JEWELED SPIDER FLIES (*EULONCHUS TRISTIS*) ARE IMPORTANT

POLLINATORS OF *IRIS BRACTEATA,* A RARE SISKIYOU MOUNTAIN ENDEMIC

By

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A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

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December 2018

ABSTRACT

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POLLINATORS OF *IRIS BRACTEATA,* A RARE SISKIYOU MOUNTAIN ENDEMIC

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Premise of the study*.* Little is known about the pollination biology of the Pacific Coast
Irises (*Iris:* series *Californicae*, hereafter PCI), especially who visits and pollinates their
flowers. In general, *Iris* flowers are considered bee-pollinated, however, flies in the genus
Eulonchus (Acroceridae) are known to visit some PCI members. Therefore, I assessed the
relative importance of *Eulonchus* and other insect visitors to the pollination of a rare PCI
species native to the Siskiyou Mountains, *I. bracteata*.

Methods*.* I quantified pollinator importance for all flower visitors at sites in northern
California and southern Oregon as the product of average visit rate and the probability of
stigma contact. I also documented the flower-handling behaviors of each visitor and
determined the average number of grains delivered to virgin stigmas by the most frequent
flower visitor.

Key results*.* A diverse set of insects visited the flowers of *I. bracteata*. Their visitation
rates, flower-handling behaviors, and probability of contacting stigmas differed
substantially. Contrary to expectations, the fly *E. tristis* was the most important
pollinator; it accounted for more than 70% of total visits and consistently contacted
stigmas when entering flowers. The species delivered an average of 185 grains per visit

(median=96), which exceeds the average number of ovules per flower (100). Some bees (*Osmia densa, Bombus vosnesenskii*) likewise contacted stigmas and thus are potential pollinators, but they seldom visited flowers at my study sites.

Implications. My findings contribute to our general understanding of the role that flies play in pollination. Specifically, they show that flies in the genus *Eulonchus* – and not bees – are the main pollinators of a rare *Iris* in northern California and southern Oregon. Resource managers concerned about the conservation status of the plant should consider the life history requirements of the flies and, especially, the vulnerability of their larval spider hosts to habitat alteration. Several other plants in the same region are known to be visited by *Eulonchus* and thus, additional studies assessing their importance as pollinators are warranted.

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INTRODUCTION

The Pacific Coast Irises (*Iris*: series *Californicae*, hereafter PCI) are well-known among wild-flower enthusiasts and gardeners for their beautiful flowers and, among botanists, for their propensity to hybridize (Lenz 1958, Young 1998, Wilson 2003). Several species are widely distributed in California, and their showy flags are familiar sights along roadsides and forest edges, but somewhat surprisingly, very little is known about their pollination biology, especially who visits and pollinates their flowers (Uno 1982, Borkent and Schlinger 2008). One explanation may be that earlier researchers assumed *a priori* that PCI species, like most other members of the genus, rely on bees (Hymenoptera) for pollination services (Rodionenko 1987, Goldblatt and Manning 2008, Guo 2015). Virtually all *Iris* have the same basic pollination mechanism (Guo 2015). A pollinator lands on one of the three sepals, and then walks beneath a petal-like style to the center of the flower in search of nectar. In the process of walking in and out, the visitor contacts stigma and anther, and pollination occurs. This system resembles what we see in plants with nototribic flowers (e.g., various mints and scrophs), and requires a degree of behavioral sophistication and consistency that are ordinarily associated with bees. Thus, pollination biologists may not have questioned who pollinates PCI because they assumed bees were the most important pollinators.

Contrary to this assumption, some evidence (Mesler unpublished, Ponte unpublished, Borkent and Schlinger 2008) suggests that the most important pollinators of PCI may instead be flies in the little-known genus *Eulonchus* (Acroceridae). This suggestion is

surprising because flies have generally been regarded as erratic flower visitors and poor pollinators (Faegri and van der Pijl 1979, Larson et al. 2001, Ssymank 2008, Borkent and Schlinger 2008). Nevertheless, recent observations suggest that *Eulonchus* frequently visits PCI flowers, exhibit foraging behaviors similar to bees, and carry *Iris* pollen on their bodies (M. Mesler unpublished, Ponte unpublished, Borkent and Schlinger 2008). Thus, *Eulonchus* flies appear to be important pollinators. However, published evidence supporting this claim is inconclusive (Borkent and Schlinger 2008).

I investigated the relative importance of *Eulonchus* and other insect visitors to the pollination of a rare PCI species native to the Siskiyou mountains, *Iris bracteata*. For each insect visitor, I measured (i) the average rate of flower visitation (*visitation rate*) and (ii) the probability of contacting stigmas during visits (an index of *visitor effectiveness*), and based on these two parameters, (iii) their relative importance as pollinators. In the process, I also estimated the average number of pollen grains a visitor delivers to stigmas during a single visit (*expected pollen delivery*). Documenting stigma contact and pollen delivery is essential because not all flower visitors contact reproductive whorls nor do they deliver the same amount of pollen grains to stigmas (King et al. 2013, Ballantyne et al. 2015). Of these parameters, measuring expected pollen delivery is more challenging technically because virgin stigmas must be presented to flower visitors (see Methods), and for this reason much more is known about variation in visitation rate than pollen deposition. The number of published estimates of expected pollen delivery is especially meager for flies (Inouye et al. 2015).

In my thesis, I present a catalog of insect visitors and report their relative importance as pollinators of *I. bracteata*. I show that the flowers attract a diverse array of insects which differ substantially in their flower visitation frequency and their probability of contacting stigmas (visitor effectiveness). My results reveal that the acrocerid fly, *Eulonchus tristis,* accounted for the majority of visits to flowers, had the highest probability of stigma contact, and delivered large loads of conspecific pollen grains.

MATERIALS AND METHODS

Study Species

Iris (Iridaceae) is a relatively large genus of more than 260 species with a northern, temperate distribution. The Pacific Coast Irises (series *Californicae*, hereafter PCI) consists of 18 taxa restricted to the Pacific Coast regions of Washington, Oregon and California (Wilson 2003). PCI are rhizomatous perennial herbs with unbranched aerial stems that produce 1-2 (rarely 3) nectar-secreting flowers (Lenz 1958, Wilson 2009). Flowers comprise three distinct floral units, each consisting of a sepal, a stamen, and a petal-like style. Each sepal subtends a style that bears a triangular flap-shaped stigma on its underside near its tip. A single stamen lines up against each style and arches over a sepal. The sepals are widely spreading, arched downward, and ornamented with colored veins that presumably guide pollinators towards nectar, which is produced at the center of the flower in a tube-like extension of the perianth (Figure 1). PCI are self-compatible (Lenz 1958, Uno 1982, Wilson 2001, Comstock and Perry unpublished), but herkogamy (spatial separation between stigma and anther) and protandry (temporal separation between stigma and anther; male to female) presumably prevents auto-pollination. Hence, PCI likely depend on animal visitors for successful pollination and seed set (Lenz 1958, Uno 1982, Wilson 2001, Mesler unpublished).

