

## The evolvability of herkogamy: quantifying the evolutionary potential of a composite trait

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### Abstract

Accurate estimates of trait evolvabilities are central to predicting the short-term evolutionary potential of populations, and hence their ability to adapt to changing environments. We quantify and evaluate the evolvability of herkogamy, the spatial separation of male and female structures in flowers, a key floral trait associated with variation in mating systems. We compiled genetic-variance estimates for herkogamy and related floral traits, computed evolvabilities, and compared these among trait groups and among species differing in their mating systems. When measured in percentage of its own size, the median evolvability of

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herkogamy was an order of magnitude greater than the evolvability of other floral size measurements, and was generally not strongly constrained by genetic covariance between its components (pistil and stamen lengths). Median evolvabilities were similar across mating systems, with only a tendency towards reduction in highly selfing taxa. We conclude that herkogamy has the potential to evolve rapidly in response to changing environments. This suggests that the extensive variation in herkogamy commonly observed among closely related populations and species may result from rapid adaptive tracking of fitness optima determined by variation in pollinator communities or other selective factors.

### **Introduction**

Herkogamy, the spatial separation of male and female structures in flowers, is a key trait promoting outcrossing and/or reducing interference between male and female functions (Webb and Lloyd 1986). Its functional importance is supported by many studies showing negative relationships between herkogamy and population-specific rates of autofertility (seed set in the absence of pollinators) and self-fertilization (e.g. Moeller 2006; Herlihy and Eckert 2007; Eckert et al. 2009; Dart et al. 2012; Opedal et al. 2016). Because herkogamy directly affects mating systems, it has been suggested to be among the first traits to evolve following changes in the reproductive environment (Mitchell and Ashman 2008; Bodbyl Roels and Kelly 2011). Therefore, understanding the evolutionary potential of herkogamy should provide insights into the evolvability of the mating system itself, and allow us to predict the fate of plant populations in the event of pollinator declines.

Evolvability of floral traits is critical for the evolution of mating systems, but trait evolvability might in turn depend on the current mating system. Indeed, evolutionary theory predicts reduced additive genetic variance and hence reduced evolvability in highly selfing

populations (Charlesworth and Charlesworth 1995; Lande and Porcher 2015). Furthermore, standing genetic variation may depend on historical patterns of selection. For mixed-mating species, temporal variation in pollinator communities may generate variation in outcrossing rates (Eckert et al. 2009) and fluctuating selection on herkogamy. In self-incompatible species and species exhibiting complete dichogamy (temporal separation of sexual functions), selection on floral architecture is more likely to promote accurate positioning of anthers and stigmas with regard to where they contact pollinators, than to act on herkogamy as a mechanism of facilitating or avoiding self-pollination. In these species, we expect stabilizing selection on flower architecture if the pollinator community is constant across years (Cresswell 2000; Armbruster et al. 2009a), while among-year variation in the pollinator community might lead to fluctuating selection (Sahli and Conner 2011; Kulbaba and Worley 2013; Campbell and Powers 2015). How these different modes of selection affect standing genetic variation is not clear and depends on specific aspects of the genetic architecture of the traits (Hermisson et al. 2003; Carter et al. 2005; Le Rouzic et al. 2013). Thus, while it seems reasonable to expect reduced evolvability under selfing, the relative evolvability of outcrossing vs. mixed-mating species remains an empirical question.

Most studies investigating the evolutionary potential of herkogamy have been conducted on self-compatible species, largely precluding any meaningful comparison of evolvabilities across mating systems. Furthermore, nearly all of these studies have measured evolutionary potentials as heritabilities. Due to correlations between additive, epistatic and environmental components of the phenotypic variance, heritability is largely uncorrelated with additive genetic variance, and is thus not a very good measure of evolutionary potential (Hansen et al. 2011). To quantify the ability of a trait to respond to selection, we will use mean-standardized genetic variance as a measure of evolvability (Houle 1992; Hansen et al.

2003b). This is interpretable as the expected evolutionary response in percentage of the trait mean under unit strength selection, i.e. the strength of selection on fitness itself (Hansen et al. 2003b).

