



Active pollination drives selection for reduced pollen-ovule ratios

Olle Pellmyr, Finn Kjellberg, Edward Allen Herre, Atsushi Kawakita, David H Hembry, J. Nathaniel Holland, Teresa Terrazas, Wendy Clement, Kari A Segraves, David M Althoff

► To cite this version:

Olle Pellmyr, Finn Kjellberg, Edward Allen Herre, Atsushi Kawakita, David H Hembry, et al.. Active pollination drives selection for reduced pollen-ovule ratios. *American Journal of Botany*, 2019, 107 (1), pp.164-170. 10.1002/ajb2.1412 . hal-02922024

HAL Id: hal-02922024

<https://hal.science/hal-02922024>

Submitted on 25 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Active pollination drives selection for reduced pollen-ovule ratios

Olle Pellmyr^{1,†}, Finn Kjellberg², Edward Allen Herre³, Atsushi Kawakita⁴, David H. Hembry^{5,6}, J. Nathaniel Holland⁷, Teresa Terrazas⁸, Wendy Clement⁹, Kari A. Segraves¹⁰, and David M. Althoff^{10,11}

1Department of Biology, University of Idaho, Moscow, Idaho 83844, USA

2CEFE, CNRS, Université Montpellier, Université Paul Valéry Montpellier, EPHE, IRD, Montpellier Cédex 5, France

3Smithsonian Tropical Research Institute, Apartado Postal, 0843-03092, Panamá, Republic of Panama

4The Botanical Gardens, Graduate School of Science, University of Tokyo, 3-7-1 Hakusan, Bonkyo-ku, Tokyo, Japan

5Department of Entomology, Cornell University, 2130 Comstock Hall, Ithaca, New York 14853, USA

6Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, USA

7School of Dentistry, University of Texas, 7500 Cambridge Street, Houston, Texas 77054, USA

8Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

9Department of Biology, The College of New Jersey, 2000 Pennington Road, Ewing, New Jersey 08628, USA

10Department of Biology, Syracuse University, 107 College Place, Syracuse, New York 13244, USA

11Author for correspondence (e-mail: dmalthof@syr.edu)

† Olle Pellmyr passed away before publication.

Citation: Pellmyr, O., F. Kjellberg, E. A. Herre, A. Kawakita, D. H. Hembry, J. N. Holland, T. Terrazas, et al. 2020. Active pollination drives selection for reduced pollen-ovule ratios. *American Journal of Botany* 107(1): 164–170. doi:10.1002/ajb2.1412

PREMISE: Variation in pollen-ovule ratios is thought to reflect the degree of pollen transfer efficiency—the more efficient the process, the fewer pollen grains needed. Few studies have directly examined the relationship between pollen-ovule ratio and pollen transfer efficiency. For active pollination in the pollination brood mutualisms of yuccas and yucca moths, figs and fig wasps, senita and senita moths, and leafflowers and leafflower moths, pollinators purposefully collect pollen and place it directly on the stigmatic surface of conspecific flowers. The tight coupling of insect reproductive interests with pollination of the flowers in which larvae develop ensures that pollination is highly efficient.

METHODS: We used the multiple evolutionary transitions between passive pollination and more efficient active pollination to test if increased pollen transfer efficiency leads to reduced pollen-ovule ratios. We collected pollen and ovule data from a suite of plant species from each of the pollination brood mutualisms and used phylogenetically controlled tests and sister-group comparisons to examine whether the shift to active pollination resulted in reduced pollen-ovule ratios.

RESULTS: Across all transitions between passive and active pollination in plants, actively pollinated plants had significantly lower pollen-ovule ratios than closely related passively pollinated taxa. Phylogenetically corrected comparisons demonstrated that actively pollinated plant species had an average 76% reduction in the pollen-ovule ratio.

CONCLUSIONS: The results for active pollination systems support the general utility of pollen-ovule ratios as indicators of pollination efficiency and the central importance of pollen transfer efficiency in the evolution of pollen-ovule ratio.

KEY WORDS mating systems; pollen production; pollination; sister-group comparisons.