Iris bracteata S. Watson, the Siskiyou Iris (Figure 1), is largely restricted to the shaded coniferous forests of the Siskiyou Mountains in northern Del Norte County,

California and southern Josephine County, Oregon (Osbrack pers. communication, Lenz 1958, Table 2). The species is listed by the California Native Plant Society as 3.3 (plant about which more information is needed), under their list of rare and endangered plants (CNDDB 2017). Populations of *I. bracteata* grow on rocky, ultramafic serpentinetic soils along shaded United States Forest Service (USFS) and county roads, forest edges, open forest patches, and hill sides (Osbrack and Mesler, personal communications). Flowers open in early/mid-May and bloom until early/mid-June, depending on elevation. Flowers spend approximately 24-hours in their male phase (mature/dehisced androecium) before stigma flaps recurve and become receptive to pollen (female phase). Stigmas are receptive for approximately two days. Each stem produces two flowers. As the stigma of the first flower (female phase) begins to senesce, the second flower (male phase) opens and anthers dehisce. Male and female phases overlap for up to 24 hours, providing an opportunity for geitonogamy (pollination between flowers on the same plant).

Study Sites

This study was conducted on US Forest Service land in northeastern Del Norte County, California, and southern Josephine County, Oregon, during the summer of 2017. I selected seven study sites, four in Six Rivers National Forest, CA and three in Rogue River-Siskiyou National Forest, OR (Figure 2). California and Oregon sites share similar weather regimes and geology, but differ in elevation and co-flowering species (Appendix Table 2).

Study site selection was determined by the number of flowers in bloom and legal accessibility to flower patches. In general, study sites were at least 1 km away from one another, but two sites were separated by only 200 meters (Figure 2, Appendix Table 2). At many of my sites, patches of flowers were distributed in a linear fashion along roads but at others, patches were scattered in forest openings and on rocky hillsides. Patches of flowers within study sites were separated by 1-50 meters. The number of flowers at a site ranged from ~300 to > 600.

Visitor Surveys

I estimated visit frequency by different insects (hereafter visitation rates), and observed visitor behavior during a series of 1-hour surveys carried out at all seven study sites (Table 2, Appendix Table 1). Surveys took place between 0900 and 1800 during optimal weather conditions for flying insects. The number of surveys made, and days spent at each study site, varied depending on day-to-day changes in the number of available flowers at each site. Most surveys were made concurrently by multiple observers. At each study site, 20 flowering patches were selected and 15 flowers within each patch were chosen for observation. Male and female phase flowers were marked with different colored tape (white or yellow). I did not count the number of male and female phase flowers within patches, but female phase clearly dominated most patches. Insect visits to focal flowers were observed from a fixed position for three-minutes at each of the 20 patches within a site. A visit was defined as any contact to a flower by an insect.

For each visit observed, I recorded visitor identity, flower handling behavior, gender phase of the visited flower, and whether stigmas were contacted. Based on previous observations, I divided handling behaviors into five categories: (a) *Sepal entry*: visitors landed on the tips of sepals, entered floral units by walking beneath petal-like styles towards the center of flowers where nectar is present, and then backed out; (b) *Pollen robbing*: visitors entered floral units from the side, flipped upside down, grasped on to anthers, and collected pollen; (c) *Nectar robbing*: visitors either landed on the top of styles and probed their proboscis at the center of flowers or bit holes at the base of the perianth tube to extract nectar; (d) *Land*: visitors landed on flowers, remained in a fixed position, and did not forage for nectar or pollen; (e) *Touch:* visitors briefly contacted a flower without landing. Neither *Land* or *Touch* visits resulted in anther or stigma contact.

I calculated the following values for each visitor type based on the surveys: (a) total number of visits pooled across all surveys; (b) average number of visits per 3-minute observation period at each site pooled across surveys; (c) average rate of visitation (number of visits/flower/hour) per 3-minute observation periods pooled across surveys; (d) the fraction of total visits made to female-phase flowers; (e) the fraction of total visits to female-phase flowers that resulted in stigma contact; and (f) the proportions of different types of flower handling behaviors (behavior proportions).

Expected Pollen Delivery

Expected pollen delivery refers to the number of conspecific pollen grains a visitor deposits during a single visit to a virgin (unvisited) stigma (King et al. 2013,

Ballantyne et al. 2015). Single-visit pollen deposition studies were conducted at CA Site 3 and 4 (Figure 2). Pollen deposition samples were not collected at Oregon sites due to low visitation rates.

Insect exclusion cages (frames covered with agricultural row cover material – Reemay®) were placed over dense patches of 30-100 flower buds (unopened flowers). Caged flowers were monitored as they developed (Appendix Figure 2). When 30 or more flowers reached their female phase (stigma obviously reflexed), the cage was removed and virgin flowers were exposed to visitors. When a visitor contacted a stigma, it was identified to the lowest taxonomic level possible, and chased from the plot. The style tip (including the stigma) was then removed, placed in a moist container, and stored in a cooler for later analysis. Insect visitors appeared unaffected and continued to visit flowers even after style tips were removed. Since the number of available virgin stigmas at each study site was limited, I used each of the three floral units of a flower as a separate pollen-deposition sample (i.e. 1 flower = 3 stigmas = 3 samples). The decision to collect stigmas only if they had been contacted by a visitor also helped increase sample size.

After collection, style tips were brought back to lab for pollen deposition analysis. Each stigma was carefully removed from its style with a razor blade, placed in a drop of 3M NaOH on a glass slide and covered with a coverslip. After 2-4 minutes, the NaOH solution dissolved pollen tubes and detached pollen grains from stigmas. Using the tip of a pencil's eraser, the top of the cover slip was delicately depressed to help separate and disperse pollen grains from stigma tissue. A compound light microscope at 100x magnification was then used to count the number of conspecific and heterospecific pollen

grains deposited on each stigma. *Iris* pollen grains are large, ellipsoid, and not easily

confused with the pollen grains of other co-flowering species in the area. I identified

heterospecific pollen to the lowest taxonomic rank possible using a reference collection

gathered from co-flowering species at my study sites. Pollen counts were made twice for

each slide and averaged.

I calculated pollen:ovule ratios to quantify the extent to which fruit and seed

production might be limited by the number of pollen grains delivered to stigmas. I

estimated the number of pollen grains delivered to stigmas (expected pollen delivery) as

the product of the expected number of stigmas contacted over the life of a flower and the

median number of grains deposited per visit:

$$\# \, stigma \, contacts \, \times \, \frac{median \, \# \, of \, grains}{contact} = total \, \# \, of \, grains \, delivered$$

The expected number of stigma contacts was calculated as the product of the expected

number of visits to female phase flowers by the probability of stigma contact:

$$\# \, visits \, to \, female \, phase \, flowers \times P(stigma \, contact)$$

$$= \# \, stigma \, contacts$$

The number of visits to female phase flowers was calculated by multiplying visitation

rates by the probability of visiting a female phase flower:

$$\frac{\# \, visits}{time} \times \, P(female \, phase \, flower) = \# \, visits \, to \, female \, phase \, flowers$$

To put the number of grains delivered during the life of a flower into context, I also

collected the ovaries of 15 flowers and counted the number of ovules. I then used the

average number of ovules per ovary to calculate an expected pollen:ovule ratio, which

can be used to infer whether fecundity was limited by insufficient pollen reception at my study sites.

Pollinator Importance

The relative contribution that a given visitor species makes to pollination (pollinator importance) can be estimated as the product of its average visitation rate and the average number of pollen grains it deposits during a single-visit (expected pollen delivery) (King et al. 2013, Ballantyne et al. 2015). Unfortunately, I was only able to obtain pollen deposition values for *Eulonchus tristis* because other taxa rarely visited flowers (and even more rarely contacted stigmas), which precluded calculating importance in the conventional way. So instead, I assessed a visitor species' *potential* importance as a pollinator as the product of its average visitation rate and its probability of contacting stigmas (visitor effectiveness). This proxy is imperfect because it does not assess the number of pollen grains a visitor species delivers to conspecific stigmas. However, the morphological features (large and hairy bodies) and flower-handling behaviors of some visitors suggest that they contact anthers and stigmas while visiting floral units, and presumably carry and deliver pollen loads comparable to *E. tristis*.

