Sex-associated differences in the network roles of pollinators

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Citation: Smith, G. P., J. Gardner, J. Gibbs, T. Griswold, M. Hauser, D. Yanega, and L. C. Ponisio. 2021. Sex-associated differences in the network roles of pollinators. Ecosphere 12(12):e03863. 10.1002/ecs2.3863

Abstract. Sex-associated differences in behavior can have large ecological consequences, especially in plant–pollinator communities where floral visitor behavior affects plant reproduction. Whether these differences are prevalent enough to impact community-level processes, however, is unknown. Using 256 plant–pollinator communities, we built networks where the floral interactions of each sex were modeled separately, comparing observations to simulated networks where sex was randomized. We found that (1) in many species the sexes differed in their network roles and visited different partners, with females tending to visit more species and more peripheral species than males; (2) more generalist species differed more in network roles between the sexes; and (3) networks where nodes were separated by sex were more specialized than simulated networks, but were similarly resistant to perturbations. These findings suggest that despite variation among species, sex-associated differences in behavior are large enough to impact the network roles of male and female pollinators and common enough to influence the interaction patterns of entire plant–pollinator communities.

Key words: intraspecific variation; plant–pollinator network; sex differences.

Received 11 June 2021; accepted 30 June 2021. Corresponding Editor: Debra P. C. Peters.

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INTRODUCTION

Intraspecific variation in behavior is ubiquitous in nature (Bolnick et al. 2003) and has the potential to impact ecological and evolutionary processes (Bolnick et al. 2011, Des Roches et al. 2017). Sex-associated differences are a major driver of this variation (Andersson 1994, West-Eberhard 2014); across many taxa, males and females differ in their diets (Barboza and Bowyer 2000, Filipiak et al. 2021), foraging strategies (Nifong et al. 2015), dispersal tendencies (Perry and Garland 2002), and habitat preferences (Levin et al. 2013). These sex differences can alter interaction strength and therefore selection regimes on interacting species (Temeles et al. 2013). Theories relating to intraspecific niche specialization predict that intraspecific competition can cause individuals to diverge in morphology or behavior (Bolnick et al. 2003)—such a division between males and females (rather than incipient species) has been shown across multiple taxa (Bolnick and Doebeli 2003, Butler et al. 2007, De Lisle and Rowe 2015). Despite these potential impacts on species interactions and evolutionary trajectories, the consequences of sex-associated variation on community-level processes such as the maintenance of ecosystem function are unknown.

Ecological network analysis provides a systematic method for representing and comparing species interaction patterns across communities for many types of ecological interactions (Pellissier et al. 2018). In ecological networks, nodes (traditionally species) are linked by observed interactions, generating patterns that describe all the direct interactions in the community. Network analyses reveal that, depending on the type of interaction under consideration, networks exhibit consistent architectures described by the nestedness, abundance, relative frequency, and redundancy of interactions (Vázquez et al. 2009). These interaction patterns have been related to ecosystem function (Kaiser-Bunbury et al. 2017) and a community's ability to withstand species loss (i.e., resistance; Memmott et al. 2004). For example, networks containing more species with narrow diet breadths and with fewer nested interactions tend to be more modular and have fewer redundant interactions; these patterns are predicted to leave species in the network more vulnerable to co-extinction cascades (Song et al. 2017). Despite the fact that sex-associated behavioral differences have the clear potential to influence species roles within networks and overall network structure, there have been few studies of network structure that partition interactions within species (Tur et al. 2014, Rumeu et al. 2018), and fewer that partition the interactions of males and females (but see Roswell et al. 2019, Kishi and Kakutani 2020).

Plant–pollinator interactions are common across ecosystems and provide crucial services to both natural and agricultural communities (Ollerton 2017). Because 87% of plants rely on animal pollen vectors for their reproduction (Ollerton et al. 2011), relatively small differences in behavior can directly influence the reproductive success of visited plants (Maruyama et al. 2016). This success in turn can affect the floral resources available to the next generation of pollinators. Sex-associated differences in pollination behavior have been reported across a diverse suite of taxa (reviewed in Smith et al. [2019]). Female pollinators generally appear to forage more frequently (Wolf and Moritz 2014) and for different resources than males (Ne'eman et al. 2006), perhaps because they are the only sex that provisions their offspring with pollen (e.g., many Hymenoptera) or searches for oviposition sites (e.g., Lepidoptera, Diptera). Further, females allocate nutrients obtained from flowers to developing eggs (Levin et al. 2017). Thus, female foraging decisions are critical to offspring success. In contrast, males tend to be more dispersive, spending their time moving longer distances between flowers (Rusterholz and Erhardt 2000, Borkent and Schlinger 2008) and patches (Ostevik et al. 2010) while they search for mates. For males, floral resources serve primarily as fuel for their own activities. Although species differ in the specific behaviors, these general differences in time investment priorities of the sexes have been observed in species across a wide range of pollinator taxa (Smith et al. 2019) and have the potential to influence the pattern of interactions each sex has with other species within a network, or their network "role."

To date, however, studies on sex differences in pollinators have primarily focused on describing patterns within single species, and precisely how these differences affect community-level processes is an open question (though see Kishi and Kakutani [2020]). For example, males looking for mates may forage opportunistically; in plant–pollinator networks, this may cause males to visit more flower species (i.e., higher network *degree*) and be less selective among available flowers (i.e., lower selectivity, measured by *d′*) while females disproportionately visit abundant flowering plants that offer large rewards. Findings consistent with these patterns have been reported in bees and moths (Ne'eman et al. 2006, Alarcón 2010) and may also result in males visiting more of the rare or under-visited flowers on the periphery of networks (i.e., lower centrality in the network). Alternatively, females need to collect more resources (Stone 1995) and more diverse resources (Rusterholz and Erhardt 2000, Eckhardt et al. 2014) from flowers than males to meet offspring nutritional requirements; these demands could cause females to visit *more* plant species than males when large or abundant resources are unavailable. In either case, species are likely to vary in the degree to which males and females differ. Just as wider niche breadth is associated with intraspecific niche variation (Bolnick et al. 2007), wider pollinator diet breadths may give males and females within generalist species more opportunity to differ, provided that the local community has sufficiently diverse floral resources to choose among.