The allocation of resources to gamete size and number has direct implications for the fitness of an organism. In particular, the trade-off between the sizes of male and female gametes has been a driving force in the evolution of separate sexes, male and female reproductive strategies, and sexual selection in plants and animals (Charnov, 1982; Kokko and Jennions, 2008; Janicke et al., 2016; Lehtonen et al., 2016). Female gametes are usually larger and less mobile than male gametes, and this difference drives much of the sexual selection dynamics in terms of mate choice and competition for mates (Janicke et al., 2016). In hermaphroditic species, there is strong selection to optimize the number of each gamete type in response to reproductive opportunities for both female and male aspects of reproduction (Beekman et al., 2016). For example, in plants, the ratio of

pollen to ovules within a plant is expected to reflect the optimal reproductive success of producing both pollen and ovules (Cruden, 1977; Charnov, 1982). In the case of ovule production, this is primarily set by the resources available for the relatively costly production of large ovules. By contrast, pollen production is under selection to maximize the number of ovules that will be fertilized. In the case of pollen, then, this creates a race for fertilization opportunities that is determined by the ability of pollen grains to reach stigmas and then compete with other pollen grains to fertilize ovules (Cruden, 2000).

The pollen-ovule ratio, a phenotype that includes changes in both pollen production and ovule production, is determined by the result of selection on many aspects of plant reproduction. Cruden (1977), for instance, used data from 28 plant families to demonstrate that mating system is highly correlated with pollen- ovule ratios. Selfing species had lower pollen-ovule ratios than outcrossing species. Moreover, pollination mode may also influence the evolution of pollen-ovule ratios. Species that use animal vectors to move pollen are more likely to have lower pollen-ovule ratios due to the higher probability of successful pollen transfer (Cruden, 2000). Shifts in pollen-ovule ratios are usually the result of changes to pollen number rather than ovule number (Cruden, 1977). In some cases, however, changes in ovule number may also occur, especially if there is large variance in the probability of fertilization (Burd et al., 2009). For example, the ovule oversupply hypothesis suggests that plants may increase ovule production in response to unpredictability in pollen availability (Rosenheim et al., 2016). Similarly, the distribution of flowers and the number of ovules across an inflorescence may also be under selection to maximize pollen deposition (Friedman and Barrett, 2011).

Although there are many factors that will influence the evolution of pollen-ovule ratios, pollen transfer efficiency is one that will likely have a major effect. Pollen transfer efficiency represents several steps and has different components, such as how much pollen is wasted loading onto the pollinator body, the probability of successful transfer to conspecific flowers, and the quantity of pollen deposited. In addition, there have been many interpretations of pollen transfer efficiency (see Ne'eman et al., 2010). Here, we focus on the probability of pollen grains reaching stigmatic surfaces of conspecific, receptive flowers. If more pollen grains successfully reach conspecific stigmas, fewer are wasted, and thus a smaller number of grains in general are needed to maximize fertilization probabilities. Many studies of the relationship between pollen-ovule ratio and the types of pollination mode with differing transfer efficiency have, by necessity, been at higher taxonomic levels in order to encompass shifts in pollination mode. One of the major assumptions is that shifts in pollination mode result in shifts in pollination efficiency. Gong and Huang (2014) provided the first empirical study to measure pollen transfer efficiency in animal-pollinated plants and its relationship to pollen-ovule ratio. They empirically

measured pollen transfer efficiency (the proportion of pollen removed from anthers that reaches conspecific stigmas) of 26 phylogenetically diverse species of meadow-dwelling plants that are insect pollinated and detected a negative correlation between pollen transfer efficiency and pollen-ovule ratio. Species with high levels of pollen transfer efficiency had low pollen-ovule ratios even when correcting for phylogeny across the diverse taxa studied. These results highlight the importance of focusing on pollen transfer efficiency itself when examining pollen-ovule ratios.

Here, we take advantage of shifts in plants from passive pollination to active pollination to test the relationship between pollen transfer efficiency and pollen-ovule ratio. In nearly all cases of insect pollination, the movement of pollen among flowers is a passive process in which insects accumulate pollen from anthers, and plants indirectly collect pollen from the body of the insect as it moves around a flower and contacts the stigma. Active pollination behavior, by contrast, occurs when the insect purposefully collects and deposits pollen on the stigmatic surface through specific behaviors not related to nectaring or resource collection within the flower. Active pollination has evolved in yucca moths (Riley, 1892; Pellmyr, 2003), fig wasps (Ramirez, 1969; Weiblen, 2002; Herre et al., 2008), leafflower moths (Kato et al., 2003; Kawakita and Kato, 2004), and senita moths (Fleming and Holland, 1998; Holland and Fleming, 1999). Recently, active pollination has also been discovered in weevils that pollinate orchid species (Nunes et al., 2018) and in heliozelid moths that pollinate *Boronia* (Rutaceae) in Australia (Milla, 2019). In all of these species, females actively collect pollen from flowers via specialized behaviors and morphologies. A female deposits eggs into or near the plant ovary and seeks out the stigmatic surface to actively place pollen. The larvae will then feed on the developing seeds fertilized by their mother. Thus, there is a high premium on females to be effective pollinators. These interactions are also highly specialized in that the pollinator species usually use a single plant species at a locality and the chances of losing pollen through interspecific pollen transfer are extremely low. In addition, most of these systems do not have any co-pollinators. For these reasons, the overall probability of pollen transfer per flower visitation will be much higher in actively than in passively pollinated species, and pollen will go to conspecific flowers. Whether active pollination translates into overall increases in the number of pollinated flowers across the whole plant in comparison to passive pollination is unclear and needs further research.