Data Analysis

Due to issues achieving parametric assumptions, a non-parametric Mann-Whitney U test was used to detect any significant differences in the number of visits in Oregon versus California (visit variation by State). In addition, I used two Kruskal-Wallis tests to

determine whether visitation rates differed significantly among study sites for each visitor

species in each state. I used a chi-square test (to test for any significance) followed by a

protected binomial GLM to test for differences in the proportion of female and male-

phase flowers used by different visitors. All statistical analyses were performed using R

version 3.1.2.

RESULTS

Total Observed Visits

I conducted 260 and 160 three-minute pollinator observations in California and Oregon, respectively, for a total of 1,260 minutes of observation (Table 2). A total of 1,689 visits were observed, with 1,346 in California and 343 in Oregon. A mixture of flies and bees visited *Iris* flowers (Table 1), but the jeweled spider fly, *Eulonchus tristis,* was the dominant visitor in both states, accounting for 72% of the observed visits. Short-tongued bees, *Dialictus* spp. and *Andrena auricoma*, were the second and third most frequent visitors, respectively, contributing to a combined 16% of total visits. The remaining 12% of visits were made by several bee species (*Apis mellifera*, *Bombus vosnesenskii*, *Osmia densa*, and *Xylocopa tabaniformis*) and fly species (*Anthrax irroratus*, *Bombylius major*, *Eupeodes fumipennis*, and *Myopa rubida*).

Visitation Surveys

Eulonchus tristis visited flowers roughly 2.7 times more frequently than all other visitor species combined (Table 3 and Figure 3), and was the most frequent visitor at all study sites except for OR site 2, where *A. auricoma* dominated (Table 2). *Dialictus* spp. and *A. auricoma* had the second and third highest visitation rates and were also present at every study site. All other visitor species were absent from at least two study sites and had relatively low visitation rates (Table 2). The number of observed visits by all taxa

combined varied by state, with significantly fewer visits in Oregon than at California sites (Mann-Whitney U test, $p < 0.001$). In both states, visitation rates were significantly different across study sites for *E. tristis*, *A. auricoma*, *Dialictus* spp., and *A. mellifera* (Kruskal-Wallis tests, p's < 0.01). Visitation rates did not vary across sites for any of the other visitor species (p's >0.05) (Table 2).

<div align="center">Flower Handling Behavior and Stigma Contact</div>

Flowers were handled differently depending on the visitor species (Table 3). *Eulonchus tristis* predominantly made *sepal entry* visits, which accounted for 77% of total visits. When visiting flowers, *E. tristis* first extended their long proboscides in flight, then landed on sepals, entered floral units, and probed for nectar. The fit between the fly and the flower was tight; during the process of walking in and out of the floral units, *E. tristis* regularly contacted anthers and stigmas. Pollen grains were deposited on the dorsal surface of the thorax. *Eulonchus tristis* was never observed consuming or grooming pollen from their bodies. When entering female phase flowers, *E. tristis* always contacted stigmas with their head and thorax. Strips bare of pollen were frequently observed on the thoraxes of *E. tristis*, where pollen grains were removed by contact with stigmas. The remaining flower handling behaviors exhibited by *E. tristis* were *land* and *touch* (23% of total visits).

Bees handled flowers differently depending on their proboscis length (Table 3). The long-tongued mason bee, *Osmia densa*, predominantly exhibited *sepal entry* flower handling behavior (67% of visits). Like *E. tristis*, *O. densa* walked in and backed out of

floral units in search of nectar, and in doing so, always contacted anthers and stigmas. Nearly one-third of *O. densa* visits were *land* and *touch* (29% of visits).

Other long-tongued bees, *A. mellifera* and *B. vosnesenskii*, primarily made *land* and *touch* visits, which accounted for 54% and 45% of total visits, respectively. Both species made *nectar robbing* visits (21% of total visits for *A. mellifera* and 36% for *B. vosnesenskii*). These visits rarely resulted in anther or stigma contact (5% for *A. mellifera* and 4% for *B. vosnesenskii*). *Sepal entry* visits accounted for 21% and 18% of total visits, respectively. Due to the relatively large body size of *A. mellifera* and *B. vosnesenskii*, these visits often resulted in anther and stigma contact.

Short-tongued bees, *A. auricoma* and *Dialictus* spp. could not access nectar. Instead, they frequently robbed flowers for pollen (39% of visits by *A. auricoma* and 49% by *Dialictus* spp. Both groups entered floral units from the side, grasped on to anthers, and collected pollen grains. Since anthers are situated slightly behind stigmas, *pollen robbing*/side visits typically did not result in stigma contact (Table 3). Occasionally, both *A. auricoma* and *Dialictus* spp. made *sepal entry* visits, but due to their relatively small body size, they often walked beneath, and missed contacting, stigmas while foraging for pollen. Nevertheless, both species occasionally contacted stigmas during *sepal entry* visits (18% for *A. auricoma* and 6% for *Dialictus* spp.). Visits by both taxa were largely land and touch (totaling together 56% and 36% of visits, respectively).

The other visitors almost exclusively made *land* and *touch* visits (90% of total visits). *Nectar robbing* and *sepal entry* behaviors comprised the remaining 10%. These visitors contacted stigmas during 3% of total visits to female flowers.

Gender Phase Preference

The proportion of visits to male and female phase flowers was similar for all

visitor species except *Dialictus* spp., which visited a significantly smaller proportion of

female phase flowers (Table 3, chi-square test, $p < 0.006$ and GLM, $p < 0.05$).

Pollen Deposition and Expected Delivery

Eulonchus tristis delivered a range of pollen grains, varying between one and as

many as 1,038 in a single visit (mean = 185 pollen grains per visit, median = 96 pollen

grains per visit) (Figure 4, Appendix Figure 1). These statistics were based on a total of

86 stigmas, each of which received a single visit by *E. tristis*. All samples consisted of

100% conspecific *Iris* pollen grains (Appendix Figure 1). The distribution of the number

of grains deposited was strongly right-skewed. The expected pollen delivery by *E. tristis*

over the life of a female phase flower is 646 grains (calculated with the equations above,

under a conservative estimate of seven hours of *E. tristis* visitation per female flower;

approximately seven visits). Because the average number of ovules I observed per *I.*

bracteata ovary is 100, the approximate ratio of pollen grains to ovules is 7:1.

Pollinator Potential

Eulonchus tristis had the highest pollinator potential value, approximately 23

times higher than the visitor with the second highest potential, *O. densa* (Figure 3).

Despite having low visitation rates, *O. densa* regularly contacted stigmas, which explains

for their slightly higher pollinator potential value compared to the rest of the visitors. The

relatively low pollinator potential values by the other visitors reflect both low visitation

and low probabilities of stigma contact.

DISCUSSION

Iris bracteata is visited by a variety of different insects. The importance of these different visitors as pollinators, however, vary markedly; a reflection of differences in visitation rate and probability of stigma contact. Flowers of most species of *Iris* are pollinated by bees (Guo 2015), but I found that the most important pollinator of *I. bracteata* was the acrocerid fly, *E. tristis*. This finding emphasizes the importance of understanding the natural history of these flies and raises questions regarding the degree of dependency between *I. bracteata* and *E. tristis*. Additionally, this finding provides further evidence that flies can be important pollinators.