Differences such as these in network role, along with observed differences in floral preferences between the sexes (Roswell et al. 2019), may also influence the architecture of networks as a whole. In particular, differences in foraging and floral preference may reduce the number of plant partners shared by the sexes, resulting in fewer redundant interactions (higher *vulnerability*). These network patterns, in turn, may reduce network resilience or increase the risk of cascading extinctions if a single species is lost (lower *robustness*; Song et al. 2017). Whether sex-associated differences are large or widespread enough to affect network architecture, however, is unknown.

To investigate how differences associated with pollinator sex affect individual network roles and overall network structure, in this study we examine networks that differentiate the interactions of males and females. To do so, we used existing data on plant–pollinator interaction observations collected over 10 yr across wild and managed systems in the western United States. (1) Based on observed differences in male and female behavior and intraspecific niche specialization theory (Bolnick et al. 2003), we predict that the sexes will show consistent differences in their network roles. In particular, we examine how the sexes differ in their (1a) specialization and (1b) centrality in the networks, testing whether the opportunism of males or the diverse resource requirements of females plays a larger part in determining network role. We also predict that (1c) males visit different floral species than females. (2) We predict that species traits will influence which species show sex differences such that a species will be more likely to show sex differences if they are more generalized. (3) We hypothesize that these differences in network roles between the sexes will affect community-level patterns by altering the identities of the pollinators' partners and their interaction frequencies. Specifically, we predict that networks accounting for differences between the sexes will show (3a) high specialization, (3b) low nestedness, and (3c) high vulnerability to species extinctions. This study represents the first large-scale test of whether sex can affect network structure and function across communities.

METHODS

Interaction observations

Plant–pollinator networks were constructed using visitation observations from three separate data sets of similarly collected networks across three geographic regions: hedgerows and weedy field margins in Yolo County, California (HR) (i.e., Ponisio et al. 2016*a*, Kremen et al. 2018), burned areas in Yosemite National Park (YNP) (Ponisio 2020), and mixed ecosystem types in the Sonoran Desert Sky Islands in California and Arizona (SI) (Ponisio et al., *unpublished data*). Within each of these data sets, floral visitors were caught at multiple sites and across 2–10 yr (HR: 63 sites between 2006 and 2016; YNP: 18 sites between 2013 and 2014; SI: 13 sites in 2012, 2017 and 2018), with sites sampled 2–5 times each season. Not all sites were sampled in all years. All insects that were observed to touch the reproductive parts of flowers were collected and considered pollinators for the purposes of this study, though here we focus on the most abundant visitors, bees, and syrphid flies. Pollinator collection at a site within each study was time-standardized, though the total sampling time per sampling round differed between studies depending on the pollinator richness of that area. Sample effort was based on flatted effort-species accumulation curves estimated from pilot sampling in each region, such that additional sampling time would be unlikely to yield additional pollinator species. These estimates were 10 h in the Sky Islands (with a exceptionally diverse pollinator community), 1.5 h in Yosemite, and 1 h in Yolo Co. Pollinator specimens were identified by expert taxonomists (see Acknowledgements and Author Contributions); while the majority were identified to species, some specimens were identified to genus or morphospecies. Plant species were identified in the field using dichotomous keys and guides (Botti 2001, Ivey 2008). Plant–pollinator interactions were organized based on their collection site and year; hereafter, we will refer to year and site combinations as a network. As the sampling period for the sites was short (<2 months in most cases), this temporal scale resulted in network sizes large enough to calculate statistics with while minimizing including species whose phenology does not overlap into the same networks. From a total of 312 networks, we restricted our analyses to the 256 networks where at least one pollinator species had both males and females present. Within these networks, we explored 2968 unique interactions between 260 plant and 393 pollinator species across 30,704 total pollinator specimens. Pollinators for which only one sex had been observed in a given network were retained in order to construct complete networks, but were not included in node-level analyses. For a list of species observed within each data set, see Appendix S1: Table S1.

Observed and randomized network generation

To construct networks, for each site-year combination, a matrix was generated with plants as rows, visitors as columns, and the value in each cell as the number of interactions between the respective plant–pollinator combination. Unlike most plant–pollinator networks, however, we modeled the interactions of males and females of the same species separately by dividing their interactions into separate visitor columns. This allowed us to generate networks where individuals within each pollinator species were divided into male and female nodes, rather than combined into a single-species node.

