

JEWELLED SPIDER FLIES (*EULONCHUS TRISTIS*) ARE IMPORTANT
POLLINATORS OF *IRIS BRACTEATA*, A RARE SISKIYOU MOUNTAIN ENDEMIC

By

Jean-Paul E. Ponte

A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

Committee Membership

Dr. Michael R. Mesler, Committee Chair

Dr. Erik S. Jules, Committee Member

Dr. Alexandru M.F. Tomescu, Committee Member

Dr. Paul E. Bourdeau, Committee Member

Dr. Erik S. Jules, Program Graduate Coordinator

December 2018

ABSTRACT

JEWELLED SPIDER FLIES (*EULONCHUS TRISTIS*) ARE IMPORTANT POLLINATORS OF *IRIS BRACTEATA*, A RARE SISKIYOU MOUNTAIN ENDEMIC

Jean-Paul E. Ponte

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.

ACKNOWLEDGEMENTS

First and foremost, I would like to dedicate this thesis to my advisor, Dr. Michael R. Mesler, whose expertise, patience, wisdom, and humor were crucial in the completion and quality of this thesis. It was an honor to work alongside Michael. His passion for the sciences was infectious and his presence was mesmerizing. I can safely say I don't know where or who I would be without him. So, here's to you, Boss. Thanks for everything; you have taught me so much and have helped me become the individual I am today.

Thank you to my committee. Special thanks to Dr. Mihai Tomescu for being so supportive and providing valuable feedback and assistance during the writing process of this thesis. Dr. Erik Jules introduced and prepared me for life in graduate school and I could not be more Grateful. Dr. Paul Bourdeau provided guidance in statistical interpretation and was an important mentor during my experience as president of the Biology Graduate Student Association. Thank you to Erik Lucas, Daniel Deeny-Miller, Scott Black, and the many other undergraduate assistants who helped in various parts of my research. Not only would this project have been nearly impossible without their participation, but it certainly would also have been far less enjoyable. Thanks to Stuart Osbrack for helping me hunt for *Iris bracteata* patches in Oregon. A world of thanks goes out to my mother and father who always had faith I would pull this off, especially since I often had my own doubts. Thank you to Alex Bippus and Mitchell Holmes for regularly rocking out and providing me with a musical outlet. And of course, thank you to Tim McClure, Kipp Pow, Kyle Brown, Drew Bost, Laura Morgan, Sarah Mason, Stefani

Brandt, and Saskia Raether for your friendship, support, and tolerance throughout this experience; I love you all. This material is based upon work supported by the North Coast Chapter: California Native Plant Society and Humboldt State University, Department of Biological Sciences.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	x
INTRODUCTION	1
MATERIALS AND METHODS	4
Study Species	4
Study Sites	5
Visitor Surveys	6
Expected Pollen Delivery	7
Pollinator Importance	10
Data Analysis	10
RESULTS	12
Total Observed Visits	12
Visitation Surveys	12
Flower Handling Behavior and Stigma Contact	13
Gender Phase Preference	15
Pollen Deposition and Expected Delivery	15
Pollinator Potential	15
DISCUSSION	17

Natural History and Pollinating Ability of <i>Eulonchus</i>	17
Relationship Between <i>E. tristis</i> and <i>I. bracteata</i>	19
Potential Parasitism by Short-tongued Bees	21
Importance of Flies	22
Conservation Implications	23
LITERATURE CITED	24
UNPUBLISHED CITATIONS	28
PERSONAL COMMUNICATIONS	29
TABLES	30
FIGURES	34
APPENDICES	39

LIST OF TABLES

Table 1. The flower visitors and the total number of visits to <i>Iris bracteata</i> for all 3-minute observation periods ($n=420$).	31
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: <i>Anthrax irroratus</i> , <i>Bombylius major</i> , <i>Eupeodes fumipennis</i> , <i>Myopa rubida</i> , and <i>Xylocopa tabaniformis</i>	32
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, $p = 0.006$).	33

