GENERALIZED VERSUS SPECIALIZED POLLINATION SYSTEMS IN *OENOTHERA* (ONAGRACEAE)

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Abstract— Although generalized and specialized plants are often discussed as alternative states, the biological reality may better be viewed as a continuum. However, estimations of pollinator specificity have been confounded in some studies by the assumption that all floral visitors are pollinators. Failure to account for pollen load can lead to inaccurate conclusions regarding the number of pollinators with which a species actually interacts. The aim of this study was to clarify the distribution of pollination-system specialization within one clade, using a more rigorous assessment of pollen flow. The genus *Oenothera* has long been used as a model system for studying reproductive biology, and it provides a diversity of pollination systems and a wealth of historical data. Both floral visitation rate and pollen-load analysis of sampled pollinators, combined into a metric of pollen flow, were used to quantify the pollination systems of 26 *Oenothera* taxa. Metric of pollinator specialization were calculated as functions of both total pollinator taxa, and as pollinator functional groups. We found that for *Oenothera*, the number of floral visitors highly overestimates the number of pollinators, and is inadequate for determining or predicting pollination system specialization. We found that that pollination systems were distributed on a gradient from generalized to specialized, with more pollinator-specialized plant taxa, especially when estimated using pollinator functional groups. These results are in conflict with previous studies that depict most plant species as generalists, and this finding may be related to how prior studies have estimated specialization.

Keywords: Pollination systems; pollinator specialization; pollinator functional groups; Oenothera

INTRODUCTION

The rapid diversification and apparent specialization of the angiosperms in the early Cretaceous is traditionally explained by the co-evolution of plants with their insect pollinators (Crane et al. 1995; Grimaldi 1999; De Bodt et al. 2005; Solds et al. 2008; Soltis et al. 2008). Studies in the latter half of the twentieth century tended focused on the tightly coupled relationships of a plant and its pollinators (Grant & Grant 1965; Faegri & Pijl 1966; Stebbins 1970) and depicted these interactions as highly specialized, meaning that a given plant species relied on a small number of pollinator species. However, beginning in the last decade of the twentieth century, pollination biology research started challenging these traditional ideas and debating the specialization of pollination systems (Ollerton 1996; Waser et al. 1996; Johnson & Steiner 2000; Bascompte et al. 2003; Fenster et al. 2004; Sahli & Conner 2006; Tripp & Manos 2008; Mitchell et al. 2009; Ollerton et al. 2009). This debate continues unabated, and often revolves on how one measures the efficacy of flower visitors (Padyšáková et al. 2013). Although pollination generalists and specialists are often discussed as alternative states, the biological reality may be better viewed as a continuum of generalization to specialization (Johnson & Steiner 2000).

A major impediment to understanding the apparent paradox of specialized plants with generalized pollination systems is the lack of a standardized method for measuring pollination system specialization (Ne'eman et al. 2010). Traditionally, one counted the number of pollinator taxa visiting a plant species (Waser et al. 1996). This method may be misleading in the case of a "generalist" plant species that is visited by multiple pollinator species, if all of the pollinators belong to a functional group defined by a single morphology or foraging behaviour. The use of pollinator functional groups, which are defined as multiple taxa that share features (such as body size or tongue length) that determine their functionality as pollinators, provides a more accurate characterization of a plant's pollination biology (Fenster et al. 2004) and can drastically alter the perceived degree of specialization. For instance, Waser et al. (1996) analyzed Robertson's (Robertson 1928) pollinator survey and reported that 91% of 375 native plants in Illinois were visited by more than one insect species and therefore were generalists. However, Waser et al. (1996) did not differentiate between insects that were visitors and insects that were pollinators. Reanalysis of the same data indicated that when the insects were grouped into functional groups and non-pollinating visitors were excluded, 75% of the flowering plants only used one pollinator type and could therefore be considered specialized by that criterion (Fenster et al. 2004).

