Herkogamy, a principal functional trait of plant reproductive biology

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Abstract

Premise of the research: Phenotypic traits that consistently mediate species' responses to environmental variation ('functional traits') provide a promising approach towards generalizing ecological and evolutionary patterns, and thereby gaining insights into the processes generating them. In the plant functional ecology literature, most trait-based studies have focused on traits mediating either resource competition or responses to variation in the abiotic environment, while traits mediating reproductive interactions have often been neglected.

Methodology: Here, I discuss the value of herkogamy (spatial separation of male and female functions in flowers) as a functional trait in plant reproductive biology and review the evidence relevant to the hypothesis that taxa exhibiting greater herkogamy have historically experienced more reliable pollination, and more outcrossed mating systems.

Pivotal results: A large body of work in the field of plant reproductive biology has identified a set of nearly ubiquitous correlations between average herkogamy and features of plant mating systems, notably autofertility (seed set in the absence of pollinators), and outcrossing rate. Herkogamy often varies extensively among populations and species, and the adaptive interpretation is that herkogamy exhibits local adaptation to the reliability of the pollination environment.

Conclusions: These results underline the value of herkogamy as a functional trait representing variation in mating histories. Many important insights are likely to emerge from studies leveraging herkogamy as an easily measured proxy of plant mating systems, as already demonstrated in comparative studies, and in studies of reproductive interactions. Going forward, greater consideration of herkogamy and other reproductive-function traits in studies

of species coexistence may provide a more complete understanding of community assembly processes.

Introduction

The dynamic complexity of biological systems has led to a focus in ecology on functional traits that consistently mediate species' responses to environmental variation (Lavorel and Garnier 2002; McGill et al. 2006; Shipley et al. 2016). In the plant functional ecology literature, most trait-based studies have focused on traits mediating either resource competition (e.g. size) or plant responses to variation in the abiotic environment (e.g. specific leaf area). Because these traits are important determinants of species distributions and species interactions, they are often used as proxies of species' ecological strategies in studies of species coexistence and community assembly (Adler et al. 2013). In parallel, a large body of work in the field of plant reproductive biology has identified traits important for reproductive interactions. Despite the dependence of most flowering plants on pollinators for sexual reproduction, traits mediating reproductive interactions have rarely been considered in studies of community assembly processes. In this essay I discuss the value of a functional trait mediating plant responses to variation in pollinator communities, an important aspect of the biotic environment.

The astonishing diversity of flowers is to a large extent the outcome of interactions with pollinators (Grant and Grant 1965; Stebbins 1970; van der Niet et al. 2014). Consequently, the morphological architecture of flowers conveys much information about the reproductive biology of the species (Ornduff 1969). This is evident, for example, from the long history of grouping species into pollination 'syndromes' based on flower color, shape and reward chemistry (Fægri and van der Pijl 1979). By considering the size and shape of self-compatible flowers, it can often also be inferred whether the species rely strongly on animal pollinators for seed production or is capable of effective self-pollination. For example, predominantly selfing species are typically characterized by a set of traits collectively referred to as the ‘selfing syndrome’ (Ornduff 1969; Sicard and Lenhard 2011), including small, short-lived flowers, low pollen-to-ovule ratios, and reduced dichogamy and herkogamy.

Herkogamy, the spatial separation of anthers and stigmas within flowers, is a key floral trait thought to promote outcrossing and/or the avoidance of interference between male and female sexual functions (Webb and Lloyd 1986). The functional importance of herkogamy is supported by studies reporting negative relationships between herkogamy and the rate of autofertility (self-fertilization in the absence of pollinators) and the rate of selfing among natural populations. In turn, several studies have demonstrated strong positive correlations between pollinator abundance and herkogamy (e.g. Moeller 2006; Opedal et al. 2016a). These observations have led to the general acceptance that variation in herkogamy among populations and species represents adaptation of the mating system to variation in the local reproductive environment.

Several functional and evolutionary aspects of herkogamy have been reviewed elsewhere. The foundational review of Webb and Lloyd (1986) includes extensive discussion of functional aspects of herkogamy, definitions of classes of herkogamy, and their distribution among taxa. Barrett (2002, 2003) offers further discussion of functional aspects related to mating strategies, and Opedal et al. (2017) synthesizes work on the quantitative genetics and evolvability of herkogamy. Here, I first outline the expected functional relationships between herkogamy and plant mating systems, and then review and synthesize evidence relevant to the hypothesis that, when two self-compatible taxa differ in average herkogamy, they also differ in their mating histories. I then go on to discuss the value of herkogamy as a mating-system proxy in comparative studies, and review examples of insights emerging from such studies. Finally, I outline the value and promise of increasingly integrating herkogamy and other reproductive-function traits into studies of species cooccurrence and community assembly.

Notes on the measurement of herkogamy

Before we can proceed, a brief discussion of measurements is required. Herkogamy is broadly defined as the spatial separation of stigmas ($x_{♀}$) and anthers ($x_{♂}$) within flowers or flower-like inflorescences. Webb and Lloyd (1986) defined several classes of herkogamy, differing among other things in the degree of 'order' in which pollinators contact floral organs. Species exhibiting ordered herkogamy can be further classified into those in which stigmas are contacted first by a visiting pollinator (*approach herkogamy*, $x_{♀}>x_{♂}$; stigmas positioned above or protruding beyond the anthers), and those in which anthers are contacted first (*reverse herkogamy*, $x_{♀}<x_{♂}$; stigmas positioned below or behind the anthers). Still others exhibit *reciprocal herkogamy*, with stigmas and anthers placed in complementary positions in two or more floral morphs. In self-compatible species approach herkogamy is by far the most common and is often associated with pollination by a limited number of pollinator species or functional groups.

How to measure herkogamy depends on the functional question to be addressed. If the aim is to quantify the effect of herkogamy on the probability of self-pollination, for example, the relevant measure in normally the shortest distance separating stigmatic surfaces from dehiscing anthers. In other cases, such as studies of constraints on the evolution of herkogamy arising from genetic covariance between pistil and stamen lengths (Opedal et al. 2017), quantifying herkogamy as the difference between pistil and stamen lengths may be more appropriate. Because anthers and stigmatic surfaces are often elongated, the absolute value of these measures will tend to differ. Furthermore, herkogamy in many species is not constant but changes during flower development due e.g. to curling of stigmatic lobes or differential rates of elongation of male and female sexual organs (see Goodwillie and Weber 2018 for review). For these reasons, great care must be taken to standardize measurements taken for comparative analyses, typically by measuring flowers at similar developmental stages. In some cases, emergent traits such as the timing of change in herkogamy may also be of key interest (e.g. Armbruster et al. 2002).

