

FLORAL SYMMETRY AFFECTS BUMBLEBEE APPROACH CONSISTENCY IN ARTIFICIAL FLOWERS

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Abstract—Bilateral symmetry has evolved from radial symmetry in several floral lineages, and multiple hypotheses have been proposed to account for the success of this floral plan. One of these hypotheses posits that bilateral symmetry (or, more generally, a reduced number of planes of floral symmetry) allows for more precise pollen placement on pollinators. Greater precision would maximize the efficacy of pollen transfer to conspecifics, while minimizing reproductive interference amongst plant species. Despite the intuitiveness of this hypothesis, it has little experimental support. Here, we tested whether a reduction in the number of floral planes of symmetry (as in the transition from radial to bilateral symmetry) increases the potential precision of pollen placement. We analyzed video recordings of bumblebees (*Bombus impatiens*) visiting artificial flowers to determine whether consistency in flower entry angle differed between radial (round) and disymmetric (rectangular) “flowers”. We observed more consistent entry angles for disymmetric flowers than for radial flowers, with entry angles to radial flowers 43% more variable on average (standard deviations of 30° vs. 21°). Bees trained on flowers with an intermediate (square) morphology exhibited a slight, non-significant preference for radial symmetry over disymmetry. Our results show that disymmetry—an evolutionarily intermediate form of floral symmetry—has the potential to increase pollen transfer to conspecific stigmas, relative to radial symmetry. Thus, evolutionary reduction in the number of planes of floral symmetry likely provides benefits in terms of pollen delivery, as suggested by the pollen-placement-accuracy hypothesis. These findings offer insight into the evolution of floral symmetry.

Keywords: artificial flowers, *Bombus*, floral symmetry, pollen placement, preference, zygomorphy

INTRODUCTION

Bilateral floral symmetry (zygomorphy) has evolved from the ancestral state of radial symmetry (actinomorphy) multiple times across the angiosperms (Donoghue et al. 1998; Damerval & Nadot 2007; Hileman 2014; reviewed by Citerne et al. 2010). Various hypotheses have been put forth to explain this evolutionary trend (reviewed by Neal et al. 1998). For example, greater distinctiveness of zygomorphic flowers may promote pollinator fidelity (Ostler & Harper 1978), or pollinators may have an innate preference for bilateral symmetry (Leppik 1953). While greater pollinator fidelity towards bilateral flowers has been observed (Fenster et al. 2004), evidence for floral-symmetry preference is mixed (e.g., West & Laverty 1998), with previous studies finding pollinator preferences for both radial (Free 1970; Wignall et al. 2006) and bilateral (Leppik 1953; Rodríguez et al. 2004; Lázaro et al. 2008) floral symmetry.

Another hypothesis, the pollen-placement-accuracy (or pollen position) hypothesis, states that flowers with fewer planes of symmetry promote more consistent entry by pollinators into the flower, such that the plant’s reproductive parts—both male and female—make more consistent contact with certain parts of pollinators’ bodies (Leppik 1972; Macior 1974). This consistency would maximize the

efficiency of pollen transfer while minimizing reproductive interference amongst pollinator-sharing floral species (Muchhala & Thomson 2010). Precise placement of reproductive parts reduces pollen waste, increases the accuracy of stigma contact with pollinators (Armbruster et al. 2009), and reduces the chances of stigma-clogging by heterospecific pollen, thereby potentially increasing reproductive output of both the pollen parent and the seed parent (Morales & Traveset 2008; Armbruster & Muchhala 2009). This should benefit plant fitness by maximizing the amount of potential reproduction per unit of floral reward produced (Muchhala et al. 2010; Muchhala & Thomson 2012; Armbruster 2014).

Despite the logic underlying the pollen-placement-accuracy hypothesis, there is little experimental evidence to support it. We therefore set out to test this hypothesis experimentally, by training laboratory-reared bumblebees to visit artificial flowers, and measuring the angle at which these bees entered both radially symmetric and disymmetric “flowers”. We used disymmetry, in which flowers possess only two planes of symmetry, to represent an evolutionary step from radial towards bilateral symmetry, in which flowers possess a single plane of symmetry. Indeed, disymmetry appears to be an evolutionarily intermediate stage between radial and bilateral floral symmetries in some plant lineages (Endress 1999; Friis et al. 2011). We expected pollinators to enter disymmetric flowers at a more consistent angle than radially symmetric flowers. We also tested for a symmetry preference in our bees, as a preference for radial symmetry

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across several pollinator groups could help explain the persistence of radial symmetry in spite of less efficient pollen transfer.