Herkogamy is a composite trait, determined by the relative positions of stigmas and anthers within flowers or flower-like inflorescences (pseudanthia). The evolvability of such complex traits depends not only on the genetic variance of the component traits (pistil and stamen lengths), but also on the genetic architecture of the multivariate phenotype, summarized by the additive genetic variance matrix,  $\mathbf{G}$  (Lande 1979). In order to estimate meaningful evolvabilities for complex traits, Hansen and Houle (2008) introduced the measurement of evolvabilities in specific directions in morphospace as the projection of the predicted evolutionary response on a hypothetical selection gradient along the direction. Here, we use this framework to measure the evolvability of herkogamy as the evolvability in the phenotypic direction of separation between male and female organs, that is, the evolvability of the difference in the lengths of the organs. To measure multivariate constraints on evolution we use the concept of conditional evolvability (Hansen et al. 2003a), which measures the evolvability of a trait when other measured correlated traits are not allowed to change, or equivalently when the directional selection on the focal trait has come to balance with stabilizing selection on the constraining traits (Hansen 2003).

To evaluate the evolutionary potential of herkogamy, we compiled estimates of genetic variances in herkogamy and its component traits (lengths or positions of female and male floral organs). Using the framework of Hansen and Houle (2008), we obtained evolvability estimates of herkogamy and related floral traits. We then compared the evolvability of herkogamy to that of male and female organs, and also to that of traits representing flower size (e.g. tube length, corolla width, petal length). We obtained

evolvabilities for species exhibiting a wide range of mating systems, and were therefore able to evaluate whether evolvabilities of floral traits depend on mating systems. To place these results in the general context of the evolution of herkogamy, we also examined studies estimating selection on herkogamy, and discuss factors likely to influence the evolvability of herkogamy.

### **Theory: Measuring the evolvability of herkogamy**

Consider a simple floral architecture, where female and male organs (e.g. pistil and stamens) vary along a single dimension (Fig. 1a), and are genetically correlated as represented by the ellipse in Fig. 1b. Here, herkogamy can be measured as pistil length minus stamen length ( $x_{\text{♀}} - x_{\text{♂}}$ ; Fig. 1c, d), which corresponds to the phenotypic distance along the direction perpendicular to the one-to-one slope between the lengths of the pistil and the stamens (indicated by the black arrow in Fig. 1b). The genetic variance in herkogamy is then given as  $\text{Var}(x_{\text{♀}} - x_{\text{♂}}) = \text{Var}(x_{\text{♀}}) + \text{Var}(x_{\text{♂}}) - 2\text{Cov}(x_{\text{♀}}, x_{\text{♂}})$ .

This measure of herkogamy ( $x_{\text{♀}} - x_{\text{♂}}$ ) is on a signed-ratio scale type, and can take both positive and negative values (Fig. 1d). Positive ( $x_{\text{♀}} - x_{\text{♂}}$ ) is referred to as approach herkogamy, and negative ( $x_{\text{♀}} - x_{\text{♂}}$ ) as reverse herkogamy (Webb and Lloyd 1986). On this scale type, scaling the variance on the trait mean is not meaningful (Hansen et al. 2011; Houle et al. 2011). To obtain a measure of evolvability that is comparable to other traits, and across species, one option is to consider the absolute value of herkogamy:  $|x_{\text{♀}} - x_{\text{♂}}|$  (Fig 1e). Because  $x = |x_{\text{♀}} - x_{\text{♂}}|$  is on a ratio scale, its evolvability can be computed as  $e_R = V_A(x) / \bar{x}^2$ , which we refer to as ratio-scale evolvability of herkogamy. This is a useful measure of evolvability, because it is interpretable as the expected evolutionary response in percentage of the trait mean under unit strength selection, when selection is measured relative to mean absolute herkogamy (Hansen et al. 2003b; Fig. 2). Importantly, the mean absolute value is

determined both by the mean departure of  $(x_{\text{♀}} - x_{\text{♂}})$  from zero, and the variance in  $(x_{\text{♀}} - x_{\text{♂}})$  (Fig. 3). Notice that the relative contribution of the variance to  $|x_{\text{♀}} - x_{\text{♂}}|$  will tend to increase as mean  $(x_{\text{♀}} - x_{\text{♂}})$  approaches zero (Fig. 3).

Alternatively, because larger organs tend to have higher variances than smaller ones, the genetic variance in  $(x_{\text{♀}} - x_{\text{♂}})$  can be scaled by the lengths of the male and female organs ( $x_{\text{♀}}$  and  $x_{\text{♂}}$ ; Fig 1c). This can be achieved by measuring the evolvability of herkogamy as  $e_{SR} = \text{Var}[\ln(x_{\text{♀}}/x_{\text{♂}})] = \text{Var}[\ln(x_{\text{♀}}) - \ln(x_{\text{♂}})]$ . This scaling gives the evolvability of herkogamy in percentage of the lengths of the male and female organs, and we refer to this measure as signed-ratio scale evolvability of herkogamy. This measure gives a simple prediction for the number of generations until  $x_{\text{♀}} = x_{\text{♂}}$ , as  $t = -\ln(x_{\text{♀}}/x_{\text{♂}})/(e_{SR}\beta_{\mu})$ , where  $\beta_{\mu}$  is the selection gradient on herkogamy scaled against mean organ size (Hereford et al. 2004). Although useful, this measure of evolvability of herkogamy is not directly comparable to standard mean-scaled evolvabilities of other traits, because it gives the expected response to selection on herkogamy in percentage of the size of the sexual organs, and not in percentage of average herkogamy.