Different kinds of measurements places herkogamy on different scale types. When quantified as the difference between pistil length and stamen length, $x_{♀} - x_{♂}$, with negative values assigned to reverse herkogamous flowers, herkogamy is on what Houle et al. (2011) referred to as a signed ratio scale. When measured as the absolute distance between stigmas and anthers, $|x_{♀} - x_{♂}|$, herkogamy is on a true ratio scale. Finally, herkogamy is sometimes divided into distinct classes, and is then on an ordinal scale. For quantitative comparative studies, these disparate scale types would represent a serious challenge (see discussion in Opedal et al. 2017), and I urge researchers to carefully consider the consequences of their choice of measurement scale in studies of herkogamy. In the following review my focus is on qualitative relationships between herkogamy and mating-system parameters, and I hence considered studies using any of the measurements of herkogamy outlined above.

The functional relationships among herkogamy, autofertility, and outcrossing

The expected negative effect of herkogamy on autofertility follows intuitively from the reduced probability of pollen transfer over longer distances. In the absence of pollinators, herkogamy-autofertility relationships are purely ‘physical’, and replicate studies of individuals, populations, or species under standardized conditions are expected to yield quantitatively similar results (Table 1). Under field conditions, some variation might nevertheless be expected due to environmental factors such as wind speed, rainfall, or variation in the resource level of maternal plants affecting seed production.

Herkogamy-autofertility relationships are always expected to be negative, yet their shapes may vary. Autofertility rates are bounded between 0 and 1 and will almost inevitably decline towards zero as herkogamy increases (Fig. 1). Furthermore, herkogamy-autofertility relationships may often be nonlinear, taking shapes ranging from negatively exponential (‘Type I’ herkogamy-autofertility relationship; Fig. 1, solid line) to logistic (‘Type II’ herkogamy-autofertility relationship; Fig. 1, dashed line). In both cases, studies comparing individuals, populations, or species at the upper end of the curve may fail to detect any relationship. In the Type II case (dashed line), the same would be true for comparisons at the lower end (see Opedal et al. 2015 for an empirical example). Note that linear herkogamy-autofertility relationships falls within the expected range between these extremes.

In addition to taxon-specific shapes of herkogamy-autofertility relationships (Type I vs. Type II vs. intermediate), the x-axis of Fig. 1 is unitless on purpose. This is because the decline in autofertility per unit (e.g. mm) increase in herkogamy may depend on the relative positions of anthers and stigmas within flowers. For example, autofertility may decline more rapidly with increasing herkogamy in approach herkogamous species than in reverse herkogamous species, and we can speculate that the relationship is often closer to Type I (solid line) for approach herkogamy, and closer to Type II (dashed line) for reverse herkogamy. Thus, in cases where herkogamy (measured as $x_{♀} - x_{♂}$) ranges from negative to positive, the fitness consequences of changing herkogamy by one unit may be asymmetric around zero. The rate of decay in autofertility with increasing herkogamy may also depend on the relative orientation of anthers and stigmas. For example, species of *Dalechampia* vines diverge in the angle between male and female flowers (Armbruster et al. 2009), and populations of *Arabis alpina* differ in the orientation of the anthers towards or away from the stigmas (Toräng et al. 2017).

The relationship between herkogamy and outcrossing rate is more complex, not least because it depends on interactions with pollinators. At the species and population level, positive herkogamy-outcrossing relationships are expected to arise due to selection for self-pollination as a mechanism of reproductive assurance when cross-pollination is unreliable, and selection for avoidance of self-pollination and/or sexual interference when cross-pollination is reliable (e.g. Moeller 2006; Opedal et al. 2016a; but see Koski et al. 2017).

Two observations help to delimit the likely shapes of herkogamy-outcrossing relationships. First, when anthers and stigmas are in direct contact (zero herkogamy), outcrossing rates will tend to be low. Second, when herkogamy becomes so large that autogamous selfing is unlikely (lower asymptote in Fig. 1), outcrossing rates should tend to stabilize at a rate close to 1 - S_G - S_B, where S_G is the rate of geitonogamy, and S_B is the rate of biparental inbreeding (Fig. 2). Between these extremes, the shapes of herkogamy-outcrossing relationships are likely to vary depending on specific aspects of floral biology. For example, outcrossing rates depend not only on the amount of self vs. cross-pollen deposited onto stigmas, but also on the timing of pollen deposition (e.g. Sorin et al. 2016). Therefore, herkogamy-outcrossing relationships may differ, for example, between those species where a female phase precedes a bisexual phase (incomplete protogyny), and those where a bisexual phase precedes a female phase (incomplete protandry) (Fig. 2). While low herkogamy combined with incomplete protandry will tend to favor selfing regardless of the reliability of pollination, incomplete protogyny will favor outcrossing during the female phase when pollination is reliable.

Importantly, while population-mean herkogamy is expected to correlate with the long-term reliability of pollination, there are several reasons why the expected relationship would not be detected in all studies. First, because outcrossing rates are bounded between 0 and 1, asymptotes are expected at least at the upper limit (Fig. 2) and comparisons among populations or species at the upper end may fail to detect differences. Second, one or more populations may experience an unusual year, and thus a mismatch between average herkogamy and current pollination reliability. In such situations, outcrossing rates will often be better predicted by current pollination reliability than by mean herkogamy. This 'stochastic' variation is one of the reasons why single-year estimates of outcrossing rates are not necessarily very good measures of the long-term mating system (see further discussion below). If all populations experience proportional increases or decreases in pollination reliability across taxa, regions, or years, this would be predicted to shift the intercept and/or slope of the herkogamy-outcrossing relationship (Fig. 2). Specifically, the solid lines in Fig. 2 might represent a 'good' year in terms of pollination reliability (many and/or efficient pollinators), while the dashed lines might represent a 'bad' year (few and/or inefficient pollinators). Similarly, variation in pollination reliability among populations will tend to add noise to herkogamy-outcrossing relationships, thus reducing the variance in current outcrossing rates explained by herkogamy. Some empirical data suggest that the impact of variation in pollination reliability on outcrossing rates is greater in more herkogamous populations (Eckert et al. 2009), but the generality of this pattern remains unknown, and is likely to depend on aspects of floral biology. Overall, more variable patterns should be expected among studies assessing herkogamy-outcrossing relationships, than among those assessing herkogamy-autofertility relationships (Table 1). While the range of patterns illustrated in Fig. 2 will probably fit a good number of species, they are unlikely to fit all.

