.8 ± 0.1 and 3.4 ± 0.1 mm, respectively). The proboscis of these flies is a sclerotized tube at least as long as the body, often reaching past the posterior tip of the abdomen when at rest or in flight (when it is held between the legs), which is used to feed on flower nectar. The body is covered in a dense pubescence that often traps pollen from the flowers visited for nectar. This species is restricted to western North America from southern British Columbia and Washington east to Idaho and south to California (Sabrosky 1948; Grant 1960). However, although specimens collected outside the Greater San Francisco Bay area key out to *E. tristis*, Schlinger (in Cole 1969) suggests that such specimens represent one or more closely related but undescribed species.

To determine if they were flower visitors, *E. tristis* were observed landing and beginning to enter flowers of *B. elegans* or *I. douglasiana* (both species have purple flowers with <105 ovules per flower (Berg 1996; Uno 1982), and pollen is principally deposited on and removed from the dorsal surface of visitors) before being collected. Specimens were killed individually in a cyanide collecting jar and then pinned with a minimum of handling to retain as much pollen as possible on the body and to not disturb the location of the pollen load. The plant species

from which each specimen was collected (focal plant) was noted.

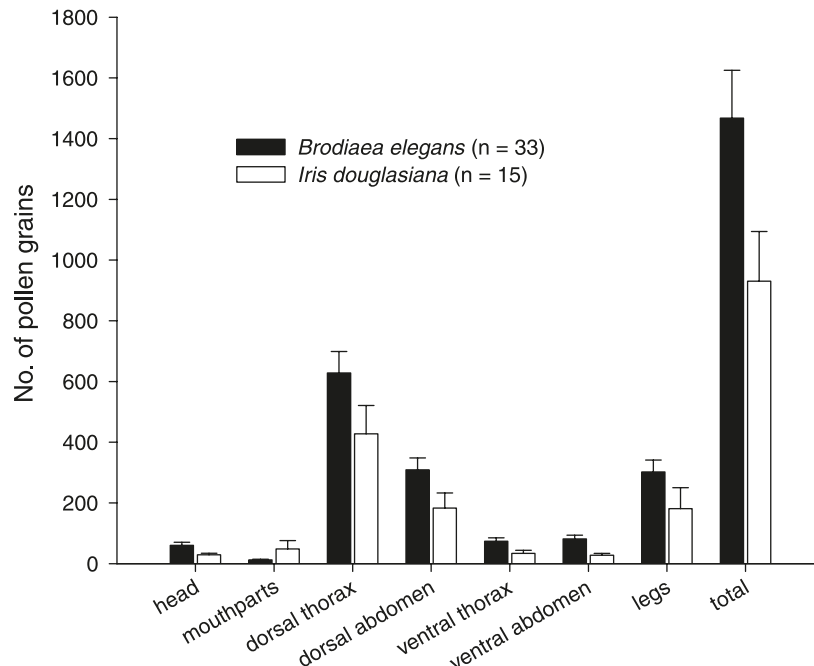
Specimens from this study are deposited at the California Academy of Sciences. All specimens were labeled with a unique E.I. Schlinger specimen number printed on green paper. Specimens from this study are numbered 011397, 011399–407, 011416–29, and 011431–54.

Pollen loads and diversity

Pollen loads were determined by counting the pollen grains present on each specimen under a dissecting microscope. The body was divided into 23 regions using obvious suture lines between sclerites or other distinct morphological regions to facilitate accurate pollen counting. The counts from the regions were then pooled to give the pollen loads on the head, mouthparts, dorsal and ventral thorax, legs, and dorsal and ventral abdomen.

The diversity of pollen loads was determined by removing the pollen from the head, legs, thorax, and abdomen of each specimen separately, using cubes of glycerin jelly containing basic fuchsin stain (Kearns and Inouye 1993). The cubes were placed on the end of an insect pin and drawn along the appropriate area of the body to pick up any pollen grains present. Once all pollen had been removed, the cubes were placed on a microscope slide, warmed to melting point on a hotplate, and covered with a glass cover slip. The stained pollen present was identified and counted along non-overlapping transects across the slide using a compound microscope at a magnification of 200 \times . A magnification of 400 \times was occasionally used to confirm pollen identity. A maximum of 300 pollen grains were counted and identified per slide. It was assumed that pollen grains were randomly distributed across the slide. The oval pollen grains of both focal-plant species are monocolpate with reticulate sculpturing of the exine. The pollen grains of *B. elegans* are smaller (62 vs. 95 μm) and have finer exine sculpturing than those of *I. douglasiana*. *Iris douglasiana* has a pollen to ovule ratio of 469:1, leading to an estimate of approximately 52 000 pollen grains per flower (Uno 1982). The amount of pollen produced per flower is not known for *B. elegans*. Pollen grains from wind-pollinated plants (grasses and gymnosperms) were rarely encountered (~1 per insect specimen) and were not included in the counts, as they were presumed to be incidental.