Natural History and Pollinating Ability of Eulonchus

The genus *Eulonchus* Gerstaecker (Diptera: Acroceridae) contains six species found primarily in the coniferous forests of northwestern North America. The larvae are internal parasitoids of fossorial mygalomorph spiders in the families Antrodiaetidae and Euctenizidae (Borkent et al. 2016). The adults are anthophilous, feed on nectar, and easily recognized by their large, hairy, golden green, blue, or purple metallic bodies (range 7-12 mm in length), and their long proboscides that extends at least the length of the body (Schlinger1960, 1987, Borkent et al. 2016). The remarkable length of the proboscis allows them to feed efficiently on flowers with deep nectar tubes. Members of the genus are fast fliers, and capable of traveling impressively long distances (up to 163 meters) (Brown 2018). Like some other anthophilous flies (e.g. Syrphidae and

Bombyliidae), *Eulonchus* use flower patches as rendezvous sites for mating. Male flies regularly scout for foraging females on or near flowers. Once a foraging female is spotted, one or more males approach her and attempt to copulate. Although the mating process is rapid, males often remain attached to the back of foraging females for some time, defending against approaches by other males. Female flies appear unaffected by the presence of males on their backs and forage as usual (Appendix Figure 3).

Eulonchus tristis provide high-quality pollination services to *I. bracteata*. These large bodied flies exclusively visited *I. bracteata* flowers and consistently performed the same flower handling behavior, *sepal entry,* which always resulted in anther and stigma contact. The body, especially the thorax, is densely covered in short hairs that regularly pick-up and remove pollen grains from anthers during floral visits. When mating pairs (male on top of female) visit floral units, they fit inside the flower even more tightly than single flies, which increases the probability of anther and stigma contact (Mesler unpublished, Ponte unpublished, Borkent and Schlinger 2008). The long distances *E. tristis* travels suggest that they regularly transfer pollen between different, often widely separated patches, which may reduce potential inbreeding depression.

Fruit and seed production by *Iris bracteata* was probably not limited by insufficient pollen reception at my study sites. I estimated that *E. tristis* delivered an average of 6.5 pollen grains per ovule, which exceeds reported values (1.8-4) for other plants that result in full fruit and seed sets (Snow 1982, Vaissiere 1991, Falque 1995).

Relationship Between E. tristis and I. bracteata

Eulonchus tristis and *I. bracteata* appeared to form a specialized relationship at my study sites. *E. tristis* accounted for the most visits to *I. bracteata* flowers at all but one of my sites (OR site 2) and likely was responsible for the majority of pollen deposition at every site – and *E. tristis* apparently restricted its visits to the *Iris*. Although I did not conduct systematic surveys, *Eulonchus tristis* was never observed visiting other co-flowering plants during my study, and stigmatic pollen loads deposited by the fly consisted entirely of *Iris* grains.

In spite of this apparent specialization, the relationship between *I. bracteata* and *E. tristis* is probably not genetically fixed, but rather a case of local, ecological specialization (Fox and Morrow 1981, Cane and Sipes 2006). *Eulonchus tristis* is known to visit a large and morphologically diverse guild of flowering plants throughout California, Oregon, and Washington (Borkent et al. 2016, Mesler unpublished). In each case, the flies adopt a local foraging specialization on a single plant species (Schlinger 1960, Borkent and Schlinger 2008, Mesler unpublished, personal observation). For instance, Cromwell (1998) observed an exclusive relationship between *E. tristis* and *Lewisia cotyledon* (Portulacaceae) in northern California, where eight other plant species were in bloom. Such local specialization is likely a reflection of *E. tristis* choosing the dominant flowering species yielding the greatest nectar reward (Borkent and Schlinger 2008). In the case of *I. bracteata*, *E. tristis* is attracted to the flowers because they are large, easily recognizable, and represent abundant sources of nectar. Other plant species

used by *Eulonchus* elsewhere also have large and showy flowers that are abundant

sources of nectar (Schlinger 1960, Cromwell 1998, Borkent and Schlinger 2008, Borkent

et al. 2016). Since nectar is produced at the base of perianth tubes, it can only be accessed

by visitors with long proboscides/tongues. Thus, *E. tristis* likely has little competition for

I. bracteata nectar, since aside from *O. densa*, which was an infrequent visitor, the other

visitors have short tongues that cannot access nectar.

      Iris bracteata likewise does not appear to have adaptations that limit visitation

and pollination to *E. tristis* alone. I observed that two native bees at my study sites – *O.*

densa and *B. vosnesenskii* – handled the flowers in the same fashion as *E. tristis* and

almost certainly would be capable of transferring substantial numbers of pollen grains to

stigmas if they visited flowers more frequently. The local specialization on *Eulonchus*

likely reflects ecological conditions favorable to the flies at my study sites (perhaps

abundant spider hosts) as well the availability of energetically more rewarding alternative

floral resources for the bees. A change in these circumstances could shift the pollinator

guild of the *Iris* to one dominated by bees. In fact, *O. densa* was the dominant visitor in

2006 at site along Knopki Creek Road near one of my sites (Mesler, unpublished

observations). Flowers of *I. bracteata* closely resemble those of other *Iris* and do not

appear to have morphological traits that are specific adaptations for pollination by

Eulonchus. Adult flies consistently emerge each year during the blooming period of *I.*

bracteata (Mesler, unpublished) and appear to vanish entirely at a particular site as

flowers begin to senesce, suggesting phenological co-adaptation, but the flowering season

also overlaps with *Osmia* and *Bombus*.

Potential Parasitism by Short-tongued Bees

Pollen-collecting bees are known to remove a great deal more pollen then primarily nectar collecting visitors in some systems (Larsson 2005). However, more pollen removal does not always lead to more pollen delivery. In fact, in some systems pollen-collecting bees are considered "parasites" because they remove pollen, and rarely contribute to pollen delivery (Thomson 2003, Padysakova et al. 2013, Parker et al. 2016). From the plants perspective, pollen grains that are removed from anthers and not deposited to stigmas are functionally lost from the system.

I suspect that both *A. auricoma* and *Dialictus* spp. fit the role of "parasites" in the *I. bracteata* system. Unable to access nectar, these short-tongued bees focused on collecting pollen and seldom contacted stigmas during floral visits. Whether these bees significantly deplete the amount of pollen in some *I. bracteata* populations is unknown. However, both taxa exhibited flower handling behaviors that suggest that their contribution to pollination is likely minimal and potentially detrimental. The propensity for both taxa to collect pollen begs the question if these bees prefer visiting male phase flowers. Several studies have documented flower gender preference in similar pollen-collecting bee species (Delph and Lively 1992, Ashman 2000, Parker et al. 2016). I found that *Dialictus* spp. visited significantly more male than female phase flowers, suggesting that they have the potential to deplete pollen loads early on and limit the amount of pollen that could otherwise be available and transferred by pollinators. If either *A. auricoma* or *Dialictus* spp. lower the effectiveness of future floral visitors, then their visits may be

more detrimental than beneficial, and potentially reduce plant fitness rather than increase it.