Within populations, more herkogamous flowers may also be more outcrossed. Such relationships may be causal, driven for example by reduced interference between male and female functions in more herkogamous flowers (Webb and Lloyd 1986). Indeed, Webb and Lloyd (1986) proposed avoidance of sexual interference as a primary function of herkogamy, at least in self-incompatible taxa. Specifically, reduced herkogamy may restrict pollinator access to stigmas, and increase autonomous and pollinator-facilitated self-pollen deposition onto stigmas. This can in turn cause 'stigma clogging' and intensify competition between self- and cross-pollen. Thus, assuming that the rate of self-fertilization increases with self-pollination (Holsinger 1991), more herkogamous flowers are expected to be more outcrossed. More herkogamous flowers may also receive more cross-pollen, if herkogamy correlates positively with traits involved in pollinator attraction (reward or advertisement). In the latter case, the herkogamy-outcrossing relationship would be non-causal.

Finally, herkogamy-outcrossing relationships (at all levels) are complicated by the fact that realized selfing rates in natural populations represent the sum of within-flower selfing (autogamy), between-flower selfing (geitonogamy), and biparental inbreeding. Among these, only the autogamous component is directly related to variation in herkogamy (Herlihy and Eckert 2004, and see below). Relationships with other components could arise indirectly if herkogamy affects, for example, the amount of pollen available for cross-pollination (including geitonogamy and biparental inbreeding).

Summary of empirical herkogamy-autofertility and herkogamy-outcrossing relationships

To evaluate the hypothesis that more herkogamous taxa have historically experienced more reliable pollination, and more outcrossed mating systems, I surveyed the literature for studies reporting empirical herkogamy-autofertility and herkogamy-outcrossing relationships. With a few exceptions, studies that have assessed herkogamy-autofertility and/or herkogamy-outcrossing relationships have detected the expected patterns: more herkogamous individuals, populations and species tend to exhibit reduced autofertility, and to be more outcrossed (Table 2). The generality of these patterns is supported by the wide geographic and taxonomic range of the focal taxa, and the diversity of growth forms and life histories represented among them. For example, Table 2 includes species from 17 families, whose habitats range from the lowland tropics (e.g. *Turnera ulmifolia*, *Eichhornia paniculata*) to high-alpine meadows in the Alps (*Primula halleri*) and Rocky Mountains (*Aquilegia caerulea*). Similarly, life histories range from annuals (*Clarkia xantiana*) to long-lived woody vines (*Dalechampia* spp.) and trees (*Nicotiana glauca*).

As expected, negative herkogamy-autofertility relationships were more consistent than were positive herkogamy-outcrossing relationships (Table 2). Interestingly, in most of the studies where results deviated from expectations, the authors offered biologically meaningful explanations. These include differences in pollinator foraging behaviour on *Aquilegia caerulea* flowers (Brunet and Sweet 2006), and strong correlations with dichogamy rather than herkogamy in *Gilia achilleifolia* (Schoen 1982) and *Campanula americana* (Koski et al. 2018). Other examples are discussed in more detail below.

Correlates of herkogamy beyond autofertility and outcrossing rate

The focus above has been on relationships among herkogamy, autofertility, and outcrossing rate, as these are the most frequently assessed. If variation in herkogamy reflects variation in the reproductive environment, we also expect correlations with other features of plant pollination and mating systems (Table 1). For example, it follows logically that individual herkogamy should correlate negatively with the number of autogamous (within-flower) pollen grains deposited onto stigmas. Such relationships have been demonstrated, for example, in *Erythronium grandiflorum* (Thomson and Stratton 1985), and in several species of *Dalechampia* (Bolstad et al. 2010; Pérez-Barrales et al. 2013), and *Ipomoea* (Murcia 1990; Parra-Tabla and Bullock 2005).

For allogamous (between-flower) pollen loads (including geitonogamous self-pollen), the opposite relationship should be expected, although with more variation among studies (Table 1). This follows from the same logic that average herkogamy should represent variation in the long-term reliability of pollination, and hence the level of outcrossing. For example, Opedal et al. (2016a) reported a strong positive correlation between population-mean cross-pollen loads and average herkogamy in *Dalechampia scandens* populations. At the individual level, allogamous pollen loads are unlikely to correlate with herkogamy, unless herkogamy is correlated with pollinator-attraction traits. If stigmatic pollen loads are limited by the size of the stigmatic surface relative to the size of pollen grains, a positive association could also arise if greater self-pollen loads of less herkogamous flowers precludes subsequent deposition of cross-pollen.

As discussed briefly above, herkogamy may not uniformly affect all functional components of selfing. A clear demonstration of this has emerged from studies of the North-American columbine *Aquilegia canadensis*. Herlihy and Eckert (2004) used floral-emasculation experiments combined with genetic analyses to disentangle the contributions of autogamy, geitonogamy, and biparental inbreeding to realized selfing rates in natural populations. As expected, only the autogamous component of selfing correlated strongly and negatively with herkogamy, while rates of geitonogamy and biparental inbreeding were instead explained by variation in ecological factors such as plant density and canopy cover.

Medrano et al. (2005, 2012) reported an interesting exception from the usual herkogamy-outcrossing relationship in the daffodil *Narcissus longipathus*. While the authors failed to detect the expected difference in outcrossing rate between plants exhibiting low, medium, and high herkogamy (Table 2), they found that the average number of fathers siring offspring in fruits increased in more herkogamous plants. Such relationships might indeed be expected to be common, and perhaps ubiquitous in species with granular pollen and where pollinators visit multiple plants per foraging bout.

Finally, plant mating systems strongly affect the population-genetic structure of populations, and herkogamy is therefore also expected to correlate positively with measures of genetic diversity within populations (e.g. Barrett and Husband 1990; Opedal et al. 2016a), and with genetic differentiation among populations as measured by F_{ST} or related metrics

(Hamrick and Godt 1996; Duminil et al. 2007). The latter relationship could arise among species, or among regions in widespread species.

On the use of herkogamy as a mating-system proxy

The consistent relationships among herkogamy, autofertility and outcrossing rates reviewed in Table 2 provides strong support for the hypothesis that when two self-compatible populations or species differ in average herkogamy, they also differ in their mating systems. An important consequence of this ‘herkogamy rule’ is that variation in population-mean herkogamy can be used as a reliable proxy of variation in mating systems. A key advantage of using mean herkogamy as a proxy of the mating system is that it is likely to reflect the mating history of a population (i.e. the long-term mean outcrossing rate) rather than the mating system in a given year or season. Indeed, outcrossing rates are known to vary from year to year within populations (Eckert et al. 2009), and even within a single season (Yin et al. 2016). Thus, using single estimates of outcrossing rates as a measure of the mating system could run the risk of interpreting an unusual year as the long-term trend, and hence bias the conclusion of comparative studies. Because measuring herkogamy is fast and can be done at essentially no cost, this provides ideal opportunities for comparative studies. In many systems, herkogamy also exhibit only limited environmental variation (Opedal et al. 2016b and references therein).

