AMERICAN JOURNAL OF BOTANY

ON THE NATURE OF THINGS: ESSAYS
New Ideas and Directions in Botany

The (relative) importance of pollinator-mediated selection for evolution of flowers[1]

Yuval Sapir[2] and Mahua Ghara

KEY WORDS adaptive peak; environmental stresses; evolvability; fitness-associated floral traits; genetic correlation; phylogenetic constraints; plant–pollinator interactions; random process

POLLINATORS AS AGENTS OF SELECTION ON FLOWERS

Perhaps the most intriguing example, surely the most aesthetic, of plant evolution is the interaction of flowers and their pollinators, which has sparked a burst of studies spanning diverse disciplines, from animal behavior to plant genetics. Starting in the late 1700s (Sprengel, 1793) and emphasized by Darwin (1862), pollination was accepted as the driver of floral variation, making pollinator-mediated selection the major paradigm for flower evolution. Indeed, numerous studies support the hypothesis that adaptation to pollinators commonly contributes to floral diversity (Harder and Johnson, 2009). Pollinators are considered to select not only for a single traits, but also for sets of correlated traits (pollination syndromes; Fenster et al., 2004). Pollinators are also thought to be agents of divergent selection that can lead to speciation and maintain species boundaries, based on divergent trait values (e.g., Schemske and Bradshaw, 1999; Hopkins and Rausher, 2012).

Plant fitness has been quantified in many ways, including pollinator visitation rate, fruit and seed production (female fitness), and seeds sired (male fitness). No matter how fitness is measured, whether female, male, or cumulative fitness, it is used for quantifying natural selection, which is the standardized covariance of a trait with fitness (selection gradient, β) (Lande and Arnold, 1983; Kingsolver et al., 2001). Harder and Johnson (2009) summarized many studies and found ample evidence for natural selection on floral traits. However, the extent to which this selection is mediated by pollinators is still in question. As an example, in their meta-analysis Harder and Johnson (2009) showed that the mean selection gradient was significantly different from zero only when fitness was quantified as seed production, rather than as pollination (see Harder and Johnson, 2009: fig. 4a). While this low average value does not mean that all individual gradients differ significantly from zero, it does raise questions regarding the quantitative value of the selection gradient, which is, on average, very small. We propose that low selection values reflect two possibilities. One possibility is that pollinator-mediated selection is relatively high but is not detected, due to masking by other agents of selection. The other possibility is that pollinator-mediated selection does not exist or is too small to be detected. In this essay, we highlight the two alternatives, and while we do not dismiss pollinators as the major putative drivers of floral evolution, we think that expanding this view to consider other agents of evolution may improve our understanding of the actual role of pollinators in shaping flowers.

NET SELECTION AND ALTERNATIVE AGENTS OF SELECTION

The net selection on pollination-relevant floral traits can be high but not apparent due to larger, contrasting effect of other agents of selection (Strauss and Whittall, 2006). For instance, selection by floral herbivores or abiotic stresses, such as water loss, may counterbalance pollinator-mediated selection and mask it (Teixido et al., 2016). Several studies have tested experimentally the net selection exerted by pollinators by subtracting estimates of selection gradients for plants receiving supplemental hand pollination from estimates obtained for open-pollinated control flowers ($\Delta\beta_{poll}$) (Sletvold et al., 2010; Lavi and Sapir, 2015). This way, the alternative hypothesis (of other selection agents) is tested through the supplementary treatment where pollinators are not affecting fitness by their behavior, and $\Delta\beta_{poll}$ is the surplus selection exerted by them. The role of alternative selection agents could be tested explicitly in a full-factorial experiment. For example, by supplementing nutrients, Sletvold et al.

