

TESTING POLLINATION SYNDROMES IN *OENOTHERA* (ONAGRACEAE)

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Abstract—Pollinators are considered a major selective force in shaping the diversification of angiosperms. It has been hypothesized that convergent evolution of floral form has resulted in “pollination syndromes” - i.e. suites of floral traits that correspond to attraction of particular pollinator functional groups. Across the literature, the pollination syndrome concept has received mixed support. This may be due to studies using different methods to describe floral traits and/or the pollination syndrome concept being supported more often in species highly reliant on pollinators for reproduction. Here, we assess the predictive ability of pollination syndromes in *Oenothera*, a species rich clade with pollination systems existing on a gradient of specialization, and in which species are either self-compatible or self-incompatible. We ask the following questions: Do *Oenothera* species follow the pollination syndrome concept using traditional, categorical floral trait descriptions and/or quantitative floral trait measurements? And, are floral traits more predictive of primary pollinators in species with specialized pollination systems and/or species that are self-incompatible? Mapping floral traits of 54 *Oenothera* species into morphospace, we do not find support for the pollination syndrome concept using either categorical or quantitative floral trait descriptions. We do not find support for specialization or breeding system influencing the prediction of primary pollinators. However, we find pollination syndromes were more predictive in *Oenothera* species with moth pollination systems. Collectively, these results suggest that the pollination syndrome concept cannot be generally applied across taxa and that evolutionary history is important to consider when evaluating the relationship between floral form and contemporary pollinators.

Keywords: floral evolution, morphospace, mutualism, pollination syndrome, *Oenothera*, Onagraceae

INTRODUCTION

The convergent evolution of floral traits among distantly related angiosperm species is a phenomenon pollination ecologists have long sought to explain (Fenster et al. 2004; Schiestl & Johnson 2013; Stebbins 1970; van der Niet & Johnson 2012). Since the late 1800s, scholars have recognized pollinator-mediated selection as a force driving floral trait convergence and divergence; Darwin proposed that pollinators are the major selective agent for floral trait evolution (Darwin 1862) and myriad botanists have documented suites of floral traits that correspond to particular pollinator groups (e.g. Delpino 1874; Knuth 1898; Faegri and van der Pijl 1979; Rosas-Guerrero et al. 2014). Over time, this work led to formalization of the pollination syndrome concept (e.g. Faegri & van der Pijl 1979; Fenster et al. 2004; Ollerton et al. 2009; Rosas-Guerrero et al. 2014; Vogel 1954), where a pollination syndrome is a suite of floral traits (e.g. color, odor, morphology, reward) that evolved in response to and are associated with a specific pollinator group (i.e. pollination system) (Faegri & van der Pijl 1979). Since its formalization, the pollination syndrome concept has played a central role in plant-pollinator studies. Pollination syndromes have been used to help explain floral diversification and plant-pollinator interactions (e.g. Fenster et al. 2004; Stebbins

1970) and in 1979, Faegri and van der Pijl outlined 11 pollination syndromes that became standard in pollination biology studies and are hereafter referred to as the *traditional pollination syndromes* (Faegri & van der Pijl 1979). However, despite its centrality in pollination research, numerous scholars have questioned the validity of the pollination syndrome concept (e.g. Gong et al. 2015; Ollerton et al. 2009; Waser et al. 1996).

Throughout the literature, the pollination syndrome concept has been critiqued for numerous reasons (e.g. Gong et al. 2015; Ollerton et al. 2009; Waser et al. 1996). First, pollination syndromes are potentially too limited an explanation of the complex relationships between a plant and a visitor. There are multiple reasons a visitor might interact with a plant other than pollination, and these interactions can affect the evolution of floral traits (Ashman & Majetic 2006; Chittka et al. 1999; Knauer & Schiestl 2017; Lehtilä & Strauss 1999; Yang & Guo 2005). Second, inherent to the concept of pollination syndromes is the idea that most plant-pollinator interactions are highly specialized (Fenster 2004; Ollerton et al. 2009; Reynolds et al. 2009); however, plant-pollinator interactions exist on a gradient of specialization, with most plant-pollinator interactions appearing more generalized. Other factors such as the habitat and biogeographical history can impact where higher rates of plant-pollinator specialization are found (Mitchell et al. 2009; Waser & Ollerton 2006; Waser et al. 1996; Johnson & Steiner 2000; Fenster et al. 2004). The existence of highly generalized plant-pollinator interactions is contrary to the idea that suites of

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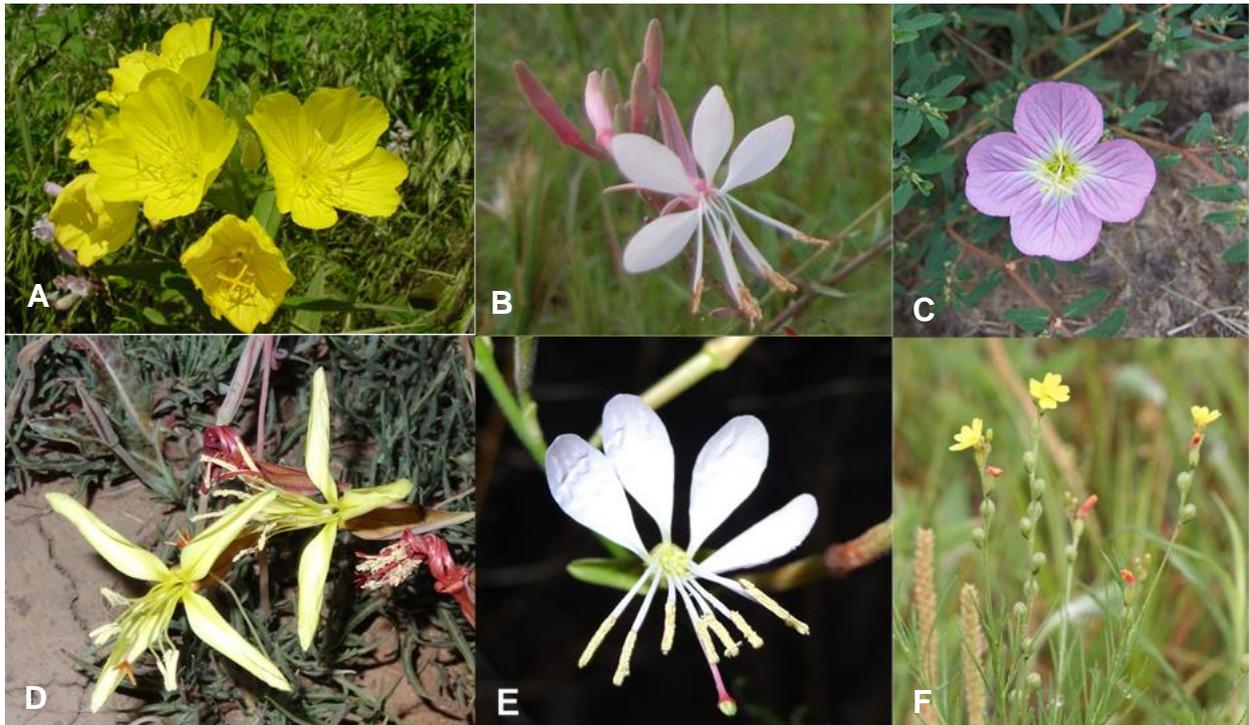


FIGURE 1. A visual overview of the floral trait diversity within Subclade B of *Oenothera*. A. *Oenothera pilosella* B. *Oenothera dodgeniana* C. *Oenothera speciosa* D. *Oenothera havardii* E. *Oenothera nealleyi* F. *Oenothera linifolia*. Photographs provided by K. N. Krakos.

floral traits attract limited types of pollinators. A final concern is that a discrepancy exists between studies that use categorical floral traits (e.g. the traditional syndromes of Faegri and van der Pijl 1979) and those that use quantitative floral traits (Smith 2010; Smith et al. 2008; Tripp & Manos 2008; Whittall & Hodges 2007) as a tool to infer a plant's pollinator. While quantitative measurements are more objective than categorical descriptions, and may have greater ability at identifying functional similarity across floral species (Abrahamczyk et al. 2017), there is a need to assess whether defining pollination syndromes using quantitative trait measurements leads to conclusions that are consistent with the conclusions that would be reached by using categorical trait descriptions.