For example, herkogamy has been repeatedly used as a mating-system proxy in comparative studies of inbreeding depression, testing the hypothesis that more inbred populations or families are subject to purging of deleterious alleles and hence experience less intense inbreeding depression than do less inbred populations or families (Carr et al. 1997; Byers and Waller 1999; Chang and Rausher 1999; Takebayashi and Delph 2000; Stone and Motten 2002; Herlihy and Eckert 2004; Weber et al. 2012; Opedal et al. 2015). Other examples include comparative studies of mating-system effects on geographical range size

(Grossenbacher et al. 2015), range overlap (Grossenbacher et al. 2016), and sexual conflict over seed provisioning (A. Raunsgard et al., manuscript in revision).

Herkogamy has also been used as a mating-system proxy in studies of the evolution of the mating system itself (Armbruster 1993). Here caution must obviously be exercised, as the argument might become circular (mating-system changes are assumed to lead to evolutionary shifts in herkogamy, and mating-system shifts are then inferred from these shifts). As should be clear from the arguments of this essay, however, herkogamy-mating-system relationships are consistent enough, at least for many groups, to place some trust in such analyses.

Reproductive-function traits and the structure of plant communities

Consistent correlations between herkogamy and mating systems also suggest that herkogamy can be informative about processes structuring natural plant communities. The role of functional traits in shaping species assemblages is a hot topic in community ecology (e.g. Adler et al. 2013; Kohli et al. 2018), and while most studies have focused on traits involved in abiotic resource competition or in response to abiotic environmental variables (‘vegetative processes’), further consideration of traits mediating reproductive interactions may provide more complete insights into the processes shaping natural plant assemblages (Armbruster 1995; Sargent and Ackerly 2008; Pauw 2013; Briscoe Runquist et al. 2016). Herkogamy and the component traits (positions of stigmas and anthers) can affect reproductive interactions through shared pollinators in several ways. First, the positions of the sexual organs can affect the position of pollen placement on the bodies of pollinators, and hence divergence in these traits can reduce interspecific pollination (e.g. Armbruster et al. 1994; Kay 2006; Keller et al. 2016). Because the position of pollen placement depends on the absolute rather than relative positions of anthers and stigmas, this process may be independent of mean herkogamy (which is defined by the relative positions of the sexual organs). The relevance of herkogamy for reproductive interactions arises from its effect on the mating system and the dynamics of pollen transfer. In selfing species with low herkogamy, large autonomous pollen loads may reduce the fitness consequences of subsequent heterospecific pollen deposition, thus providing a buffer against the negative effects of pollinator sharing. For example, *Ipomoea purpurea* and *I. hederacea* commonly occur in sympatry, and share bumblebee pollinators. Smith and Rausher (2007, 2008) have demonstrated experimentally that reduced herkogamy in *I. hederacea* acts as a ‘shield’ reducing interspecific pollination and thus increasing fitness. A role of mating-system divergence mediated by herkogamy has also been invoked to explain species coexistence in other systems, including *Arenaria* (Fishman and Wyatt 1999), *Mimulus* (Grossenbacher and Whittall 2011; Briscoe Runquist et al. 2016), and *Centaurium* (Brys et al. 2014; Brys et al. 2016; Schouppe et al. 2017). Taken together, these observations suggest that the probability of coexistence is greater for species pairs that share pollinators if they differ in mean herkogamy, and the effect would be stronger if one of the species exhibit very low herkogamy. Similarly, pairs of highly selfing species with very low average herkogamy might be more likely to coexist, because reproductive interactions through shared pollinators are unlikely in such species. The kind of mating-system-related structure of plant communities described above may result from ecological ‘sorting’ of species into communities, or at least in part be caused by selection for reproductive character displacement among sympatric species (Brown and Wilson 1956; Armbruster and Muchhala 2009).

Recent methodological developments of joint species distribution models are beginning to consider species traits as predictors of species cooccurrences (e.g. Abrego et al. 2017). While traits mediating vegetative processes are likely to be informative about cooccurrence explained by shared or contrasting responses to the abiotic environment, herkogamy and other traits mediating reproductive interactions may increase the net explanatory power by explaining patterns of variation unexplained by vegetative processes.

Thus, increased consideration of ‘reproductive-function traits’ will allow us to move beyond purely vegetative processes in predictive models of community assembly.

Concluding remarks

Here, I have compiled a large body of evidence supporting the hypothesis that, when a pair of self-compatible taxa differ in their average anther-stigma separation (herkogamy), they will also differ predictably in their mating systems. This nearly ubiquitous pattern provides a valuable tool for a wide range of comparative studies, and have already provided important insights into the ecological, genetical, and evolutionary causes and consequences of variation and evolution of plant mating systems. Going forward, increased use of herkogamy as a functional trait in studies of community structure and assembly might yield new and important insights allowing us to predict the consequences of changes in the reproductive environment.

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References

Abrego N, A Norberg, O Ovaskainen 2017 Measuring and predicting the influence of traits on the assembly processes of wood-inhabiting fungi. J Ecol 105: 1070-1081.

Adler PB, A Fajardo, AR Kleinhesselink, NJ Kraft 2013 Trait-based tests of coexistence mechanisms. Ecol Lett 16: 1294-1306.

Armbruster WS 1988 Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. Ecology 69: 1746-1761.

Armbruster WS 1993 Evolution of plant pollination systems - hypotheses and tests with the neotropical vine *Dalechampia*. Evolution 47: 1480-1505.

Armbruster WS 1995 The origins and detection of plant community structure: reproductive versus vegetative processes. Folia Geobotanica & Phytotaxonomica 30: 483-497.

Armbruster WS, ME Edwards, EM Debevec 1994 Floral character displacement generates assemblage structure of western Australian triggerplants (*Stylidium*). Ecology 75: 315-329.

Armbruster WS, N Muchhala 2009 Associations between floral specialization and species diversity: cause, effect, or correlation? Evol Ecol 23: 159-179.

Armbruster WS, CPH Mulder, BG Baldwin, S Kalisz, B Wessa, H Nute 2002 Comparative analysis of late floral development and mating-system evolution in Tribe Collinsieae (Scrophulariaceae SL). Am J Bot 89: 37-49.

Armbruster WS, C Pélabon, TF Hansen, GH Bolstad 2009 Macroevolutionary patterns of pollination accuracy: a comparison of three genera. New Phytol 183: 600-617.

Barrett SCH 2002 The evolution of plant sexual diversity. Nat Rev Genet 3: 274-284.

Barrett SCH 2003 Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 358: 991-1004.

Barrett SCH, BC Husband 1990 Variation in outcrossing rates in *Eichhornia paniculata*: the role of demographic and reproductive factors. Plant Species Biol 5: 41-56.

Barrett SCH, JS Shore 1987 Variation and evolution of breeding systems in the *Turnera ulmifolia* L. complex (Turneraceae). Evolution 41: 340-354.