To generate a distribution for the null expectation that females and males have the same partners and network roles, we created 10,000 simulated data set. Simulated data sets were generated by randomly reshuffling the sexes within each pollinator species within each network using the sample command in base R without replacement, thereby keeping the total number of males and females of that species constant (Fig. 1). Simulated networks were then constructed from these data sets using the same methods described above. To control for differences in network size, species abundance, and sampling intensity, all comparisons were made

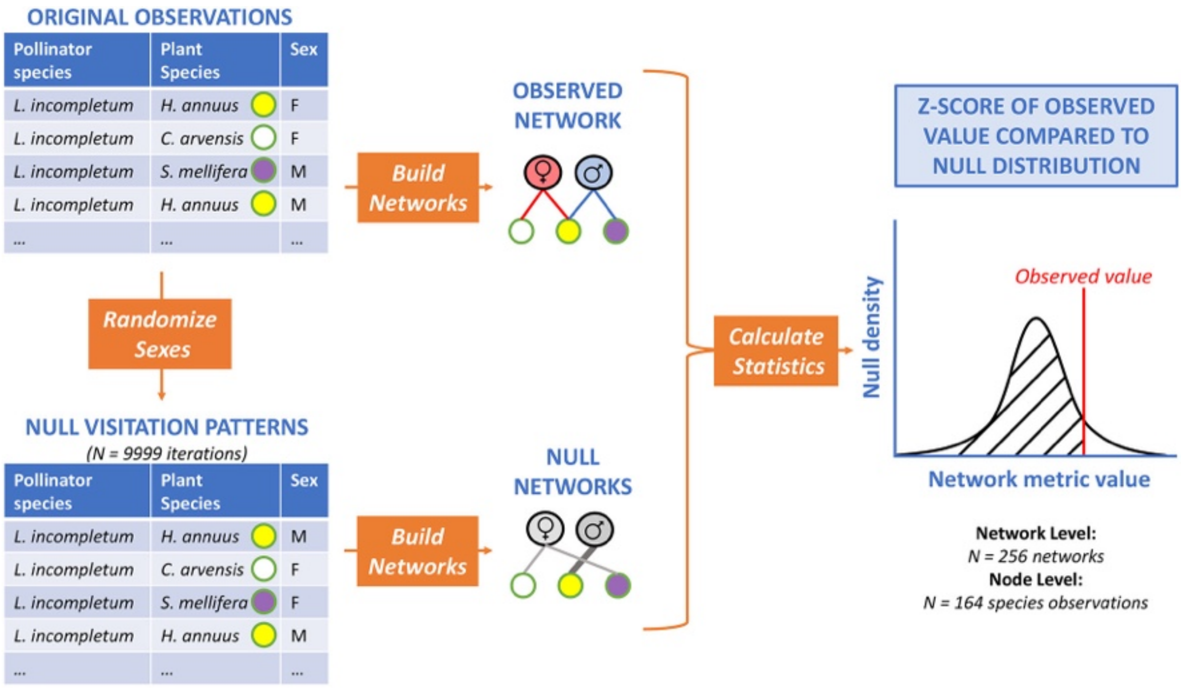

Fig. 1. Summary of the methods used to generate null networks and test hypotheses. First, the sexes within each species within each network of the observed data set (top left) were reshuffled randomly to generate null visitation patterns (bottom left). Both the observed and simulated data sets were used to generate networks at the sex level (i.e., where each pollinator sex was its own node). Node-level and network-level statistics from the observed network were compared to null network values to test hypotheses.

between observed patterns and the distribution of patterns from simulated data for the same network or species. All plant–pollinator network generation and analyses were performed in R version 3.6.3 R (R Core Team 2020).

Node-level analyses

To examine whether male and female pollinators occupied different network roles, we calculated node-level metrics related to specialization and network centrality for both our observed and simulated networks using the specieslevel function in the bipartite package bipartite. For a summary of metrics used and their meanings, see Table 1. To test the prediction that the sexes differ in their specialization (hypothesis 1a), we calculated two network metrics to capture different aspects of specialization: *degree* and *d′*. *Degree* is the number of plant species that each sex of each pollinator species interacted with, representing diet breadth. Each sex's selectivity of floral species relative to floral abundance in a given network is represented by *d′* (Blüthgen et al. 2006), with floral abundance calculated from the number of visits each plant species received in the network. Together, these metrics can describe different patterns of specialization; for example, a node with low *degree* and low *d′* visits few species but visits them equally, as may be the case for a pollinator that specializes on plants in a given genus but is not picky among them. High *degree* and high *d′* would represent a node that samples many flowers, but "specializes" on or primarily visits one species. For each species within each network, the values generated for females were subtracted from the values generated for males to produce a single role difference score for each network metric. Thus, more positive values would indicate that males had a higher metric value than females, while more negative values would indicate females had a higher value.

To test the prediction that the sexes would differ in how centrally connected they were within their networks (1b), we calculated network *closeness* as the number of links separating a given node from other nodes in the network (i.e., path length between the nodes). Pollinator nodes interacting with frequently visited or generalist flowers will show high *closeness*, while those interacting with rare or peripheral flowers will show low *closeness*. As above, the metric values generated for females were subtracted from values generated for males to produce difference scores.

To test the prediction that the sexes would differ in the identity of the plants they visited within each network (1c), we also calculated the

Table 1. Summary of network metrics calculated at both the node and network levels.

Analysis level	Network metric	How it's calculated	What a high value means
Node	Degree	Number of unique interaction partners of the focal node	The node interacts with many plant species
	d′	The proportion of interactions by the focal node to each of its partners is compared to the "abundance" of each of those partners (i.e., the number of interactions those partners receive across the whole network)	The node is very selective in the plant species it visits (relative to other pollinators in the network)
	closeness	Average path length between the focal node and all others	The node interacts with generalist/well-connected plant species
	M-H	Dissimilarity of community matrices, taking proportional differences into account	The males and females are dissimilar in the identity and/or relative frequency with which they interact with plants
Network	H2′	As *d′*, but averaged across the whole network	There are many highly selective nodes in the network (both plants and pollinators)
	NODF	The degree of overlap and decreasing fill of network matrix cells	Generalists interact with specialists in the network
	Redundancy	Mean effective plants per pollinators (and vice versa), weighted by marginal totals	The pollinators (or plants) in the network have multiple redundant partners
	Robustness	Number of nodes that lose all of their partners after simulated extinctions of single species	The network is resilient to change: Single-species extinctions do not domino into further extinctions