To quantify the constraints imposed on the evolution of herkogamy by genetic covariance between the lengths of female and male organs, it is useful to consider the evolvabilities of female and male organs conditioned on each other. The conditional evolvability of trait  $y$  relative to trait  $x$  is defined as the evolvability of  $y$  while  $x$  is under stabilizing selection (Hansen et al. 2003a). For female organ length conditional on male organ length, we have  $c(x_{\text{♀}}|x_{\text{♂}}) = \text{Var}(x_{\text{♀}}) - \text{Cov}(x_{\text{♀}}, x_{\text{♂}})^2 / \text{Var}(x_{\text{♂}})$ , and vice versa for male organ length conditioned on female organ length. With our measure of herkogamy,  $c(x_{\text{♀}}|x_{\text{♂}})$  equals the evolvability of herkogamy conditional on the length of the male organ (along the vertical grey arrow in Fig. 1b), that is the evolvability of herkogamy while the male organ is

under stabilizing selection. The degree of genetic constraint can also be measured by the autonomy, defined as the conditional evolvability divided by the evolvability ( $a = c/e$ ; Hansen and Houle 2008).

Finally, we note that the measures outlined above are not specific to herkogamy, but apply to similar traits on a signed-ratio scale defined as differences, such as sexual size dimorphism or directional asymmetry. These measures are not valid, however, for traits on an interval scale (such as egg laying date).

## Methods

### *Literature survey*

Starting from the 74 studies considered by Ashman and Majetic (2006), and four additional studies involving relevant floral-architecture traits cited in Hansen et al. (2011), we compiled estimates of quantitative-genetic variation in herkogamy and related floral traits (linear size measures of male and female organs) and genetic covariances among them. To facilitate comparisons, we also included measures of genetic variances in traits representing overall flower size. We searched the ISI Web of Science database using the keywords *quantitative genetic, genetic variance, evolvability, heritability, floral trait, herkogamy, anther, stigma, and pistil*, in various combinations, and traced references within studies. Our survey yielded 12 additional studies not included in the existing databases. Most studies were conducted on natural populations, but we also included a few studies on cultivated species, and on hybrids obtained from interpopulation or interspecific crosses (e.g. QTL studies). We included both measures of additive genetic variances (or narrow-sense heritabilities) and total genetic variances (or broad-sense heritabilities). We paid attention to potential confusion of sire variances with additive genetic variances, which appear to be a common error in quantitative-genetic studies (Garcia-Gonzalez et al. 2012). For each study (or population, morph or

environment analyzed separately), we included only one estimate per trait group (herkogamy, male organs, female organs, and flower size). Thus, we avoided non-independence arising from repeated measures of floral structures, when for example both petal length and corolla diameter were measured in the same study.

To assess whether evolvabilities are influenced by mating systems, we classified species as self-compatible or self-incompatible based on the authors' descriptions or related publications, and we compiled estimates of outcrossing rates when these were available. Then, we classified species as predominantly selfing (outcrossing rate  $< 0.2$ , or described as predominantly selfing), mixed-mating (outcrossing rate between 0.2 and 0.8, or described as mixed mating) or predominantly outcrossing (self-incompatible, outcrossing rate  $> 0.8$ , or described as predominantly outcrossing). Self-incompatible species were assigned an outcrossing rate of 1 in the analyses. The type of evidence used to infer the mating system of each study population or species is listed in Appendix 2.

#### *Data treatment and evolvability measures*

Many studies did not report genetic variances and covariances, only heritabilities ( $h^2$ ) and genetic correlations ( $r_A$ ). We excluded studies from which phenotypic means or variances ( $V_P$ ) were unavailable (Appendix 3), because this precludes calculating evolvabilities from heritabilities. In a few cases, we contacted authors to obtain the necessary information.

Whenever possible, we back-calculated genetic variances as  $V_A = h^2 V_P$ , and genetic covariances as  $\text{Cov}(x, y) = r_A \sqrt{\text{Var}(x)\text{Var}(y)}$ .