Belaoussoff S, JS Shore 1995 Floral correlates and fitness consequences of mating-system variation in *Turnera ulmifolia*. Evolution 49: 545-556.

Bolstad GH, WS Armbruster, C Pélabon, R Pérez-Barrales, TF Hansen 2010 Direct selection at the blossom level on floral reward by pollinators in a natural population of *Dalechampia schottii*: full-disclosure honesty? New Phytol 188: 370-384.

Breese EL 1959 Selection for differing degrees of out-breeding in *Nicotiana rustica*. Ann Bot 23: 331-344.

Briscoe Runquist R, D Grossenbacher, S Porter, K Kay, J Smith 2016 Pollinator-mediated assemblage processes in California wildflowers. J Evol Biol 29: 1045-1058.

Brown WL, EO Wilson 1956 Character displacement. Systematic Zoology 5: 49-64.

Brunet J, CG Eckert 1998 Effects of floral morphology and display on outcrossing in Blue Columbine, *Aquilegia caerulea* (Ranunculaceae). Funct Ecol 12: 596-606.

Brunet J, HR Sweet 2006 Impact of insect pollinator group and floral display size on outcrossing rate. Evolution 60: 234-246.

Brys R, B Geens, T Beeckman, H Jacquemyn 2013 Differences in dichogamy and herkogamy contribute to higher selfing in contrasting environments in the annual *Blackstonia perfoliata* (Gentianaceae). Ann Bot 111: 651-661.

Brys R, H Jacquemyn 2011 Variation in the functioning of autonomous self-pollination, pollinator services and floral traits in three *Centaurium* species. Ann Bot 107: 917-925.

Brys R, H Jacquemyn 2012 Effects of human-mediated pollinator impoverishment on floral traits and mating patterns in a short-lived herb: an experimental approach. Funct Ecol 26: 189-197.

Brys R, J van Cauwenberghe, H Jacquemyn, N Rafferty 2016 The importance of autonomous selfing in preventing hybridization in three closely related plant species. J Ecol 104: 601-610.

Brys R, A Vanden Broeck, J Mergeay, H Jacquemyn 2014 The contribution of mating system variation to reproductive isolation in two closely related *Centaurium* species (Gentianaceae) with a generalized flower morphology. Evolution 68: 1281-1293.

Byers DL, DM Waller 1999 Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annu Rev Ecol Syst 30: 479-513.

Carr DE, CB Fenster 1994 Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. Heredity 72: 606-618.

Carr DE, CB Fenster, MR Dudash 1997 The relationship between mating-system characters and inbreeding depression in *Mimulus guttatus*. Evolution 51: 363-372.

Chang SM, MD Rausher 1998 Frequency-dependent pollen discounting contributes to maintenance of a mixed mating system in the common morning glory *Ipomoea purpurea*. Am Nat 152: 671-683.

Chang SM, MD Rausher 1999 The role of inbreeding depression in maintaining the mixed mating system of the common morning glory, *Ipomoea purpurea*. Evolution 53: 1366-1376.

Chen XS, S Marten-Rodriguez, QJ Li, CB Fenster 2009 Potential autonomous selfing in *Gesneria citrina* (Gesneriaceae), a specialized hummingbird pollinated species with variable expression of herkogamy. J Integr Plant Biol 51: 973-978.

Dart SR, KE Samis, E Austen, CG Eckert 2012 Broad geographic covariation between floral traits and the mating system in *Camissoniopsis cheiranthifolia* (Onagraceae): multiple stable mixed mating systems across the species range? Ann Bot 109: 599-611.

de Vos JM, B Keller, ST Isham, S Kelso, E Conti 2012 Reproductive implications of herkogamy in homostylous primroses: variation during anthesis and reproductive assurance in alpine environments. Funct Ecol 26: 854-865.

de Vos JM, B Keller, L-R Zhang, MD Nowak, E Conti 2018 Mixed mating in homostylous species: genetic and experimental evidence from an alpine plant with variable herkogamy, *Primula halleri*. Int J Plant Sci 179: 87-99.

Dole JA 1992 Reproductive assurance mechanisms in 3 taxa of the *Mimulus guttatus* complex (Scrophulariaceae). Am J Bot 79: 650-659.

Duminil J, S Fineschi, A Hampe, P Jordano, D Salvini, GG Vendramin, RJ Petit 2007 Can population genetic structure be predicted from life-history traits? Am Nat 169: 662-672.

Duncan TM, MD Rausher 2013 Evolution of the selfing syndrome in *Ipomoea*. Frontiers in Plant Science 4.

Eckert CG, B Ozimec, CR Herlihy, CA Griffin, MB Routley 2009 Floral morphology mediates temporal variation in the mating system of a self-compatible plant. Ecology 90: 1540-1548.

Eckert CG, A Schaefer 1998 Does self-pollination provide reproductive assurance in *Aquilegia canadensis* (Ranunculaceae)? Am J Bot 85: 919-924.

Elle E, JD Hare 2002 Environmentally induced variation in floral traits affects the mating system in *Datura wrightii*. Funct Ecol 16: 79-88.

Ennos RA 1981 Quantitative studies of the mating system in two sympatric species of *Ipomoea* (Convolvulaceae). Genetica 57: 93-98.

Epperson BK, MT Clegg 1987 First-pollination primacy and pollen selection in the morning glory, *Ipomoea purpurea*. Heredity 58: 5-14.

Fishman L, R Wyatt 1999 Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution 53: 1723-1733.

Fægri K, L van der Pijl 1979 The principles of pollination ecology. Pergamon Press, Oxford, UK.

Ganders FR, SK Denny, D Tsai 1985 Breeding systems and genetic variation in *Amsinckia spectabilis* (Boraginaceae). Canadian Journal of Botany 63: 533-538.

Goodwillie C, JM Ness 2005 Correlated evolution in floral morphology and the timing of self-compatibility in *Leptosiphon jepsonii* (Polemoniaceae). Int J Plant Sci 166: 741-751.

Goodwillie C, JJ Weber 2018 The best of both worlds? A review of delayed selfing in flowering plants. Am J Bot 105: 641-655.

Grant V, KA Grant 1965 Flower pollination in the *Phlox* family. Columbia University Press, New York.

Grossenbacher D, RD Briscoe Runquist, EE Goldberg, Y Brandvain 2016 No association between plant mating system and geographic range overlap. Am J Bot 103: 110-117.

Grossenbacher D, RB Runquist, EE Goldberg, Y Brandvain 2015 Geographic range size is predicted by plant mating system. Ecol Lett 18: 706-713.

Grossenbacher DL, JB Whittall 2011 Increased floral divergence in sympatric monkeyflowers. Evolution 65: 2712-2718.