LIST OF FIGURES

Figure 1. Iris flower morphology. a) Each flower consists of three functional units (floral units). b) Detail of one of these units.....	35
Figure 2. Study site locations in Del Norte County, CA (4 sites) and Josephine County, OR (3 sites).	36
Figure 3. Comparison of performance by visitors to <i>Iris bracteata</i> 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).....	37
Figure 4. Number of grains deposited on virgin stigmas by <i>Eulonchus tristis</i> during a single visit ($n=86$ stigmas). All stigmas received at least one pollen grain.....	38

LIST OF APPENDICES

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.	40
APPENDIX B. Comparison of the seven study sites: coordinates, elevation, site description, general location, and co-flowering species. Plant codes: ACMA = <i>Acer macrophyllum</i> , ARME = <i>Arbutus menziesii</i> , ARNO = <i>Arctostaphylos nortensis</i> , COSE = <i>Cornus sessilis</i> , CEAN = <i>Ceanothus</i> spp., HOGR = <i>Hosackia crassifolia</i> , LICA = <i>Lithospermum californicum</i> , LUTR = <i>Lupinus tracyi</i> , MAEL = <i>Madia elegans</i> , NODE = <i>Notholithocarpus densiflorus</i> , ROGY = <i>Rosa gymnocarpa</i> , THGR = <i>Thermopsis gracilis</i> , TODI = <i>Toxicodendron diversilobum</i>	42
APPENDIX C. Photo of one pollen deposition sample. <i>I. bracteata</i> pollen grains and stigma tissue focused at 50x magnification.	43
APPENDIX D. Photo of an insect exclusion cage.	44
APPENDIX E. Mating pair of <i>Eulonchus tristis</i> visiting a floral unit of <i>Iris bracteata</i> ..	45

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 (CNDDDB 2017). Populations of *I. bracteata* grow on rocky, ultramafic serpentinitic 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/dehiscid 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:

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

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:

$$\begin{aligned} \# \text{ visits to female phase flowers} \times P(\text{stigma contact}) \\ = \# \text{ stigma contacts} \end{aligned}$$

The number of visits to female phase flowers was calculated by multiplying visitation rates by the probability of visiting a female phase flower:

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

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).

Flower Handling Behavior and Stigma Contact

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 (Schlinger 1960, 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 than 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*.

LITERATURE CITED

- Ashman, T. L., Swetz, J., and Shivitz, S. 2000. Understanding the basis of pollinator selectivity in sexually dimorphic *Fragaria virginiana*. *Oikos* 90:347-356.
- Ballantyne, G., Baldock, K. C., and Willmer, P. G. 2015. Constructing more informative plant–pollinator networks: visitation and pollen deposition networks in a heathland plant community. *Proc. R. Soc. B* 282: 20151130.
- Borkent, C. J., Gillung, J. P., and Winterton, S. L. 2016. Jewelled spider flies of North America: a revision and phylogeny of *Eulonchus* Gerstaecker (Diptera, Acroceridae). *ZooKeys* 619: 103.
- Borkent, C. J., and Schlinger, E. I. 2008a. Pollen loads and pollen diversity on bodies of *Eulonchus tristis* (Diptera: Acroceridae): implications for pollination and flower visitation. *The Canadian Entomologist* 140: 257-264.
- Borkent, C. J., and Schlinger, E. I. 2008b. Flower-visiting and mating behaviour of *Eulonchus sapphirinus* (Diptera: Acroceridae). *The Canadian Entomologist* 140: 250-256.
- Cane, J. H., and Sipes, S. 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. *Plant-pollinator interactions: from specialization to generalization*, 99-122. University of Chicago Press, Chicago, Illinois, USA.
- CNDDDB. 2017. California Natural Diversity Database On-line. California Department of Fish and Wildlife. Sacramento CA. [LIST OF RARE AND ENDANGERED PLANTS AND ANIMALS](#). Downloaded on 16 July 2017.
- Cromwell, J. E. 1998. Heterogeneity in Pollinator Visitation and Importance of a Sympatric Population of *Lewisia Cotyledon* and *L. Leana* (Portulacaceae). Master's thesis, Humboldt State University, Arcata, CA, USA.
- Delph, L. F., and Lively, C. M. 1992. Pollinator visitation, floral display, and nectar production of the sexual morphs of a gynodioecious shrub. *Oikos* 63:161-170.
- Faegri, K. and van der Pijl. 1979. *The Principles of Pollination Ecology*. Elsevier, Amsterdam, Netherlands.