Fig. 1. Numbers of pollen grains (mean \pm SE) on the bodies of *Eulonchus tristis* visiting *Brodiaea elegans* and *Iris douglasiana*, showing both total pollen grains and number of pollen grains present in each body region.



Statistical analysis

We compared pollen loads among visitors to *I. douglasiana* or *B. elegans* and between the sexes of visitors to *B. elegans* using *t* tests (*t* test procedure of SPSS®, release 15.0; SPSS Inc. 2006). The mean and standard error for each value is given for each comparison. The numbers of pollen types (species or recognizable taxonomic units) present on the bodies of visitors were compared between visitors to the two plant species and between the sexes of visitors to *B. elegans* by means of contingency tables. Because of a high frequency of expected values <5 , we used exact χ^2 tests (Crosstabs procedure of SPSS®, release 15.0; SPSS Inc. 2006).

Results

Forty-eight *E. tristis* individuals were collected for pollen-load analysis: 33 from *B. elegans* (12 females and 21 males) and 15 from *I. douglasiana* (1 female and 14 males). The visitors to *B. elegans* carried more pollen on their bodies (1467 ± 158 pollen grains) than visitors to *I. douglasiana* (930 ± 164 pollen grains; $t_{46} = 2.1$, $P < 0.05$). There was no difference in the amount of pollen carried with

respect to sex for visitors to *B. elegans* ($t_{31} = 0.57$, $P > 0.5$). For both plant species most of the pollen was carried on the dorsal thorax, followed by the dorsal abdomen and the legs (Fig. 1).

No difference was found in the numbers of pollen types carried by visitors to each of the two plant species (8.77 ± 0.28 ; exact $\chi^2_7 = 7.99$, $n = 48$, $P > 0.3$), although the fewest types were carried on the head and the most types on the abdomen (Fig. 2). There was also no difference in the numbers of pollen types carried by male and female *E. tristis* visiting *B. elegans* (9.06 ± 0.33 ; exact $\chi^2_6 = 4.77$, $n = 33$, $P > 0.6$). Pollen from *Mimulus aurantiacus* W. Curtis (Scrophulariaceae), species of *Linanthus* Benth. and *Leptosiphon* Benth. (Polemoniaceae), and three species of Asteraceae were present on visitors, along with a large number of species that were unidentifiable. The majority of non-focal-plant pollen grains present in pollen loads were found in low numbers (<3), with a couple of types in each case accounting for the abundance of the non-focal-plant pollen.

The percentage of focal-plant pollen present on *E. tristis* bodies was higher in visitors to *B. elegans* than in those to *I. douglasiana* ($91.1 \pm 1.5\%$ and $38.3 \pm 9.4\%$, respectively; Fig. 3). The

Fig. 2. Numbers of pollen types (mean \pm SE) on the bodies of *Eulonchus tristis* visiting *Brodiaea elegans* and *Iris douglasiana*, showing both total pollen types and number of pollen types present in each body region.