Calculating the degree of pollination specialization based solely on visitation, meaning the animals that land on the

flower, can also be misleading because not all flower visitors are pollinators. A plant may be visited by dozens of potential pollinators, but critical pollen transfer may be accomplished by few pollinators (King et al. 2013). In addition, a frequent visitor may carry a small pollen load, while a less frequent visitor may carry a large pollen load (Mayfield et al. 2001). In *Oenothera cinerea* (Onagraceae), when both visitation and pollen load were examined, Clinebell et al. (2004) found a high degree of specialization to a few major pollinators: of 45 species of floral visitor, only 5 carried major pollen loads, and 32 carried little or no pollen. However, relatively few recent studies evaluate pollination based on both visitation and pollen load (Armbruster 1985; Armbruster et al. 1989; Clinebell et al. 2004; Forup et al. 2008; Reynolds & Fenster 2008; Bosch et al. 2009; Reynolds et al. 2009; Jacobs et al. 2010; Popic et al. 2013), and failure to account for pollen load can lead to inaccurate assumptions regarding the number of pollinators with which a plant species actually interacts.

Furthermore, many angiosperm traits, including pollination system, are shared due to common ancestry, and results from comparative studies can be biased by phylogenetic constraint and niche conservatism (Sanderson & Donoghue 1996; Sakai et al. 1997; Freckleton 2000; Vamosi et al. 2003; Machado & Lopes 2004). A well-resolved phylogeny can provide a framework for comparing pollination systems while controlling for shared evolutionary history (Armbruster 1992; Nosil & Mooers 2005). Furthermore, performing an analysis of pollination system across one sub-generic clade will help shed light on the degree of evolutionary lability of pollinator specialization.

Onagraceae, specifically the genus *Oenothera*, has long served as a model system for the evolution of flowering plant reproductive biology (Raven 1979; Raven 1988; Hoch et al. 1993; Clinebell et al. 2004). The diversity of pollination systems within *Oenothera* make it ideal for testing hypotheses of pollination system specialization. Recent molecular phylogenetic studies have clarified phylogenetic relationships within *Oenothera* (Levin et al. 2003; Hoggard et al. 2004; Levin et al. 2004; Wagner et al. 2007; Wagner et al. 2013), notably, the once segregate genera *Gaura, Calylophus* and *Stenisiphon* now appear within a monophyletic *Oenothera* (Raven & Gregory 1972; Raven 1988). We focused on 26 species in sections *Kneiffia, Megapterium, Peniophyllum, Paradoxus* and *Gaura,* hereafter referred to as the "*Gaura* clade." The 26 species of the *Gaura* clade are widely-distributed in North America and Mexico (Raven & Gregory 1972; Straley 1977; Raven 1979), and they exhibit a broad array of floral form, both diurnal and nocturnal flowering, and diverse pollinators, including noctuid moths, antlions, bees, flies, wasps, butterflies, and hawkmoths (Raven & Gregory 1972; Straley 1977; Raven 1979; Nonnenmacher 1999; Moody-Weis & Heywood 2001; Clinebell et al. 2004). The *Gaura* clade provides a system in which we can make a more rigorous assessment of *Oenothera* pollination systems to clarify the degree of specialization while controlling for similarity due to shared ancestry.

The aim of this study is to examine the pollination systems of 26 species of taxa in the *Gaura* clade of *Oenothera*, and identify pollination specialization within the clade. We use these 26 focal species to test the hypothesis that insect visitation rate is sufficient to characterize pollination system specialization. We also examine the distribution of the pollination system of these *Oenothera* to explore the hypothesis that most flower species have generalized pollination systems. We predict that defining pollination systems using pollinator functional groups will result in a distribution that shows that most pollination systems for these *Oenothera* are specialized, and that functional groups will be informative about which pollinator group interacts with the plant most often. To place our findings and metric in the broader literature, we also review recent pollination literature in order gain a broad view of how pollination specialization is measured. We determine if they use visitation rate alone as a measurement of pollination, or visitation and a secondary assessment such as stigma contact, pollen load transferred, etc.

MATERIALS AND METHODS

Characterization of pollination system in contemporary published studies

Using the Boolean search terms "pollination AND ecology", we searched in Web of Science (Thompson Reuters 2010) for all publications from 2004 to 2012. We examined the research objectives of 978 records and found that 326 of these records measured pollination systems as part of their study (Tab. S1). For these 326 records, we read the methods and determined whether the number of animal visitors alone was used to characterize the pollination systems, or whether both visitation and pollen load were used to determine the pollination system.