- Falque, M., Vincent, A., Vaissiere, B. E., and Eskes, A. B. 1995. Effect of pollination intensity on fruit and seed set in cacao (*Theobroma cacao* L.). *Sexual Plant Reproduction* 8: 354-360.
- Fox, L. A., and Morrow, P. A. 1981. Specialization: species property or local phenomenon?. *Science* 211: 887-893.
- Goldblatt, P., and Manning, J. C. 2008. *The Iris family: natural history & classification*. Timber Press, Portland, Oregon.
- Guo, J. 2015. Comparative micromorphology and anatomy of crested sepals in *Iris* (Iridaceae). *International Journal of Plant Sciences* 176: 627-642.
- Inouye, D. W., Larson, B. M., Ssymank, A., and Kevan, P. G. 2015. Flies and flowers III: ecology of foraging and pollination. *Journal of Pollination Ecology* 16:115-133.
- Kearns, C. A., and Inouye, D. W. 1994. Fly pollination of *Linum lewisi* (Linaceae). *American Journal of Botany* 81:1091-1095.
- Kearns, C. A. 2001. North American dipteran pollinators: assessing their value and conservation status. *Conservation Ecology* 5:1.
- King, C., Ballantyne, G., and Willmer, P. G. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4: 811-818.
- Larson, B. M. H., Kevan, P. G., and Inouye, D. W. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist* 133: 439-465.
- Larsson, M. 2005. Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (Dipsacaceae). *Oecologia* 146: 394-403.
- Lenz, L. W. 1958. A revision of the Pacific Coast Irises. *Aliso* 4: 1-72.
- Orford, K. A., Vaughan, I. P., and Memmott, J. 2015. The forgotten flies: the importance of non-syrphid Diptera as pollinators. *Proc. R. Soc. B* 282: 20142934.
- Padyšáková, E., Bartoš, M., Tropek, R., & Janeček, Š. 2013. Generalization versus specialization in pollination systems: visitors, thieves, and pollinators of *Hypoestes aristata* (Acanthaceae). *PloS One* 8: e59299.

- Parker, A. J., Williams, N. M., & Thomson, J. D. 2016. Specialist pollinators deplete pollen in the spring ephemeral wildflower *Claytonia virginica*. *Ecology and evolution* 6: 5169-5177.
- Rader, R., Bartomeus, I., Garibaldi, L. A., Garratt, M. P., Howlett, B. G., Winfree, R., and Bommarco, R. 2016. Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences* 113: 146-151.
- Rodionenko, G. I. 1987. The genus *Iris* L.(questions of morphology, biology, evolution and systematics). London, British Iris Society.
- Schlinger, E. I. 1960. A review of the genus *Eulonchus* Gerstaecker. Part I. The species of the smaragdinus group (Diptera: Acroceridae). *Annals of the Entomological Society of America* 53: 416-422.
- Schlinger, E. I. 1987. The biology of Acroceridae (Diptera): true endoparasitoids of spiders. In *Ecophysiology of spiders*: pp. 319-327. Springer, Berlin, Heidelberg.
- Snow, A. A. 1982. Pollination intensity and potential seed set in *Passiflora vitifolia*. *Oecologia* 55: 231-237.
- Ssymank, A., Kearns, C. A., Pape, T., and Thompson, F. C. 2008. Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity*, 9: 86-89.
- Thomson, J. 2003. When Is It Mutualism?. *The American Naturalist* 162: S1-S9.
- Uno, G. E. 1982. Comparative reproductive biology of hermaphroditic and male-sterile *Iris douglasiana* Herb.(Iridaceae). *American Journal of Botany* 69: 818-823.
- Vaissière, B. E. 1991. Honey bees, *Apis mellifera* L. Hymenoptera: Apidae, as pollinators of upland cotton, *Gossypium hirsutum* L.(Malvaceae), for hybrid seed production. PhD, Texas A&M University.
- Vincent, L.S. 1993. The natural history of the California turret spider *Atypoides Riversi* (Araneae, Antrodiaetidae): Demographics, growth rates, survivorship, and longevity. *Journal of Arachnology* 21: 29-39.
- Wilson, C. A. 2009. Phylogenetic relationships among the recognized series in *Iris* section *Limniris*. *Systematic Botany* 34: 277-284.
- Wilson, C. A. 2003. Phylogenetic relationships in *Iris* series *Californicae* based on ITS sequences of nuclear ribosomal DNA. *Systematic Botany* 28: 39-46.