Hamrick JL, MJW Godt 1996 Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 351: 1291-1298.

Herlihy CR, CG Eckert 2004 Experimental dissection of inbreeding and its adaptive significance in a flowering plant, *Aquilegia canadensis* (Ranunculaceae). Evolution 58: 2693-2703.

Herlihy CR, CG Eckert 2005 Evolution of self-fertilization at geographical range margins? A comparison of demographic, floral, and mating system variables in central vs. peripheral populations of *Aquilegia canadensis* (Ranunculaceae). Am J Bot 92: 744-751.

Herlihy CR, CG Eckert 2007 Evolutionary analysis of a key floral trait in *Aquilegia canadensis* (Ranunculaceae): genetic variation in herkogamy and its effect on the mating system. Evolution 61: 1661-1674.

Holsinger KE 1991 Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. Am Nat 138: 606-622.

Holtsford TP, NC Ellstrand 1992 Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). Evolution 46: 216-225.

Houle D, C Pélabon, GP Wagner, TF Hansen 2011 Measurement and meaning in biology. Q Rev Biol 86: 3-34.

Karron JD, RT Jackson, NN Thumser, SL Schlicht 1997 Outcrossing rates of individual *Mimulus ringens* genets are correlated with anther-stigma separation. Heredity 79: 365-370.

Kay KM 2006 Reproductive isolation between two closely related hummingbird-pollinated neotropical gingers. Evolution 60: 538-552.

Keller B, JM de Vos, AN Schmidt-Lebuhn, JD Thomson, E Conti 2016 Both morph- and species-dependent asymmetries affect reproductive barriers between heterostylous species. Ecol Evol 6: 6223-6244.

Kohli BA, RC Terry, RJ Rowe 2018 A trait-based framework for discerning drivers of species co-occurrence across heterogeneous landscapes. Ecography in press.

Koski MH, D Grossenbacher, JW Busch, LF Galloway 2017 A geographic cline in the ability to self-fertilize is unrelated to the pollination environment. Ecology 98: 2930-2939.

Koski MH, L Kuo, KM Niedermaier, LF Galloway 2018 Timing is everything: dichogamy and pollen germinability underlie variation in autonomous selfing among populations. Am J Bot in press.

Lavorel S, E Garnier 2002 Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct Ecol 16: 545-556.

Lennartsson T, JGB Oostermeijer, J van Dijk, HCM den Nijs 2000 Ecological significance and heritability of floral reproductive traits in *Gentianella campestris* (Gentianaceae). Basic Appl Ecol 1: 69-81.

Li XX, Y Zou, CL Xiao, RW Gituru, YH Guo, CF Yang 2013 The differential contributions of herkogamy and dichogamy as mechanisms of avoiding self-interference in four self-incompatible *Epimedium* species. J Evol Biol 26: 1949-1958.

McGill BJ, BJ Enquist, E Weiher, M Westoby 2006 Rebuilding community ecology from functional traits. Trends Ecol Evol 21: 178-185.

Medrano M, CM Herrera, SCH Barrett 2005 Herkogamy and mating patterns in the self-compatible daffodil *Narcissus longispathus*. Ann Bot 95: 1105-1111.

Medrano M, R Requerey, JD Karron, CM Herrera 2012 Herkogamy and mate diversity in the wild daffodil *Narcissus longispathus*: beyond the selfing-outcrossing paradigm in the evolution of mixed mating. Plant Biol 14: 801-810.

Moeller DA 2006 Geographic structure of pollinator communities, reproductive assurance, and the evolution of self-pollination. Ecology 87: 1510-1522.

Motten AF, J Antonovics 1992 Determinants of outcrossing rate in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). Am J Bot 79: 419-427.

Motten AF, JL Stone 2000 Heritability of stigma position and the effect of stigma-anther separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). Am J Bot 87: 339-347.

Murcia C 1990 Effect of floral morphology and temperature on pollen receipt and removal in *Ipomoea trichocarpa*. Ecology 71: 1098-1109.

Ollerton J, S Watts, S Connerty, J Lock, L Parker, I Wilson, S Schueller, J Nattero, AA Cocucci, I Izhaki, S Geerts, A Pauw, JC Stout 2012 Pollination ecology of the invasive tree tobacco *Nicotiana* *glauca*: comparisons across native and non-native ranges. Journal of Pollination Ecology 9: 85-95.

Opedal ØH, E Albertsen, WS Armbruster, R Pérez-Barrales, M Falahati-Anbaran, C Pélabon 2016a Evolutionary consequences of ecological factors: pollinator reliability predicts mating-system traits of a perennial plant. Ecol Lett 19: 1486-1495.

Opedal ØH, WS Armbruster, C Pélabon 2015 Inbreeding effects in a mixed-mating vine: effects of mating history, pollen competition and stress on the cost of inbreeding. AoB Plants 7: plv133.

Opedal ØH, GH Bolstad, TF Hansen, WS Armbruster, C Pélabon 2017 The evolvability of herkogamy: quantifying the evolutionary potential of a composite trait. Evolution 71: 1572-1586.

Opedal ØH, J Listemann, E Albertsen, WS Armbruster, C Pélabon 2016b Multiple effects of drought on pollination and mating-system traits in *Dalechampia scandens*. Int J Plant Sci 177: 682-693.

Ornduff R 1969 Reproductive biology in relation to angiosperm systematics. Taxon 18: 121-133.

Parra-Tabla V, SH Bullock 2005 Ecological and selective effects of stigma-anther separation in the self-incompatible tropical tree *Ipomoea wolcottiana* (Convolvulaceae). Plant Syst Evol 252: 85-95.

Pauw A 2013 Can pollination niches facilitate plant coexistence? Trends Ecol Evol 28: 30-37.

Pérez-Barrales R, GH Bolstad, C Pélabon, TF Hansen, WS Armbruster 2013 Pollinators and seed predators generate conflicting selection on *Dalechampia* blossoms. Oikos 122: 1411-1428.

Rick CM, JF Fobes, M Holle 1977 Genetic variation in *Lycopersicon pimpinellifolium*: evidence of evolutionary change in mating systems. Plant Syst Evol 127: 139-170.

Rick CM, M Holle, RW Thorp 1978 Rates of cross-pollination in *Lycopersicon pipinellifolium*: impact of genetic variation in floral characters. Plant Syst Evol 129: 31-44.

Ritland C, K Ritland 1989 Variation of sex allocation among 8 taxa of the *Mimulus guttatus* species complex (Scrophulariaceae). Am J Bot 76: 1731-1739.

Robertson AW, DG Lloyd 1991 Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). Evolutionary Trends in Plants 5: 53-63.

Sargent RD, DD Ackerly 2008 Plant-pollinator interactions and the assembly of plant communities. Trends Ecol Evol 23: 123-130.