MATERIALS AND METHODS

Experimental design

Colonies of captive-reared bumblebees (*B. impatiens* Cresson) were connected to a $60 \times 60 \times 180$ cm flight cage by a gated tunnel that allowed for controlled entry and exit of individual bees. During initial training, bees were allowed to forage freely on artificial flowers until consistent foraging began and reliable foragers could be individually marked using dots of coloured paint applied to the dorsum of the thorax. Flowers consisted of 1.5 mL Eppendorf tubes with the lids removed, ringed by blue plastic 11.4 cm² “corollas” (details below). Training flowers were filled with 30% (*w/w*) sucrose solution (“nectar”) and were presented in the horizontal plane (i.e., corollas parallel, and Eppendorf tubes perpendicular, to the floor of the cage) to allow us to fill Eppendorf tubes to the brim during initial training. Training flowers were replaced every second day to avoid build-up of sucrose residue or bee scent marks on corollas.

Two colonies were used in this study. The first colony was used to compare entry-angle variability between radial and disymmetric flowers. The second colony was used both to measure entry-angle variability and to assess floral symmetry preference. For the first colony, training flowers were identical to our radially symmetric flowers (described below). For the second colony, we used 3.4×3.4 cm square corollas for training in an effort to minimize bee bias towards a particular symmetry before completion of the preference test. Pollen was provided to the colonies daily.

For testing entry-angle consistency, 5.5×2.1 cm blue plastic rectangles were used to represent corollas of disymmetric flowers, and circles with radii of 1.9 cm represented corollas of radially symmetric flowers (Fig. 1). The surface area of all corollas (both experimental and training) was the same (11.4 cm²). Flowers (corollas and Eppendorf tubes) were washed with warm water and detergent, rinsed, and dried prior to each foraging bout. The first colony, of which 6 bees were individually observed (15 Dec. 2014–30 Jan. 2015), foraged on a setup consisting of 9 flowers that were either entirely radial or entirely disymmetric. The centre of each flower was 8–10 cm from the centre of the closest neighbouring flower. The second colony, of which 10 bees were individually observed (13 Mar.–2 Apr. 2015), foraged on a setup consisting of a combination of radial and disymmetric flowers (10 of each symmetry) in a 5×4 matrix. Flowers were set up in a pattern that allowed for an equal ratio of neighbouring radial and disymmetric flowers. For both colonies, flowers were presented in a single vertical plane (i.e., corollas perpendicular, and Eppendorf tubes parallel, to the floor of the cage) (Fig. 1). Each flower contained 3.5 μ L of nectar at the start of a foraging bout and was refilled as soon as it was emptied by a bee. For the first colony, each bee performed 2–3 foraging bouts per flower symmetry (approximately 60 visits per symmetry per bee; Appendix 1), with alternation of