Wilson, C. A. 2001. Floral stages, ovule development, and ovule and fruit success in *Iris tenax*, focusing on var. *gormanii*, a taxon with low seed set. *American Journal of Botany* 88: 2221-2231.

Young, N. D. 1998. Pacific Coast *Iris* species delimitation using three species definitions: biological, phylogenetic and genealogical. *Biological Journal of the Linnean Society* 63: 99-120.

UNPUBLISHED CITATIONS

Brown, K. 2018. Determining the flight distance and behavior of a flower-visiting: Implications for pollination success in a fragmented habitat. Unpublished manuscript. Department of Biology, Humboldt State University.

Comstock, R.B., and Perry S.L. 2005. Self-compatibility, autogamy, and pollen limitation in *Iris tenuissima* on Horse Mountain; and the role of the Acrocerid fly *Eulonchus*. Unpublished manuscript. Department of Biology, Humboldt State University.

Mesler, M.R. 2006. *Iris bracteata* visitation study. Unpublished manuscript. Department of Biology, Humboldt State University.

Ponte, J.E. 2016. *Iris bracteata* visitation pilot-study. Unpublished manuscript. Department of Biology, Humboldt State University.

PERSONAL COMMUNICATIONS

Borkent, C.J. 2015. Personal communication. Plant Pest Diagnostics Branch, California Department of Food & Agriculture, 3294 Meadowview Road, Sacramento, California, 95832-1448.

Mesler, M.R. 2015. Personal communication. Department of Biological Sciences, Humboldt State University, Arcata, California, 95521.

Osbrack, S. 2016. Personal communication. Rogue River –Siskiyou National Forest, Wild Rivers, Ranger District, 2164 N.E. Spalding Avenue, Grants Pass, Oregon, 97526.

TABLES

[THIS PAGE INTENTIONALLY LEFT BLANK]

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

<u>Order</u>	<u>Family</u>	<u>Species</u>	<u>No. of visits</u>	<u>Percentage of Visits</u>
Diptera	Acroceridae	<i>Eulonchus tristis</i>	1,222	72.4%
	Bombyliidae	<i>Bombylius major</i>	61	3.6%
		<i>Anthrax irroratus</i>	10	0.6%
	Conopidae	<i>Myopa rubida</i>	5	0.3%
	Syrphidae	<i>Eupeodes fumipennis</i>	7	0.4%
Hymenoptera	Andrenidae	<i>Andrena auricoma</i>	101	6.0%
	Apidae	<i>Apis mellifera</i>	24	1.4%
		<i>Bombus vosnesenskii</i>	11	0.6%
		<i>Xylocopa tabaniformis</i>	1	0.1%
	Halictidae	<i>Lasioglossum (Dialictus) spp.</i>	168	10.0%
Megachilidae	<i>Osmia densa</i>	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	<i>Andrena auricoma</i>	<i>Apis mellifera</i>	<i>Bombus vosnesenskii</i>	<i>Eulonchus tristis</i>	<i>Dialictus</i> spp.	<i>Osmia densa</i>	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, $p = 0.006$).