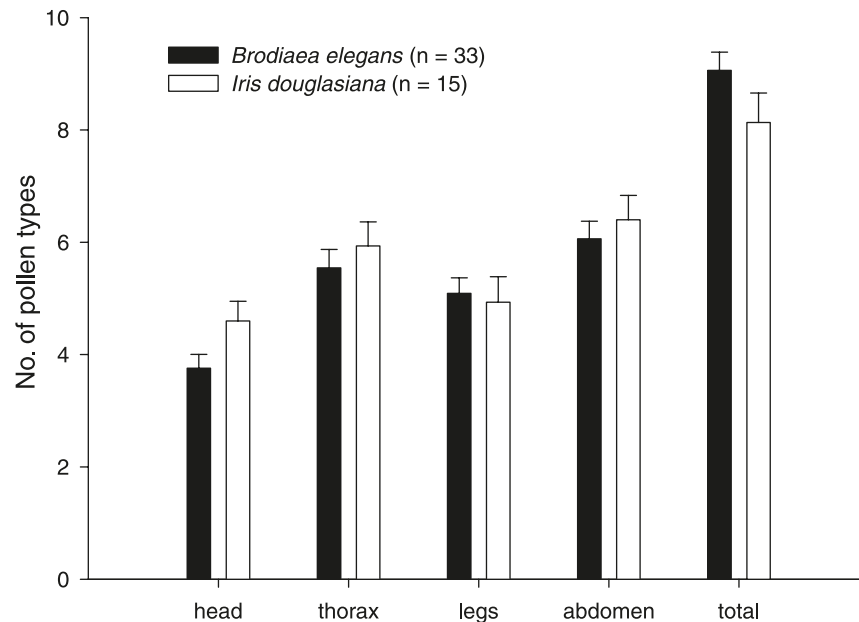
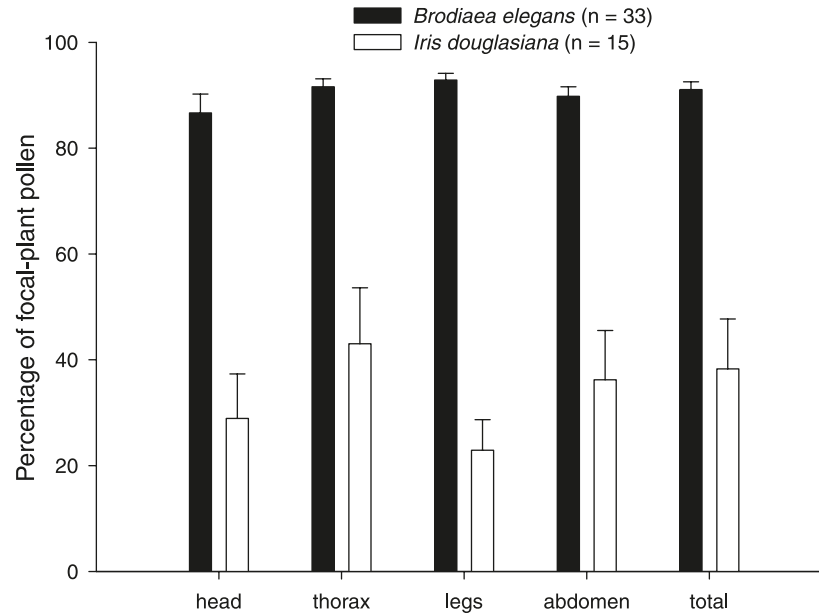


Fig. 3. Percentages of focal-plant pollen (mean \pm SE) on the bodies of *Eulonchus tristis* visiting *Brodiaea elegans* and *Iris douglasiana*, showing both total pollen and pollen present in each body region.



lowest percentage of focal-plant pollen was present on the legs and head of visitors to *I. douglasiana* (Fig. 3). There was no difference between the percentages of focal-plant pollen present on male and female visitors to *B. elegans*.

Additional observations

Eulonchus tristis individuals were also seen visiting flowers of *M. aurantiacus* in the Angelo Coast Range Reserve. Only a few flowers of this mass-flowering plant were visited per bush. Individuals were also frequently found late in the day

or early in the morning resting inside the flowers of *M. aurantiacus* (where they commonly spend the entire night). The few individuals observed visiting more than one flower flew long distances between them, often disregarding flowers of the same focal species that were along their path. Individuals of the related species *Eulonchus smaragdinus* Gerstaecker and *Eulonchus sapphirinus* Osten Sacken were also seen visiting flowers in areas of the reserve, although the numbers of collected individuals were not large enough to allow for inclusion in the analysis. The few *E. smaragdinus* seen were visiting flowers of *B. elegans*, *M. aurantiacus*, and *Leptosiphon bicolor* Nutt. (Polemoniaceae). These individuals carried, on average, >1800 pollen grains, and one individual carried >4400 pollen grains, more than any other specimen of *Eulonchus* spp. collected. Collected *E. sapphirinus* were visiting *Trientalis borealis* Raf. ssp. *latifolia* (Hook.) Hultén (Primulaceae) or *Vaccinium ovatum* Pursh (Ericaceae) and carried, on average, <100 pollen grains. No *Eulonchus* spp. were observed eating pollen when visiting flowers, unlike behaviour seen in some Bombyliidae (Deyrup 1988).