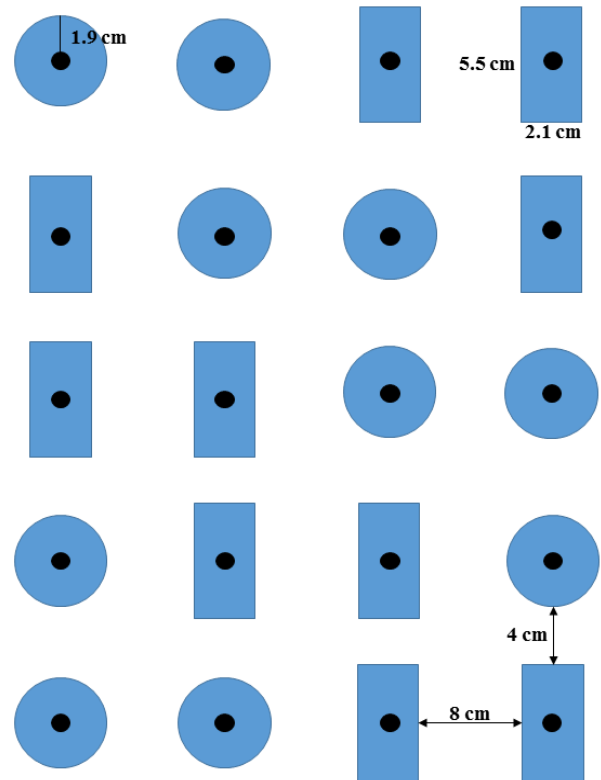


FIGURE 1. Experimental setup of artificial flowers for the second *Bombus impatiens* colony. Note the presence of both radial and disymmetric (rectangular) “flowers”. The array was set up in a vertical configuration (i.e., the “flowers” in the top row of the array were closest to the roof of the flight cage; those in the bottom row were closest to the floor; and rectangular flowers were oriented so as to be longest top-to-bottom).

symmetries between foraging bouts and alternation of starting symmetries among individual bees. For the second colony, three foraging bouts per bee were performed overall (approximately 44 visits per symmetry per bee; Appendix 1). Each individual bee’s foraging bouts were recorded by video camera and still photographs were extracted as the bee entered a flower. Entry was identified as insertion of the entire thorax of the bee into the Eppendorf tube. Any entry attempts or movements that did not meet this criterion were ignored and not measured. ImageJ (National Institutes of Health, Version 1.49k, 2014) was used to measure the angle at which bees entered flowers by drawing two lines (one for the vertical midline of the flower and one for the midline of the bee) and measuring the angle between these lines (Fig. 2). The midline from the centre of the flower to the bottom of the flower was considered to be 0° , and arbitrary designations of positive for left of 0° and negative for right of 0° were used to measure directionality. This meant that entries left of the midline theoretically ranged from 0° – 180° , and entries right of the midline could range from -180° – 0° . For testing flower symmetry preference, we counted the number of flower entries per symmetry by the 10 bees (from the second colony) that foraged on the mixed (10 radial: 10 disymmetric) array.

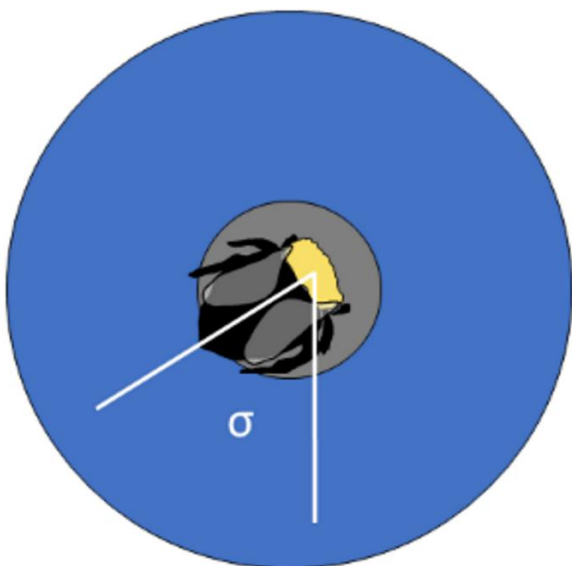


FIGURE 2. Schematic representation of an entry-angle measurement, as performed for each flower visit. Entry angle was measured as the bee’s thorax completely entered the flower.

Statistical analyses

For each bee on each symmetry, the standard deviation of entry angle was calculated separately for each foraging bout. We ran linear mixed models using the R (R Core Team, Version 3.1.1, 2014) package lme4 (Bates et al. 2015) to test for an effect of floral symmetry on these standard deviation values, which were normally distributed. We included colony and bee nested within colony as random factors in the models. To test for symmetry preference, we modelled the number of flower visits received (per foraging bout) from bees in the second colony, as a function of floral symmetry and bout number (a categorical factor). Because these visit counts were normally distributed, we again used linear mixed-effects models, with bee as the random factor. For all models, we tested the significance of predictor variables using likelihood-ratio tests of nested models.