Schoen DJ 1982 The breeding system of *Gilia achilleifolia*: variation in floral characteristics and outcrossing rate. Evolution 36: 352-360.

Schouppe D, R Brys, M Vallejo-Marin, H Jacquemyn 2017 Geographic variation in floral traits and the capacity of autonomous selfing across allopatric and sympatric populations of two closely related *Centaurium* species. Sci Rep 7: 46410.

Schueller SK 2004 Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). Am J Bot 91: 672-681.

Shipley B, F De Bello, JHC Cornelissen, E Laliberte, DC Laughlin, PB Reich 2016 Reinforcing loose foundation stones in trait-based plant ecology. Oecologia 180: 923-931.

Sicard A, M Lenhard 2011 The selfing syndrome: a model for studying the genetic and evolutionary basis of morphological adaptation in plants. Ann Bot 107: 1433-1443.

Smith RA, MD Rausher 2007 Close clustering of anthers and stigma in *Ipomoea hederacea* enhances prezygotic isolation from Ipomoea purpurea. New Phytol 173: 641-647.

Sorin YB, RJ Mitchell, DW Trapnell, JD Karron 2016 Effects of pollination and postpollination processes on selfing rate in *Mimulus ringens*. Am J Bot 103: 1524-1528.

Stebbins GL 1970 Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. Annu Rev Ecol Syst 1: 307-326.

Stone JL, AF Motten 2002 Anther-stigma separation is associated with inbreeding depression in *Datura stramonium*, a predominantly self-fertilizing annual. Evolution 56: 2187-2195.

Takebayashi N, LF Delph 2000 An association between a floral trait and inbreeding depression. Evolution 54: 840-846.

Takebayashi N, DE Wolf, LF Delph 2006 Effect of variation in herkogamy on outcrossing within a population of *Gilia achilleifolia*. Heredity (Edinb) 96: 159-165.

Tedder A, S Carleial, M Golebiewska, C Kappel, KK Shimizu, M Stift 2015 Evolution of the selfing syndrome in *Arabis alpina* (Brassicaceae). Plos One 10: e0126618.

Thomson JD, DA Stratton 1985 Floral morphology and cross-pollination in *Erythronium grandiflorum* (Liliaceae). Am J Bot 72: 433-437.

Toräng P, L Vikström, J Wunder, S Wötzel, G Coupland, J Ågren 2017 Evolution of the selfing syndrome: anther orientation and herkogamy together determine reproductive assurance in a self-compatible plant. Evolution 71: 2206-2218.

van der Niet T, R Peakall, SD Johnson 2014 Pollinator-driven ecological speciation in plants: new evidence and future perspectives. Ann Bot 113: 199-211.

Webb CJ, DG Lloyd 1986 The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. N Z J Bot 24: 163-178.

Weber JJ, C Goodwillie 2009 Evolution of the mating system in a partially self-incompatible species: reproductive assurance and pollen limitation in populations that differ in the timing of self-compatibility. Int J Plant Sci 170: 885-893.

Weber JJ, SG Weller, AK Sakai, A Nguyen, ND Tai, CA Dominguez, FE Molina-Freaner 2012 Purging of inbreeding depression within a population of Oxalis alpina (Oxalidaceae). Am J Bot 99: 923-932.

Yin G, SC Barrett, YB Luo, WN Bai 2016 Seasonal variation in the mating system of a selfing annual with large floral displays. Ann Bot 117: 391-400.

Figure legends

Fig. 1. Range of expected functional relationships between herkogamy (anther-stigma distance) and autofertility (autonomous seed-set in the absence of pollinators). The solid line represents a 'Type I' herkogamy-autofertility relationship, and the dashed line a 'Type II' herkogamy-autofertility relationship. The units on the x-axis are expected to be taxon-specific and are therefore not shown.

Fig. 2. Examples of expected functional relationships between herkogamy (anther-stigma distance) and outcrossing rate for species exhibiting incomplete protandry (a) and incomplete protogyny (b). The dotted lines indicate the patterns expected in the absence of geitonogamy (S_G) and biparental inbreeding (S_B), and the solid and dashed lines indicate the patterns expected in the presence of geitonogamy and biparental inbreeding under conditions of high (solid line) and low (dashed line) pollination reliability. The units on the x-axis are expected to be taxon-specific and are therefore not shown.

Table 1. Expected correlates of herkogamy (anther-stigma distance) at the level of individuals (I), populations (S), and species (S). The 'Variance' column indicates whether relationships are expected to be stable or variable across different studies. See text for details.

Correlate	Definition	Sign of correlation	Level	Variance
Autofertility (a)	Seed set in absence of pollinators	Negative	I, P, S	Low
Outcrossing rate (t)	Proportion of seeds outcrossed	Positive	I, P, S	High
Autogamous pollen load (P_S)	Number of self-pollen grains on stigma	Negative	I, P, S	Low
Allogamous pollen load (P_C)	Number of cross-pollen grains on stigma	Positive	P, S	High
Genetic diversity (e.g. H_E)	Among-individual allelic variation	Positive	P, S	High
Genetic differentiation (F_{ST})	Proportion of genetic diversity among populations	Negative	S	High
Sire number	Number of sires per fruit	Positive	I, P, S	High

Table 2. List of studies testing for relationships between herkogamy and autofertility (*a*) or outcrossing rate (*t*) among individuals, populations, or species. + and – indicate strong relationships, (+) and (-) indicate weaker relationships, and 0 indicate no detectable relationship.

Species	Family	Level	*a*	*t*	Evidence	Reference
Amsinckia spectabilis	Boraginaceae	Population		+	Positive relationship between herkogamy and outcrossing rate among 10 populations, including heterostylous, homostylous and mixed populations.	Ganders et al. 1985
Aquilegia caerulea	Ranunculaceae	Individual	**-**	+	Herkogamy negatively correlated with autofertility, and positively with outcrossing rate, among three groups within a population.	Brunet and Eckert 1998
		Population		**(-)**	Weak negative relationship between herkogamy and outcrossing rate across 10 population-year combinations.	Brunet and Sweet 2006
Aquilegia canadensis	Ranunculaceae	Individual	**-**		Negative relationship between individual herkogamy and autofertility among plants from two populations.	Eckert and Schaefer 1998
		Population		**(+)**	Weak positive relationship between herkogamy and outcrossing rate among 10 populations. Negative relationship with autogamous selfing in isolation.	Herlihy and Eckert 2004
		Population		**0**	No detectable difference in mating system between central and northern population despite difference in average herkogamy.	Herlihy and Eckert 2005
		Individual		+	Greater outcrossing rate of high vs. low herkogamy groups of plants in 13/19 populations.	Herlihy and Eckert 2007
		Population		+	Outcrossing rate increases with increasing herkogamy among 18 populations. Positive relationship between herkogamy and between-year variation in outcrossing rates.	Eckert et al 2009
Arabis alpina	Brassicaceae	Population		+	Reduced herkogamy in three selfing populations compared to three more outcrossing populations.	Tedder et al. 2015
Blackstonia perfoliata	Gentianaceae	Population	**-**		Greater autofertility in pollinator-poor environment with smaller flowers and reduced herkogamy.	Brys et al. 2013
Camissoniopsis cheiranthifolia	Onagraceae	Population	**-**	+	Positive relationship between herkogamy and outcrossing rate among 16 populations.	Dart et al. 2012
Campanula americana	Campanulaceae	Population	**0**		No detectable relationship between herkogamy and autofertility among 24 populations. Autofertility explained by dichogamy.	Koski et al. 2018