[1] Manuscript received 14 August 2017; revision accepted 16 October 2017.
The Botanical Garden, School of Plant Sciences and Food Security, Tel Aviv University, Tel Aviv 69978 Israel
[2] Author for correspondence (e-mail: sapiry@post.tau.ac.il); ORCID id 0000-0001-9685-8076
https://doi.org/10.3732/ajb.1700321

(2017) could exclude the net effect of nutrient-mediated fitness, which is a possible contrasting factor that may masks pollinator-mediated selection. Despite the scarcity of such experimental studies, the evidence does suggest that alternative agents of selection on floral traits exert complementary or contradicting selection, reducing the power to measure pollinator-mediated selection. Taking into account other agents of selection and experimentally controlling for their effects on fitness (Caruso et al., 2017) will strengthen our ability to estimate accurately the net selection mediated by pollinators.

CONSTRAINTS ON SELECTION

Constraints on variance or evolvability of floral traits may limit the opportunity for pollinator-mediated selection. Low values or lack of pollinator-mediated selection may be explained by phylogenetic constraints on the values or the range of floral traits (Smith, 2010). For example, number and organization of anthers in the Brassicaceae family is a diagnostic trait that has hardly evolved even when other flower traits changed, suggesting that past mechanisms, other than selection, constraint evolvability (Royer et al., 2016). Genetic linkage between a pollination-relevant floral trait and another trait, which is under nonpollinator-mediated selection, can also constrain the capacity of the floral phenotype to respond to selection (Ashman and Majetic, 2006).

Low phenotypic (and genetic) trait variation can also reduce the potential detection of pollinator-mediated selection. It is plausible that many studies that failed to detect pollinator-mediated selection captured only the low phenotypic variation within the adaptive peak of the trait, which is selectively neutral (Hansen et al., 2003). While it may seem that pollinators do not exert selection on these floral traits, pollinator-mediated stabilizing selection, apparent only in the variance "margins", actually maintains floral traits around their adaptive value. This stabilizing selection could be difficult to detect and assess due to very small effect size. The challenge is to find a plant system that has not arrived at an evolutionary steady state and where selection is still acting. Anthropogenic disturbances, such as climate change or expanding distributions (as in invasion), may provide such opportunities (Thomann et al., 2015).

BACK TO THE NULL HYPOTHESIS

The above ecological or genetic explanations for the lack of strong pollinator-mediated selection assume that pollinator-mediated selection exists but is undetectable. However, there still is the null hypothesis that no selection acts on the floral trait in question and that variance is random or nonadaptive. While direct proof of randomness is challenging, indirect evidence, such as random spatial distribution of a floral trait (Wang et al., 2016), may assist in rejecting all other alternatives. Likely, null results of pollinator-mediated selection studies are scarcely published (e.g., Lavi and Sapir, 2015). We think that random processes have been overlooked in evolutionary ecology studies of flower evolution and call for more publications that emphasize both significant and nonsignificant results.

FUTURE DIRECTIONS

We propose that future experiments that incorporate designs aimed at controlling for other possible agents of selection may allow partitioning of the net selection gradients mediated by pollinators. Experimental designs that manipulate nonpollinator factors, such as nutrient availability or herbivory will enhance understanding of the possible agents of selection. Because controlling all possible agents of selection is practically impossible, we suggest using at least supplementary pollination to estimate $\Delta\beta_{poll}$ (Sletvold et al., 2010), to assess the net selection gradient mediated by pollinators.

Studies involving other agents of selection may yield more realistic results if they are performed in synergy with expansion of natural variation. Increased variation is enabled by, for example, crosses of distinct phenotypes (Schemske and Bradshaw, 1999), manipulating floral traits (as was done in many studies, e.g., Campbell et al., 2014), or artificial selection that pushes traits beyond their natural range (Sapir et al., 2017). Nonetheless, one should keep in mind that pollinators might also be limited in their sensory modalities (Schiestl and Johnson, 2013), resulting in biased reaction if correlated traits (such as in pollination syndromes) are disrupted, or if variation is extended beyond pollinator's sensory range. Another option of testing directly pollinators' reaction to floral traits is the use of artificial flowers that test phenotypes beyond the natural