RESULTS

Observed flower-entry angles ranged from -140° to 159° for radially symmetric flowers and from -60° to 169° for disymmetric flowers. However, bees typically entered flowers from below: only 12 of 1585 entry angles ($< 1\%$) had absolute values $\geq 90^{\circ}$. Entry angles were more consistent for disymmetric flowers than radial flowers for 15 of the 16 bees tested (Fig. 3). Standard deviations of entry angles were approximately 9° lower (95% C.I. = 6.59–11.32) for bees entering the disymmetric flowers (mean SD = 21°) than for bees entering radial flowers (mean SD = 30° ; LMM, $X_1^2 = 24.1$, $N = 16$ bees, $P < 0.0001$). Excluding visits with extreme entry angles (those with absolute values $\geq 90^{\circ}$) and recalculating the standard deviations only strengthened this result (LMM, $X_1^2 = 47.4$, $N = 16$ bees, $P < 0.0001$). There was no significant difference in entry-angle variability between the two colonies, despite their different training regimens ($X_1^2 = 0.10$, $N =$

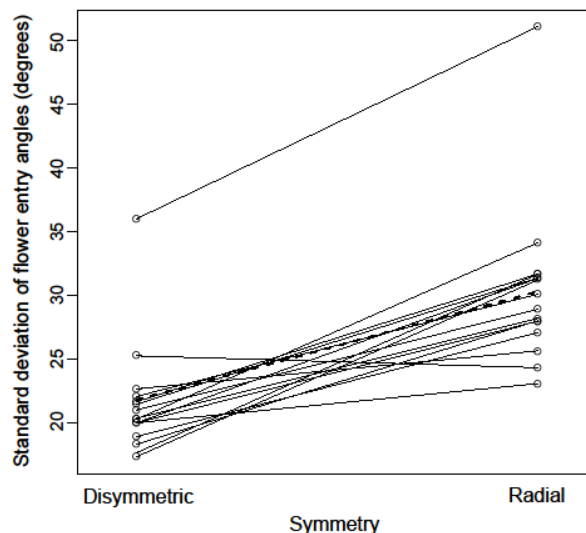


FIGURE 3. Standard deviations of flower entry angles for all bees on disymmetric and radial symmetries. Lines connect radial and disymmetric values for a given individual. The average trend across all bees is depicted by the thick, dashed line. $N = 16$ bees; note overlap between several points and lines.

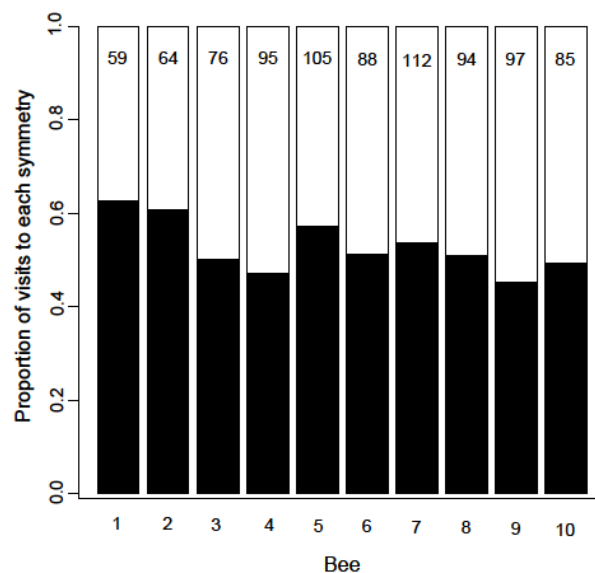


FIGURE 4. Proportion of visits made by bees to flowers of each symmetry. The lower portion of each column (black) represents visits to radial flowers; the upper portion of each column (white) represents visits to disymmetric flowers. Total number of flower visits made by each bee is given at the top of the appropriate column.

16 bees, $P = 0.75$), nor did mean entry angles differ significantly between floral symmetries (1.8° for disymmetric; 3.0° for radial; LMM, $X_1^2 = 0.06$, $N = 16$ bees, $P = 0.80$). Bees showed a non-significant preference for radial flowers (average proportion of visits to radial flowers = 0.53; $X_1^2 = 0.9$, $N = 10$ bees, $P = 0.34$; Fig 4).