Morisita-Horn dissimilarity index (M-H) of the sexes within each species using the vegdist function in the package vegan (Oksanen et al. 2019). This index was chosen because it takes into account proportional differences in community composition and is relatively independent of sample size (Magurran 2013), which makes it ideal for use across many networks of varying size. For M-H, higher values represent more dissimilar communities of plants visited by the sexes. For each species, both the M-H values and the difference scores described above were then compared to the values of that species across all simulated networks for that site and year to generate a *z*-score. Linear mixed-effects models (LMEs) were used to confirm whether the means of the *z*-score distributions differed from 0 (i.e., whether the model intercept differed significantly from 0). In these models, data set was included as a fixed effect and network (site and year) was included as a random effect. Mean raw metric values for the observed networks are reported as a representation of the magnitude of differences between the sexes (rather than the relative difference magnitude compared to simulated networks), but raw values are otherwise not used for analysis.

To ensure that there were enough observations for reshuffling to thoroughly mix the sexes during randomization, these node-level analyses were restricted to pollinator species for which at least five males and five females had been observed in a given network. After removing species below this threshold, 40 pollinator species and 93 sites were retained, combinations of which resulted in 164 species observations. As all of our sites separated by longer distances than typical pollinator foraging ranges and had discrete community compositions (Ponisio et al. 2016*a*, *b*), we believe that each of these observations was independent even within data sets. To confirm that data set source did not significantly affect the metrics we calculated, however, we included data set as a fixed effect in the models described below.

Pollinator trait analyses

To examine whether species-level diet breadth influenced the probability that the sexes would differ in their network roles (2), we first estimated diet breadth for each pollinator species for the whole data set by rarefying all visitation observations for that species using the chao1 function (Vavrek 2011). We then ran LME models with the response being the metric in question (*degree*, *d′*, *closeness* and *betweenness*), the fixed effects being rarefied degree, the number of plants in the network, and the data set source. The network itself (site + year) was a random effect.

Network-level analyses

To examine whether behavioral differences between sexes affect the structure and function of networks as a whole, we calculated four metrics for each observed and randomized network using the networklevel function in the bipartite package (Dormann et al. 2008). To test the prediction that networks accounting for differences between males and females show higher specialization (3a), we calculated H2′ as a representation of partner selectivity relative to abundance at the whole-network level (Blüthgen et al. 2006). Networks where each pollinator node is relatively specialized (i.e., interacts with few plants and overlaps little with other pollinators) would have high H2′. To test the prediction that networks accounting for differences between males and females show lower nestedness (3b), we calculated negative association of nestedness (NODF) as a measure of the degree to which specialist species interact with generalists (Almeida-neto et al. 2008), with high NODF values indicating highly nested network architectures. This type of architecture where specialists interact with generalists has been associated with resistance to species extinctions (Gómez et al. 2011). To test the prediction that sex-associated differences would cause networks to be more vulnerable to species extinctions (3c), we calculated interaction *redundancy* and network *robustness*. *Redundancy*, which is typically referred to as generality for pollinators and vulnerability for plants, estimates the average number of redundant interactions in the network (i.e., the weighted mean number of links per node Bersier et al. 2002). *Robustness* was used as an estimate of how sensitive each network would be to the extinction of plants and pollinators (Memmott et al. 2004, Burgos et al. 2007). We simulated the extinction of species, eliminating nodes based on their *degree*, and then tallied the number of species of the other trophic level that subsequently went extinct. To account for the fact that males and females were separate columns in our networks, extinctions of females also caused the extinction of males of the same species. Extinction of male nodes did not cause the extinction of female nodes of the same species, representing the ability of females to lay eggs and recover local populations. The simulated extinctions were used to generate a curve representing proportion of species remaining after the extinction of their partner species, and the area below the extinction curve was used as an estimate of network resistance to co-extinction cascades (*network robustness* Burgos et al. 2007). While the utility of this metric in predicting actual extinctions is unclear in light of real biological processes such as re-wiring (CaraDonna et al. 2017) that buffer against species loss, robustness is still a commonly used representation of the stability of a network to perturbations (Maia et al. 2021).

All 256 networks that included at least one pollinator species with both males and females present were included in these network-level analyses. As with the node-level analyses, network-level metric values from each observed network were compared to the values across all simulated null networks to generate *z*-scores. Linear models (LMEs) were used to confirm whether the means of the *z*-score distributions differed from 0 (i.e., whether the model intercept differed significantly from 0). In these models, data set was included as a fixed effect.

Model validation

Due of the discrete nature of the sites we sampled (hedgerows surrounded by agriculture, burned forest patches, and alpine meadows), many of our networks were small and had relatively few plant species blooming concurrently during sampling (mean 8.14 plant species per network). While our methods control for network size by comparing observed networks to simulations with the same dimensions, the range of values in our simulations may have been constrained for network metrics that are dependent on network size or sampling (e.g., *degree*, M-H, *redundancy*; Dormann et al. 2009; Blüthgen 2010; Ulrich et al. 2017). Therefore, to validate our approach, we repeated the reshuffling, network building, and network comparison procedure described above on an artificially modified visitation data set as a starting point for the simulations rather than the original observations. In this data set, males and females of each species within each network were modified to share the same plant visitation vector, mimicking a scenario in which the sexes did not differ in their floral visitation patterns. With this validation model, none of the metrics tested at either the node or network level had *z*-score distributions that differed significantly from 0. Mean raw metric values for the validation data are reported along side the mean raw values of the observed data for comparison; interpretations were made based on the observed mean value minus the validation mean value. For more details and statistical results obtained using this validation model, see Appendix S2.