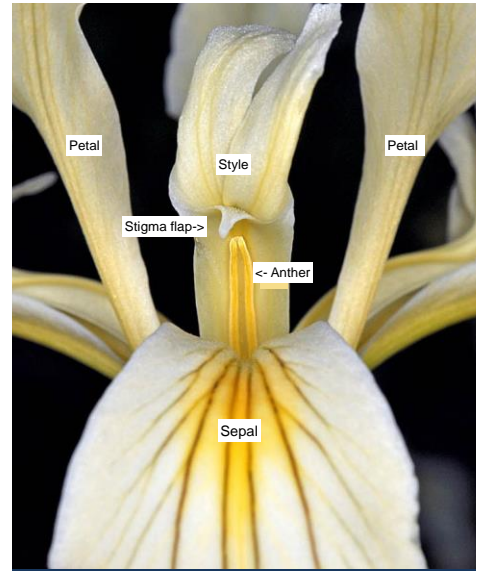
Taxon	N	Count	Female phase	Stigma contact	Visitation Rates		Behavior Proportions				
					Mean	SD	Pollen robbing	Nectar robbing	Sepal entry	Land	Touch
<i>Apis mellifera</i>	24	3	0.79	0.26	0.03	0.17	0.04	0.21	0.21	0.38	0.15
<i>Bombus vosnesenskii</i>	11	13	0.82	0.22	0.01	0.15	0.00	0.36	0.18	0.18	0.27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

[THIS PAGE INTENTIONALLY LEFT BLANK]



a)



b)

Figure 1. Iris flower morphology. a) Each flower consists of three functional units (floral units). b) Detail of one of these units.