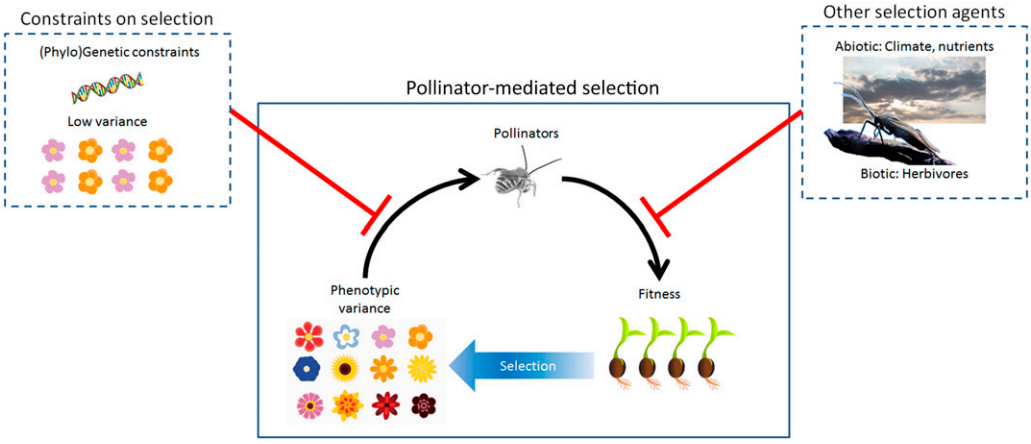

FIGURE 1 Pollinator-mediated selection and competing and complementary factors that also affect floral evolution. Central panel: pollinator-mediated selection is the effect of phenotypic flower variance on pollinators' behavior, which, in turn, affects a plant's fitness. Right panel: Other selection agents affect a plant's fitness, regardless of floral phenotypic variance. These agents can be abiotic factors such as climatic stresses and soil nutrients, or biotic factors such as floral herbivory. Left panel: Constraints on evolutionary potential change through selection. These constraints can be genetic such as epistasis, phylogenetic such as evolutionary stasis, or resulting from low variance, possibly due to past selection that fixed the population in an adaptive peak. Altogether, selection on floral traits is combination of all the factors, and to detect pollinator-mediated selection, other effects should be controlled.

variation (Fenster et al., 2015). New technologies, such as 3-D printing of flowers, or using synthetic fragrance (Policha et al., 2016), as well as utilizing new technologies for accurate tracking of pollen grains can make a breakthrough in this field. Finally, understanding the genetic basis of floral traits and measuring the indirect effect of genetic variation on pollinators' behavior and plant's fitness (Sapir, 2009) may enable determining the level of evolutionary potential of pollinator-mediated selection.

To summarize, we argue that taking pollinator-mediated selection as the sole explanation for flower evolution may limit our understanding of the processes that underlie diversification; the real picture is more complex (Fig. 1). Although the paradigm of pollinator-mediated floral evolution is strongly rooted in the perception of plant evolutionary ecology, and many decades of studying pollination ecology and pollinator-mediated selection have provided a wealth of information, we think that pollinator-mediated selection should be evaluated in the light of alternative possible drivers of flower evolution.

ACKNOWLEDGEMENTS

The authors thank P. Diggle for the invitation to write this essay and for her many efforts to improve the manuscript. Members of the Sapir laboratory provided useful discussions and comments on an early version of this essay. S. Rencus-Lazar helped with English editing. The authors thank two anonymous reviewers for constructive comments of our ideas. Y.S. was supported by a grant from the Israel Science Foundation (#336/16). M.G. was supported by an Israeli Inter-University Planning and Budgeting Commission (PBC) postdoctoral fellowship.

LITERATURE CITED

Ashman, T. L., and C. J. Majetic. 2006. Genetic constraints on floral evolution: A review and evaluation of patterns. *Heredity* 96: 343–352.

Campbell, D. R., M. Forster, and M. Bischoff. 2014. Selection of trait combinations through bee and fly visitation to flowers of *Polemonium foliosissimum*. *Journal of Evolutionary Biology* 27: 325–336.

Caruso, C. M., R. A. Martin, N. Sletvold, M. B. Morrissey, M. J. Wade, K. E. Augustine, S. M. Carlson, et al. 2017. What are the environmental determinants of phenotypic selection? A meta-analysis of experimental studies. *American Naturalist* 190: 363–376.