Discussion

Most pollen was deposited dorsally on *E. tristis* bodies by both *B. elegans* and *I. douglasiana*, as was expected from the morphology of the flowers. The amount of pollen deposited on *E. tristis* was substantial (>900 grains) and was more than sufficient to ensure effective pollination in both plant species (at least 8 times the number of ovules). *Eulonchus tristis* visiting *B. elegans* carried more pollen on their bodies, and more of this pollen was from the focal plant (*B. elegans*) than was seen in visitors to *I. douglasiana*. The difference in pollen loads between the plant species may solely reflect the pollen-carrying capacity of the dorsal surface of *E. tristis*. Although the pollen grains of the two species are similar in shape and ornamentation, those of *I. douglasiana* are much larger than those of *B. elegans* (95 and 62 µm, respectively), so fewer would be required to cover the available surface. It is also possible that *I. douglasiana* produces less pollen per flower because its pollen grains are larger or it has fewer flowers open per plant; in either case this would lead to smaller pollen loads on visitors.

The high percentage of focal-plant pollen present on the visitors to *B. elegans* suggests that

E. tristis is probably highly constant to this plant species and might act as an important pollinator. The low percentage of *I. douglasiana* pollen carried by visitors to this plant species is puzzling. The variance between specimens was high and pollen loads contained from 10% to 90% *I. douglasiana* pollen, suggesting that some individuals may have been incidental visitors to *I. douglasiana* and others were fairly constant to this species. Possible reasons for this high variability are discussed below.

The large number of pollen types present on the bodies of *E. tristis* visiting both plant species was unexpected, as other research on both behaviour and pollen loads of acrocerids has shown visitation to only a few plant species (Goldblatt *et al.* 1998; Borkent and Schlinger 2008). There are a couple of possible reasons for this difference. Firstly, *E. tristis* may be more opportunistic in its flower-visitation behaviour than previously studied acrocerids, with this reduced constancy leading to more pollen types being present. Secondly, these flies are essentially nongrooming and the large number of pollen types present may reflect the learning ability of the individual as it samples the available flowering species. This possibility is supported in the case of *B. elegans* visitors, which carried >91% focal-plant pollen, although, on average, eight other pollen types were also present. The low numbers of pollen grains from other plant species could indicate residual pollen trapped in relatively inaccessible areas of a fly's body from its initial visits to flowers after emergence, when the individual was determining which flowering plant species yielded the greatest nectar rewards. This effect could be compounded if the flies are long-lived (*i.e.*, 3–4 weeks), as they may have to select another plant species to feed on when the plant species initially selected ends its flowering season. This could lead to a second learning period that would increase the number of pollen types present on the body of the nongrooming fly. This option may explain the high variability of the percentage of focal-plant pollen on the bodies of *I. douglasiana* visitors, as they may have been in a transition stage in switching from *I. douglasiana* to a new plant species. This is also supported by the low number of non-focal-plant pollen grains (<3) for most types present in the pollen loads. A final possibility is that the flower-visiting behaviours of acrocerids are dependent on the diversity and abundance of the flowering plants in bloom at a given time.

This option is unlikely, as this behaviour should be reflected in the pollen load composition and abundance being relative to the number of flowers available from each flowering species.

Unlike findings for other *Eulonchus* species (Schlinger 1960; Borkent and Schlinger 2008), no difference in flower-visiting behaviour and ability between the sexes of *E. tristis* was apparent, based on pollen-load analysis. The sexes could still show behavioural differences that would not be apparent from the pollen load (e.g., in the duration of flower visits or the distance between flowers visited). However, the sexes carry the same amount and number of types of pollen and should therefore show no difference in constancy to a given flowering plant species. Further study of the pollination behaviours of *E. tristis* may show differences between the sexes that were not apparent in the pollen loads.

The findings from this study suggest that *E. tristis* may be an important and relatively constant pollinator, carrying large pollen loads with a high percentage of focal-plant pollen. *Eulonchus tristis* carries a percentage of focal-plant pollen and number of types of pollen similar to the long-proboscis species of Nemestrinidae and Bombyliidae discussed by Goldblatt *et al.* (1995), Williams and Adam (1998), and Potgieter *et al.* (1999), and therefore probably has similar pollination abilities.

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