RESULTS

Node-level analyses

Males and females differed in their network roles across many species and sites (Fig. 2). (1a) Compared to the null expectation that the sexes would not differ in their network roles, females tended to be less specialized than males. Females had wider diet breadths (i.e., higher *degree*; see Table 2a for model details) and were less selective in their partners (i.e., lower *d′*; Table 2a) than males. The mean raw *degree* and *d′* across the whole data set were 3.29 and 0.258, respectively. Averaged across the data set, females visited 0.482 more species than males (14.6% of mean; observed mean *degree* difference value = −0.738; validation mean *degree* difference value = −0.256), and their *d′* was 0.046 lower (17.9% of mean; observed mean *d′* difference value = 0.0379; validation mean *d′* difference value = −0.00815).

(1b) Females were also less centrally connected in the networks (i.e., had lower *closeness*; Table 2a). The mean raw *closeness* across the whole data set was 0.0613. Averaged across the data set, females' closeness values were 0.00441 higher than males (7.20% of mean; observed mean *closeness* difference value = −0.0043; validation mean *degree* difference value = −0.0088).

(1c) Finally, males and females tended to be dissimilar in the community of plants they visited (i.e., higher M-H; Table 2a). Averaged across the

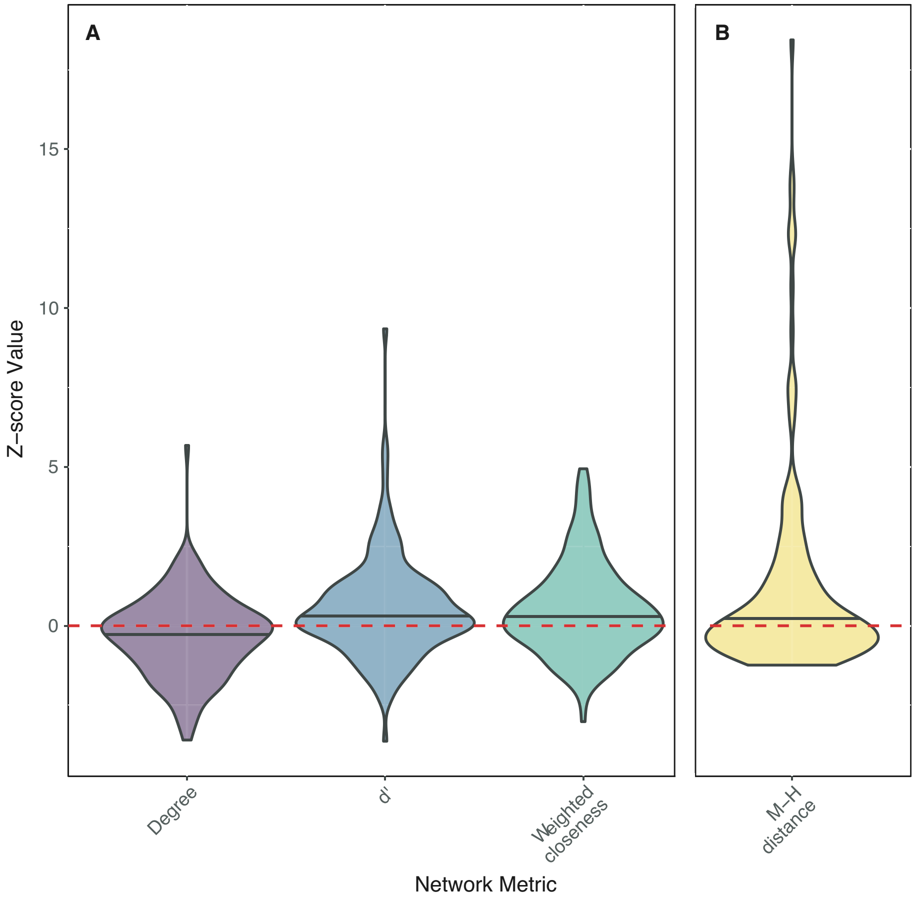

Fig. 2. Distribution of *z*-scores of node-level metrics comparing male and female network roles in networks where individuals within species were split by sex relative to networks where individuals were split randomly. The solid black lines within each distribution represent the mean *z*-score value for that metric ($N = 152$ species observations). The dashed red line is at zero. A: Metrics were calculated by subtracting the female value from the male value; more positive *z*-scores suggest that males have larger values, while more negative *z*-scores suggest that females have larger values. B: More positive *z*-scores of M-H suggest that the community of plants visited by males and females are more different than the null expectation; negative scores suggest that the sexes differed less than expected.

data set, the mean dissimilarity attributable to sex is 0.0545 (30.1% of mean; observed mean M-H value = 0.181; validation mean M-H value = 0.126). Data set source did not significantly affect any of these metrics.

Pollinator trait analyses

(2) Females in taxa with higher rarefied degree across all networks had higher *degree* than males of the same species (for model details, see Table 2b). Females from these more generalized species also had lower *closeness* than males and differed more from males in the identity of plants visited compared to more specialized species, but relative selectivity of the sexes (*d′*) did not vary by rarefied degree (Table 2b). The number of plant species in the network did not affect *degree* or M-H, but females in species in more diverse networks were less selective in their visitation (lower *d′*) but more central in their network position (higher *closeness*). Data set source had no significant effect on *degree*, *d′*, or M-H, but males were had relatively higher *closeness* within SI networks (Table 2b).

Table 2. Model details for analyses of (a) node-level network metrics and (b) specialization analyses.