Across the pollination biology literature, the predictive power of pollination syndromes has received mixed support (e.g. Danieli-Silva et al. 2012; Guzmán et al. 2017; Johnson & Wester 2017; Lázaro et al. 2008; Liu & Huang 2013; Mayfield et al. 2001; Quintero et al. 2017; Rodrigues et al. 2018). Many comparative studies have found support for the pollination syndrome concept (e.g. Abrahamczyk et al. 2017; Armbruster et al. 2011; Danieli-Silva et al. 2012; Fenster et al. 2015; Johnson 2013; Lagomarsino et al. 2017; Martín-Rodríguez et al. 2009; Murúa & Espíndola 2015; Reynolds et al. 2009; Wilson et al. 2004; Wolfe & Sowell 2006), while others have not supported the predictive power of pollination syndromes across taxa (e.g. Fishbein & Venable 1996; Kingston & McQuillan 2000; Li & Huang 2009; Maruyama et al. 2013; Ollerton et al. 2009; Prieto-Benítez et al. 2015; Valdivia & Niemeyer 2006; Waser et al. 1996). A potential reason for this mixed support may be that pollination syndromes are predictive in species highly dependent on pollinators for reproduction, but not in those species less

dependent on pollinators. For example, pollinator-mediated selection on floral traits may be stronger in self-incompatible (SI) species, than in self-compatible (SC) species, as autogamy can provide SC plants with reproductive assurance when pollinator reliability is low (Rosas-Guerrero et al. 2014; Moriera-Hernandez & Muchhala 2019; also see Anderson et al. 2010). As a result, floral traits may be more predictive of pollinators in SI species than in SC species (Rosas-Guerrero et al. 2014). Furthermore, as plants with high pollinator specialization (i.e. plant species that use one or a small number of pollinator functional groups) may have floral traits more closely adapted to their pollinator type than generalist species (i.e. plant species that utilize multiple pollinator functional groups), the pollination syndrome concept may be more consistently predictive in specialist compared to generalist species (Ashworth et al. 2015; Johnson 2013). Given the numerous factors that may affect the ability of floral traits to predict pollinators, more research on the predictive ability of the pollination syndrome concept is needed, especially in species rich clades with a diversity of pollination systems.

Onagraceae - the evening primrose family, one of the major plant radiations in western North America (Raven 1979; Raven & Gregory 1972; Straley 1977) - is an ideal group in which to test the predictive ability of pollination syndromes. The genus *Oenothera* is a model system for studying plant reproductive biology and floral evolution, with a wealth of literature describing *Oenothera* taxonomy and phylogenetics (Krakos 2011; Krakos et al. 2014; Krakos et al. accepted; Raven 1988; Raven & Gregory 1972; Wagner et al. 2007; Wagner et al. 2013). *Oenothera* is a species rich, monophyletic group, with a diversity of floral traits (e.g. Fig. 1) and a wide range of pollination systems (e.g. bee, bird, butterfly, wasp, moth, antlion, fly, beetle, and hawkmoth

pollination), despite ancestral *Oenothera* likely being primarily moth pollinated (Clinebell et al. 2004; Krakos & Fabricant 2014; Moody-Weiss & Heywood 2001; Nonnenmacher 1999; Raven 1979; Raven & Gregory 1972; Straley 1977; Wagner et al. 2007). Furthermore, *Oenothera* pollination systems exist on a gradient of specialization (KraKos & Fabricant 2014), and the breeding systems of *Oenothera* species have undergone multiple independent transitions from SI to SC (KraKos et al. 2014; Krakos et al. accepted; Raven & Gregory 1972; Wagner et al. 2007). Numerous studies have provided detailed empirical data on the pollination systems of *Oenothera* taxa that show diverse floral forms (Clinebell et al. 2004; Krakos & Fabricant 2014; Moody-Weiss & Heywood 2001; Nonnenmacher 1999; Raven 1979; Raven & Gregory 1972; Straley 1977), thus providing an opportunity to rigorously test the pollination syndrome concept comparatively across *Oenothera* species.

Here, we test the predictive ability of pollination syndromes by evaluating the correspondence between floral traits and pollinators in 54 *Oenothera* species. Toward this aim, we map *Oenothera* floral traits into morphospace (i.e. phenotype space) - a technique increasingly recognized as having applicability to angiosperm eco-evolutionary studies (Chartier et al. 2014) - and compare observed pollination data to pollinators predicted by the pollination syndrome concept. We ask the following questions:

1. Do *Oenothera* species fit the traditional, categorical pollination syndromes of Faegri and van der Pijl (1979)? We predict that *Oenothera* species will form groups that correspond to their main pollinators when using categorical floral trait measurements, consistent with the traditional pollination syndromes of Faegri and van der Pijl (1979).

2. When using quantitative floral trait measurements, do *Oenothera* species form groups that correspond to their main pollinators? We predict that *Oenothera* species will form groups that correspond to their main pollinators when using quantitative floral trait measurements.

3. Does pollinator specialization affect (a) how close *Oenothera* species are to traditional pollination syndromes in morphospace and (b) how accurately these traditional pollination syndromes predict primary pollinators? We predict greater pollinator specialization will be associated with *Oenothera* species falling closer to traditional pollination syndromes in morphospace and these syndromes accurately predicting primary pollinators more frequently.

4. Does breeding system affect (a) how close *Oenothera* species are to traditional pollination syndromes in morphospace and (b) how accurately these traditional pollination syndromes predict primary pollinators? We predict that SI *Oenothera* species will fall closer to traditional pollination syndromes in morphospace and that these syndromes will accurately predict primary pollinators more frequently, compared to SC *Oenothera* species.

Through asking these questions, this study helps elucidate the applicability of the pollination syndrome concept across taxa, by (i) assessing whether traditional, categorical floral trait descriptions and quantitative floral trait measurements similarly predict main pollinators in *Oenothera* and (ii) testing

whether certain ecological characteristics promote adherence to the traditional pollination syndrome concept in *Oenothera*. Determining the predictive ability of pollination syndromes across diverse taxa is essential for understanding the role of pollinators in driving floral trait convergence.

MATERIALS AND METHODS

Pollinator Data and Floral Traits

We used the 54 species of *Oenothera* in Subclade B (Levin et al. 2004) for this study. This clade has a diversity of floral forms and species that are characterized by multiple pollination systems. Krakos and Fabricant (2014) give detailed pollination data for 26 of these species - including visitation, pollen load, and stigma contact of visitors - that we use here. For the remainder of these species, the main pollinator group comes from published pollination studies (Clinebell et al. 2004; Moody-Weiss & Heywood 2001; Nonnenmacher 1999) and data on plant-pollinator associations at the Missouri Botanical Garden (RR Clinebell *unpublished data*, same methodology as Krakos & Fabricant 2014). Pollinators were determined using both visitation and pollen load data, and main pollinators were considered those that contributed to 95% of the total pollen flow. These pollinators were then grouped into functional groups of similar species and sizes following Fenster et al. (2004) and Krakos and Fabricant (2014), for use in the pollination syndrome analyses described below.