Study system and field sites

We studied 26 species of *Oenothera*, all within the *Gaura* clade, in sites throughout the Northeast, Midwest, and Southwest of the United States. Fieldwork was conducted from April 2007 to August 2010. Our survey data were combined with archived data and captured pollinators stored at the Missouri Botanical Garden, especially data collected between 1999 and 2004 by R. Clinebell from sites in the Midwest United States and Mexico, and data from P. Raven and D. Gregory between 1964 and 1966 from Texas and Mexico. Each of the 26 species of Oenothera had between one and three study sites, for a total of 44 sites spread over 11 U.S. states and 3 Mexican states (Tab. S2). For each population, we conducted pollination observations and collected insects for later pollen load analyses. Vouchers of the *Oenothera* species were collected from each site and deposited with the Missouri Botanical Garden herbarium.

Measuring Pollination

Pollination system was determined based on both visitation rates and pollen-load analysis. For each population of *Oenothera,* we conducted a series of 20 min observation periods at multiple randomly-chosen inflorescences,

recording the total number of visits, type of visitor, and behaviour of visitors, including physical contact between an insect and stigma. Observations were conducted on four occasions during each species' flowering season, and took place at peak pollinator activity times of the day or night. For species whose flowers remained viable across both day and night periods, a series of observations were carried out for both night and day. We recorded between 63 and 1110 (median: 231) observations per species (Tab. 1). A sample of insect visitors was collected after observation periods to determine average pollen load. Insects were collected only after observed stigma contact to maximize the probability of collecting true pollinators. The insect visitors to the flower were collected using a net and a killing jar charged with ethyl acetate vapor, and pinned for later pollen analysis. The sampling of the insects included at least five collections per species.

To assess the identity and number of pollen grains carried by each visitor to an *Oenothera* species, we made a library of pollen grains from flowering plants at each study site. Dehiscent stamens were placed on labeled glass slides and the pollen was teased out with probes. Pollen was then stained with 1-2 drops of Calbera's fluid to make a semi-permanent mount (Goldblatt et al. 1998; Bernhardt et al. 2003). In the lab, each insect collected on the *Oenothera* species was placed on a separate glass slide and washed with a few drops of 70% ethanol. Any remaining pollen was scraped off the insect, including the scopal loads in the bees. The insect specimen was removed from the slide and the slide was allowed to air dry. Washed insect specimens were then dried, pinned, and saved for identification by regional entomologists. The pollen on the slide was stained with 1-2 drops of Calbera's fluid (Goldblatt et al. 1998) and mounted with a cover slip. Slides were then viewed with a light microscope to count pollen grains and compare to the pollen library for identification. Foreign pollen was identified and counted as well; however, most loads were homogenous. Data on the location, amount, and type of pollen were all recorded; however, only the load from the contact area of the insect with the stigma was used for these analyses.

Earlier collections of insect visitors collected on various *Oenothera* species are stored at the Missouri Botanical Garden. These include collections by R. Clinebell, P. Raven, and D. Gregory. We conducted the pollen-load analysis on these insect collections. Visitation rates for these visitors are found in records kept at the Missouri Botanical Garden (unpublished data).

Quantifying specialization: "S-score" and "F-score"

In quantifying specialization, we focused on the movement of pollen, and therefore the male reproductive success of the plant. Pollinators were characterized by their rate of visitation and pollen carrying efficiency. The degree of pollinator specialization of a plant species, which we have termed the S-score, is defined as the minimum number of pollinator taxa that account for 95% of the *Oenothera* pollen flow. Pollen flow is a measure to correct for the disparity between frequency and efficacy of pollinators. To calculate pollen flow, we multiplied the visitation rate

(visits/inflorescence/20min) by the pollen load (number of *Oenothera* pollen grains carried by an animal visitor) for each visitor species, and then summed this across visitor species:

$$\text{Pollen Flow} = \sum (\text{Visitation Rates}_{px} * \text{PollenLoads}_{px})$$