DISCUSSION

Floral symmetry affects the consistency with which bees enter flowers. We found more consistent floral entry angles by bumblebees on disymmetric artificial flowers than on radially symmetric flowers. Assuming that more consistent floral entry allows plants to more precisely place pollen onto (and receive pollen from) their pollinators (Armbruster et al. 1994; Wang et al. 2014), disymmetric flowers should have a fitness advantage over radial flowers—perhaps especially in species-rich plant communities where plants share pollinators. If, more generally, flowers with fewer planes of symmetry exhibit more precise pollen placement and therefore more efficient deployment of resources, then the evolution of bilateral symmetry should be favoured. Thus, our results may help explain the evolution, in many plant lineages, of bilateral floral symmetry from ancestrally radial symmetry.

Our artificial flowers did not have reproductive structures, and we did not measure actual pollen transfer. Artificial flowers with male and female “reproductive organs” and dye as a pollen analogue (see Fenster et al. 1996; Adler & Irwin 2006; Thomson et al. 2012) could be developed in the future to determine whether transfer rates differ between radial and bilateral flowers. In this study, we have imagined our flowers as having few or bilaterally arranged reproductive structures (e.g., the conjoined stamens or staminal columns of orchids, lobelioids, and many legumes), such that consistent pollinator entry angles would allow consistent dorsal or ventral pollen placement. (Disymmetric corollas combined with numerous, radially arranged reproductive organs would not be expected to increase pollen-placement precision, regardless of their effect on pollinator behaviour.) In fact, Rudall & Bateman (2004) have noted that the evolution of bilateral symmetry is associated with stamen suppression (the development of fewer stamens per flower), and suggested that the latter was a preliminary step towards the development of bilateral symmetry. Stamen suppression frequently results in flowers with a single functional stamen, most commonly positioned at the top of the flower. Alternatively, the evolution towards zygomorphy may have been initiated by a shift in placement of stamens to one side of the flower (e.g., as seems to have occurred in *Passiflora* and in *Schlumbergera* cacti). This evolutionary scenario (reduction or rearrangement of reproductive organs first; selection on corolla symmetry second) was the premise for our current study and would be the logical starting point for future studies measuring actual pollen transfer.

Although several angiosperm lineages have evolved bilateral symmetry, many others have not, and some may have even reverted to radial from bilateral symmetry (Donoghue et al. 1998). This suggests there are benefits to radial symmetry. One possible explanation for the persistence of radial symmetry is that pollinators have a preference for radial flowers. Our bees did show a slight, though non-significant, preference for radial flowers (Fig. 4); but our bees were not completely naïve (having been trained on flowers with square corollas, which they may have perceived as more similar to our disymmetric flowers). In general,

although many types of bees have a strong preference for symmetric over asymmetric flowers (Møller 1995; Møller & Eriksson 1995; but see West & Lavery 1998), preferences for a particular symmetry type seem to be idiosyncratic. Previous studies have found no innate preference for radial over bilateral symmetry in *Bombus impatiens* (West & Lavery 1998); a preference for bilateral symmetry in *B. terrestris* (Rodríguez et al. 2004) and other bumblebee species (Leppik 1953; Lázaro et al. 2008); and a preference for radial symmetry in honeybees (*Apis mellifera*) (Free 1970; Wignall et al. 2006).

Even if bumblebees do not consistently prefer radial symmetry, radial flowers may attract more functional groups of pollinators (Fenster et al. 2004). Radially symmetrical flowers may therefore benefit from high and relatively stable visitation rates, at the expense of low pollination efficiency. For this reason, it has been suggested that plants with radially symmetric flowers might be less pollen limited (McLernon et al. 1996; Wolowski et al. 2014); however, syntheses of the available data show no general trend for greater pollen limitation in plants with zygomorphic flowers (Knight et al. 2005; Vamosi et al. 2013). Regardless, radial flowers may have advantages over bilateral flowers that would be apparent only in a more diverse field setting.

In nature, radial flowers are generally oriented in the horizontal plane (parallel to the ground), whereas bilateral flowers are oriented in the vertical plane (Neal et al. 1998). In our study, both symmetries were presented in the vertical plane. It is therefore possible that radial flowers have a pollen-transmission advantage when oriented horizontally, such that pollinators may approach from any direction (Ushimaru & Hyodo 2005; Fenster et al. 2009; Ushimaru et al. 2009; Wang 2010; Wang et al. 2014a; Wang et al. 2014b). It seems likely that bilateral symmetry would have evolved on a background of vertical floral orientation—the background we simulated here. However, floral orientation is an additional factor that could be manipulated in future studies to investigate its functional interaction with floral symmetry. It should also be noted that since we deployed our disymmetric flowers in a vertical orientation, they presented an enhanced “landing platform”, or lower lip, compared to the radial flowers. The presence of this landing platform may have contributed to the increased entry-angle consistency that we observed for disymmetric flowers. Bilateral symmetry is often associated with presence of a landing platform in nature—an association we mimicked in this study—but any landing-platform effect could be experimentally decoupled in the future from the effect of floral symmetry by rotating the corollas 90°, such that they are wider than long.