Importance of Flies

Traditionally, flies have received little attention from pollination biologists in spite of the fact that they are frequent flower visitors. This neglect likely reflects the entrenched canon that flies are poor pollinators (Faegri and van der Pijl 1979), a generalization that is suspect for both theoretical and empirical reasons. Flower-visiting flies are diverse. Depending on the taxonomic group, they can differ strongly in size, degree of hairiness, proboscis length, and flower handling behavior, and as such, their performance as pollinators is likely to vary in a corresponding fashion. Empirically, flies are known to pollinate many crop and wild plant species, and can be especially important in habitats where cool temperatures and/or moist conditions limit bee activity (Inouye et al. 2015). Moreover, like bees, their relative importance as pollinators is likely to depend on ecological context as well as their taxonomic group (Kearns and Inouye 1994). This newer, more expansive view about the global role of flies as pollinators is reviewed in several recent studies (Kearns 2001, Larson et al. 2001, Ssymank et al. 2008, Inouye et al. 2015, Orford et al. 2015, Rader *et. al* 2016). Consistent with these studies, the findings of my work provide additional evidence that flies can be important pollinators and emphasize the importance of continued studies aimed at documenting the pollinating ability of acrocerid flies.

Conservation Implications

Resource managers concerned with the reproductive success of *I. bracteata* should consider the habitat requirements of *E. tristis*. These flies require nectar to fuel their flight behavior, but they also require suitable spider host species to complete their life cycles (Borkent et al. 2016). Effective management for successful *I. bracteata* pollination would thus require an understanding of the habitat requirements of the spider hosts and, especially, the impacts of habitat alteration (e.g. prescribed fires, logging, road construction/maintenance, recreational activities, etc.) on their abundance. For example, prescribed fires and logging might impact the prey (small insects) availability and nesting substrate (soft soil and conifer needles) necessary for these relatively shallow-burrowing (max. 30 cm.) fossorial spider hosts, and consequently reduce the number of suitable hosts for *E. tristis* (Vincent 1993). A reduction in *E. tristis* abundance will presumably have negative effects on the pollination of *I. bracteata*. However, several other visitors of *I. bracteata* have the potential to be effective pollinators, and perhaps their roles will shift and become more important in the absence of *E. tristis*. Further research regarding the impacts of these forest management practices on *E. tristis* and their spider hosts is warranted to determine whether these disturbances will negatively affect the fecundity of *I. bracteata*.

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TABLES

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Table 1. The flower visitors and the total number of visits to *Iris bracteata* for all 3-minute observation periods (*n*=420).

Order	Family	Species	No. of visits	Percentage of Visits
Diptera	Acroceridae	*Eulonchus tristis*	1,222	72.4%
	Bombyliidae	*Bombylius major*	61	3.6%
		Anthrax irroratus	10	0.6%
	Conopidae	*Myopa rubida*	5	0.3%
	Syrphidae	*Eupeodes fumipennis*	7	0.4%
Hymenoptera	Andrenidae	*Andrena auricoma*	101	6.0%
	Apidae	*Apis mellifera*	24	1.4%
		Bombus vosnesenskii	11	0.6%
		Xylocopa tabaniformis	1	0.1%
	Halictidae	*Lasioglossum (Dialictus)* spp.	168	10.0%
	Megachilidae	*Osmia densa*	79	4.7%
Total			1,689	100%

Table 2. Average visitation rates (#visits/#flowers/hour) per study site for each visitor. Study sites are listed by state and site location (see study map). The number of 3-minute observation periods, survey days, insect visits varied for each study site. Standard deviations for average visitation rates are given in parentheses. Species with low visitation rates were grouped together and categorized as 'Other.' The 'Other' includes: *Anthrax irroratus*, *Bombylius major*, *Eupeodes fumipennis*, *Myopa rubida*, and *Xylocopa tabaniformis*.

Study site	No. of 3 – minute observations	No. of days	No. of visits	*Andrena auricoma*	*Apis mellifera*	*Bombus vosnesenskii*	*Eulonchus tristis*	*Dialictus* spp.	*Osmia densa*	Other
CA – 1	60	3	269	0.09 (0.32)	0	0	1.54 (1.75)	0.22 (0.49)	0.14 (0.54)	0.03 (0.11)
CA – 2	60	2	303	0.16 (0.50)	0.01 (0.06)	0	1.91 (2.22)	0.1 (0.23)	0.04 (0.25)	0.01 (0.11)
CA – 3	120	3	546	0.01 (0.08)	0.01 (0.08)	0	1.69 (1.65)	0.16 (0.42)	0.04 (0.21)	0.12 (0.30)
CA – 4	20	1	228	0.02 (0.70)	0	0.02 (0.10)	4.29 (1.88)	0.44 (0.61)	0	0.07 (0.22)
OR – 1	20	1	16	0.07 (0.22)	0	0	0.16 (0.60)	0.02 (0.10)	0	0.07 (0.30)
OR – 2	40	2	110	0.51 (0.91)	0.17 (0.43)	0	0.36 (0.67)	0.10 (0.56)	0	0.04 (0.17)
OR - 3	100	3	217	0.03 (0.12)	0.21 (0.46)	0.04 (0.32)	0.33 (0.61)	0.16 (0.64)	0.004 (0.04)	0.13 (0.38)

Table 3. Comparison of performance and behavior of flower visitors. N is the total number of visits by a given taxon across all observation periods. Count is the number of 3-minute observations where a taxon was observed. Female phase is the proportion of female phase flowers visited. Stigma contact is the proportion of visits to female-phase flowers that resulted in stigma contact. Visitation rates (#visits/#flowers/#hour) are means calculated for all 3-minute observations pooled across sites and dates. Flower handling behaviors are described in the text. Visitors showed an overall significant difference in the proportion of female phase flowers they visited (chi-square test, $p = 0.006$).

| Taxon | N | Count | Female phase | Stigma contact | Visitation Rates | | Behavior Proportions | | | | |
					Mean	SD	Pollen robbing	Nectar robbing	Sepal entry	Land	Touch
Apis mellifera	24	3	0.79	0.26	0.03	0.17	0.04	0.21	0.21	0.38	0.15
Bombus vosnesenskii	11	13	0.82	0.22	0.01	0.15	0.00	0.36	0.18	0.18	0.27
Andrena auricoma	101	41	0.72	0.18	0.11	0.42	0.39	0.06	0.01	0.23	0.33
Eulonchus tristis	1,222	260	0.74	0.87	1.30	1.76	0.003	0.01	0.77	0.13	0.1
Dialictus spp.	168	93	0.57	0.06	0.18	0.43	0.49	0.07	0.08	0.26	0.1
Osmia densa	79	23	0.72	0.67	0.08	0.44	0.03	0.01	0.67	0.14	0.15
Other	84	44	0.81	0.03	0.08	0.27	0.00	0.06	0.04	0.51	0.39

FIGURES

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a)
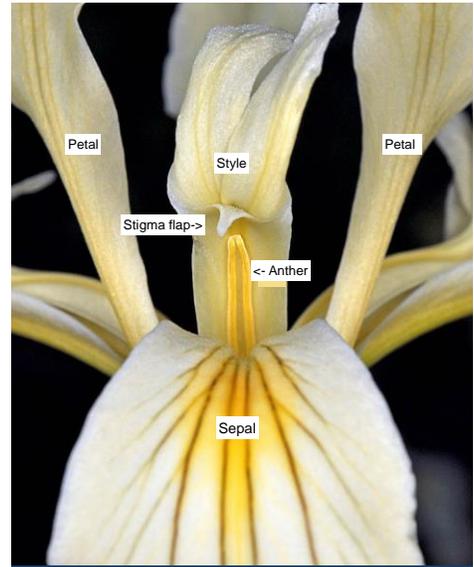
b)