We then determined the number of visiting species that accounted for 95% of the total pollen flow, and designated that as the "S-score" for those specific *Oenothera* taxa. We also measured generalization by placing the visitors into functional groups based on family and size (Tab. 1). For example, all noctuid moths of a similar size that visited during the same time period were considered as one functional group. We then determined the number of functional groups that accounted for 95% of the total pollen flow and designated that as the "F-Score". We designated 95% of the total pollen flow as our metric in order to capture the broadest set of pollinators that are moving pollen. It is also the 95% probability that pollen will be carried by a member of the designated pollinators/functional group members.

Statistical Analyses

To test whether visitation alone was sufficient to characterize pollination systems for a plant taxon, we compared the total number of all floral visitors with the number of pollinators, defined as those visitors that carried the plant species pollen and made stigma contact. To assess if visitors were significantly more numerous than pollinators, we used a Wilcoxon signed rank test, as well as a paired t-test after log transformation for normality. A Wilcoxon signed rank test was also used to test if F-scores were significantly lower than S-scores. A linear regression between visitors and pollinators was calculated using log-transformed data. This linear model, after diagnostic checks for patterns and normality in fitted values and residuals, was used to create a prediction interval for S-score based on number of visitors.

RESULTS

Characterization of pollination systems in contemporary published studies

Of the 326 records examined, 66.6% used only observed visitation rates of insects or birds to plants as a method to characterize pollination (Tab. SI). The remaining 33.4% used various techniques of measuring pollen load, flow, or deposition. There was no trend of increasing reliance on secondary pollen measurement over time.

Pollination System

Of the 26 *Oenothera* species examined, *O. curtiflora*, *O. sessilis*, and *O. simulans* were completely autogamous. *Oenothera simulans* and *O. sessilis* had visitors, but none that carried any pollen and contacted a stigma. *Oenothera macrocarpa*, *O. nealleyi*, *O. filiformis*, *O. dodgeniana*, and *O. gaura* all used both night and day pollinators. *Oenothera linifolia*, *O. pilosella*, *O. perennis*, *O. riparia*, *O. glaucifolia*, *O. demareei* and *O. lindheimeri* were all day pollinated. *Oenothera patriciae*, *O. triangulata*, *O. xenogaura*, *O.*

Table 1. Species measured, number of flowers observed for 20-minute periods, number of insects with pollen loads counted, and functional groups identified per species.

Species name	Species code	Flowers observed	Pollen loads counted	Functional groups
O. anomala	A	176	71	Hawk moth; Antlion
O. arida	B	63	25	Noctuid moth
O. cinerea ssp. *cinerea*	C	904	330	Noctuid moth; Halictid bee; Bumble bee
O. dodgeniana	D	308	66	Halictid bee; Noctuid moth
O. curtiflora	E	400	0	Autogamous
O. demareei	F	311	132	Honeybee; Bumble bee
O. filiformis	G	1110	212	Halictid bee; Noctuid moth
O. gaura	H	241	126	Noctuid moth; Fly; Wasp; Halictid bee
O. glaucifolia	I	228	182	Halictid bee; Wasp; Fly; Syrphid
O. harvardii	J	125	30	Hawk moth
O. hexandra	K	236	108	Noctuid moth; Honeybee; Fly
O. lindheimeri	L	180	72	Honeybee; Wasp
O. linifolia	M	152	52	Fly; Halictid bee
O. macrocarpa ssp. *macrocarpa*	N	331	155	Noctuid moth; Hawk moth; Halictid bee; Wasp; Honeybee
O. patriciae	O	228	29	Noctuid moth
O. perennis	P	236	56	Halictid bee; Honeybee; Bumble bee
O. pilosella ssp. *pilosella*	Q	185	69	Honeybee; Halictid bee
O. riparia	R	285	41	Halictid bee; Honeybee; Bumble bee
O. sessilis	S	137	0	Autogamous
O. simulans	T	290	19	Autogamous
O. sinuosa	U	330	50	Noctuid moth
O. suffrutescens	V	373	100	Noctuid moth; Honeybee; Halictid bee; Bumble bee
O. nealleyi	W	296	46	Noctuid moth; Halictid bee
O. suffulta	X	133	109	Noctuid moth
O. triangulata	Y	155	19	Noctuid moth
O. xenogaura	Z	349	41	Noctuid moth

suffulta, *O. sinuosa*, *O. cinerea* ssp. *cinerea*, *O. hexandra*, *O. havardii*, *O. arida*, *O. anomala*, and *O. suffrutescens* were all exclusively night pollinated. The main functional groups for all 26 species are listed in Tab. 1.