In conclusion, bumblebees (*Bombus impatiens*) have a more consistent angle of entry into disymmetric flowers than radially symmetric artificial flowers. They do not, however, have a significant preference for one symmetry over the other. Our findings provide the first experimental support for the pollen-placement-accuracy hypothesis, and shed light on why bilateral symmetry has evolved from radial symmetry so frequently.

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APPENDICES

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

APPENDIX I. Summary of entry angle standard deviation (SD) and preference data for *B. impatiens* on artificial flowers.

REFERENCES

- Adler LS, Irwin RE (2006) Comparison of pollen transfer dynamics by multiple floral visitors: Experiments with pollen and fluorescent dye. *Annals of Botany* 97:141-150.
- Armbruster WS, Edwards ME, Debevec EM (1994) Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology* 75:315-329.
- Armbruster WS, Hansen TF, Pélabon C, Pérez-Barralés R, Maad J (2009) The adaptive accuracy of flowers: measurement and microevolutionary patterns. *Annals of Botany* 103:1529-1545.
- Armbruster WS, Muchhala N (2009) Associations between floral specialization and species diversity: cause, effect, or correlation? *Evolutionary Ecology* 23:159-179.
- Armbruster WS (2014) Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *Annals of Botany Plants* 6:1-24.
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1-48.
- Citerne H, Jabbour F, Nadot S, Damerval C (2010). The evolution of floral symmetry. *Advances in Botanical Research* 54:85-137.
- Damerval C, Nadot S (2007) Evolution of perianth and stamen characteristics with respect to floral symmetry in Ranunculales. *Annals of Botany* 100:631-640.
- Damerval C, Citerne H, Le Guilloux M, Domenichini S, Dutheil J, Ronse De Craene L, Nadot S (2013) Asymmetric morphogenetic cues along the transverse plane: shift from disymmetry to zygomorphy in the flower of Fumarioideae. *American Journal of Botany* 100:391-402.
- Donoghue MJ, Ree RH, Baum DA (1998) Phylogeny and the evolution of flower symmetry in the Asteridae. *Trends in Plant Science* 3:311-317.
- Endress, PK (1999) Symmetry in flowers: diversity and evolution. *International Journal of Plant Sciences* 160:S3-S23.
- Fenster CB, Hassler CL, Dudash MR (1996) Fluorescent dye particles are good pollen analogs for hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *Canadian Journal of Botany* 75:189-193.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics* 35:375-403.
- Fenster CB, Armbruster WS, Dudash MR (2009) Specialization of flowers: is floral orientation an overlooked first step? *New Phytologist* 183:502-506.
- Free JB (1970) Effect of flower shapes and nectar guides on the behaviour of foraging honeybees. *Behaviour* 37:269-285.
- Friis EM, Crane PR, Pedersen KR (2011) Patterns of structural diversification in angiosperm reproductive organs. In: *Early Flowers and Angiosperm Evolution*. Cambridge University Press, Cambridge, pp 387-414.
- Hileman LC (2014) Trends in flower symmetry evolution revealed through phylogenetic and developmental genetic advances. *Philosophical Transactions of the Royal Society B* 369:20130348.
- Jabbour F, Nadot S, Damerval C (2009) Evolution of floral symmetry: a state of the art. *Comptes Rendus Biologies* 332:219-231.
- Knight TM, Sheets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman TL (2005) Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36:467-497.
- Lázaro A, Hegland SJ, Totland Ø (2008) The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. *Oecologia* 157:249-257.
- Leppik, EE (1953) The ability of insects to distinguish number. *American Naturalist* 87:229-236.
- Leppik EE (1972) Origin and evolution of bilateral symmetry in flowers. *Evolutionary Biology* 5:49-85.
- Macior LW (1974) Behavioral aspects of coadaptations between flowers and insect pollinators. *Annals of the Missouri Botanical Garden* 61:760-769.
- McLernon SM, Murphy SD, Aarssen LW (1996) Heterospecific pollen transfer between sympatric species in a midsuccessional old-field community. *American Journal of Botany* 83:1168-1174.
- Møller AP (1995) Bumblebee preference for symmetrical flowers. *Proceedings of the National Academy of Sciences of the United States of America* 92:2288-2292.
- Møller AP, Eriksson M (1995) Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos* 73:15-22.
- Morales CL, Traveset A (2008) Interspecific pollen transfer: Magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences* 27:221-238.
- Muchhala N, Thomson JD (2010) Fur versus feathers: pollen delivery by bats and hummingbirds and consequences for pollen production. *American Naturalist* 175:717-726.
- Muchhala N, Brown Z, Armbruster WS, Potts MD (2010) Competition drives specialization in pollination systems through costs to male fitness. *American Naturalist* 176:732-743.
- Muchhala N, Thomson JD (2012) Interspecific competition in pollination systems: costs to male fitness via pollen misplacement. *Functional Ecology* 26:476-482.
- Neal PR, Dafni A, Giurfa M (1998) Floral symmetry and its role in plant-pollinator systems: Terminology, distribution, and hypotheses. *Annual Review of Ecology and Systematics* 29:345-373.
- Ostler WK, Harper KT (1978) Floral ecology in relation to plant species diversity in the Wasatch Mountains of Utah and Idaho. *Ecology* 59:848-861.
- R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>
- Rodríguez I, Gumbert A, Hempel de Ibarra N, Kunze J, Giurfa M (2004) Symmetry is in the eye of the 'beeholder': innate preference for bilateral symmetry in flower-naïve bumblebees. *Naturwissenschaften* 91:374-377.