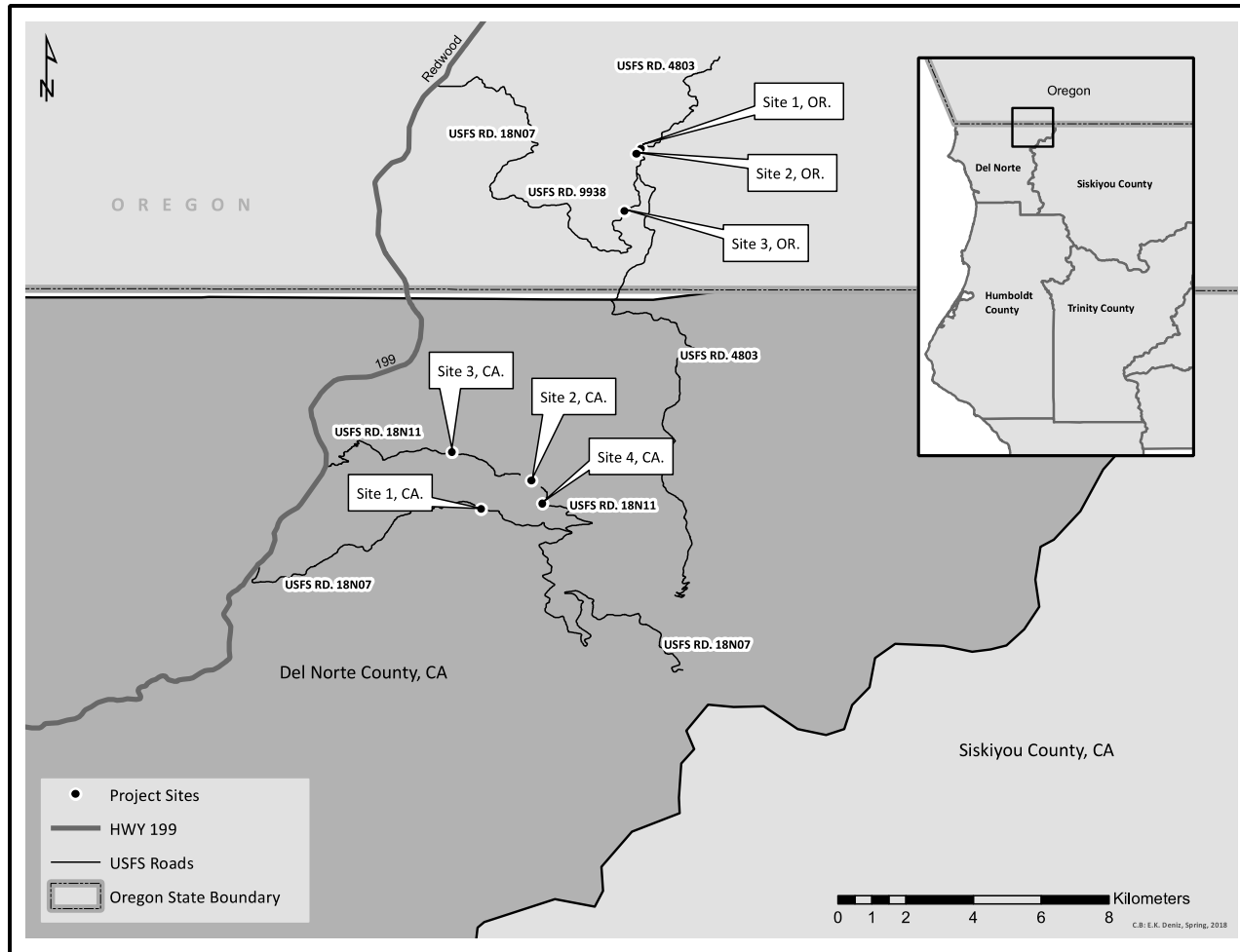


Figure 2. Study site locations in Del Norte County, CA (4 sites) and Josephine County, OR (3 sites).