	Fixed effects							Random effects			
			Output							Output	
Metric	Model	Term	Value	SE	df	t	P	Model	Term	Intercept	Residual
(a)											
Degree	degree ~ data set	(Intercept)	−0.3994	0.12036	92	−3.318	**0.0013**	~1 | SiteYear	SD	0.325949	1.22688
		data set	0.2414	0.23947	92	1.0081	0.3161				
d′	*d′* ~ data set	(Intercept)	0.5232	0.16553	92	3.1611	**0.0021**	~1 | SiteYear	SD	0.99638	1.25266
		data set	0.019	0.37792	92	0.0503	0.96				
Weighted closeness	closeness ~ data set	(Intercept)	0.4997	0.14129	91	3.5366	**0.0006**	~1 | SiteYear	SD	0.545769	1.33606
		data set	0.0428	0.29291	91	0.146	0.8843				
M-H	distance ~ data set	(Intercept)	1.1978	0.32648	91	3.6689	**0.0004**	~1 | SiteYear	SD	1.529442	2.90101
		data set	−0.0389	0.71445	91	−0.0545	0.9567				
(b)											
Degree	degree ~ rareDeg	(Intercept)	0.6798	0.3195	91	2.1277	**0.0361**	~1 | SiteYear	SD	0.374293	1.15393
	+ data set	rareDeg	−0.0145	0.00345	69	−4.2189	**0.0001**				
	+ plants	plants	−0.0126	0.02064	91	−0.6104	0.5431				
		data set	−0.1041	0.33254	91	−0.3129	0.7551				
d′	*d′* ~ rareDeg	(Intercept)	−0.2934	0.42249	91	−0.6944	0.4892	~1 | SiteYear	SD	0.886735	1.28822
	+ data set	rareDeg	0.004	0.00423	69	0.9561	0.3424				
	+ plants	plants	0.0595	0.02965	91	2.0065	**0.0478**				
		data set	−0.4	0.47604	91	−0.8403	0.403				
Weighted closeness	closeness ~ rareDeg	(Intercept)	0.5867	0.35958	90	1.6317	0.1062	~1 | SiteYear	SD	0.409906	1.30366
	+ data set	rareDeg	0.01	0.00388	69	2.5728	**0.0122**				
	+ plants	plants	−0.0819	0.02319	90	−3.5291	**0.0007**				
		data set	1.1801	0.37386	90	3.1565	**0.0022**				
M-H	distance ~ rareDeg	(Intercept)	−1.5761	0.84779	90	−1.8591	0.0663	~1 | SiteYear	SD	1.494109	2.77466
	+ data set	rareDeg	0.0336	0.0088	68	3.8219	**0.0003**				
	+ plants	plants	0.0574	0.0575	90	0.9979	0.321				
		data set	0.5103	0.93828	90	0.5438	0.5879				

Note: Values in boldface are significant at $P < 0.05$.

Network-level analyses

Many of the networks split by sex also differed from networks where individuals were split randomly within each species (Fig. 3). (3a) Networks split by sex were more selective (higher H2′) than the null expectation (Table 3). (3b) Networks split by sex were also less nested than networks split randomly, with NODF being significantly lower than the null expectation (Table 3). (3c) The *redundancy* of partners was lower for both pollinators and plants in simulated networks compared to observed networks. The stability (*robustness*) of observed networks, however, did not differ for either plants or pollinators from the null expectation (Table 3).

DISCUSSION

Here, we demonstrate that males and females in many species interact with different partners and occupy distinct network roles. Compared to the null expectation that males and females behave similarly, we found that females tended to visit more plant species (higher *degree*), fewer generalist plants (lower *closeness*), and visited flowers more in proportion to their abundance (lower *d′*) compared to males, who focused more of their foraging effort on a few generalist plants. Furthermore, consistent with our predictions and with prior observations (Roswell et al. 2019, Smith et al. 2019), males and females tended to show larger floral partner composition differences than the null expectation. This difference is also consistent with the finding that dividing the sexes reduced the interconnectedness of subgroups within pollinator networks (Kishi and Kakutani 2020). On average, females visited 0.5 more species than males and were 18% less selective among those resources (*d′*) after correcting