- Rudall PJ, Bateman RM (2004) Evolution of zygomorphy in monocot flowers: Iterative patterns and developmental constraints. *New Phytologist* 162:25-44.
- Thomson JD, Ogilvie JE, Makino TT, Arisz A, Raju S, Rojas-Luengas V, Guo Rui Tan M (2012) Estimating pollination success with novel artificial flowers: Effects on nectar concentration. *Journal of Pollination Ecology* 9:108-114.
- Ushimaru A, Hyodo F (2005) Why do bilaterally symmetric flowers orient vertically? Flower orientation influences pollinator landing behaviour. *Evolutionary Ecology Research* 7:151-160.
- Ushimaru A, Dohzono I, Takami Y, Hyodo F (2009) Flower orientation enhances pollen transfer in bilaterally symmetrical flowers. *Oecologia* 160:667-674.
- Vamosi JC, Sheets JA, Ashman TL (2013) Drivers of pollen limitation: macroecological interactions between breeding systems, rarity, and diversity. *Plant Ecology & Diversity* 6:171-180.
- Wang Y, Meng LL, Yang YP, Duan YW (2010) Change in floral orientation in *Anisodus luridus* (Solanaceae) protects pollen grains and facilitates development of fertilized ovules. *American Journal of Botany* 97:1618-1624.
- Wang H, Tie S, Yu D, Guo YH, Yang CF (2014a) Change of floral orientation within an inflorescence affects pollinator behavior and pollination efficiency in a bee-pollinated plant, *Corydalis shearerii*. *PLoS ONE* 9:e95381.
- Wang H, Xiao CL, Gitruru RW, Xiong Z, Yu D, Guo YH, Yang CF (2014b) Change of floral orientation affects pollinator diversity and their relative importance in an alpine plant with generalized pollination system, *Geranium refractum* (Geraniaceae). *Plant Ecology* 215:1211-1219.
- West EL, Lavery TM (1998) Effect of floral symmetry on flower choice and foraging behaviour of bumble bees. *Canadian Journal of Zoology* 76:730-739.
- Wignall AE, Heiling AM, Cheng K, Herberstein ME (2006) Flower symmetry preferences in honeybees and their crab spider predators. *Ethology* 112:510-518.
- Wolowski M, Ashman TL, Freitas L (2014) Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic forest of Brazil. *PLoS ONE* 9:e89498.