Visitation versus Pollination

For 3 of the *Oenothera* species, the number of visitors equalled the number of pollinators. In all other species, visitation alone was not sufficient to characterize accurately the pollination system (Fig. 1). There was a significant difference between visitation and S-score using both non-parametric (Wilcoxon V = 253, $P < 0.0001$, two-tailed), and transformed parametric ($t_{25} = 7.382$, $P < 0.0001$) analyses. There was also a significant difference in how specialized the pollination systems are when calculated using taxa (S-score) versus when using functional groups (F-score) (Wilcoxon V = 120, $P = 0.0006$). There was a significant relationship between number of visitors and S-score for *Oenothera* plants (S-score = Visitors0.55, $F_{1,24} = 48.13$, $P < 0.0001$). However, number of total visitors could only explain a moderate amount of the variation in S-score ($R^2 = 0.67$), and this scatter results in a very wide prediction interval (Fig. 1). The majority of non-autogamous species (56.5%) were found to have between 1 and 3 pollinators, suggesting more specialization than generalization in this system (Fig. 2). This number increases to 82.6% when pollinators are considered as functional groups, while the majority (65.2%) only required the services of 1 or 2 functional groups to achieve pollen flow (Fig. 3).
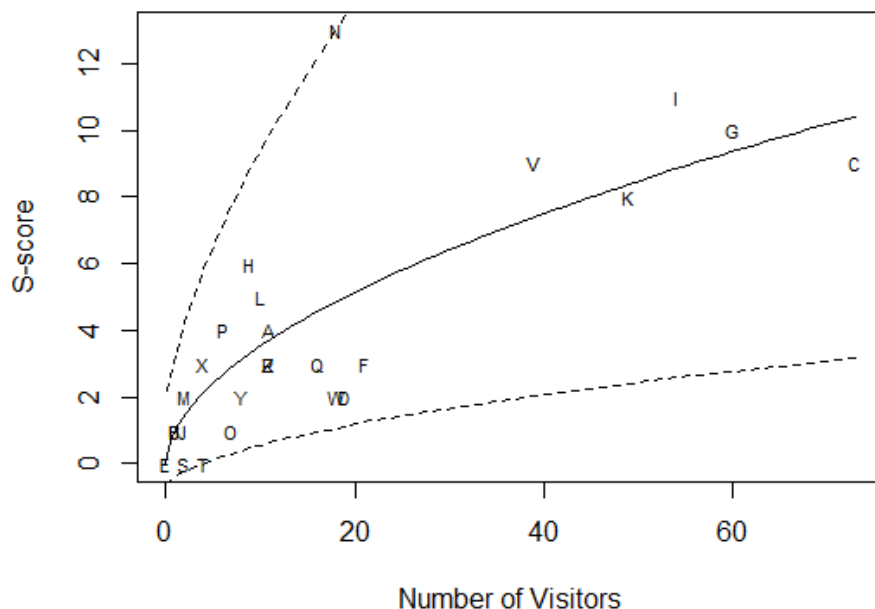
FIGURE 1. Scatterplot of S-score versus number of visitors for each of the 26 *Oenothera* taxa measured. The solid line is a linear regression of log-transformed visitor count versus S-score. The dotted lines demarcate the 95% prediction interval for S-score given a novel visitor count. For species names, see the species codes in Tab. I. For an extended definition and calculation of S-score (pollinator specialization score), see text.
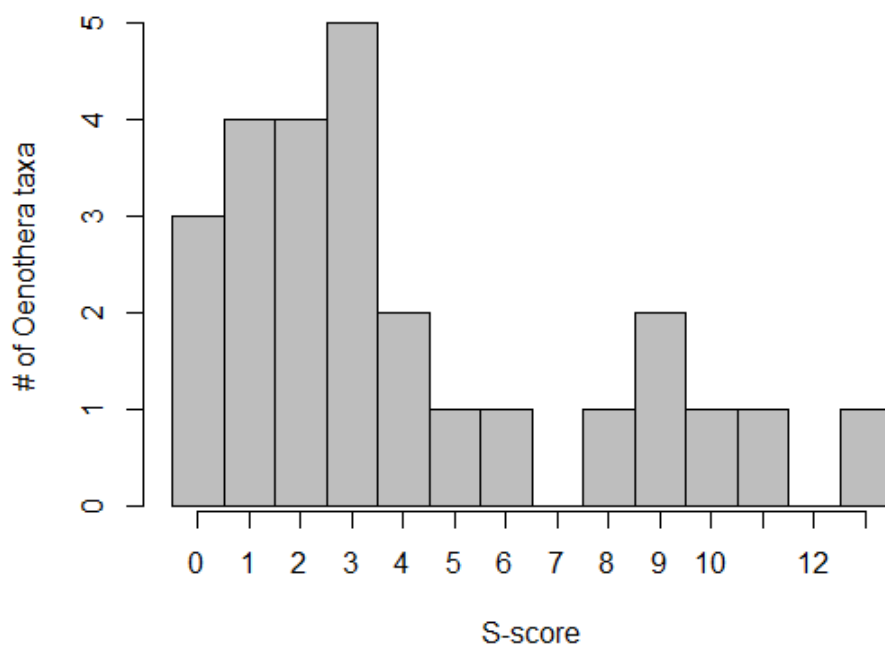