Because herkogamy was not always measured in the original studies, various pre-treatments and transformations were necessary. General methods are outlined here, and the specifics of data treatment for each study are summarized in Table S1. For studies where

genetic variances and covariances of male and female organ lengths were available, we compiled **G**-matrices and computed genetic variances of herkogamy ( $x_{\text{♀}} - x_{\text{♂}}$ ) using  $\text{Var}(x_{\text{♀}} - x_{\text{♂}}) = \text{Var}(x_{\text{♀}}) + \text{Var}(x_{\text{♂}}) - 2\text{Cov}(x_{\text{♀}}, x_{\text{♂}})$ . This equals the genetic variance in multivariate trait space along the vector  $\beta = [1, -1]$ , which represent the phenotypic direction separating the two traits (Fig. 1b). To obtain evolvabilities scaled by organ sizes (signed-ratio scale evolvability,  $e_{SR}$ ), we mean-scaled the **G**-matrices with the ‘meanStdG’ function of the *evolvability* R-package (Bolstad et al. 2014) before computing  $\text{Var}(x_{\text{♀}} - x_{\text{♂}})$ . To obtain conditional evolvabilities of male and female organs conditioned on each other, we calculated the conditional evolvabilities of the respective bivariate **G**-matrices along the selection gradient  $\beta = [1, 0]$  using the ‘evolvabilityBeta’ function of the *evolvability* package. Using the same function, we also obtained the joint autonomy of the male and female organs conditional on each other (in the bivariate case considered here, the autonomy is  $1 - r_A^2$ , where  $r_A$  is the genetic correlation).

To transform  $(x_{\text{♀}} - x_{\text{♂}})$  to a ratio scale, we computed the mean ( $\mu$ ) and variance ( $\sigma^2$ ) of a chi distribution (i.e. the distribution of the absolute values of a normal variate; Fig. 1e)

using equations 7 and 8 in Morrissey (2016),  $\bar{\mu}_{|x|} = \sqrt{\frac{2}{\pi}} \sigma(x) e^{-\mu_x^2/2\sigma^2(x)} + \mu_x \left( 1 - 2\Phi\left(\frac{-\mu_x}{\sigma(x)}\right) \right)$ ,

and

$\sigma^2(|x|) = \mu_x^2 + \sigma^2(x) - \bar{\mu}_{|x|}^2$ , where  $\Phi$  is the standard normal cumulative distribution function.

For traits on a ratio scale, including herkogamy after transformation, we computed evolvability as  $e = V_A / \bar{x}^2$ , where  $\bar{x}$  is the trait mean. If genetic variances were estimated for traits on a natural log scale, we used the genetic variance directly as a measure of evolvability (the variance of natural-log transformed data is almost identical to that of mean-standardized data).

## *Analyses*

Because of the heterogeneous nature of the data, limited sample sizes for individual trait groups, and incomplete reporting of standard errors, we chose not to perform a formal meta-analysis (Morrissey 2016). Throughout this paper, we present median evolvabilities with 95% confidence intervals obtained from 10000 non-parametric bootstrap estimates drawn from the original data points.

## **Results**

### *Patterns of evolvability across trait groups*

Overall, 36 studies were included in the database (Appendix S2). For herkogamy, we obtained evolvability estimates for 27 natural populations of 17 species representing 10 families, and three hybrid populations obtained by crosses between natural populations, or species (Table 1). A few populations were measured in several environments, yielding a total of 34 ratio-scale evolvability estimates, and 20 signed-ratio scale estimates. For flower size and sexual organs, we obtained 114 evolvability estimates from 31 species representing 17 families (Appendix S2). Studies that reported analyses of relevant traits, but that we did not include in the database, are listed in Appendix S3 with the reason for their exclusion.

The median ratio-scale evolvability of herkogamy ( $e_R = 9.07\%$ ; Table 1, 2) was an order of magnitude greater than the median (ratio-scale) evolvabilities of male organs ( $e = 0.42\%$ ), female organs ( $e = 0.50\%$ ), and flower size ( $e = 0.44\%$ ). In contrast, the median signed-ratio scale evolvability of herkogamy ( $e_{SR} = 0.42\%$ ; Table 1, 2) was similar to the medians for male and female organs. Median evolvabilities remained similar when we restricted the analysis to additive genetic variances only (Table 2), and when hybrid populations were excluded. Except for herkogamy on a ratio scale, these estimates are comparable to the median evolvability for a large variety of traits reported in Hansen et al.