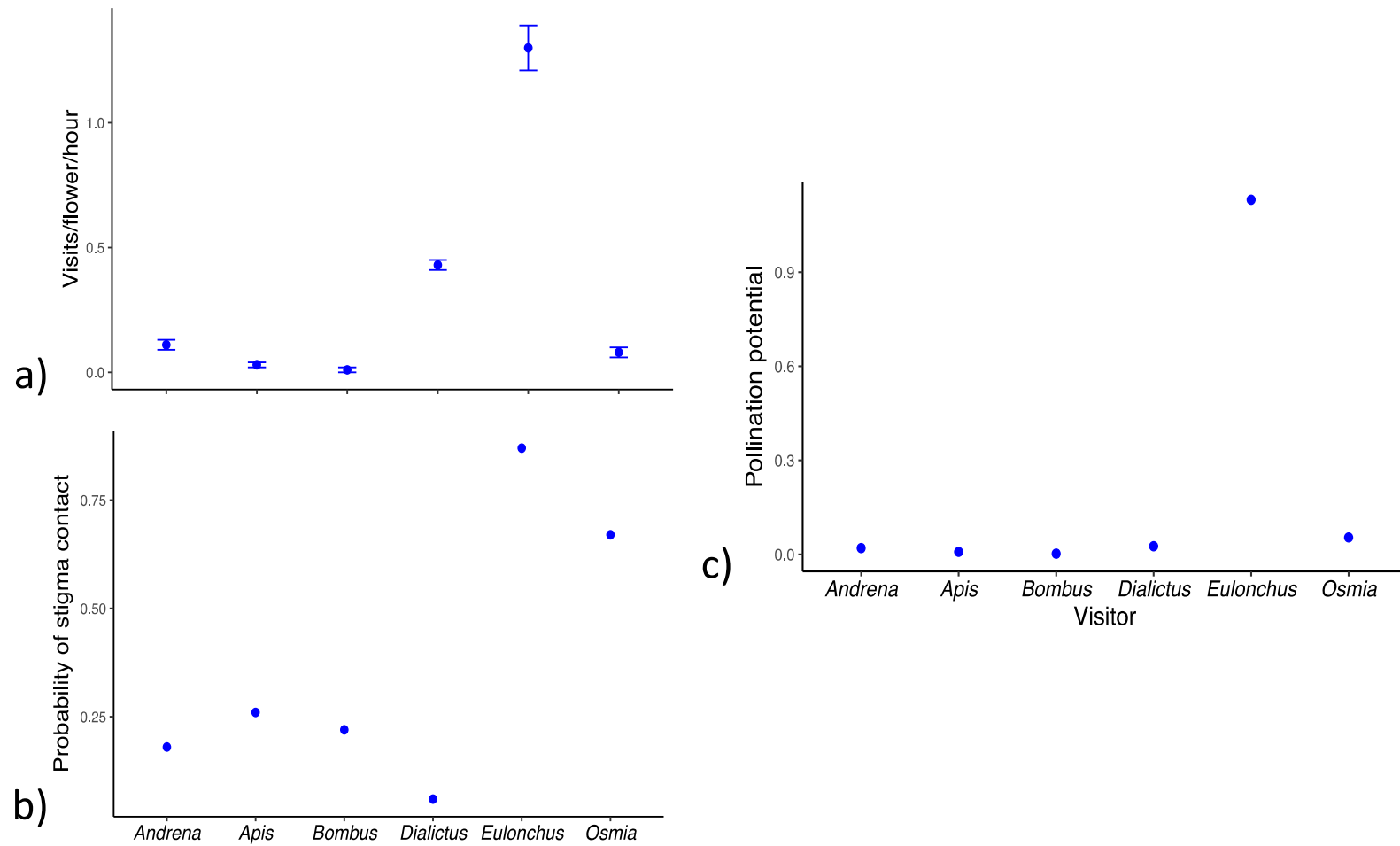


Figure 3. Comparison of performance by visitors to *Iris bracteata* flowers. a) Mean visitation rates and standard errors. b) Probability of stigma contact during visits to female phase flowers. c) Pollination potential (visitation rate x probability of stigma contact).