FIGURE 2. Histogram of the number of 26 *Oenothera* taxa in this study with a particular S-score (the number of pollinating species responsible for 95% of the pollen flow).

DISCUSSION

The pollination systems of *Oenothera* have been studied for several decades and serve as a model system for studying plant reproduction (Raven & Gregory 1972; Raven 1979; Raven 1988; Wagner et al. 2007). This study has furthered our understanding of *Oenothera* as a model system for studying pollination biology and provided insight into how specialization of pollination systems is measured. We examined the pollination systems of 26 taxa of *Oenothera* and focused on how to most accurately define the degree of specialization of these pollination systems. We found that these species attract a wide range of major pollinator groups including fly, bee, moth, hawk moth, wasp, and antlion.

These species cover a broad range of pollination system types, both in temporal and spatial variation, and are a good representation of North American pollination. However, most taxa studied had large disparities between number of visiting insect species and number of important contributors to pollen flow, and this is likely to have implication for selection on floral traits. In contrast to Waser et al. (1996), we find that *Oenothera* pollination systems, as representative of North American pollination systems, are more specialized than generalized, similar to findings by Fenster et al. (2004).

It has been suggested that visitation is still an accurate way to measure specialization of pollination systems because the number of visitors is proportional to the number of actual pollinators (Cayenne Engel & Irwin 2003), and so one
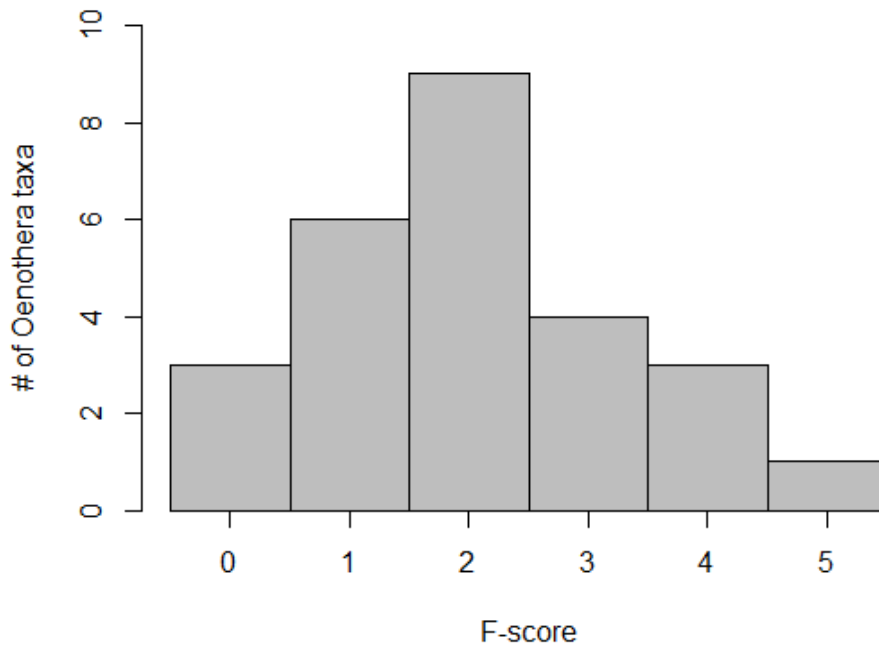
could make relative comparisons between plant species based on just visitation. Of the 23 non-autogamous *Oenothera* species we studied, for only 3 of those species did the numbers of visitors equal the number of pollinators, while in the other 20 species, the number of actual pollinators was lower than the number of visitors. We found that while visitors were proportional to pollinators on a log scale, the high variation in visitors for a given S-score results in a very wide prediction interval (Fig. 1). Therefore, visitation rate is a very blunt instrument, and is not reliable for predicting degree of pollinator specialization (as inferred from pollen flow). For example, species with an S-score of 1 can have between 1 and 7 visitor species, while an S-score of 2 is associated with between 2 and 19 visiting insect species, and this variability increases with S-score. Visiting species richness explained approximately 67% of the variance in S-score; these results are similar to the correlations found by Salhi & Connor (2006). We suggest that not only does visitation highly over-estimate the number of pollinators, but it is also not a sufficiently accurate measurement of specialization of pollination systems in a proportional or comparative way either.