Figure 1. Iris flower morphology. a) Each flower consists of three functional units (floral units). b) Detail of one of these units.
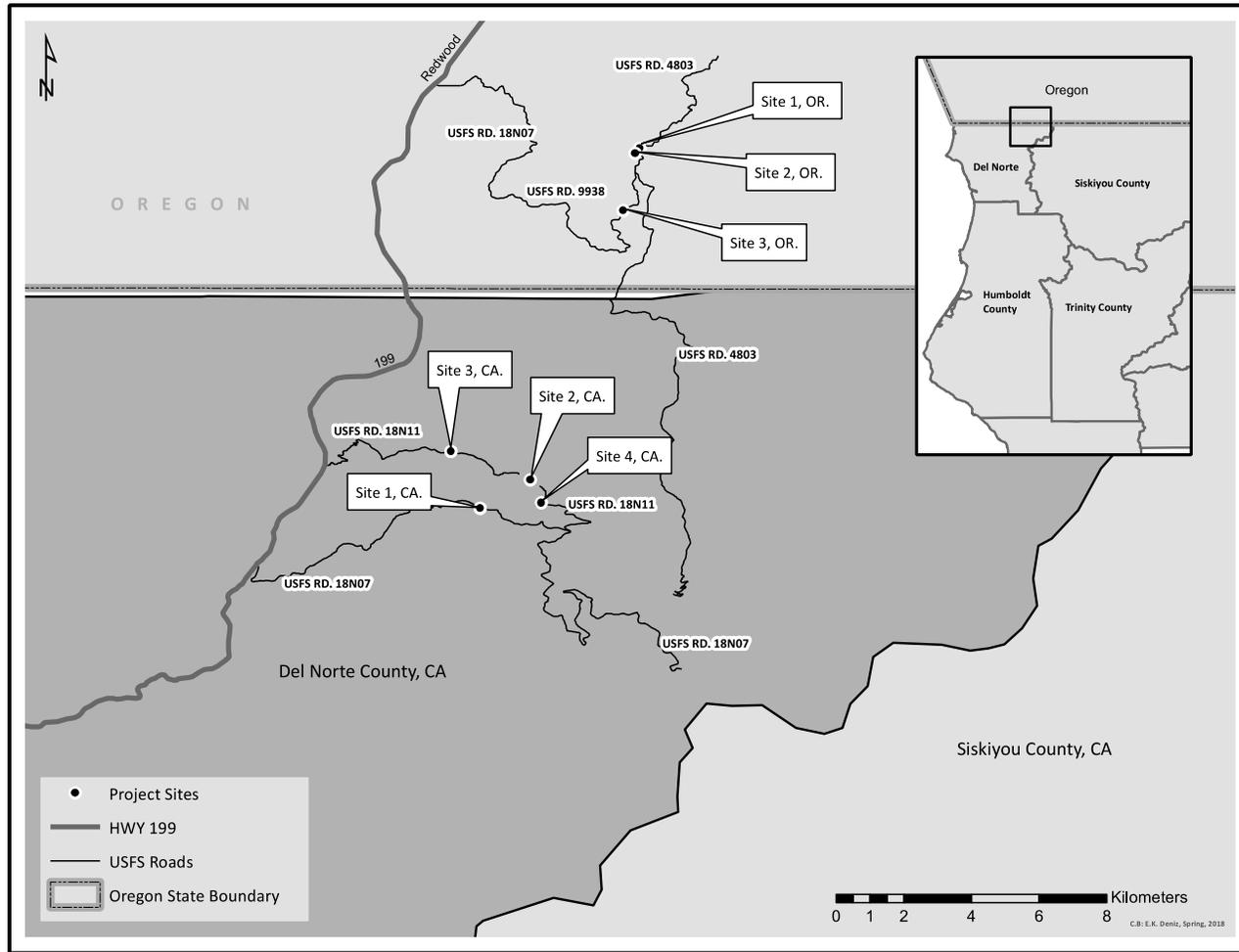
Figure 2. Study site locations in Del Norte County, CA (4 sites) and Josephine County, OR (3 sites).
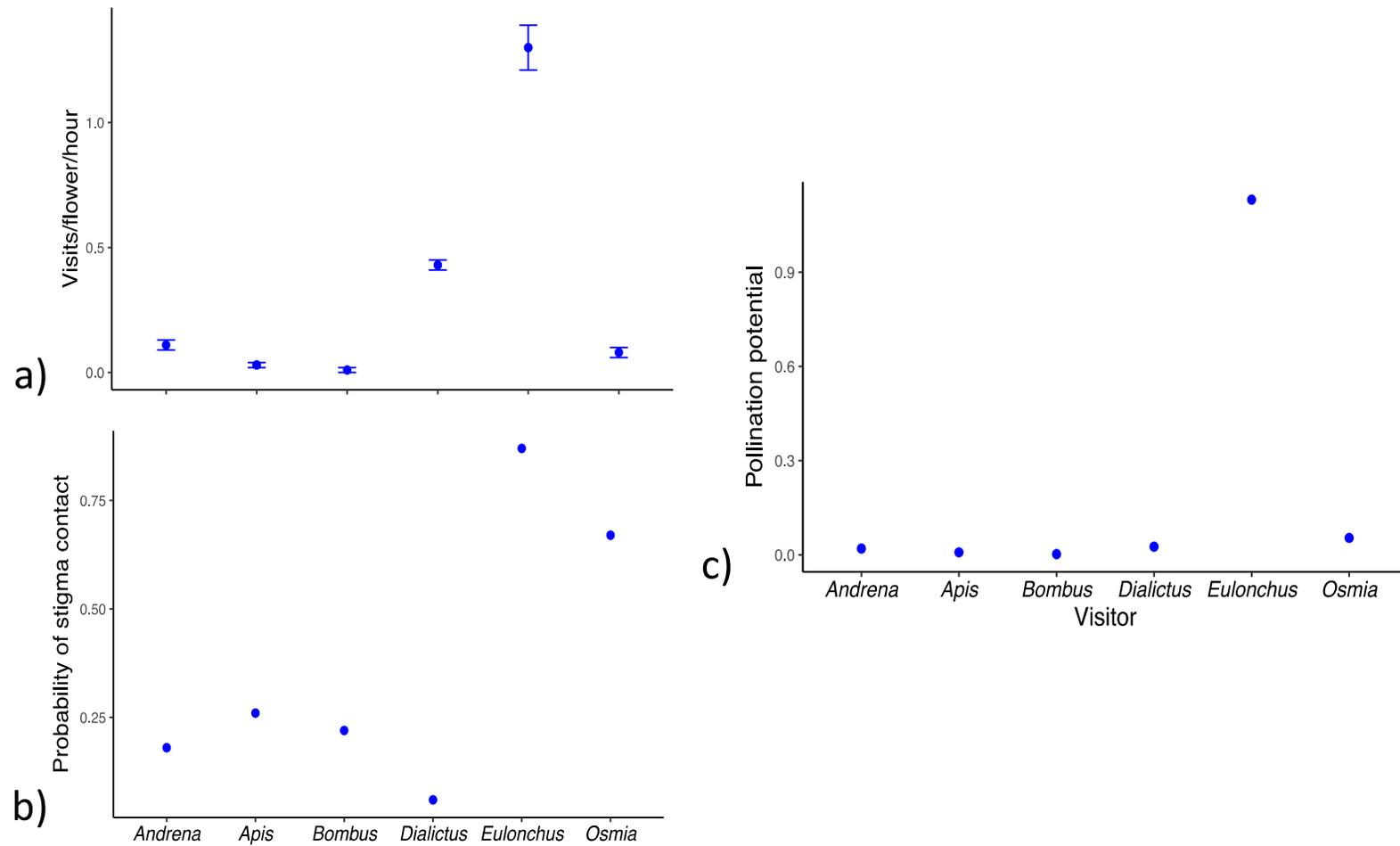
Figure 3. Comparison of performance by visitors to *Iris bracteata* flowers. a) Mean visitation rates and standard errors. b) Probability of stigma contact during visits to female phase flowers. c) Pollination potential (visitation rate x probability of stigma contact).
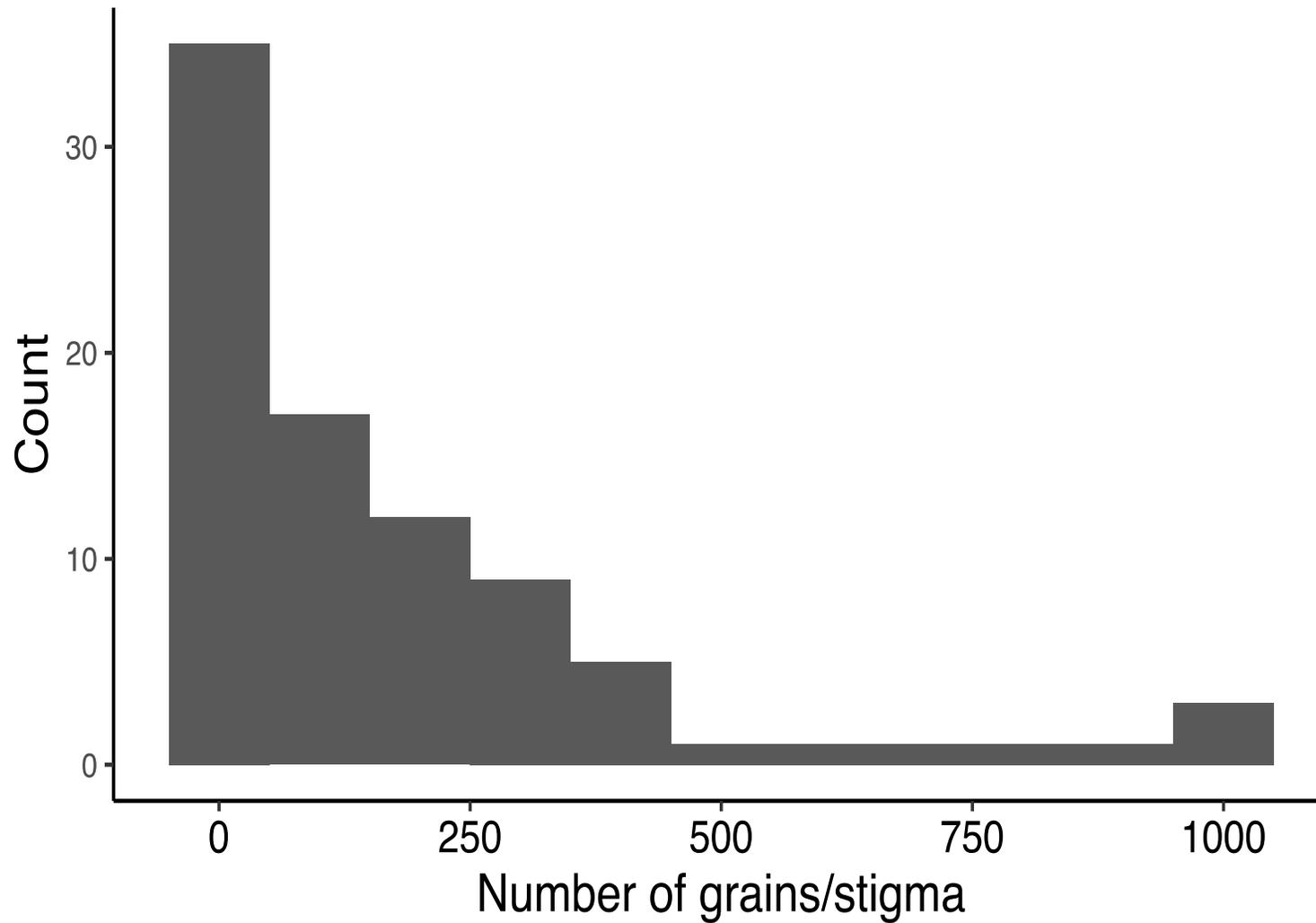
Figure 4. Number of grains deposited on virgin stigmas by *Eulonchus tristis* during a single visit (*n*=86 stigmas). All stigmas received at least one pollen grain.