(2011) ( $e = 0.36\%$ ,  $n = 1465$ ), but greater than the median for linear size measures ( $e = 0.12\%$ ,  $n = 512$ ).

Among self-incompatible species, 89% of populations exhibited approach herkogamy ( $x_{\text{♀}} > x_{\text{♂}}$ ). For self-compatible species, approach and reverse herkogamy were equally common. There was no significant relationship between signed-ratio scale herkogamy ( $x_{\text{♀}} - x_{\text{♂}}$ ) and signed-ratio scale evolvability ( $r^2 = 5\%$ ). Thus, evolvabilities did not differ systematically between approach and reverse herkogamous populations.

The median conditional evolvabilities of male and female organs conditioned on each other were  $c = 0.20\%$  and  $c = 0.32\%$ , respectively, corresponding to a median autonomy of 85.5% (mean = 66.1%, range = 3.0 - 99.8%; Table 1). This indicates that in most cases the evolution of herkogamy would only be mildly constrained by genetic covariance. In a few cases, however, there were indications of strong constraints. The strongest genetic constraints were detected for two self-incompatible species of *Nicotiana*, where floral organs are apparently organized in tightly integrated modules (Bissel and Diggle 2010).

#### *Genetic vs. residual phenotypic variances*

Hansen et al. (2011) showed that, across trait groups, heritabilities and evolvabilities are poorly correlated due to strong positive correlations between additive genetic variances and other variance components. Our data on floral traits confirm this pattern for all traits combined. If we estimate the mean-scaled residual variance as  $I_R = e(1-h^2)/h^2$ , we obtain a moderately strong correlation between  $I_R$  and  $e$  on log scale ( $r^2 = 47\%$ ), which is weaker than the 60% reported by Hansen et al. (2011). For each trait group separately, however, this correlation largely disappears: herkogamy, 0.8%; flower size, 3.9%; male and female organs, 0.4%. Consequently, the correlation between heritabilities and evolvabilities for all traits combined was only  $r^2 = 6\%$ , with a 95% confidence interval from 1% to 15%, while trait-

specific correlations were much stronger: herkogamy,  $r^2 = 53\%$ ; 95% CI = 26% - 73%, male and female organs,  $r^2 = 40\%$ ; 95% CI = 19% - 59%, flower size,  $r^2 = 45\%$ ; 95% CI = 24% - 63%.

### *Relationships with mating systems*

For all traits combined, we found only weak relationships between mating systems and evolvability. Median evolvabilities tended to be slightly greater for self-compatible taxa ( $e = 0.64\%$ ;  $n = 103$ ; Fig. 4) than for self-incompatible taxa ( $e = 0.56\%$ ;  $n = 45$ ), but median evolvabilities were somewhat greater for mixed-mating ( $e = 0.69\%$ ;  $n = 67$ ; Fig. 4) and outcrossing taxa ( $e = 0.56\%$ ;  $n = 49$ ) than for primarily selfing taxa ( $e = 0.35\%$ ;  $n = 28$ ). For herkogamy (both scale-types), and male and female organs separately, median evolvabilities tended to increase from selfing to mixed-mating and outcrossing taxa, while for flower size there was no such trend (Fig. 4). Focusing on the subset of studies for which outcrossing rates were available (Fig. 5), we found even weaker relationships between mating system (outcrossing rate) and evolvability. Outcrossing rates never explained more than 14% of the variation in evolvability, and the correlation was zero for the full data set.

### **Discussion**

Herkogamy, classically measured as the distance between anthers and stigmas within a flower, is a key trait mediating transitions in plant mating systems. Our understanding of the evolutionary potential of herkogamy is, however, limited. Measuring the evolvability of anther-stigma distance on two different scales, we find that herkogamy has a median evolvability an order of magnitude greater than other linear floral measurements when measured in percent of its own size, but of a comparable level when measured in percent of pistil and stamen length. Thus, changing herkogamy by 1 mm is not easier than changing the length of the pistil or stamens by 1 mm, but given that mean herkogamy is generally much

smaller than the sizes of its component traits (pistils and stamens), it can be changed more rapidly in percentage of the trait mean (Fig. 2). Considering changes in percent of the trait mean might be biologically the more relevant, if small changes in herkogamy have larger effects on autofertility or flower-pollinator fit at small compared to large average herkogamy. In the following, we evaluate the patterns emerging from available data on floral-trait evolvabilities, examine patterns of selection on herkogamy, and discuss factors likely to affect the evolvability of herkogamy and related traits.