Question 1 - Traditional Pollination Syndromes of Faegri and van der Pijl

To evaluate the predictive power of Faegri and van der Pijl's (1979) traditional pollination syndromes, we first created a morphospace using the categorical floral traits of these syndromes. While Faegri and van der Pijl (1979) originally described 11 pollination syndromes, the traits for their hawkmoth and moth syndromes have been found to be indistinguishable (Ollerton et al. 2009). Accordingly, we combined Faegri and van der Pijl's (1979) hawkmoth and moth syndromes, and used the remaining 10 syndromes in our analyses: bat, bee, beetle, bird, butterfly, fly, hawkmoth/moth, carrion fly, small non-flying mammal, and wasp. The resultant matrix of these traditional pollination syndrome traits is a modified version of Ollerton et al. (2009), which gives different versions of each traditional pollination syndrome (e.g. bee 1, bee 2, etc.). This creates a broader, more realistic definition of each traditional pollination syndrome by capturing the variability of floral traits associated with a syndrome (e.g. a bee syndrome can have white or yellow flowers). Following Ollerton et al. (2009), we used a multiple trait vector approach of 537 vectors across 10 syndromes, with each trait scored as present (score of 1) or absent (score of 0). However, as several traits scored by Ollerton et al. (2009) are not applicable to *Oenothera*, we modified how the syndromes are characterized to include only the following nine traits, which are all applicable to *Oenothera*: color at anthesis (yellow, white, red, pink, green, purple, brown, blue, orange), scent (sweet, fruity, fresh, musty, sour, decay, none), flower shape (dish, bell/funnel, trumpet, tube), symmetry (actinomorphic, zygomorphic), orientation (pendant, upright,

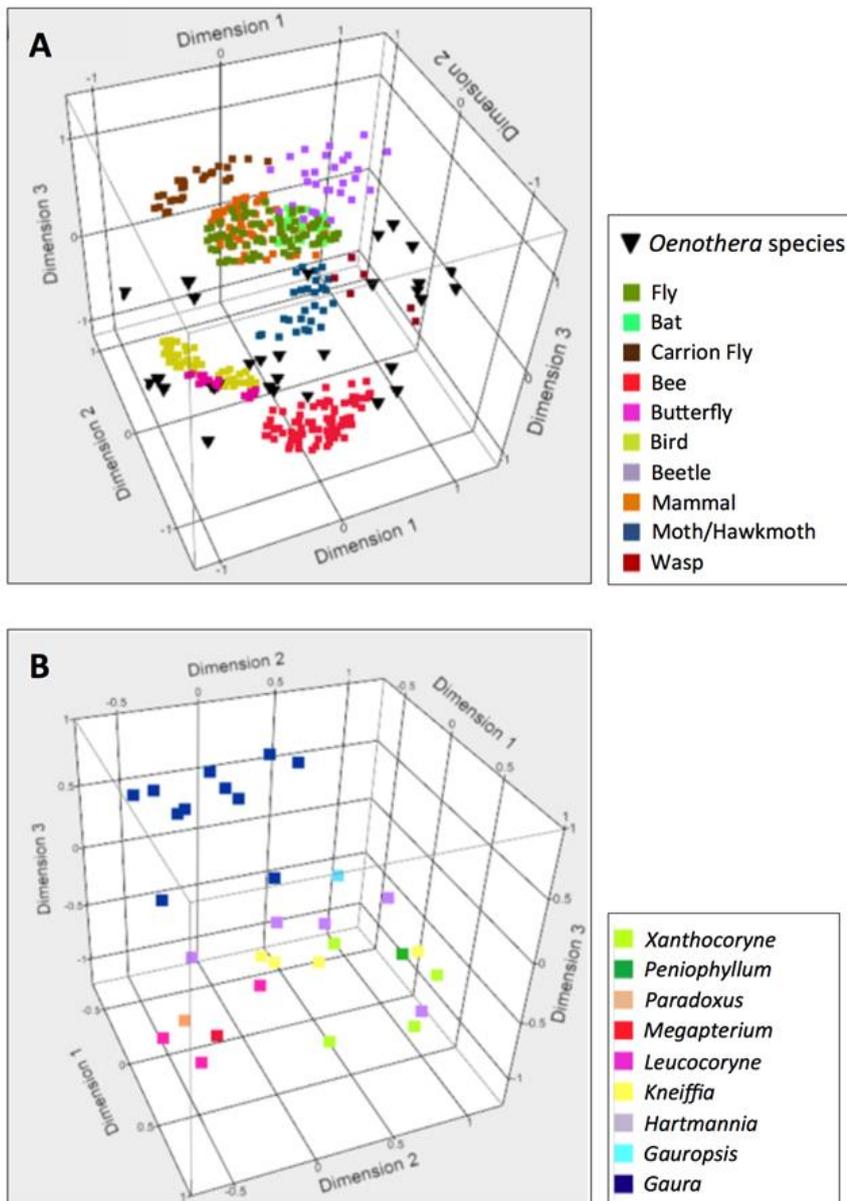


FIGURE 2. Non-metric multidimensional scaling (NMDS) ordination of *Oenothera* floral traits. (A) Rectangular points are the 537 traditional pollination syndromes, colored by pollination system; black triangular points are the 54 *Oenothera* species. (B) The 54 *Oenothera* species, colored by section, to depict phylogenetic clustering in morphospace. Plots are rotated to increase visibility of points.

horizontal), brightness (dull, vivid), anthesis time (day, night), nectar presence (present, absent), and nectar location (hidden, accessible) (see Supplementary Data Table 1 for full matrix). We then used these traditional pollination syndrome traits to score the 54 *Oenothera* species such that each species was described by a vector of 35 ones and zeroes (see Supplementary Data Table 2 for full matrix). These analyses were carried out in PC-ORD (McCune & Mefford 2006).

To determine whether the different vectors for each of the 10 traditional syndromes grouped into discrete groups, we used formal ordinations using non-metric multidimensional scaling (NMDS) (Ollerton et al. 2009). We used NMDS for this analysis because it is appropriate for binary data, makes no assumptions about the distribution of variables, and creates multivariate space in which similar objects are close to one another (McCune & Mefford 2006; McCune & Grace 2002). We used a Sorensen's index (Bray-Curtis) to express the distance relationships between the traditional pollination

syndromes described by the binary data set. NMDS was used to find the best dimensional representation of the distance matrix. The NMDS analyses started with 250 runs of real data, which were then compared with a Monte Carlo test with 250 ordinations of randomized data. Mean stress did not decline after three dimensions, and so a three-dimensional space was selected for the analyses (McCune & Grace 2002). We then ran the final solution and assessed the stability of this solution by examining a Scree plot (final stress versus the number of dimensions), and the final stability reported from the NMDS output. We assessed the final stress from the NMDS using Kruskal's stress formula and Clarke's rule of thumb (McCune & Grace 2002).

This ordination of the traditional pollination syndromes created a three-dimensional space with each traditional syndrome represented by a cluster of the multiple trait combinations (Fig. 2A). Using these results and the matrix that scored the floral traits of the 54 *Oenothera* species, we

used NMDS Scores algorithm in PC-ORD 5.14 to calculate co-ordinates for the *Oenothera* species in that morphospace. Then, we calculated the Euclidean distance for each species, which is the distance between each *Oenothera* species and the nearest traditional pollination syndrome. A shorter Euclidean distance indicates greater correspondence between an *Oenothera* species' floral traits and a traditional pollination syndrome. To determine how accurately the traditional pollination syndromes of Faegri and van der Pijl (1979) predict observed pollinators, we compared the predicted pollinators from these analyses to the primary pollinators observed in our ecological data (i.e. predicted pollinator does or does not match observed primary pollinator).

Question 2 - Pollination Syndromes Using Quantitative Floral Trait Measurements

It may be that quantitative morphological floral measurements are better predictors of pollinator type than Faegri and van der Pijl's (1979) traditional, categorical floral traits. To determine whether *Oenothera* species form groups based on quantitative floral traits that correspond to their main pollinators, we measured floral tube length, floral tube mouth width, corolla span, stamen length, and style length of individuals from each of the 54 *Oenothera* species. We took these measurements on 10-15 flowers per species, with each flower sampled from a different plant, and represented each trait per species as an average. For *O. deserticola*, *O. canescens*, *O. rosea*, *O. speciosa*, *O. texensis*, *O. epilobiifolia*, *O. multicaulis*, *O. seifrizii*, *O. dissecta*, *O. kunthiana*, *O. orizabae*, *O. tetraptera*, *O. brachycarpa*, *O. coryi*, *O. howardii*, *O. spachiana*, *O. anomala*, *O. boquillensis*, *O. cinerea ssp. parksii*, *O. filipes*, and *O. mckelveyae*, we used herbarium sheets from the Missouri Botanical Garden to make these measurements. All other measurements were taken from the plant populations used for pollinator data collection (KraKos & Fabricant 2014; KraKos et al. 2014) or with greenhouse populations. We tested whether *Oenothera* form quantitative floral trait groups using principle component analyses (PCA), a method commonly employed by studies that infer pollination syndromes using quantitative traits (e.g. Lagomarsino et al. 2017; Muchhala 2006; Murúa & Espindola 2015; Tripp & Manos 2008; Whittall & Hodges 2007). We log transformed our data and conducted a PCA using JMP, Version 8.0 (JMP 2009).