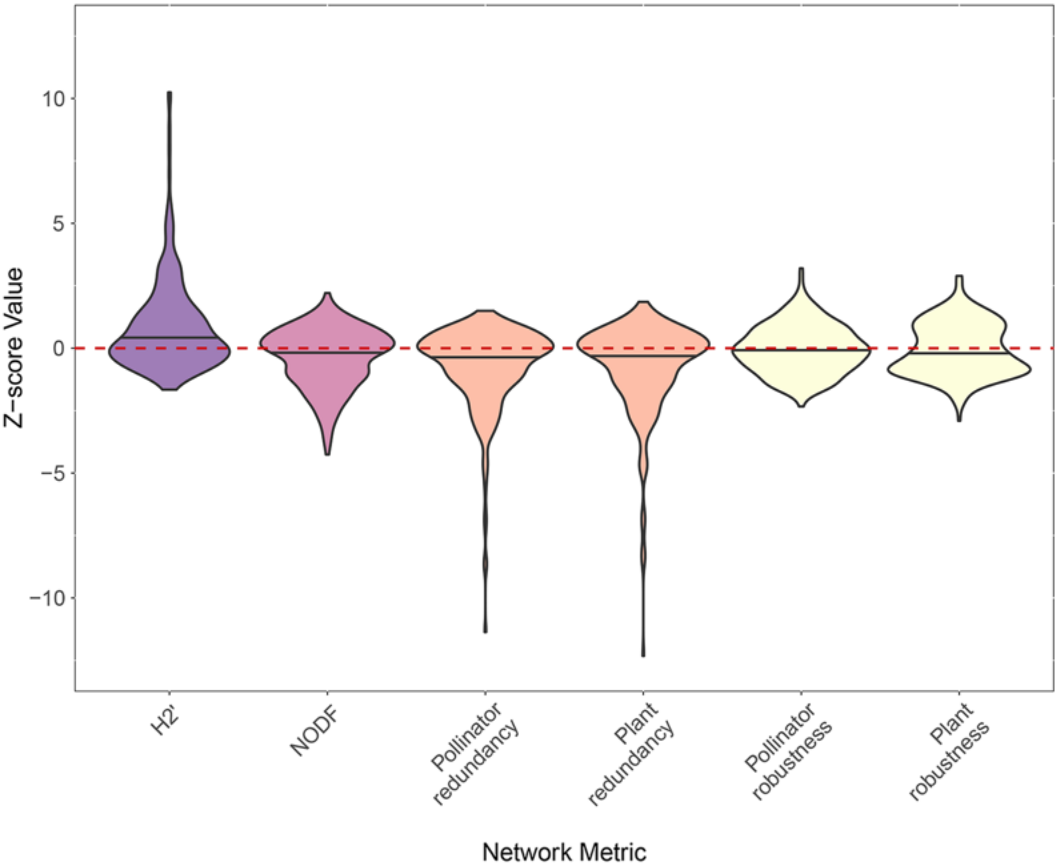

Fig. 3. Distribution of *z*-scores of network-level metrics comparing networks where individuals within species were split by sex to networks where individuals were split randomly. The solid black lines within each distribution represent the mean value for that metric ($N = 256$ networks). The dashed red line is at zero. Positive *z*-score values suggest that that metric is larger than expected based on the distribution of null networks; negative *z*-scores suggest that the metric is smaller than would be expected.

for differences in the validation model. This may suggest that that offspring nutritional demands on females may be a stronger driver of generalization than opportunism is for foraging males. Although contrary to previous studies showing that females are more specialized than males in some pollinator taxa (Ne'eman et al. 2006, Ostevik et al. 2010), our results are consistent with expectations of higher demands for floral rewards by females due to offspring provisioning requirements. These nutritional demands cause females to gather more rewards per visit and per foraging bout (Wolf and Moritz 2014), and gather specific nutrients from flowers for their developing offspring (e.g., Rusterholz and Erhardt 2000). Given that pollen can differ in nutrient composition between plant species (Roulston and Cane 2000), gathering appropriately mixed nutrients may also require females to visit more flower species than males who are primarily interested in nectar as flight fuel (Svensson 1979). Female pollinators also had 7% lower *closeness* than males, suggesting that they visited less well-connected plants than males. This pattern may be the result of males focusing more of their attention on abundant, easily accessible, and frequently visited flowers.

The differences in male and female network roles were also associated with differences in the structure of the networks as a whole. As all comparisons were made within a given network and to simulated networks where species identities and abundances were maintained, and no significant differences were observed with the

Table 3. Model details for analyses of network-level metrics.

Metric	Model Fixed effects	Output				
		Term	Estimate	SE	*t*	*P*
NODF	NODF ~ data set	(Intercept)	−0.38222	0.07763	−4.924	**1.53E-06**
		Data set (SI)	−0.03882	0.27718	−0.14	0.889
		Data set (YO)	0.38222	1.1925	0.321	0.749
H2′	H2 ~ data set	(Intercept)	0.9081	0.1397	6.5	**4.26E-10**
		Data set (SI)	0.8325	0.4989	1.669	0.0964
		Data set (YO)	−0.9081	2.1463	−0.423	0.6726
Redundancy—Pollinators	Generality ~ data set	(Intercept)	−0.752	0.126	−5.97	**7.97E-09**
		Data set (SI)	−0.7984	0.4497	−1.775	0.0771
		Data set (YO)	0.752	1.9349	0.389	0.6979
Redundancy—Plants	Vulnerability ~ data set	(Intercept)	−0.7165	0.1202	−5.963	**8.29E-09**
		Data set (SI)	−0.6721	0.4291	−1.566	0.118
		Data set (YO)	0.7165	1.8459	0.388	0.698
Robustness—Pollinators	robust.HL ~ data set	(Intercept)	−0.05865	0.06846	−0.857	3.92E-01
		Data set (SI)	0.10566	0.24445	0.432	0.666
		Data set (YO)	−1.25715	1.05169	−1.195	0.233
Robustness—Plants	robust.LL ~ data set	(Intercept)	−0.04814	0.06332	−0.76	4.48E-01
		Data set (SI)	0.12046	0.22609	0.533	0.595
		Data set (YO)	−0.75485	0.97272	−0.776	0.438

Note: Values in boldface are significant at $P < 0.05$.

validation data, these patterns reflect true structural differences driven by sex-associated behavior and are not simply the consequence of dividing species nodes or differences in network size. Networks where species were separated by sex tended to be more specialized than networks where species were separated randomly, with nodes showing high selectivity in their partners (H2′). These patterns reflect the differences in the identity of plants visited by males and females (M-H): Each sex focused their foraging effort on non-overlapping plant species, resulting in higher network-level specialization than expected by chance. Networks accounting for sex were also less nested (NODF) than the randomized networks, presumably because the more specialized nodes interacted with other specialists rather than with a core group of generalists. Networks with lower nestedness and higher specialization have been predicted to be more vulnerable to perturbations (Song et al. 2017). Indeed, we saw less *redundancy* in interaction partners for both plant- and pollinator-level nodes, leaving them with fewer redundant partners if any species went locally extinct. Despite this, we found no strong differences between observed and simulated networks in the *robustness* of either plants or pollinators to secondary extinction cascades. While other studies have also found little support for the connections between network nestedness and robustness (CaraDonna et al. 2017), our results suggest that differences in floral foraging by males and females do not make plant–pollinator networks more (or less) prone network architecture change in the face of disturbance. Given that the presence and abundance of males and females of a given species are inherently linked, this may be because the observed differences in partners between the sexes intrinsically complement each other, reducing the destabilizing effects of higher specialization.