Pollination systems, in particular their degree of generalization/specialization, are most often measured as the number of taxa involved in the plant-pollinator interaction. However, the use of functional groups, in which the visiting taxa are grouped by some morphological characteristic that defines how pollen load is delivered to a plant species, is perhaps a more informative way to examine pollination systems. This may be especially important for studies that examine pollination syndromes, given that pollination syndromes are predicting the selective agent on a floral trait. One definition is that a pollination system is considered specialized when a single functional group is responsible for greater than 75% of the pollination visits (Fenster et al. 2004). In this study, when we grouped the insect visitors by major taxon groups and size, we found that the *Oenothera*

pollination systems were more specialist than generalist. By the definition of Fenster et al. (2004), 17 of the *Oenothera* species have specialized pollination systems. Of the remaining species, 8 used only 2 functional groups of pollinators to reach 75% of the pollination visits, and only one species, *O. gaura*, uses 3 functional groups.

We decided to measure pollination specialization in a way that would show the continuous nature of pollination systems. The F-score calculated was the number of functional groups responsible for 95% of pollen flow, and thus is somewhat more stringent than the metric of Fenster et al. (2004). The majority of the *Oenothera* species were toward the specialist end of the continuum, i.e. having a functional group score (F-score) of 1 or 2 (Fig. 3). The highest F-score was a 5, and only two species, *O. macrocarpa* and *O. glaucifolia*, had this score. While F-scores were statistically lower than S-scores, the number of functional groups was not just a proportionally smaller set of the pollinators; of the 26 *Oenothera* pollination systems we studied, 8 species had the same number of pollinating taxa as they did functional groups. Furthermore, some taxa do not contribute sufficiently to the pollen flow to be included in the S-score; however, when the taxa are grouped by functional groups, they can become a dominant contributor to the pollen flow. For example, *O. cinerea ssp. cinerea* is pollinated by several species of small halictid bees, bumble bees and noctuid moths. As individual species, the noctuid moths contribute little to total pollen flow, but when all 14 species of noctuid are considered one functional group, they collectively become the second most important group contributing to pollen flow. Overall, we found that the use of functional groups gives the most accurate representation of how specialized these *Oenothera* pollination systems are, with respect to morphological specialization to a specific type of pollinator.