Darwin, C. R. 1862. On the various contrivances by which British and foreign orchids are fertilised by insects. John Murray, London, UK.

Fenster, C. B., W. S. Armbuster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology Evolution and Systematics* 35: 375–403.

Fenster, C. B., R. J. Reynolds, C. W. Williams, R. Makowsky, and M. R. Dudash. 2015. Quantifying hummingbird preference for floral trait combinations: The role of selection on trait interactions in the evolution of pollination syndromes. *Evolution* 69: 1113–1127.

Hansen, T. F., W. S. Armbruster, M. L. Carlson, and C. Pélabon. 2003. Evolvability and genetic constraint in *Dalechampia* blossoms: Genetic correlations and conditional evolvability. *Journal of Experimental Zoology. Molecular and Developmental Evolution* 296B: 23–39.

Harder, L. D., and S. D. Johnson. 2009. Darwin's beautiful contrivances: Evolutionary and functional evidence for floral adaptation. *The New Phytologist* 183: 530–545.

Hopkins, R., and M. D. Rausher. 2012. Pollinator-mediated selection on flower color allele drives reinforcement. *Science* 335: 1090–1092.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, et al. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157: 245–261.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226.

Lavi, R., and Y. Sapir. 2015. Are pollinators the agents of selection for the extreme large size and dark color in *Oncocyclus* irises? *The New Phytologist* 205: 369–377.

Policha, T., A. Davis, M. Barnadas, B. T. M. Dentinger, R. A. Raguso, and B. A. Roy. 2016. Disentangling visual and olfactory signals in mushroom-mimicking *Dracula* orchids using realistic three-dimensional printed flowers. *The New Phytologist* 210: 1058–1071.

Royer, A. M., C. Kremer, K. George, S. G. Pérez, D. W. Schemske, and J. K. Conner. 2016. Incomplete loss of a conserved trait: Function, latitudinal cline, and genetic constraints. *Evolution* 70: 2853–2864.

Sapir, Y. 2009. Effects of floral traits and plant genetic composition on pollinator behavior. *Arthropod-Plant Interactions* 3: 115–129.

Sapir, Y., K. Karoly, V. A. Koelling, H. F. Sahli, F. N. Knapczyk, and J. K. Conner. 2017. Effect of expanded variation in anther position on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Annals of Botany* doi:10.1093/aob/mcx041.

Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences, USA* 96: 11910–11915.

Schiestl, F. P., and S. D. Johnson. 2013. Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution* 28: 307–315.

Sletvold, N., J. M. Grindeland, and J. Ågren. 2010. Pollinator-mediated selection on floral display, spur length and flowering phenology in the deceptive orchid *Dactylorhiza lapponica*. *The New Phytologist* 188: 385–392.

Sletvold, N., M. Tye, and J. Ågren. 2017. Resource- and pollinator-mediated selection on floral traits. *Functional Ecology* 31: 135–141.

Smith, S. D. 2010. Using phylogenetics to detect pollinator-mediated floral evolution. *The New Phytologist* 188: 354–363.

Sprengel, C. K. 1793. Das Entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen. Vieweg, Berlin, Germany.

Strauss, S. Y., and J. B. Whittall. 2006. Non-pollinator agents of selection on floral traits. *In* L. D. Harder and S. C. H. Barrett [eds.], Ecology and evolution of flowers, 120–138. Oxford University Press, Oxford, UK.

Teixido, A. L., M. Barrio, and F. Valladares. 2016. Size matters: Understanding the conflict faced by large flowers in Mediterranean environments. *Botanical Review* 82: 204–228.

Thomann, M., E. Imbert, R. C. Engstrand, and P. O. Cheptou. 2015. Contemporary evolution of plant reproductive strategies under global change is revealed by stored seeds. *Journal of Evolutionary Biology* 28: 766–778.

Wang, H., M. Talavera, Y. Min, E. Flaven, and E. Imbert. 2016. Neutral processes contribute to patterns of spatial variation for flower colour in the Mediterranean *Iris lutescens* (Iridaceae). *Annals of Botany* 117: 995–1007.