We would expect that if pollen transfer efficiency is an important factor driving the evolution of pollen-ovule ratios, actively pollinated plant species should have significantly reduced ratios in comparison to passively pollinated species. The origins of active pollination in insects have been accompanied by multiple shifts from

the ancestral condition of passive pollination to active pollination in the host plant lineages (Hartmann et al., 2002; Arias and Terrazas, 2006, 2009; Smith et al., 2008; Kawakita and Kato, 2009; Cruaud et al., 2012). To test this idea, we used sister-group comparisons and phylogenetically controlled comparisons among actively and passively pollinated plant groups for each of the independent shifts to active pollination in yuccas, figs, leafflowers, and senita cactus.

MATERIALS AND METHODS

SPECIES STUDIED

The major groups of plants that have shifted to active pollination are yuccas (*Yucca* and *Hesperoyucca*), figs (single genus), leafflowers (multiple genera), and senita cactus (*Lophocereus schottii*). Based on both phylogenetic data and gamete production data, all of these groups except senita cactus contain multiple independent shifts to active pollination. For yuccas, there were independent shifts to active pollination for the genera *Yucca* and *Hesperoyucca* and we analyzed these separately. Within figs, there have been at least five transitions from passive to active pollination (Cruaud et al., 2012). We analyzed these in a single phylogenetically corrected analysis, because we had single-point estimates for each species (see below). For leafflowers, we had limited data for species in three independent shifts to active pollination and we examined each shift separately.

We used flower buds to make direct counts of pollen-ovule ratios for actively pollinated species of *Yucca* (30 species) and *Hesperoyucca whipplei* and passively pollinated species from their sister groups, *Agave* (four species) and *Hesperaloe* (three species) (Appendix S1). We used the phylogenetic analyses of Smith et al. (2008) as the basis for identifying sister groups. Similarly, we obtained pollen-ovule ratios from flower buds for the actively pollinated *L. schottii* and its passively pollinated sister species *L. marginatus* as identified by the phylogenetic analyses of Hartmann et al. (2002) and Arias and Terrazas (2006, 2009).

For leafflower plants, we used direct counts from flower buds to obtain pollen-ovule ratios. For three inferred independent shifts to active pollination according to the phylogenetic analyses of Kawakita and Kato (2009), we sampled pairs of one actively pollinated and one passively pollinated species: (1) *Glochidion acuminatum* (active) and *Phyllanthus roseus*, (2) *Breynia vitis-idaea* (active) and *Sauropus quadrangularis*, and (3) *P. reticulatus* (active) and *P. flexuosus* (Appendix S2). We also obtained pollen-ovule ratios from the passively pollinated species *P. liukiuensis* and *S. garettii*.

We used data on figs from Cruden (1997) and Kjellberg (unpublished data) to obtain pollen-ovule ratios for 15 actively pollinated *Ficus* species and six passively pollinated species. This is a phylogenetically diverse set of *Ficus* species, and it was not possible to make phylogenetically corrected comparisons because the phylogenetic relationships among the species are unclear (due to lack of data) and they are likely very distantly related. Instead, we used the anther-ovule ratio for 142 *Ficus* species reported by Kjellberg et al. (2001) and the *Ficus* phylogeny from Cruaud et al. (2012) to make phylogenetically corrected comparisons of anther-ovule ratios. There were 75 fig species that overlapped between the data in Kjellberg et al. (2001) and the phylogeny from Cruaud et al. (2012).