#### *Patterns of evolvability across mating systems*

Ashman and Majetic (2006) reported a tendency for floral traits to be more heritable in self-incompatible than in self-compatible species, a finding the authors interpreted as support for reduced genetic variation under inbreeding. If this pattern is real, it would be in accordance with Stebbins' classic hypothesis that selfing constitutes an evolutionary dead-end (Stebbins 1957; see reviews in Takebayashi and Morrell 2001; Igic and Busch 2013; Wright et al. 2013). This inference, however, was based on the premise that heritability reliably reflects genetic variation, an assumption that has been criticized (Hansen et al. 2011). Indeed, across all traits in our database, heritabilities and evolvabilities were poorly correlated. Although we found stronger correlations within trait groups (and see Hoffmann et al. 2016 for a similar pattern in domesticated animals), the variation in evolvability explained by variation in heritability rarely exceeded 50%, confirming that heritability is not a good predictor of evolutionary potential. Overall, our survey of floral-trait evolvabilities suggests that there is only a weak relationship between mating system and evolutionary potential for these traits.

Additionally, in Ashman and Majetic's (2006) comparison of heritabilities between self-compatible and self-incompatible species, predominantly selfing species were pooled with mixed-mating species, and even with predominantly outcrossing but self-compatible

species. This is problematic because, although some evidence supports a pattern of reduced additive genetic variances in highly selfing taxa (Charlesworth and Charlesworth 1995; Bartkowska and Johnston 2009; Lande and Porcher 2015), expectations for mixed-mating vs. outcrossing species are far from clear. We found limited differences in evolvabilities of floral traits across mating systems, with only a tendency for reduced evolvabilities of some traits in predominantly selfing taxa (Fig. 4). This supports theoretical models predicting that quite high rates of selfing are necessary for a reduction of genetic variation to occur (Lande & Porcher 2015), a prediction also supported by Bartkowska and Johnston (2009), who found reduced nuclear genetic variation in a highly selfing population of *Amsinckia spectabilis* compared to mixed-mating populations. In our literature survey, reduced evolvabilities in selfing taxa was observed only for sexual organs (male and female organs, and herkogamy), and not for flower size (Fig. 4). If these patterns hold up with additional data, they may reflect differences in selection. For floral traits functionally involved in pollen transfer, temporal variation in pollinator communities may generate fluctuating selection that might maintain genetic variation in mixed-mating and outcrossing taxa. Nevertheless, the observed relationship between mating system and evolvability of floral traits remains weak, and calls for further studies comparing evolvabilities across mating systems. Studies comparing conspecific populations exhibiting wide ranges of selfing rates will be particularly valuable, because these avoid many of the confounding variables involved in interspecific comparisons.

#### *Strength of selection and expected evolutionary response in herkogamy*

To predict trait evolution in specific cases, evolvabilities must be combined with estimates of natural selection (Fig. 2). Although many studies have documented phenotypic selection on floral traits (Harder and Johnson 2009; Siepielski et al. 2009; Siepielski et al. 2013),

surprisingly few studies have considered selection on herkogamy (Table 3). In open-pollinated, natural populations, mean-scaled directional selection gradients on herkogamy range from -34% to 33% of the strength of selection on fitness as a trait, with a median of 4% and a median absolute value of 16%. Considering that mean absolute values are intrinsically biased upwards due to sampling error (Hereford et al. 2004; Morrissey 2016), this suggests that directional selection on herkogamy is often weak. For example, several studies have failed to detect selection on herkogamy in *Mimulus guttatus* under natural conditions (Table 3). Fenster and Ritland (1994) did detect negative selection in one of the three populations they studied, however, a finding the authors attributed to strong pollen limitation in this population. This observation was further supported by experimental studies on *M. guttatus* (Fishman and Willis 2008), and *M. luteus* (Carvallo and Medel 2010), showing that when pollinators were excluded, selection strongly favored reduced herkogamy (Table 3). Similarly, Moeller and Geber (2005) detected stronger negative selection on herkogamy in experimental populations of *Clarkia xantiana* when pollinators were less abundant.