Question 3 - Specialist versus Generalist *Oenothera*

Floral species with specialist pollination systems may have more targeted selection on floral traits than species with generalist pollination systems (Ashworth et al. 2015). To evaluate whether degree of pollinator specialization affects adherence to the pollination syndrome concept in *Oenothera*, we first assessed whether pollinator specialization affects how close *Oenothera* species fall to the traditional pollination syndromes of Faegri and van der Pijl (1979) in morphospace. To accomplish this, we assigned each *Oenothera* species a specialization score (i.e. S-score), which equals the minimum number of pollinating taxa that account for 95% of an *Oenothera* species' pollen flow; S-scores were taken from KraKos and Fabricant (2014), as well as an unpublished data set at the Missouri Botanical Garden for *O. coloradoensis* (see Wagner et al. 2013). We then ran a linear regression, with S-

score as the predictor variable and Euclidean distance from the nearest pollination syndrome as the response variable, to test whether pollinator specialization impacts how close *Oenothera* species are to traditional pollination syndromes in morphospace. Prior to analysis, we normalized residuals by transforming S-score and Euclidean distance by adding 1 and subsequently calculating the natural logarithm (ln) of each value.

Following analysis of how specialization affects the distance between *Oenothera* species and traditional pollination syndromes in morphospace, we assessed whether specialization affects how accurately an *Oenothera* species' closest pollination syndrome predicts its observed primary pollinator. To accomplish this, we scored each *Oenothera* species for traditional syndrome predictability, i.e. whether its closest traditional pollination syndrome accurately predicts its primary pollinator. Specifically, we used a binary scoring system, whereby an *Oenothera* species received a score of 1 if its closest traditional pollination syndrome accurately predicted its main pollinator (i.e. the pollinator type contributing at least 95% of pollen flow) or a score of 0 if its closest traditional syndrome did not predict its main pollinator. We then ran a logistic regression, with S-score as the predictor variable and traditional syndrome predictability as the response variable. All question 3 analyses were performed with 27 *Oenothera* species, as specialization data was not available for all 54 species used in the first two questions. Analyses were performed with RStudio (ver. 0.99.902).

Question 4 - Self-Incompatible versus Self-Compatible *Oenothera*

As floral species that are self-incompatible (SI) are more reliant on pollinators for reproduction than are self-compatible (SC) species, breeding system may affect adaptation of floral traits to certain pollinator groups (Rosas-Guerrero et al. 2014). To evaluate whether breeding system affects adherence to the pollination syndrome concept in *Oenothera*, we first assessed whether SI *Oenothera* species fall closer to the traditional pollination syndromes of Faegri and van der Pijl (1979) in morphospace than do SC *Oenothera* species. To accomplish this, we ran a Welch's *t*-test, with breeding system (SI or SC) as the predictor variable and Euclidean distance as the response variable. Prior to analysis, we transformed Euclidean distance by adding 1 and subsequently calculating the ln of each value.

Following analysis of how breeding system affects the distance between *Oenothera* species and traditional pollination syndromes in morphospace, we assessed whether breeding system affects how accurately an *Oenothera* species' closest pollination syndrome predicts its primary pollinator. To accomplish this, we performed a two-proportions *z*-test, with breeding system as the predictor variable and traditional syndrome predictability (1 = accurate prediction; 0 = inaccurate prediction) as the response. All question 4 analyses were performed with all 54 *Oenothera* species used in the first two questions. Analyses were performed with RStudio (ver. 0.99.902).

RESULTS

Question 1 - Traditional Pollination Syndromes of *Faegri* and van der Pijl

Ordination using non-metric multidimensional scaling (NMDS) of the traditional pollination syndromes of Faegri and van der Pijl (1979) produced a well-resolved three-dimensional morphospace that accounted for nearly 75% of the variance of the among-syndrome variation (axis 1 $R^2 = 0.16$, axis 2 $R^2 = 0.28$, axis 3 $R^2 = 0.29$, cumulative $R^2 = 0.74$). After 279 iterations the instability was 0.00, and the final stress for the three-dimensional solution was 15.31. Most ecological community data sets have solutions with stress between 10 and 20, and this data set falls within this range (Clarke 1993). In agreement with the results for the traditional pollination syndromes used by Ollerton et al. (2009), we also find that the traditional syndromes, which had multiple versions for each pollinator type, group into discrete areas of morphospace without overlap (Fig. 2A). For example, all of the “bee” syndrome vectors group together, while all of the “moth” syndrome vectors group together and do not overlap with the “bee” syndrome. However, some syndrome groups are closer together, for example, non-flying mammal and bat.

If *Oenothera* species conform to the traditional pollination syndromes of Faegri and van der Pijl (1979), with the floral trait combination for a given species being similar to one of the defined traditional syndromes, we expect that *Oenothera* species to fall within the cluster of a traditional syndrome. These results show that these 54 *Oenothera* species do not fall within any of the morphospace clusters that represent traditional pollination syndromes (Fig. 2A). There is no grouping in the morphospace between the sections of *Oenothera* that reflects phylogenetic relatedness. Sections that are sister to one another are not near each other in morphospace. However, *Oenothera* do show some clustering within the sections of the genus (Fig. 2B). For instance, 24 of the 26 species in section *Gaura* cluster together and the four species in section *Megapterium* occupy the same morphospace.

When we calculated the nearest traditional pollination syndrome for each *Oenothera* species, and compared that to current ecological pollinator data, we found that observed primary pollinators were accurately predicted by traditional pollination syndromes for 48.2% of species (Table I). Several species had equal Euclidean distance values from multiple traditional pollination syndrome vectors, in which case, we scored their pollination syndrome as not accurately predicting their observed primary pollinator. The predictability of traditional pollination syndromes varied (Table I). Plants observed to be primarily moth-pollinated and primarily butterfly-pollinated were the most accurately predicted (moth 69.7% accuracy, 23/33 species; butterfly 100.0% accuracy, 1/1 species). Bird-pollinated plants were accurately predicted 25.0% of the time (1/4 species), while bee-pollinated plants were accurately predicted 26.7% of the time (4/15 species). Fly-pollinated and beetle-pollinated plants were never accurately predicted (0/2 and 0/1 species respectively).

Furthermore, pollinator predictability was more successful in some sections of *Oenothera* than others. In subsection *Megapterium*, three of the four (75.0%) taxa were accurately predicted by the traditional pollination syndromes, and 18 of the 26 (69.2%) taxa in subsection *Gaura* were accurately predicted by the traditional pollination syndromes. The one species in subsection *Paradoxus* had its pollination system accurately predicted. The pollination systems for sections *Kneiffia*, *Gauopsis*, and *Peniophyllum* were never predicted accurately (0/7, 0/1, and 0/1 species respectively) and only one of five (20.0%) species in section *Hartmannia* had their pollination system accurately predicted. One in four (25.0%) taxa of section *Xanthocoryne* and two in five (40.0%) taxa of section *Leucocoryne* had accurately predicted pollination systems.

Question 2 - Pollination Syndromes Using Quantitative Floral Trait Measurements

For all 54 *Oenothera* species examined, the floral trait measurement data (i.e. floral tube length, floral tube mouth width, corolla span, stamen length, style length) used in the principle component analyses (PCA) are given in Table 2. The first two PCA axes explained 78.87% and 10.87% of the variance in the data (Fig. 3). Although approximately 90% of the data is explained with the first two axes, the PCA is unable to give sufficient resolution to discern any grouping of the *Oenothera* species that might correspond to a pollination syndrome. The eigenvector coefficients of axis 1 are all positive, which suggests an allometric relationship among the variables. The correlations of variables on PCA axes are given in Table 3. Axis 1 shows some differentiation between species with long floral tubes and those without. The species that demonstrate some clustering are in section *Megapterium*, which are taxa that all have much longer floral tubes than the other *Oenothera*. Most of the variance for axis 2 is explained by “floral tube mouth width”; however, there is no discernable grouping of species by this quantitative trait. Despite this partial differentiation, unlike the NMDS results, the PCA overall does not provide resolution sufficient to discern groupings of taxa that correspond to pollination syndromes.