POLLEN COUNTS

Pollen samples were prepared by placing the contents of one anther sac in polyvinyl alcohol mounting medium (BioQuip, Gardena, California, USA) on a glass slide and performing a visual count using a binocular light microscope. The number of pollen grains per anther sac was then multiplied by the number of anthers within the flower. We also counted the total number of ovules for the flower through visual counts after dissection of the pistil. *Yucca*, *Hesperoyucca*, *Hesperaloe*, *Agave*, and *Lophocereus* have hermaphroditic flowers. In the leafy flower species studied, separate male and female flowers occur on the same plant and all female flowers have six ovules. We counted the male and female flowers on each plant and multiplied the pollen-ovule ratio per flower by the male-to-female flower ratio to determine the plant-level pollen-ovule ratio. In actively pollinated figs, the fig is a compound inflorescence that can contain both male and female flowers. We counted the number of male flowers and the number of pollen grains per male flower and divided the total pollen production per fig by the number of female flowers. Anther-ovule ratios were calculated by Kjellberg et al. (2001) by counting the number of anthers and female flowers (each with one ovule) within a fig.

TABLE 1. Comparisons of pollen-ovule ratios across shifts to active pollination. Results for *Yucca*, *Hesperoyucca*, *Lophocereus*, and leafflowers are from sister-group comparisons. Results for both uncorrected and phylogenetically corrected comparisons (PGLS) are given for figs. Sampling within leafflowers precluded statistical tests between sister species, but results are presented for completeness.

Taxa	Pollination mode	Species	Pollen-ovule ratio	Statistical test
Yuccas and allies	Active	<i>Yucca</i> (30 spp.)	44 ± 1.9	$F_{1, 31.62} = 6.00, P < 0.02$
	Passive	<i>Agave</i> (4 spp.)	128 ± 6.7	
	Active	<i>Hesperoyucca whipplei</i>	28 ± 1.5	$F_{1, 1.80} = 35.04, P < 0.01$
Senita cactus	Passive	<i>Hesperaloe</i> (3 spp.)	134 ± 6.1	
	Active	<i>Lophocereus schottii</i>	607 ± 120	$F_{1, 32} = 5.97, P < 0.02$
	Passive	<i>Lophocereus marginatus</i>	3311 ± 345	
Leafflowers	Active	<i>Glochidion acuminatum</i>	542 ± 103	Active 2.4× lower
	Passive	<i>Phyllanthus roseus</i>	1308 ± 249	
	Active	<i>Breynia vitis-idaea</i>	469 ± 51	Active 6× lower
	Passive	<i>Sauropus</i> spp.	3012 ± 1739	
Figs	Active	<i>Phyllanthus reticulatus</i>	663 ± 469	Active 11× lower
	Passive	<i>Phyllanthus flexuosus</i>	7504 ± 690	
	Phylogenetically uncorrected analyses from across <i>Ficus</i> genus			$\chi^2 = 7.85, P < 0.005$
	Active	15 species	8013 ± 8012	
	Passive	6 species	36,262 ± 6883	
	Anther-ovule ratio for 75 <i>Ficus</i> species			$F_{1, 139} = 397.5, P < 0.001$
	Active	56 species	0.077 ± 0.003	
	Passive	19 species	0.625 ± 0.040	
	Phylogenetically corrected test:		PGLS, $\beta_{\text{passive}} = 0.51$	$F_{1, 73} = 9.4, P = 0.003$

ANALYSES

For the sister-group comparisons of *Yucca* with *Agave* and *Hesperoyucca* with *Hesperaloe*, we treated species as a random factor and pollination mode as a fixed factor and used REML to test for the effects of pollination mode (sample sizes are presented in Table 1 for all plant species). For the *L. schottii* and *L. marginatus* comparison, we tested for differences with analysis of variance. For leafflowers, the data did not contain sufficient replication to run statistical tests between sister species within each shift to active pollination, but we

present these results for completeness. We used a Wilcoxon test to examine pollen-ovule ratios in figs for which we could find data. We examined the changes in anther-ovule ratio for figs with phylogenetic generalized least squares as outlined in Paradis (2012) and a Brownian model of trait evolution as the correlation matrix. The fig phylogeny was trimmed to include only the taxa for which we had pollen and ovule production data. Analyses were performed in JMP version 9.0.2 (SAS 2010) and the package “ape” in R (R Core Team, 2017).

RESULTS

Sister-group comparisons for yuccas, *Hesperoyucca*, and senita cactus demonstrated that actively pollinated species had significantly lower pollen-ovule ratios than closely related passively pollinated species (Table 1; Fig. 1; Appendix S2). For leafflowers, actively pollinated species paired with their closest passively pollinated species had more than a twofold reduction in pollen-ovule ratios. Actively pollinated figs also had lower pollen-ovule ratios than passively pollinated species (phylogenetic correction was not possible). Similarly, phylogenetically corrected analyses demonstrated that actively pollinated fig species had lower anther-ovule ratios than passively pollinated species (Fig. 1). Across the plant lineages, actively pollinated species had a mean (\pm SE) reduction in the pollen-ovule ratios of $76.5 \pm 0.01\%$ (Fig. 2). The anther-ovule ratio in figs was reduced by 88%.