Magnitude of differences and variability

Together, the results of our simulations imply that there are meaningful differences associated with pollinator sex that influence network role and that these differences are large or common enough to influence network structure. It is also clear from the shape of the *z*-score distributions (Fig. 2); however, that pollinator species vary extensively in the degree to which males and females differ, with many species showing differences between the sexes that were relatively small and did not differ significantly from null expectations. A variety of factors, such as floral specialization and taxonomy (Minckley and Roulston 2006), likely contribute to the magnitude of sex differences within each species. Indeed, we found that females in species that were more generalized (i.e., had higher *degree* across *all* interactions within a data set) had higher *degree*, lower *closeness*, and larger differences in visit composition compared to males. This result is consistent with the general expectation of higher within-species variability in more generalized species (Bolnick et al. 2007) and implies for our data sets that females being more generalized than males is most common among already generalist species. This also makes intuitive sense, as specialists can have adaptations to preferentially collect or digest certain resources (Praz et al. 2008), and therefore may have fewer of the nutritional incentives to gather rewards from diverse plants than females in generalist species. The pattern remains, however, that many pollinator species in our sample show no significant differences between the sexes despite expectations that these patterns would be common (Smith et al. 2019). While the specific mechanisms underlying this pattern remain to be answered in future work, we propose two non-mutually exclusive hypotheses.

First, in natural plant communities with limited floral richness the sexes may be constrained to behave, or appear to behave, in similar ways. If there are few plant species blooming, intuitively there may be little room for male and female network roles to be measurably different, especially when the number of males and females captured is low. Pollinator sampling in many networks was limited, and many of the communities we investigated had limited floral richness (mean 8.14 plant species). While our methods control for network size by comparing observed networks to simulations with the same dimensions, dissimilarity metrics such as M-H are positively related to sample size, and plant number had small but significant effects on *closeness* and *d′*. Lower pollinator sample size within each of our networks (a single site within a single year) may explain why the magnitude of sex-associated differences we found in M-H distance was smaller than those found by Roswell et al. (2019) in New Jersey (USA) meadows, where multiple sites were pooled together to form networks. It is important to note, however, that these potential sampling effects on the *magnitude* of differences between the sexes do not discount that a clear difference is present which would likely appear even stronger with increased sampling. We would also argue that the small networks used in this study accurately represent the plant–pollinator communities at our sites, which were often small discrete communities (hedgerows, meadows, and burned forest patches) that had few floral species blooming concurrently. Thus, in many natural plant communities the sexes may differ little in their realized foraging patterns, even if they would differ if given adequate choices. Studies of sex differences in larger natural networks would be an important test of this possibility. Interestingly, male pollinators from the most diverse region (SI) tended to have higher relative *closeness* than females, but data set source otherwise did not significantly affect network role.

Alternatively, some of the sex differences that affect foraging may affect behavior at the bout-level or across bouts. These differences would be less apparent when networks are based on single-visit observations, where each individual pollinator was observed only on the one flower on which it was collected. For example, one frequently observed difference between the sexes is that males are more dispersive than females, moving between patches more frequently and flying longer distances (Wolf et al. 2012). This difference may influence the quality of pollination service provided by males (e.g., frequency of outcrossing or heterospecific pollen transfer; Smith et al. 2019), but would not be apparent if each individual is observed visiting only a single flower. Furthermore, the visitation patterns and specialization of individuals may differ over time (i.e., within-individual variation, Bolnick et al. 2003), such as in bees where individuals focus on gathering specific resources during a single bout or run of bouts, but occasionally switch the focal resource between bouts (Russell et al. 2017). This type of bout-level specialization could allow individuals (e.g., females) to gather diverse resources while still gaining the efficiency benefits of specializing (Smith et al. 2019). Future work that complements our community-level approach by examining different facets of foraging, such as these bout-level behaviors, with direct experiments would be particularly valuable. Furthermore, integrating this information into networks, such as by constructing networks using strings of visits for each individual, rather than a single visit, would be a critical step understand how sex-associated differences influence their interactions with plants.

CONCLUSIONS

Our results suggest that accounting for sex-associated differences, and including males in networks, can have effects on plant–pollinator network structure, and is critical to accurately capture natural interactions. Future work examining taxonomic patterns and the context dependency of sex differences more deeply would also greatly improve our understanding of when differences in network roles are likely to have the largest impacts. More broadly, our results suggest that intraspecific variation can affect community function by changing the identity and strength of interactions within networks. The importance of intraspecific variation on community processes is increasingly apparent, but to our knowledge such effects have not been reported for mutualistic communities or for variation associated with sex. Empirically examining the consequences of sex-level interaction differences will greatly improve our ability to accurately estimate network resistance to perturbations, and thereby improve our ability to conserve plant–pollinator communities in the face of environmental change.

ACKNOWLEDGMENTS

We would like to thank Magda Argueta, Lindsay Cook, Julie Fowler, Marilia Gaiarsa, Kelly Kulhanek, Jess Mullins, Hanna Scardina, Kaysee Arrowsmith, and the Kremen laboratory for their help gathering field samples. We would also like to thank Robbin Thorp and John Asher for help identifying specimens. This work was supported by funding from the Foundation for Food and Agriculture grant CA18-SS-0000000009 to LCP. Author Contributions: GPS and LCP designed the study and simulations. GPS wrote the simulations and analyzed the results. LCP collected the data. J. Gardener, J. Gibbs, TG, MH, and DY identified the 30,704 insect specimens to species or morpho species. GPS wrote the first version of the manuscript, and all authors contributed to revisions.

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DATA AVAILABILITY

Data are available from Zenodo: https://doi.org/10.5281/zenodo.5517942

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3863/full