APPENDICIES

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APPENDIX A. Visitation rates (#visits/#flowers/hour) for each visitor group organized by study site, location, date, and time. Total number of visits are given for each 1-hour observation period. Standard deviations are in parentheses.

Study Site, Location, Date, & Time	No. of Visits	*Eulonchus*	*Bombus*	*Andrena*	*Apis*	*Dialictus*	*Osmia*	*Syrphid*	*Other*
Site 1 – CA – (5/24) 5:00 - 6:43 p.m.	67	1.28 (1.6)	0	0	0	0.08 (0.002)	0.22 (0.02)	0.02 (0.0004)	0
Site 1 – CA – (5/28) 10:05 - 12:11 p.m.	81	1.38 (1.8)	0	0.08 (0.2)	0	0.06 (0.2)	0.02 (0.6)	0.02 (0.1)	0
Site 1 – CA – (5/29) 12:40 - 2:26 p.m.	121	1.96 (1.8)	0	0.18 (0.4)	0	0.52 (0.8)	0	0.04 (0.2)	0
Site 2 – CA – (5/28) 9:30 - 11:14 a.m.	103	1.84 (2.6)	0	0.32 (0.8)	0	0.04 (0.2)	0.08 (0.4)	0	0
Site 2 – CA – (5/29) 10:16 - 11:57 a.m.	68	1.20 (1.6)	0	0.08 (0.4)	0	0.12 (0.2)	0.04 (0.2)	0	0.04 (0.2)
Site 2 – CA – (5/29) 12:21 - 1:40 p.m.	132	2.68 (2.4)	0	0.08 (0.4)	0.02 (0.2)	0.14 (0.2)	0	0	0
Site 3 – CA – (5/28) 1:15 - 3:00 p.m.	63	1.14 (1.4)	0	0	0	0	0.12 (0.2)	0	0.16 (0.2)
Site 3 – CA – (5/28) 1:16 - 2:43 p.m.	112	2.04 (1.2)	0	0	0	0.36 (0.6)	0	0	0.06 (0.2)
Site 3 – CA – (5/28) 3:38 - 5:06 p.m.	33	0.64 (0.8)	0	0	0.04 (0.2)	0.06 (0.2)	0	0	0.02 (0.2)
Site 3 – CA – (5/28) 3:30 - 4:48 p.m.	82	1.74 (1.4)	0	0	0	0	0	0.0 (0.2)	0.06 (0.2)
Site 3 – CA – (5/29) 10:10 -11:37 a.m.	82	1.28 (1.4)	0	0	0	0.38 (0.6)	0.04 (0.2)	0	0.12 (0.2)
Site 3 – CA – (6/3) 1:16 - 2:15 p.m.	174	3.26 (2.2)	0	0.04 (0.2)	0	0.18 (0.4)	0.08 (0.4)	0	0.28 (0.6)
Site 4 – CA – (6/18) 1:17 - 2:37 p.m.	228	4.28 (1.8)	0.02 (0.1)	0.22 (0.6)	0	0.44 (0.6)	0	0	0.06 (0.2)
Site 1 – OR – (5/25) 10:47 - 12:23 p.m.	16	0.16 (0.6)	0	0.06 (0.2)	0	0.02 (0.1)	0	0	0.06 (0.2)