DISCUSSION

Cruden (1977) noticed that pollen-ovule ratios can vary dramatically across plants with different pollination syndromes and do so in an apparently consistent way. Animal-pollinated plants generally exhibit lower pollen-ovule ratios than wind-pollinated plants, and inbred plants generally exhibit lower pollen-ovule ratios than outcrossed plants. Cruden (1977) interpreted this general pattern as reflecting the response to selection for reduced investment in pollen in species with higher efficiency in pollen transfer to conspecific stigmatic surfaces. Although this interpretation seems to be largely consistent with observations, there are exceptions. For example, Friedman and Barrett (2009) measured pollen transfer in several wind-pollinated herbs and demonstrated that transfer efficiencies were equal to those in many animal-pollinated species, even though the herbs had high pollen-ovule ratios. They argued that high pollen-ovule ratios in these wind-pollinated taxa may be due to mate competition through pollen, not low pollen transfer efficiency. Several authors have correctly emphasized the fact that there are potentially confounding factors, and more rigorous tests that provide tighter control of such factors are needed (Ackerman, 2000; Erbar and Langlotz, 2005; Burd, 2011).

Here, we provide such control by focusing exclusively on plant taxa that possess obligate, highly specialized, animal pollinators and that differ in whether pollination is passive or active. We used the independent shifts from passive pollination to active pollination within yuccas and allies, senita cactus, figs, and leafy flowers to examine if transfer efficiency has a significant effect on pollen-ovule ratio. The evolution of active pollination produced pollen vectors that are highly specific and that purposefully place the pollen grains on the stigmatic surface of conspecific flowers (Riley, 1892; Ramirez, 1969; Fleming and Holland, 1998; Kato et al., 2003). This process represents the most directed animal pollination system known in nature and should select for reduced plant investment in pollen production. Given that the shift to active pollination

in plants has arisen at least 10 separate times, these independent origins provide the ability to test whether active pollination has selected for reduced investment in pollen production compared to ovule production.

As expected, all instances of active pollination resulted in reduced pollen-ovule ratios for actively pollinated species in comparison to closely related passively pollinated species. This result was statistically significant for all lineages in which tests could be conducted. The reduction in pollen-ovule ratios across all lineages was large, with a more than 76% reduction for each shift. For the hermaphroditic flowers of yuccas, *Hesperoyucca*, and senita cactus, the decrease in pollen-ovule ratio was due to a significant decrease in pollen production per flower, as much as a threefold reduction, with little change in ovule number. The reductions in figs and leafy flowers are more complex, given the separate male and female flowers on many species in both lineages. Both of these groups, however, have a consistent and low ovule number per flower, one for figs and six for the leafy flowers used in this study. Thus, changes in pollen number by either production per anther, the number of anthers, or the number of male flowers led to reductions in the pollen-ovule ratio.

While our conclusions are limited by the number of independent shifts to active pollination, the reduction in pollen production in actively pollinated plant species is substantial and supports the idea that pollen transfer efficiency has a major effect on pollen-ovule ratios. As more data on the phylogenetic breadth of the weevil–orchid system and the heliozelid moth–*Boronia* accumulate, these systems can also be tested for shifts in pollen-ovule ratios. Even so, the results raise the question of why actively pollinated plant species still produce relatively large amounts of pollen, particularly in figs and leafy flowers in which ovule number is very low but thousands of pollen grains are still produced per flower (>23,000 and >26,000, respectively). We might expect that active pollen collection and substantial, directed pollen deposition per pollination event might select for very low numbers of pollen grains produced per flower. For figs, previous work has shown that pollen may be limiting on a per fig basis, partly due to the number of female flowers per fig, the amount of pollen that each

pollinator can carry, and the number of female wasps that enter a fig (Kjellberg et al., 2014). For leafflowers, however, the low ovule number per each flower would suggest that pollen production could be quite low and still be large enough for fertilizing a large number of ovules, all else being equal.