One reason that pollination systems are often seen as generalized is because they are defined only using visitation rates of all potential pollinators to a plant. In our literature review, we found that the majority of pollination studies in a recent nine-year period used only visitation rate to characterize a pollination system. However, we found that visitation rate highly over-estimates the number of taxa pollinating a plant species. Insects visit flowers for a variety of reasons (Buchmann & Nabhan 1996). The flower may be a mating site or a source of food or shelter, and such visits may not involve anther or stigma contact.

This current study has several important strengths and limitations. The greatest strengths are in its number of species, their respective diversity of pollination systems, and broad geographic range, all phylogenetically controlled by studying members of one clade within one genus. This is compared to other recent studies which focus on few taxa (Adler & Irwin 2006; Fumero-Cabán & Meléndez-Ackerman 2007) or unrelated taxa (Bosch et al. 2009; King et al. 2013). However, this study focuses on pollen flow, and does not address pollen deposition as those studies do (Adler & Irwin 2006; Fumero-Cabán & Meléndez-Ackerman 2007; King et al. 2013). This is because we have utilized archived data on visitation rate and pollen flow to maximize our taxonomic coverage. By only considering visitors that make stigma contact, we partially account for this discrepancy. Furthermore, *Oenothera* species have low ovule count per flower, rendering voluminous pollen transfer unnecessary (Raven & Gregory 1972). However, as effective pollinators will be a subset of total pollinators, our measure is intrinsically conservative. Therefore, *Oenothera* plants may be even more specialized in pollination than we have ascertained.

For several of the species, we have multiple years of pollination data, but with such a broad study, this was not available with all the taxa. This is a second potential limitation of the study. We have sufficient data to gauge the stability of pollination systems between years for two species, *O. filiformis* and *O. macrocarpa*. We found that, although the taxa or functional group of pollinator differed, the total number of pollinator species or functional groups active in a single year did not change. This is in agreement with recent pollination network studies that show that, while the type of species interacting may change from year to year, the overall number of interactions tends to remain constant (Memmott et al. 2004; Petanidou et al. 2008). When looking at functional groups, a snapshot approach can be sufficient to characterize a pollination system in terms of how specialized it may be (Alarcon et al. 2008). Therefore, we made an informed assumption that a 'snapshot' approach, involving a single season of detailed pollination data, is sufficient for the broad scale comparison of this project.

One further difficulty in applying these results to other floral systems is that *Oenothera* pollen grains are large compared to other flowering species. Pollen size could be a trait that limits the number of pollinators that can manipulate and carry pollen. This could possibly filter out smaller visitors that would be pollinators if the pollen were smaller. Perhaps smaller pollen may encourage more generalized pollination systems. In addition, the viscine threads that hold together *Oenothera* pollen could potentially affect the size of pollen load carried by a pollinator. Future studies should look at a comparison of specialization of pollination systems between different floral systems.

Understanding the degree of specialization of pollination systems is important when making inferences about a plant's evolutionary history. If pollinators are a selective pressure that has led to such a diversity of floral form, then plant-pollinator interactions are expected to be highly specialized, and specialized pollination systems should be seen for a majority of flowering species (Ollerton 1996). Of course, pollinators are not the only factor in the adaptation of floral forms. For example, life history, breeding system, successional status, and abundance all play roles, but pollinators are considered a dominant influence in the evolution of floral specialization (Endress 1994; Crane et al. 1995; De Bodt et al. 2005; Soltis et al. 2008). How specialized a pollination system is also plays a critical role when making conservation decisions for plant species (Johnson & Steiner 2000; Biesmeijer et al. 2006; Winfree 2008; Ashworth et al. 2009; Bascompte 2009). Concluding that a plant has a generalized pollination system, when it is actually highly specialized, could lead to poor management decisions and result in a loss of plant diversity in the face of a changing climate or habitat loss. Therefore, this study may serve as an example of a more informative way to determine pollination system in future research.

ACKNOWLEDGEMENTS

APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I: References used for literature review on pollination study methodologies.

APPENDIX II: Table of field sites used in pollinator surveys.

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