The emerging pattern is that directional selection on herkogamy is context dependent, and most readily detected following sudden changes in the reproductive environment (Mitchell and Ashman 2008; Bodbyl Roels and Kelly 2011; Brys and Jacquemyn 2012; Brys et al. 2013). The potential strength of selection on herkogamy in the absence of pollinators is illustrated by our experiment with *Dalechampia scandens* in a pollinator-free greenhouse. Opedal et al. (2015) measured herkogamy on 120 plants from four populations, and left these plants to produce seeds by autonomous selfing. Treating seed set as a measure of fitness, the selection gradient on herkogamy was -134% relative to mean anther-stigma distance. Carvallo *et al.* (2010) reported selection of similar strength on *M. luteus* in the absence of pollinators (Table 3). If we assume for simplicity that selection on herkogamy in the event of a drastic pollinator decline is as strong as selection on fitness ( $\beta = 100\%$ ), and a ratio-scale

evolvability at our median of  $e_R = 9.1\%$ , herkogamy would be expected to be reduced by half in as few as  $t = [\ln(0.5)/0.091 \times 1] \approx 7.6$  generations (Fig. 2). For an average species in our database, this means that herkogamy would change from 2.45 mm to 1.23 mm in less than 8 generations. Given the strong effect of herkogamy on autofertility in many systems, such a change might be expected to have a large effect on fitness in terms of seed set. Using signed-ratio scale evolvabilities, the median time until herkogamy reaches zero under a selection strength of 100% relative to the sizes of male and female organs for species in our database would be  $t = 48$  generations. Thus, if selection is indeed strong, typical evolvabilities of herkogamy are unlikely to represent strong constraints, even on microevolutionary timescales.

The difference between our two measures of evolvability illustrates the consequences of the choice of measurement scale and standardization, two important but often neglected aspects of measurements in biological studies (Hansen et al. 2011; Houle et al. 2011; Armbruster et al. 2017). The two measures make different assumptions about what aspects of variation and selection that stays constant as evolution proceeds, and yield complementary insights into the evolutionary potential of the trait.

Ratio-scale evolvabilities of herkogamy ( $e_R$ ) predict evolutionary responses in percentage of the trait mean when combined with mean-scaled selection gradients (i.e.  $\beta$  multiplied with the trait mean, Hereford et al. 2004). On this scale type, we therefore expect the evolvability of herkogamy to dramatically increase when herkogamy approaches zero, while the mean-scaled selection gradient will become increasingly smaller. Signed-ratio scale evolvabilities of herkogamy ( $e_{SR}$ ), on the other hand, predict evolutionary responses in percentage of the sizes of the male and female organs. With this standardization, the strength of selection relative to organ size should stay relatively constant for small changes in herkogamy.

Notice also that when herkogamy ranges from positive to negative, taking absolute values confounds two aspects of herkogamy that we can define as bias (average difference in length of the male and female organs), and imprecision (variances in male and female organ lengths, Fig. 3). Thus, selection measured on this scale also confounds directional selection acting on the bias, and stabilizing selection against imprecision (Hansen et al. 2006; Armbruster et al. 2017). Which measure to consider therefore depends on the scope of the study, and we want to emphasize here that this choice is not just a matter of removing units or statistical convenience, but should be done based on explicit biological assumptions.

#### *Floral architecture and the evolvability of herkogamy*

With our measure of herkogamy, we assumed a simple floral architecture where male and female organs are oriented in a single dimension, and there is only one stigma and one rank of anthers (Fig. 1). Alternative floral architectures might constrain or increase the realized evolvability of herkogamy. For example, Smith and Rausher (2008) reported an interesting constraint on the evolution of herkogamy in *Ipomoea hederacea*, wherein some anthers are positioned above the stigma, others below, and selection favors the clustering of anthers around the stigma (i.e. reduction of herkogamy from two sides). The constraint arises because the lengths of the long and short stamens are positively correlated genetically. The evolution of herkogamy may also be in conflict with selection for pollination accuracy (Armbruster et al. 2009a). Efficient pollination requires placing pollen on the body of pollinators in a location that is likely to contact the stigmas of subsequently visited flowers, and the adaptive optimum for stigma and anther positions are thus often where the population-mean distance from the site of reward secretion to the stigma equals the population-mean distance to the anthers (Armbruster et al. 2009a). Consequently, there is a possible conflict between herkogamy and pollination accuracy, both of which are expected to promote cross-pollination

(Armbruster et al. 2009b). Some species have apparently resolved this conflict by evolving herkogamy in higher dimensions (Armbruster et al. 2009b). The resulting floral architecture might provide ‘additional’ evolvability of herkogamy through the ability to change the spatial orientation of male and female organs, while keeping their lengths, and hence the sites of pollen placement on and pickup from pollinators, constant. These considerations illustrate some of the complexities involved in understanding the evolutionary potential of composite traits, and more of these are likely to emerge as more species are studied.