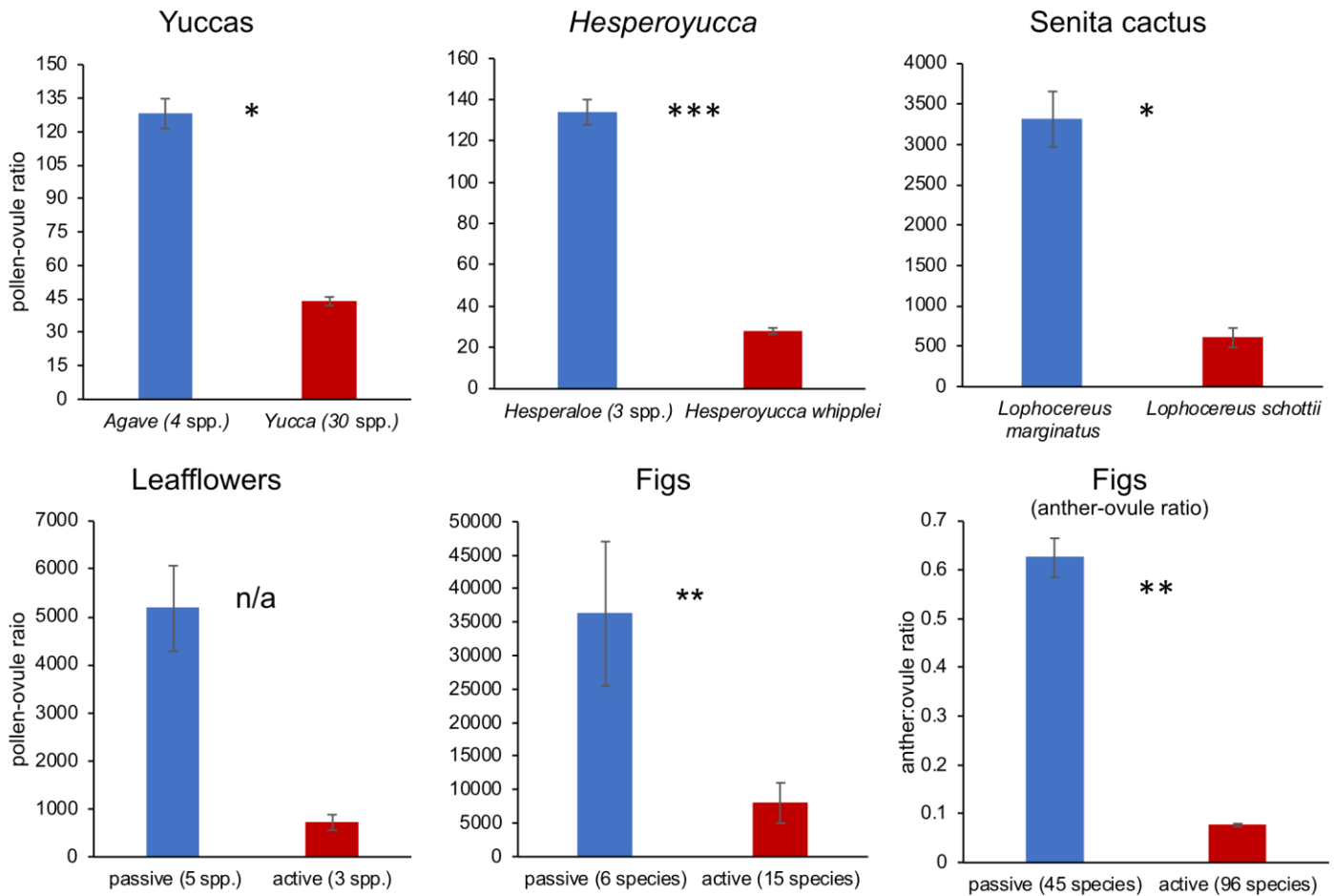


FIGURE 1. Comparisons of pollen-ovule ratios for each of the actively pollinated plant lineages. Results for yuccas, *Hesperoyucca*, and senita cactus are from sister-group comparisons. Sampling for leafflowers was too incomplete to run statistical tests, but results combined for all three shifts are presented for completeness. The result of the phylogenetically corrected test for fig anther-ovule ratio is presented in Table 1.



FIGURE 2. Comparison of pollen-ovule (P:O) ratios from actively pollinated taxa and passively pollinated taxa for each major plant group with active pollination. Line represents equality of ratios. nated plant species still produce relatively Actively pollinated taxa have more than a 75% reduction in pollen-ovule ratios.