Question 3 - Specialist versus Generalist *Oenothera*

We did not find support for pollinator specialization (i.e. S-score) affecting how close *Oenothera* species fall to traditional pollination systems in morphospace. Our linear regression explained none of the variance in Euclidean distance scores ($R^2 = 0.000$) and was not statistically significant ($F_{1,26} = 0.003$, $p = 0.958$) (Fig. 4A). Furthermore, we did not find support for pollinator specialization affecting how accurately traditional pollination syndromes predict primary pollinators of *Oenothera*. Our logistic regression was not statistically significant ($p = 0.707$) (Fig. 4B).

Question 4 - Self-Incompatible versus Self-Compatible *Oenothera*

We did not find support for breeding system affecting how close *Oenothera* species fall to traditional pollination systems in morphospace. Our Welch's t -test did not find statistically significant differences in Euclidean distance between self-incompatible (SI) and self-compatible (SC)

TABLE I. A comparison of the predicted pollinator and the observed primary pollinators for the 54 *Oenothera* species. Predicted pollinators are determined by the closest traditional pollination syndrome to the *Oenothera* species in morphospace. The primary pollinators are determined by ecological data.

Section	Species	Predicted Pollinator	Current Main Pollinator
<i>Gauropsis</i>	<i>O. canescens</i>	bird	moth/hawkmoth
<i>Hartmannia</i>	<i>O. deserticola</i>	butterfly	bee
<i>Hartmannia</i>	<i>O. platanorum</i>	bird	bee
<i>Hartmannia</i>	<i>O. rosea</i>	butterfly	bee
<i>Hartmannia</i>	<i>O. speciosa</i>	moth/hawkmoth	moth/hawkmoth
<i>Hartmannia</i>	<i>O. texensis</i>	bird	bee
<i>Xanthocoryne</i>	<i>O. epilobiifolia s. epilobiifolia</i>	fly	bird
<i>Xanthocoryne</i>	<i>O. epilobiifolia s. cuphrea</i>	bird	bird
<i>Xanthocoryne</i>	<i>O. multicaulis</i>	fly	bird
<i>Xanthocoryne</i>	<i>O. seifrizzii</i>	fly	bird
<i>Leucocoryne</i>	<i>O. dissecta</i>	moth/hawkmoth	moth/hawkmoth
<i>Leucocoryne</i>	<i>O. kunthiana</i>	beetle	moth/hawkmoth
<i>Leucocoryne</i>	<i>O. luciae-julianae</i>	moth/hawkmoth	moth/hawkmoth
<i>Leucocoryne</i>	<i>O. orizabae</i>	beetle	moth/hawkmoth
<i>Leucocoryne</i>	<i>O. tetraptera</i>	beetle	moth/hawkmoth
<i>Paradoxus</i>	<i>O. havardii</i>	moth/hawkmoth	moth/hawkmoth
<i>Megapterium</i>	<i>O. brachycarpa</i>	moth/hawkmoth	moth/hawkmoth
<i>Megapterium</i>	<i>O. coryi</i>	moth/hawkmoth	moth/hawkmoth
<i>Megapterium</i>	<i>O. howardii</i>	moth/hawkmoth	moth/hawkmoth
<i>Megapterium</i>	<i>O. macrocarpa s. macrocarpa</i>	bird	moth/hawkmoth
<i>Peniophyllum</i>	<i>O. linifolia</i>	bird	none/fly/bee
<i>Kneiffia</i>	<i>O. fruticosa s. fruticosa</i>	butterfly	bee
<i>Kneiffia</i>	<i>O. fruticosa s. glauca</i>	butterfly	bee
<i>Kneiffia</i>	<i>O. riparia</i>	butterfly	bee
<i>Kneiffia</i>	<i>O. perennis</i>	bird	bee
<i>Kneiffia</i>	<i>O. pilosella</i>	butterfly	bee
<i>Kneiffia</i>	<i>O. sessilis</i>	butterfly	none
<i>Kneiffia</i>	<i>O. spachiana</i>	bird	none
<i>Gaura</i>	<i>O. anomala</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. glaucifolia</i>	fly	bee/fly/beetle
<i>Gaura</i>	<i>O. curtiflora</i>	beetle	none
<i>Gaura</i>	<i>O. arida</i>	beetle	moth/hawkmoth
<i>Gaura</i>	<i>O. suffrutescens</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. boquillensis</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. cinerea s. cinerea</i>	moth/hawkmoth/beetle	moth/hawkmoth
<i>Gaura</i>	<i>O. cinerea s. parksii</i>	moth/hawkmoth	antlion
<i>Gaura</i>	<i>O. calcicola</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. filipes</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. mckelveyae</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. sinuosa</i>	moth/hawkmoth/beetle	moth/hawkmoth
<i>Gaura</i>	<i>O. hispida</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. coloradoensis</i>	moth/hawkmoth	moth/hawkmoth

Tab. continued

Section	Species	Predicted Pollinator	Current Main Pollinator
<i>Gaura</i>	<i>O. dodgeniana</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. demareei</i>	bee	bee
<i>Gaura</i>	<i>O. filiformis</i>	moth/hawkmoth	moth/hawkmoth/bee
<i>Gaura</i>	<i>O. gaura</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. lindheimeri</i>	bee	bee/butterfly
<i>Gaura</i>	<i>O. hexandra</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. podocarpa</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. patriciae</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. simulans</i>	beetle	moth/hawkmoth
<i>Gaura</i>	<i>O. suffulta</i>	moth/hawkmoth	moth/hawkmoth
<i>Gaura</i>	<i>O. nealleyi</i>	moth/hawkmoth	moth/hawkmoth/bee
<i>Gaura</i>	<i>O. triangulata</i>	beetle	moth/hawkmoth

species ($p = 0.112$) (Fig. 5A); mean Euclidean distance of SC and SI species is $0.363 (\pm 0.018 \text{ SE})$ and $0.407 (\pm 0.021 \text{ SE})$ respectively. Furthermore, we did not find support for breeding system affecting how accurately traditional pollination syndromes predict primary pollinators in *Oenothera*. Our two-proportions z-test was not statistically significant ($p = 0.107$) (Fig. 5B); mean traditional syndrome predictability of SC and SI species is $0.367 (\pm 0.089 \text{ SE})$ and $0.625 (\pm 0.101 \text{ SE})$ respectively.

DISCUSSION

The main goal of this study was to assess the predictive ability of pollination syndromes in *Oenothera*, using both the traditional pollination syndromes of Faegri and van der Pijl (1979) and quantitative floral trait measurements. Across the literature, the predictive ability of pollination syndromes has received mixed support; the pollination syndrome concept has been supported in some taxa (e.g. Rosas-Guerrero et al. 2014; Johnson & Wester 2017) and has failed to be supported in others (e.g. Ollerton et al. 2009). By evaluating the correspondence between floral traits and primary pollinator in 54 *Oenothera* species, we do not find support for the pollination syndrome concept across *Oenothera* following the traditional pollination syndromes of Faegri and van der Pijl (1979) (*Question 1*). Furthermore, we do not find support for the pollination syndrome concept in *Oenothera* when quantitative floral trait measurements are used, as such measurements did not cluster in morphospace (*Question 2*). This study adds to the pollination biology literature by testing the pollination syndrome concept in a species rich, monophyletic clade with high ecological diversity. *Oenothera* is comprised of species across the Americas with high variation in pollination systems and pollinator specialization (Kraeos & Fabricant 2014), some of which are self-incompatible (SI), while others are self-compatible (SC) (Kraeos et al. 2014). While we do not find support for pollinator specialization (*Question 3*) or breeding system (*Question 4*) affecting adherence to the pollination syndrome concept using the traditional syndromes of Faegri and van der Pijl (1979), we do find that floral traits - as defined by these traditional

syndromes - are predictive of primary pollinators more frequently in certain *Oenothera* clades and for *Oenothera* species that are predominantly pollinated by moths. Collectively, these results suggest that the pollination syndrome concept cannot be generalized across taxa and that evolutionary history should be considered when evaluating pollination syndromes in different clades.