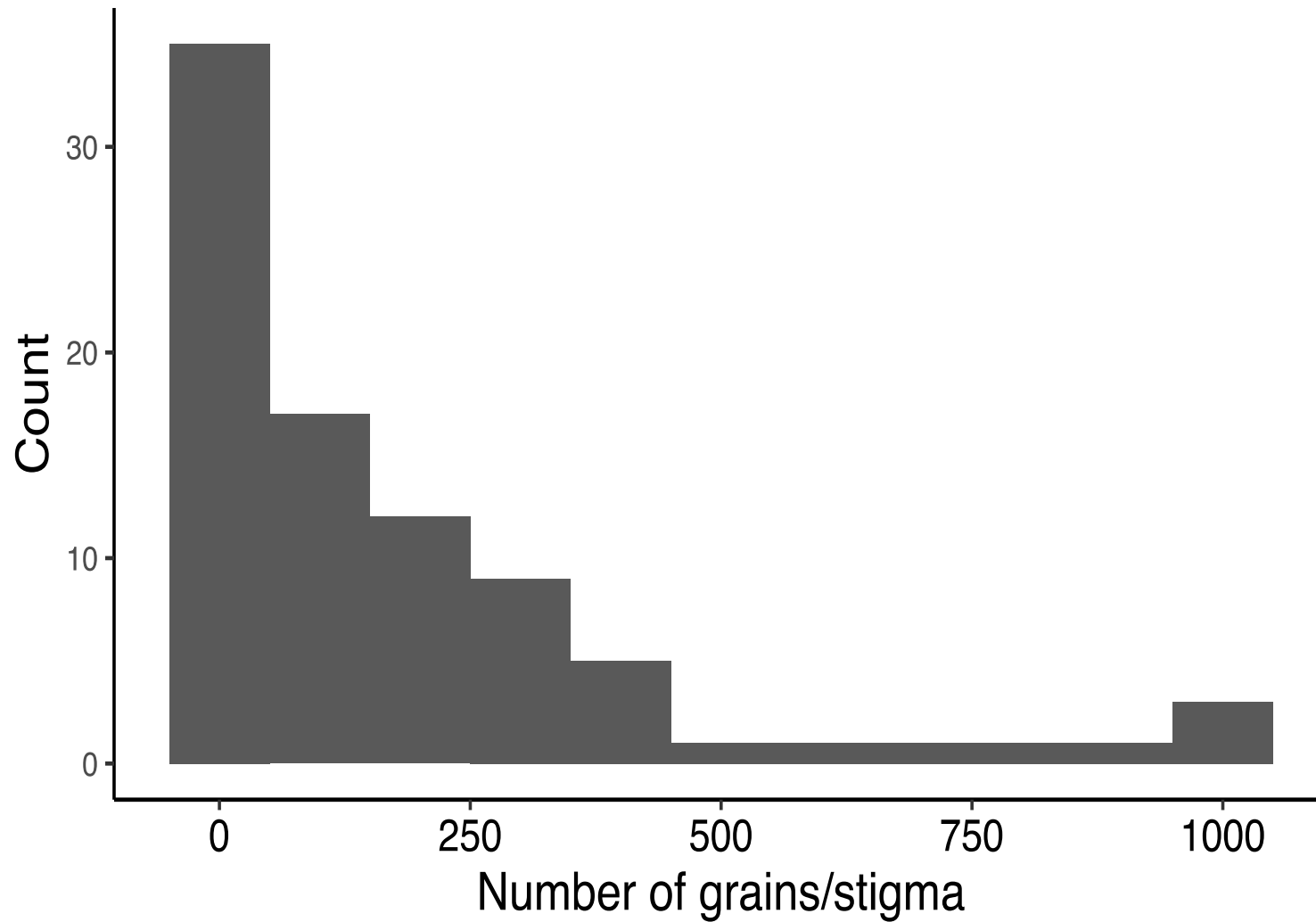


Figure 4. Number of grains deposited on virgin stigmas by *Eulonchus tristis* during a single visit ($n=86$ stigmas). All stigmas received at least one pollen grain.

APPENDICIES

[THIS PAGE INTENTIONALLY LEFT BLANK]

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.

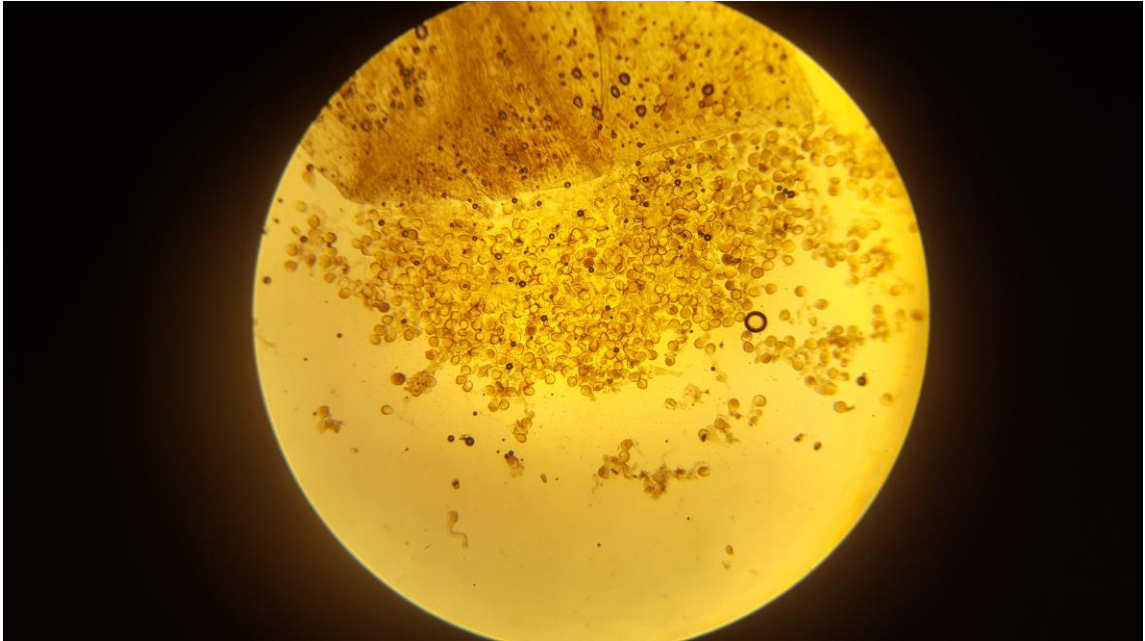
Study Site, Location, Date, & Time	No. of Visits	<i>Eulonchus</i>	<i>Bombus</i>	<i>Andrena</i>	<i>Apis</i>	<i>Dialictus</i>	<i>Osmia</i>	<i>Syrphid</i>	<i>Other</i>
Site 1 – CA – (5/24) 5:00 - 6:43 p.m.	67	1.28 (1.6)	0	0	0	0.08 (0.002)	0.22 (0.02)	0.02 (0.0004)	0
Site 1 – CA – (5/28) 10:05 - 12:11 p.m.	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	<i>Eulonchus</i>	<i>Bombus</i>	<i>Andrena</i>	<i>Apis</i>	<i>Dialictus</i>	<i>Osmia</i>	<i>Syrphid</i>	<i>Other</i>
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., HO CR = *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, HO CR, 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*