The copious amounts of pollen production per flower in actively pollinated plant species further highlight the role of additional factors in determining pollen-ovule ratios. For example, sexual selection might contribute to greater pollen production even though transfer efficiency is high in actively pollinated species, similar to the conclusions of Friedman and Barrett (2009). In addition, multiple insects may pollinate the same flower, which could lead to male–male competition for ovules and female choice for pollen quality. Again, deposition of extra pollen grains may be advantageous. There are currently no studies of male–male competition or female choice in any actively pollinated plant species, but such studies could help shed light on the evolution of pollen-ovule ratios in these plant lineages. In addition, there are no data on the longevity of pollen grains once they are collected by the insects, the mortality rates of the pollinators, or the loss of pollen to other insects that may feed on pollen. If pollinators with collected pollen are likely to perish before completing pollen transfer or if the pollen loses viability quickly, this may select for plants that produce more pollen grains than expected based on the pollen transfer efficiency. Similarly, pollen loss due to other biotic or abiotic sources could select for increased pollen production.

CONCLUSIONS

In conclusion, comparisons of closely related passively and actively pollinated plant species indicate that increases in pollen transfer efficiency lead to the evolution of lower pollen-ovule ratios. This factor alone,

however, does not explain the evolution of pollen-ovule ratios in actively pollinated plant species. Once pollen transfer efficiency reaches its maximum for a species, other factors will begin to have more of an effect. Burd (2011) argues that understanding the evolution of pollen-ovule ratios is going to require integrating the results of selection across many traits rather than relying on a single explanation. This is also true for actively pollinated plant species.

ACKNOWLEDGMENTS

This work was the result of Olle Pellmyr's interest in the evolutionary and ecological consequences of active pollination systems. He conceived the idea for the manuscript in 2009 and continued to collect specimens and data from colleagues across the globe until 2011. Unfortunately, he was not able to analyze the data before passing away in 2018. All the authors will fondly remember Olle for demonstrating the importance of natural history, his insightfulness and razor-sharp wit, and his continued support and camaraderie as we explored insects and pollination biology together over the years. We thank T. Anneberg, A. Cure, M. Vidal, and S. Wang for discussions and comments on the manuscript. We also thank J. Williams and three anonymous reviewers for their excellent comments for improving the manuscript. This work was partially supported by National Science Foundation DEB 1556568 and 1655544 to D.M.A. and K.A.S.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. List of taxa and sample sizes used to examine pollen-ovule ratio in actively pollinated plant lineages.

APPENDIX S2. List of taxa and pollen number, ovule number, and pollen-ovule ratio (mean \pm SE) used to examine pollen-ovule ratio in actively pollinated and passively pollinated plant lineages.

LITERATURE CITED

- Ackerman, J. D. 2000. Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Systematics and Evolution* 222: 167–185.
- Arias, S., and T. Terrazas. 2006. Análisis cladístico del género *Pachycereus* (Cactaceae) con caracteres morfológicos. *Brittonia* 58: 197–216.
- Arias, S., and T. Terrazas. 2009. Taxonomic revision of *Pachycereus* (Cactaceae). *Systematic Botany* 34: 68–83.
- Beekman, M., B. Nieuwenhuis, D. Ortiz-Barrientos, and J. P. Evans. 2016. Sexual selection in hermaphrodites, sperm and broadcast spawners, plants and fungi. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences* 371: 20150541.
- Burd, M. 2011. Are relationships between pollen-ovule ratio and pollen and seed size explained by sex allocation? *Evolution* 65: 3002–3005.