When applying the pollination syndrome concept, pollination biologists have often used the traditional pollination syndromes described by Faegri and van der Pijl (1979). While we find that these syndromes segregate into discernable clusters in morphospace, our 54 *Oenothera* species do not fall within or near these traditional syndrome clusters (Fig. 2A). This is in agreement with Ollerton et al. (2009), who also found that plant species do not fall within these traditional pollination syndrome clusters in morphospace. Comparing observed pollination data to the primary pollinator predicted by the nearest traditional pollination syndrome, we find that the predicted pollinator matches the observed primary pollinator in less than half (48.2%) of our *Oenothera* species. This is slightly more successful than the results of Ollerton et al. (2009), who found that the primary pollinator was successfully predicted by the nearest traditional syndrome one-third of the time. One reason for the higher predictability with our data may be that our pollination data are based on both visitation and pollen load, whereas Ollerton et al. (2009) used only flower visitor observations to determine pollinators. Flower visitation alone is an unreliable indicator of pollinator effectiveness and visitor observations can overestimate the number of actual pollinators (Kraeos & Fabricant 2014; Ashworth et al. 2015; King et al. 2013). Moving beyond the traditional pollination syndromes of Faegri and van der Pijl (1979), many studies have tested the pollination syndrome concept using quantitative floral trait measurements (e.g. Lagomarsino et al. 2017; Muchhala 2006; Murúa & Espindola 2015; Tripp & Manos 2008; Whittall & Hodges 2007). When applying this approach to *Oenothera*, our quantitative floral traits also did not form discernable clusters (Fig. 3). This suggests that, for *Oenothera*, the categorical floral traits of traditional pollination syndromes better represent floral form

TABLE 2. Mean measurements of flower morphology (mm) for 54 species of *Oenothera*.

Section	Species	Floral Tube Length	Floral Tube Mouth Width	Corolla Span	Stamen Length	Style Length
<i>Gauropsis</i>	<i>O. canescens</i>	12.50	2.00	17.00	24.50	7.00
<i>Hartmannia</i>	<i>O. deserticola</i>	7.75	2.00	25.50	16.50	6.75
<i>Hartmannia</i>	<i>O. platanorum</i>	11.50	2.25	23.00	15.50	6.50
<i>Hartmannia</i>	<i>O. rosea</i>	6.00	2.00	14.00	10.25	5.00
<i>Hartmannia</i>	<i>O. speciosa</i>	18.50	4.00	60.00	37.50	16.00
<i>Hartmannia</i>	<i>O. texensis</i>	18.00	3.50	33.00	28.00	11.00
<i>Xanthocoryne</i>	<i>O. epilobiifolia s. epilobiifolia</i>	11.00	5.25	14.50	10.25	2.25
<i>Xanthocoryne</i>	<i>O. epilobiifolia s. cuphrea</i>	11.00	5.25	14.50	10.25	2.25
<i>Xanthocoryne</i>	<i>O. multicaulis</i>	5.75	2.75	10.50	7.25	3.25
<i>Xanthocoryne</i>	<i>O. seifrizii</i>	13.00	4.75	20.00	18.00	5.75
<i>Leucocoryne</i>	<i>O. dissecta</i>	38.50	4.50	60.00	58.50	13.50
<i>Leucocoryne</i>	<i>O. kunthiana</i>	19.50	4.00	26.00	23.00	10.00
<i>Leucocoryne</i>	<i>O. luciae-julianae</i>	16.50	4.00	42.00	25.00	8.50
<i>Leucocoryne</i>	<i>O. orizabae</i>	12.00	4.00	40.00	24.00	5.00
<i>Leucocoryne</i>	<i>O. tetraptera</i>	34.00	4.75	50.00	43.00	11.50
<i>Paradoxus</i>	<i>O. havardii</i>	52.50	3.85	51.00	75.50	16.50
<i>Megapterium</i>	<i>O. brachycarpa</i>	165.00	7.50	103.00	155.00	26.00
<i>Megapterium</i>	<i>O. coryi</i>	87.50	6.50	78.00	120.00	21.00
<i>Megapterium</i>	<i>O. howardii</i>	85.00	7.00	100.00	127.50	31.50
<i>Megapterium</i>	<i>O. macrocarpa s. macrocarpa</i>	105.00	8.00	115.00	147.50	35.00
<i>Peniophyllum</i>	<i>O. linifolia</i>	1.50	0.10	8.00	1.50	1.50
<i>Kneiffia</i>	<i>O. fruticosa s. fruticosa</i>	10.00	1.00	40.00	15.00	10.00
<i>Kneiffia</i>	<i>O. fruticosa s. glauca</i>	12.50	1.00	35.00	16.00	10.00
<i>Kneiffia</i>	<i>O. riparia</i>	13.72	1.95	31.96	13.30	11.08
<i>Kneiffia</i>	<i>O. perennis</i>	6.50	1.00	15.00	3.50	3.50
<i>Kneiffia</i>	<i>O. pilosella</i>	17.50	1.00	45.00	15.00	11.00
<i>Kneiffia</i>	<i>O. sessilis</i>	12.50	1.00	29.23	11.00	8.00
<i>Kneiffia</i>	<i>O. spachiana</i>	7.00	1.00	19.00	5.00	5.00
<i>Gaura</i>	<i>O. anomala</i>	3.40	0.50	37.00	5.25	11.50
<i>Gaura</i>	<i>O. glaucifolia</i>	9.50	0.10	10.00	6.50	6.50
<i>Gaura</i>	<i>O. curtiflora</i>	3.25	0.10	5.50	6.00	2.25
<i>Gaura</i>	<i>O. arida</i>	11.00	0.25	15.00	20.00	4.00
<i>Gaura</i>	<i>O. suffrutescens</i>	7.50	1.00	10.00	15.50	4.75
<i>Gaura</i>	<i>O. boquillensis</i>	5.75	0.25	14.00	10.75	3.25
<i>Gaura</i>	<i>O. cinerea s. cinerea</i>	3.50	0.25	21.50	14.25	8.00
<i>Gaura</i>	<i>O. cinerea s. parksii</i>	2.75	1.00	19.00	12.50	6.75
<i>Gaura</i>	<i>O. calcicola</i>	6.00	1.00	17.50	14.25	5.00
<i>Gaura</i>	<i>O. filipes</i>	4.25	0.50	15.00	13.75	5.75
<i>Gaura</i>	<i>O. mckelveyae</i>	2.75	0.25	17.50	12.50	7.00
<i>Gaura</i>	<i>O. sinuosa</i>	3.75	1.67	21.50	15.25	8.00
<i>Gaura</i>	<i>O. hispida</i>	9.00	1.00	16.00	19.00	6.25
<i>Gaura</i>	<i>O. coloradoensis</i>	8.00	1.00	20.50	22.00	7.75
<i>Gaura</i>	<i>O. dodgeniana</i>	8.00	1.00	24.30	25.00	7.75

Tab. 2 continued

Section	Species	Floral Tube Length	Floral Tube Mouth Width	Corolla Span	Stamen Length	Style Length
<i>Gaura</i>	<i>O. demareei</i>	8.50	1.00	28.00	24.50	12.50
<i>Gaura</i>	<i>O. filiformis</i>	8.75	1.00	21.50	22.75	9.00
<i>Gaura</i>	<i>O. gaura</i>	9.25	1.95	18.50	13.50	7.50
<i>Gaura</i>	<i>O. lindheimeri</i>	6.50	0.25	25.50	21.25	9.75
<i>Gaura</i>	<i>O. hexandra</i>	6.00	0.25	11.50	11.75	2.90
<i>Gaura</i>	<i>O. podocarpa</i>	8.00	1.70	15.00	15.00	5.00
<i>Gaura</i>	<i>O. patriciae</i>	9.25	0.25	20.50	19.50	6.50
<i>Gaura</i>	<i>O. simulans</i>	5.50	0.80	12.50	13.25	4.25
<i>Gaura</i>	<i>O. suffulta</i>	10.25	2.00	10.00	24.00	7.50
<i>Gaura</i>	<i>O. nealleyi</i>	15.00	2.00	13.00	29.00	10.50
<i>Gaura</i>	<i>O. triangulata</i>	4.75	0.25	8.50	9.50	2.75