Centaurium erythraea	Gentianaceae	Population	-		Greater autofertility in pollinator-poor environment with smaller flowers and reduced herkogamy.	Brys and Jacquemyn 2012
Centaurium	Gentianaceae	Species	-		Negative relationship between herkogamy and autofertility across three species.	Brys and Jacquemyn 2011 (and see Schouppe et al. 2017)
Clarkia parviflora	Onagraceae	Population	-		Negative relationship between herkogamy and autofertility among 15 populations.	Moeller 2006
Clarkia tembloriensis	Onagraceae	Population		+	Positive relationship between herkogamy and outcrossing rate among eight populations. Herkogamy positively correlated with dichogamy.	Holtsford and Ellstrand 1992
Clarkia xantiana	Onagraceae	Population	-		Negative relationship between herkogamy and autofertility among 15 populations.	Moeller 2006
Dalechampia scandens	Euphorbiaceae	Individual, Population	-		Negative relationship between herkogamy and autofertility rate within and among four populations.	Opedal et al. 2015
		Population, Species	-		Negative relationship between herkogamy and autofertility across two populations of each of two species.	Opedal et al. 2016a
		Population		+	Positive relationship between herkogamy and outcrossing rate among four populations.	Opedal et al. 2016b
Dalechampia	Euphorbiaceae	Species	-		Negative relationship between herkogamy and autofertility across nine species.	Armbruster 1988
Datura stramonium	Solanaceae	Individual		+	Positive relationship between herkogamy and outcrossing rate among plants within two populations.	Motten and Stone 2000 (and see Motten and Antonovics 1992)
Datura wrightii	Solanaceae	Individual	-	+	Positive relationship between herkogamy and outcrossing rate in a field experiment, and negative relationship between herkogamy and autofertility in a greenhouse experiment.	Elle and Hare 2002
Eichhornia paniculata	Pontederiaceae	Population		+	Positive relationship between "frequency of selfing variants" and outcrossing rate among 32 populations.	Barrett and Husband 1990
Epimedium	Berberidaceae	Species	-		Greater autofertility in two low-herkogamy species compared to two high-herkogamy species.	Li et al. 2013
Gentianella campestris	Gentianaceae	Individual	**(-)**		Greater autofertility in 'iso-stigmatic' and 'hypo-stigmatic' groups than in 'hyper-stigmatic groups'.	Lennartsson et al. 2000
Gesneria citrina	Gesneriaceae	Individual	-		Negative relationship between herkogamy and autofertility within a population.	Chen et al. 2009

Gilia achilleifolia	Polemoniaceae	Individual		+	Higher outcrossing rate within a population for the group of plants with greater herkogamy.	Takebayashi et al. 2006
		Individual	**-**		Negative relationship between herkogamy and autofertility within a population.	Takebayashi and Delph 2000
		Population		**0**	Dichogamy, but not herkogamy, positively correlated with outcrossing rate among six populations. Autofertility correlated with outcrossing rate.	Schoen 1982
Ipomoea cordatotriloba/ Ipomoea lacunosa	Convolvulaceae	Species, Population		+	Positive relationship between herkogamy and selfing rate between, and to some extent within, species.	Duncan and Rausher 2013
Ipomoea purpurea	Convolvulaceae	Individual	**-**		Negative relationship between herkogamy and autofertility within a population.	Ennos 1981
		Individual		+	Positive relationship between herkogamy and outcrossing among individuals in a population.	Epperson and Clegg 1987
		Individual		+	Higher outcrossing rate of high-herkogamy group in experimental arrays.	Chang and Rausher 1998
Leptosiphon jepsonii	Polemoniaceae	Population		+	Positive relationship between herkogamy and 'SI index' among 17 populations. Outcrossing rate correlated with SI index in four populations.	Goodwillie and Ness 2005 (and see Weber and Goodwillie 2009)
Lycopersicon pimpinellifolium	Solanaceae	Population		+	Positive relationships between anther length, stigma exsertion and outcrossing rate among 43 populations.	Rick et al. 1977, 1978
Mimulus	Phrymaceae	Species		+	Positive relationship between herkogamy and 'outcrossing indicators' across eight species.	Ritland and Ritland 1989
		Population, Species	**-**		Negative relationship between herkogamy and autofertility among 10 populations of three species, and among five populations of *M. guttatus*.	Dole 1992
Mimulus guttatus	Phrymaceae	Individual	**(-)**		Weak negative relationship between herkogamy and autofertility among families within a population.	Carr and Fenster 1994
Mimulus ringens	Phrymaceae	Individual		+	Positive relationship between herkogamy and outcrossing rate among genets in two populations.	Karron et al. 1997
Myosotis	Boraginaceae	Species	**-**		Reduced autofertility in 'always herkogamous' species compared to 'initially herkogamous' and 'never herkogamous' species.	Robertson and Lloyd 1991
Narcissus longipathus	Amaryllidaceae	Individual		**0**	No detectable relationship between herkogamy-class and outcrossing rate within a population.	Medrano et al. 2005 (but see Medrano et al. 2012)

Nicotiana glauca	Solanaceae	Individual, Population	**-**		Negative relationship between herkogamy and autofertility among sites and plants.	Schueller 2004 (and see Ollerton et al. 2012)
Nicotiana rustica	Solanaceae	Individual		+	Positive relationship between ‘heterostathmy’ (=herkogamy) and outcrossing in experimental array.	Breese 1959
Primula halleri	Primulaceae	Individual	**-**		Negative relationship between herkogamy-class and seed set following pollinator exclusion in three populations.	de Vos et al. 2012
		Individual		**(+)**	Weak positive relationship between herkogamy-class and family-level outcrossing rates, averaged over four populations.	de Vos et al. 2018
Turnera ulmifolia	Turneraceae	Population		+	Positive relationship between herkogamy and outcrossing rate among 13 populations.	Belaoussoff and Shore 1995 (and see Barrett and Shore 1987)