- Burd, M., T. Ashman, D. R. Campbell, M. R. Dudash, M. O. Johnston, T. M. Knight, S. J. Mazer, et al. 2009. Ovule number per flower in a world of unpredictable pollination. *American Journal of Botany* 96: 1159–1167.
- Charnov, E. L. 1982. The theory of sex allocation. Princeton University Press, Princeton, NJ.
- Cruaud, A., N. Ronsted, B. Chantarasuwan, L. S. Chou, W. L. Clement, A. Couloux, B. Cousins, et al. 2012. An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. *Systematic Biology* 61: 1029–1047.
- Cruden, R. W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.
- Cruden, R. W. 1997. Implications of evolutionary theory applied to pollination ecology. *Acta Horticulturae* 437: 27–51.
- Cruden, R. W. 2000. Pollen grains: why so many? *Plant Systematics and Evolution* 222: 143–165.
- Erbar, C., and M. Langlotz. 2005. Pollen to ovule ratios: standard or variation—a compilation. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 126: 71–132.
- Fleming, T. H., and J. N. Holland. 1998. The evolution of obligate pollination mutualisms: senita cactus and senita moth. *Oecologia* 114: 368–375.
- Friedman, J., and S. C. Barrett. 2009. Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* 103: 1515–1527.
- Friedman, J., and S. C. Barrett. 2011. The evolution of ovule number and flower size in wind-pollinated plants. *American Naturalist* 177: 246–257.
- Gong, Y. B., and S. Q. Huang. 2014. Interspecific variation in pollen-ovule ratio is negatively correlated with pollen transfer efficiency in a natural community. *Plant Biology (Stuttg)* 16: 843–847.
- Hartmann, S., J. D. Nason, and D. Bhattacharya. 2002. Phylogenetic origins of *Lophocereus* (Cactaceae) and the senita cactus-senita moth pollination mutualism. *American Journal of Botany* 89: 1085–1092.
- Herre, E. A., K. C. Jandér, and C. A. Machado. 2008. Evolutionary ecology of figs and their associates: Recent progress and outstanding Puzzles. *Annual Review of Ecology, Evolution, and Systematics* 39: 439–458.
- Holland, J. N., and T. H. Fleming. 1999. Mutualistic interactions between *Upiga virescens* (Pyralidae), a pollinating seed-consumer, and *Lophocereus schottii* (Cactaceae). *Ecology* 6: 2074–2084.
- Janicke, T., I. K. Haderer, M. J. Lajeunesse, and N. Anthes. 2016. Darwinian sex roles confirmed across the animal kingdom. *Science Advances* 2: e1500983.
- Kato, M., A. Takimura, and A. Kawakita. 2003. An obligate pollination mutualism and reciprocal diversification in the tree genus *Glochidion* (Euphorbiaceae). *Proceedings of the National Academy of Sciences of the United States of America* 100: 5264–5267.
- Kawakita, A., and M. Kato. 2004. Evolution of obligate pollination mutualism in New Caledonian *Phyllanthus* (Euphorbiaceae). *American Journal of Botany* 91: 410–415.
- Kawakita, A., and M. Kato. 2009. Repeated independent evolution of obligate pollination mutualism in the Phyllanthaceae-Epiccephala association. *Proceedings of the Royal Society of London Series B-Biological Sciences* 276: 417–426.
- Kjellberg, F., E. Jousselin, J. L. Bronstein, A. Patel, J. Yokoyama, and J. Y. Rasplus. 2001. Pollination mode in fig wasps: the predictive power of correlated traits. *Proceedings of the Royal Society of London Series B-Biological Sciences* 268: 1113–1121.
- Kjellberg, F., N. Suleman, S. Raja, A. Tayou, M. Hossaert-McKey, and S. G. Compton. 2014. Some pollinators are more equal than others: Factors influencing pollen loads and seed set capacity of two actively and passively pollinating fig wasps. *Acta Oecologica* 57: 73–79.
- Kokko, H., and M. D. Jennions. 2008. Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* 21: 919–948.
- Lehtonen, J., H. Kokko, and G. A. Parker. 2016. What do isogamous organisms teach us about sex and the two sexes? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 371: 20150532.

- Milla, E. 2019. Evolution and ecology of the Australian Heliozelidae (Adeloidea, Lepidoptera). Ph.D. dissertation, University of Melbourne, Melbourne, Australia.
- Ne'eman, G., A. Jürgens, L. Newstrom-Lloyd, S. G. Potts, and A. Dafni 2010. A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews* 85: 435–451.
- Nunes, C. E. P., P. K. Maruyama, M. Azevedo-Silva, and M. Sazima. 2018. Parasitoids turn herbivores into mutualists in a nursery system involving active pollination. *Current Biology* 28: 980–986.
- Paradis, E. 2012. Analysis of Phylogenetics and Evolution with R. Second ed. Springer, New York.
- Pellmyr, O. 2003. Yuccas, Yucca moths, and coevolution: a review. *Annals of the Missouri Botanical Garden* 90: 35–55.
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org/>.
- Ramirez, B. W. 1969. Fig wasps: mechanisms of pollen transport. *Science* 163: 580–581.
- Riley, C. 1892. The yucca moth and yucca pollination. *Annals of the Missouri Botanical Garden* 3: 99–158.
- Rosenheim, J. A., S. J. Schreiber, and N. M. Williams. 2016. Does an ‘oversupply’ of ovules cause pollen limitation? *New Phytologist* 210: 324–332.
- Smith, C. I., O. Pellmyr, D. M. Althoff, M. Balcazar-Lara, J. Leebens-Mack, and K. A. Segraves. 2008. Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. *Proceedings of the Royal Society of London Series B-Biological Sciences* 275: 249–258.
- Weiblen, G. D. 2002. How to be a fig wasp. *Annual Review of Entomology* 47: 299–330.