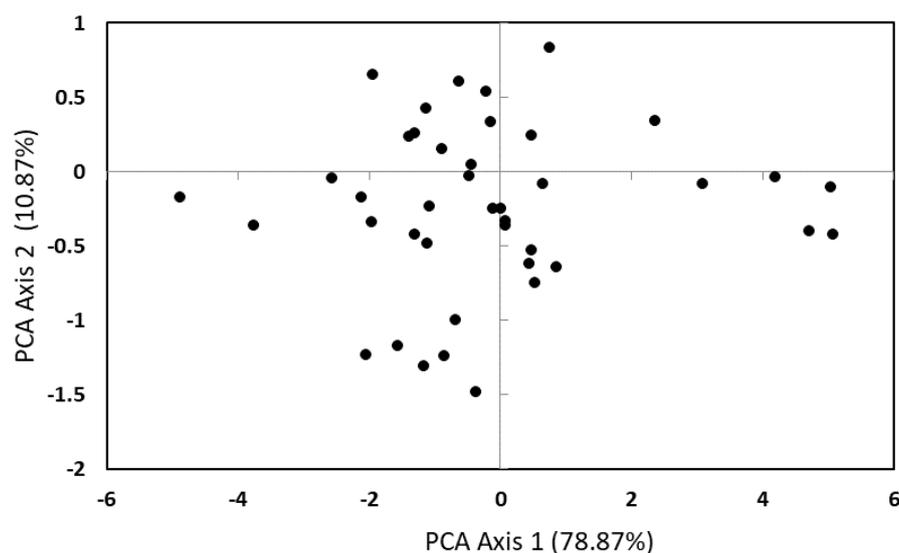


FIGURE 3. Principle component analysis (PCA) of five *Oenothera* floral trait measurements.

convergence, when compared to our quantitative floral traits. However, it is important to note that, if syndromes exist in *Oenothera*, the five quantitative floral traits we analyzed may not fully capture the breadth of syndrome traits in this group. Indeed, it is possible that pollination syndromes in *Oenothera* may be best represented by quantitative floral traits, but will only be realized with the inclusion of a greater number of functional traits. Nonetheless, given the clustering of Faegri and van der Pijl's (1979) traditional syndromes in morphospace, and the absence of clustering when using our five measured floral traits, our results suggest that the traditional syndromes are more appropriate to utilize when testing the pollination syndrome concept compared to our quantitative floral traits.

The mixed support for the pollination syndrome concept found across the literature may result from floral traits being predictive of pollinators in certain species, but not in others. Of the *Oenothera* species examined here, we find that some subsections had higher traditional pollination syndrome predictability, namely *Paradoxus* (100.0%, 1/1 species),

Megapterium (75.0%, 3/4 species), and *Gaura* (69.2%, 18/26 species). Furthermore, we find species predicted to have moth and butterfly syndromes - which are close to one another in morphospace, typically sharing traits such as long, narrow corolla tubes - were predicted with the highest accuracy (moth 69.7%, 23/33 species; butterfly 100.0%, 1/1 species), while species with other predicted syndromes were predicted with low accuracy (e.g. bird 33.3%, 1/4 species; bee 26.7%, 4/15 species; fly 0.0%, 0/2 species; beetle 0.0%, 0/1 species). This is notable as (1) the majority of *Oenothera* species are moth pollinated (e.g. 33 of the 54 focal *Oenothera* species in this study), and (2) *Oenothera* generally have many floral traits that are classically associated with moth pollination (e.g. flower at night, long floral tubes) (Raven & Gregory 1972; Raven 1988; Wagner et al. 2007), including most species within *Paradoxus*, *Megapterium*, and *Gaura*. In agreement with the broad literature on *Oenothera* evolutionary history (e.g. Raven 1988; Raven & Gregory 1972; Wagner et al. 2007; Wagner et al. 2013), these suggest that ancestral *Oenothera* were moth pollinated. It is possible

TABLE 3. Eigenvector coefficients for the morphological characters used in the PCA analysis.

Floral Trait	Axis 1	Axis 2
Floral tube length	0.467	0.158
Floral tube mouth width	0.392	0.801
Corolla span	0.461	-0.253
Length of stigma	0.465	-0.093
Length of stamen	0.448	-0.510

that our results reflect an evolutionary lag between ancestral and contemporary pollinators. In other words, contemporarily moth pollinated *Oenothera* may follow the pollination syndrome concept more frequently than *Oenothera* that are pollinated by a different primary pollinator, as sufficient evolutionary time may not have passed to allow divergence in floral traits from the ancestral moth syndrome characters. Fitting with this interpretation, most *Gaura* are moth pollinated and cluster in morphospace (Fig. 2B); however, certain *Gaura* are primarily pollinated by bees, flies, or beetles and the *Gaura* with the least predictability have the most generalist pollination systems. Additionally, the sections with the lowest predictability were those that have floral traits suggestive of moth pollination, but that are pollinated by a different primary pollinator. For example, *Kneiffia* (0.0%, 0/7 species) have many floral traits that suggest a moth pollination syndrome; however, *Kneiffia* open in the morning and are bee pollinated (Krakos & Fabricant 2014; Krakos et al. 2014). This interpretation, that our results reflect an evolutionary lag between ancestral and contemporary pollinators, is concordant with the argument of Li and Huang (2009) that pollination syndromes may not reflect contemporary pollinators when historic pollinators have been replaced.

We further tested whether the mixed support for the pollination syndrome concept seen across the literature may result from floral traits predicting primary pollinators more often in species that are highly reliant on certain pollinator groups for reproduction (e.g. highly specialized and/or SI species) as opposed to species less reliant on specific pollinator groups for reproduction (e.g. generalist and/or SC species) (Rosas-Guerrero et al. 2014). In *Oenothera*, we do not find support for either pollinator specialization or breeding system affecting adherence to the pollination syndrome concept, when using the traditional syndromes of Faegri and van der Pijl (1979) (Figs. 4 and 5). *Oenothera* is a well-studied clade that is hypothesized to have originated in Central America and then radiated to North and South America (Raven 1988, Munz 1938, Dietrich et al. 1997 Evans et al. 2005 Wagner et al. 2007). It has been found to have highly labile breeding systems; in Subclade B (54 species total), there have been 12 to 15 independent transitions from SI to SC (Wagner et al. 2007; Krakos et al. accepted). Evolutionary transitions to SC often occur when pollinators do not reliably transfer conspecific pollen between flowers, thus providing reproductive assurance to plants that have low pollinator fidelity (Moreira-Hernandez & Muchhala 2019). Consequently, such transitions to SC are thought to promote the diversification of angiosperms, as SC can be a mechanism of reproductive isolation, when all seed set is from self-pollen (Baker 1955; Barrett 2002). Fitting with the interpretation that our results may reflect an evolutionary lag between ancestral and contemporary pollinators, it is possible that sufficient evolutionary time has not passed since transitions to SC to allow divergence in floral traits from their SI ancestors. Alternatively, as SC *Oenothera* are capable of outcrossing, evolutionary pressure may still exist to maintain floral traits for pollinator attraction.