Study Site, Location, Date, & Time	No. of Visits	*Eulonchus*	*Bombus*	*Andrena*	*Apis*	*Dialictus*	*Osmia*	*Syrphid*	*Other*
Site 2 – OR – (5/25) 1:27 - 3:20 p.m.	74	0.48 (0.8)	0	0.56 (1.0)	0	0.28 (0.6)	0.20 (0.8)	0	0.08 (0.2)
Site 2 – OR – (5/27) 10:15 - 11:51 a.m.	36	0.22 (0.6)	0	0.46 (0.8)	0.04 (0.2)	0.06 (0.2)	0	0	0
Site 3 – OR – (5/26) 12:53 - 3:09 p.m.	59	0.40 (0.8)	0	0	0.02 (0.1)	0.34 (0.6)	0.24 (0.6)	0.02 (0.1)	0.26 (0.6)
Site 3 – OR – (5/26) 1:52 - 4:03 p.m.	46	0.48 (0.6)	0	0.07 (0.2)	0.18 (0.6)	0.22 (0.4)	0.02 (0.1)	0	0.04 (0.2)
Site 3 – OR – (5/27) 10:07 - 11:51 a.m.	12	0	0	0.04 (0.2)	0.12 (0.4)	0.08 (0.2)	0.02 (0.1)	0	0.02 (0.1)
Site 3 – OR – (5/27) 12:27 - 2:39 p.m.	33	0.36 (0.1)	0.16 (0.6)	0	0.04 (0.2)	0.08 (0.2)	0.06 (0.2)	0	0.02 (0.1)
Site 3 – OR – (5/27) 1:17 – 2:51 p.m.	67	0.38 (0.6)	0.02 (0.1)	0.02 (0.1)	0.06 (0.2)	0.32 (0.8)	0.42 (1.2)	0	0.26 (0.6)

APPENDIX B. Comparison of the seven study sites: coordinates, elevation, site description, general location, and co-flowering species. Plant codes: ACMA = *Acer macrophyllum*, ARME = *Arbutus menziesii*, ARNO = *Arctostaphylos nortensis*, COSE = *Cornus sessilis*, CEAN = *Ceanothus* spp., HOCR = *Hosackia crassifolia*, LICA = *Lithospermum californicum*, LUTR = *Lupinus tracyi*, MAEL = *Madia elegans*, NODE = *Notholithocarpus densiflorus*, ROGY = *Rosa gymnocarpa*, THGR = *Thermopsis gracilis*, TODI = *Toxicodendron diversilobum*.

Study sites	Coordinates (DMS)	Elevation (Ft.)	Site description	General location	Co-flowering species (plant codes)
CA - 1	41°56'24.72" N, -123°42'06.48" W	3,160	≈ 100 meters of large linear patches.	4 mi. up from Hwy 199 on Knopki Creek rd. (18N07)	ACMA, ARME, ARNO, COSE, VAOV
CA - 2	41°56'51.72" N, -123°41'18.96" W	2,934	Large and small patches in open forest clearings and steep rocky west-facing slopes along entire road (18N11)	6 mi. up 18N07 from US 199, turn left on 18N11 and continue ≈ 3 mi.	ARME, ARNO, LUTR, MAEL, THGR, VAOV
CA - 3	41°57'19.08" N, -123°42'34.92" W	3,110	≈ 40x40 meter forest opening of large patches	≈ 2 mi. past CA - 2	ACMA, ARME, ARNO, CEAN, LUTR, MAEL, ROGY, ROSP, THGR, VAOV
CA - 4	41°56'30.12" N, -123°41'08.88" W	2,926	Population with large patches growing on a steep and rocky west-facing slope	≈ 2mi. up 18N11, about a mile before CA - 2	ARME, ARNO, LUTR, MAEL, THGR, VAOV
OR - 1	42°02'09.24" N, -123°39'33.84" W	2,493	Small and patchy linear population ≈ 80 meters long. Covered by a thick forest canopy and only experienced a couple hours of sunlight a day	≈ 3mi. up Sanger Peak rd. (NF 4803)	ACMA, LICA, NODE, TODI
OR – 2	42°02'04.20" N, -123°39'38.52" W	2,500	Large opening near bend in road ≈ 25x25 meter plot of small patches distributed in a non-linear fashion	≈ 200 meters passed OR – 1 on 4803.	ACMA, CEAN, LICA, LUTR, NODE, TODI
OR - 3	42°01'09.48" N, -123°39'50.04" W	2,513	Large population with dense patches mottled throughout a 50x50 meter forest clearing and all along the margins of the road.	≈ 4mi. up 4803 or ¾ mi. up from OR – 2, turn right on 9938, and continue ≈ 2mi.	ARME, ARNO, CEAN, HOCR, LICA, LUTR, ROGY, TODI

APPENDIX C. Photo of one pollen deposition sample. *I. bracteata* pollen grains and stigma tissue focused at 50x magnification.
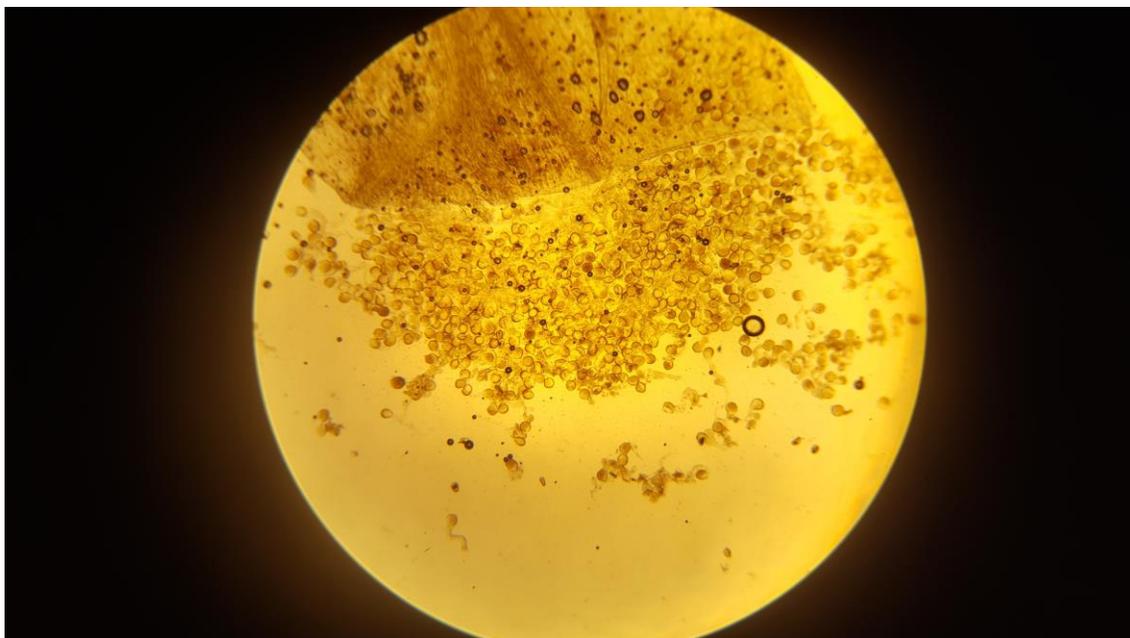
APPENDIX D. Photo of an insect exclusion cage.

APPENDIX E. Mating pair of *Eulonchus tristis* visiting a floral unit of *Iris bracteata*