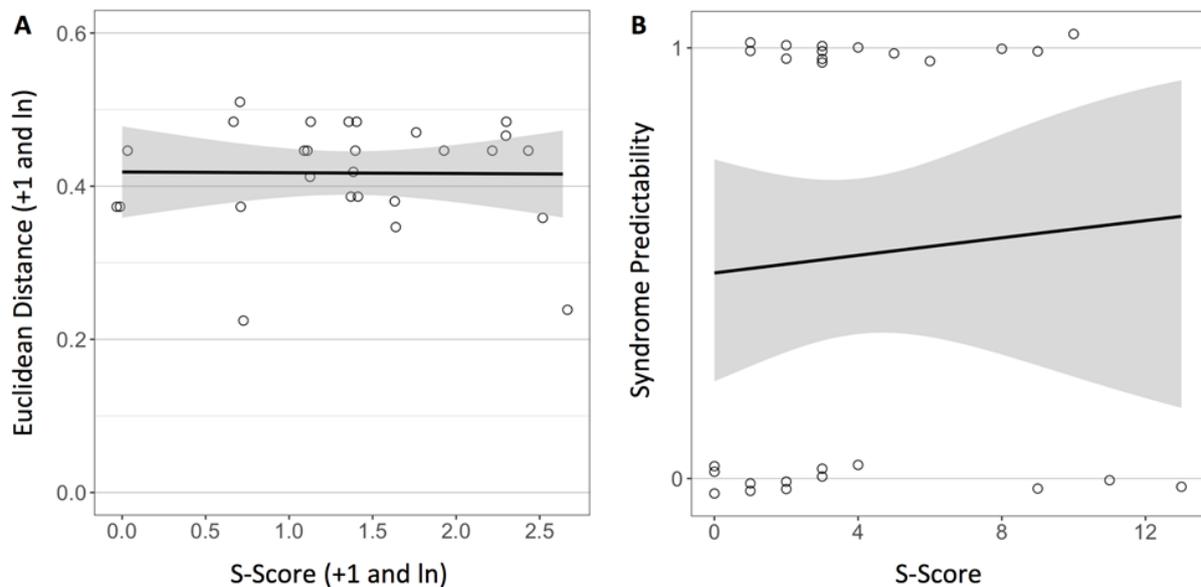


FIGURE 4. Specialization (i.e. S-score) regressed against (A) Euclidean distance (i.e. how close *Oenothera* floral traits are to a traditional pollination syndrome in morphospace) and (B) whether an *Oenothera*'s closest pollination syndrome accurately predicts its primary pollinator (1 = accurate prediction; 0 = inaccurate prediction). Gray areas are 95% confidence intervals. Points are slightly offset to show when multiple points exist for a value. In panel A, the regression depicts the relationship between S-score and Euclidean distance, with both variables +1 and ln transformed.

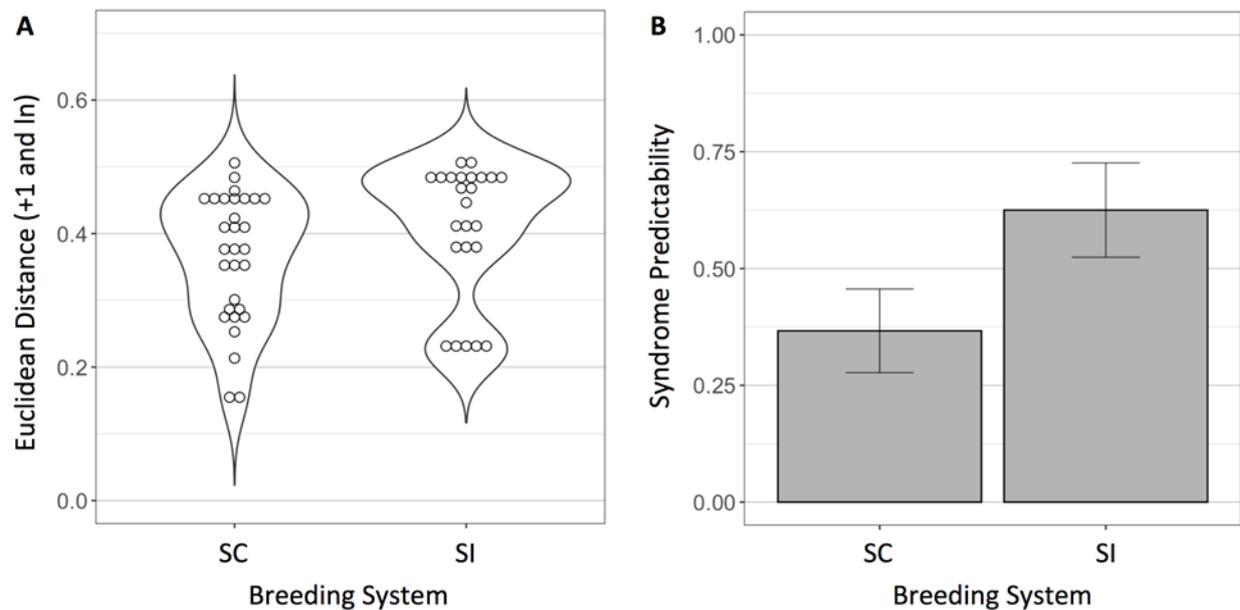


FIGURE 5. Breeding system effects on (A) Euclidean distance (i.e. how close *Oenothera* floral traits are to a traditional pollination syndrome in morphospace) and (B) whether an *Oenothera*'s closest pollination syndrome accurately predicts its primary pollinator (1 = accurate prediction; 0 = inaccurate prediction). In panel A, Euclidean distance is +1 and ln transformed. In panel B, bars are \pm SE. SC = self-compatible; SI = self-incompatible.

In addition to an evolutionary lag in pollinators, our results may be partially explained by pollinator stochasticity over shorter temporal intervals. Across time and space, pollinator community composition may change due to multiple factors (e.g. habitat disturbances, range shifts, alterations to phenological timing), thus inducing spatiotemporal variation in the identity of pollinators visiting particular species of flowering plant (e.g. Davila & Wardle 2008; Burkle & Alarcon 2011; Lázaro et al. 2008). As these pollinators may vary along multiple trait axes (e.g. functional traits, sensory perception), such spatiotemporal variation can produce high variation in pollination network structure from season to season (Burkle & Alarcon 2011; Burkle et al. 2013). While sustained shifts in pollination network structure may promote floral trait evolutionary change, pollinator stochasticity over short periods may result in plant-pollinator associations that do not follow predictions of the pollination syndrome concept. Thus, defining a plant's primary pollinator from a relatively short time window could lead to a documented plant-pollinator association that does not represent a plant's true primary pollinator that would be captured by long-term monitoring. While we believe our methods for defining observed primary pollinators constituted sufficient sampling across time and space to capture representative associations between our *Oenothera* species and their true primary pollinators (Krakos & Fabricant 2014), we acknowledge that pollinator spatiotemporal stochasticity may have influenced these measures. This consideration raises an important, but often overlooked, question for studies on plant-pollinator interactions; namely, how does the reliability of primary pollinators affect pollination networks and floral evolution. Numerous studies have considered how unreliable transfer of conspecific pollen between flowers (e.g. interspecific pollen transfer) affects floral evolution (e.g. Hildesheim et al. 2019; Kalisz & Vogler

2003; Moreira-Hernandez & Muchhala; Opedal et al. 2016) and studies have examined how reliability of floral rewards affects pollinator behavior (e.g. Austin et al. 2019; Dunlap et al. 2017); however, how stochasticity in the presence of pollinators affects floral evolution and the delineation of pollination systems remains a largely understudied topic. Future studies testing the pollination syndrome concept should utilize plant-pollinator data with broad spatiotemporal coverage when defining primary pollinators and consider how pollinator reliability across time and space may influence the interpretation of pollination syndromes (or the lack thereof) in their focal species.

Conclusion

Overall, we do not find support for the pollination syndrome concept in *Oenothera*; i.e. fewer than 50% of species' primary pollinators were predicted by the traditional pollination syndromes of Faegri and van der Pijl (1979) and *Oenothera* did not cluster in morphospace using our quantitative floral trait measurements. While we do not find that floral traits predict current pollinators using either categorical or quantitative data, and there is no difference in adherence to the traditional pollination syndromes based on breeding system or pollinator specialization, our results add to the pollination biology literature by testing the pollination syndrome concept in a monophyletic clade with a diversity of pollination systems and ecotypes. Given that (1) ancestral *Oenothera* were likely moth pollinated and (2) extant *Oenothera* species with moth pollination systems were predicted most accurately, our results suggest that evolutionary history is important to consider when evaluating the relationship between floral form and contemporary pollinators. While pollinators are important selective forces that influence the evolution of floral traits, there are also multiple factors that influence floral form, such as multiple

primary pollinator groups, antagonistic interactions, and pleiotropic effects on other plant traits (Reynolds et al. 2009; Strauss & Irwin 2004). As pollination syndromes may result from convergent evolution of floral form due to pollinator mediated selection, the pollination syndrome concept is useful for guiding hypothesis development on the eco-evolutionary effects of pollination system interactions. However, to determine the current pollination system for a species, direct observation and data collection are still necessary. Collectively, our results demonstrate that in certain taxa, such as *Oenothera*, the pollination syndrome concept may not accurately predict contemporary associations between floral species and their primary pollinators.

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