### *Conclusions and perspectives*

To predict evolutionary responses to pollinator declines, we need to understand the evolvability of traits mediating variation in the mating system, among which herkogamy is arguably the most important. The available data indicate that herkogamy is usually able to respond rapidly to selection under a variety of assumptions. However, further studies of floral quantitative genetics using theoretically relevant measurements of evolvability are needed. A key task will be to obtain the data needed to evaluate rigorously how both pollination and mating systems affect evolvability. There is also a need to understand better the dynamics of phenotypic selection on herkogamy and other traits related to plant mating systems. We note that there are few long-term studies of selection in natural plant populations (but see Campbell and Powers 2015), which, combined with surveys of pollinator communities over time, could provide valuable insights into plant responses to changes in pollinator reliability.

High evolvability of herkogamy compared to other floral traits leads to interesting expectations of macroevolutionary patterns in herkogamy. The link between micro- and macroevolution is subject to a long-standing debate in evolutionary biology (Gould 2002; Hansen 2012; Bolstad et al. 2014), but if trait evolvability is important in constraining or facilitating trait divergence, we expect divergence of a given trait, or the direction of

divergence in morphospace, to be positively associated with evolvability (Schluter 1996; Hansen and Voje 2011; Bolstad et al. 2014). This leads to the prediction that herkogamy should have diverged proportionally more than other floral traits in many systems, which appears to be the case (e.g. Herlihy and Eckert 2007; Stock et al. 2014; Opedal et al. 2016). Further studies combining accurate estimates of quantitative-genetic parameters with data on trait divergence among populations and species seems a promising avenue toward understanding the links between micro- and macroevolution (Bolstad et al. 2014).

### Data accessibility

The database analyzed will be supplied as supplementary materials or deposited on Dryad.

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Table 1. Genetic variances ( $V_G$ ), heritabilities ( $h^2$ ) and evolvabilities ( $e$ ) of herkogamy, and conditional evolvabilities ( $c$ ) of male ( $x_\delta$ ) and female ( $x_\ominus$ ) organs conditional on each other. Abbreviations: SC = self-compatible, SI = self-incompatible. S = predominantly selfing, M = mixed mating, O = predominantly outcrossing, ASD = anther-stigma distance (on a ratio scale).  $V_{G(R)}$  and  $e_R$  is the genetic variance and evolvability of herkogamy on a ratio scale (scaled by mean absolute herkogamy),  $V_{G(SR)}$  and  $e_{SR}$  is the genetic variance and evolvability of herkogamy on a signed-ratio scale (scaled by the lengths of male and female organs). Evolvabilities are given as percentages (i.e.  $\times 100$ ).  $a$  is the autonomy of  $x_\ominus$  and  $x_\delta$ .

Species	Population	S	M	Measure	$h^2$	$V_G$ -type	$V_{G(R)}$ (mm <sup>2</sup> )	$e_R$	$V_G$ (mm <sup>2</sup> )	$e_{SR}$	$c(x_\ominus x_\delta)$	$c(x_\delta x_\ominus)$	$a$	Reference		
<i>Aquilegia canadensis</i>	QFP1	S	M	ASD	0.	Broad	2.43	27.	2.	0.4	4	3	92.	Herlihy and Eckert 2007		
					7			04								
					8			%								
<i>Aquilegia canadensis</i>	QLL3	S	M	ASD	0.	Broad	2.66	11.	2.	56	6	6	92.	Herlihy and Eckert 2007		
					3			6								
					8			%								
<i>Aquilegia canadensis</i>	QOR1	S	M	ASD	0.	Broad	0.53	6.7	0.	6%	5	3	92.	Herlihy and Eckert 2007		
					2			5								
					1			3								
<i>Aquilegia canadensis</i>	VACB W1	S	M	ASD	0.	Broad		8.4		1%			92.	Herlihy and Eckert 2007		
					5			1								
					4											
<i>Aquilegia canadensis</i>	VANP T1	S	M	ASD	0.	Broad		3.2		4%			92.	Herlihy and Eckert 2007		
					2			4								
					0											
<i>Dalechampia scandens A*</i>	Tulum	S	M	ASD	0.	Additive	0.25	1.9	0.	0.3	0.34	0.06	92.	Hansen et al. 2003, Bolstad et al. 2014		
					2			1%							2	5%
					6			5								
<i>Dalechampia scandens B*</i>	Tovar	S	S	$x_\ominus - x_\delta$	0.	Additive	0.23	4.5	0.	1.1	0.72	0.77	89.	Bolstad et al. 2014		
					3			8%							2	1%
					3			3								
<i>Datura stramonium</i>	Infinite road: Botany plot	S	S	$x_\ominus - x_\delta$	0.	Additive	1.41	14.	1.	0.8	4	3	92.	Motten and Stone 2000		
					3			08								
					0			%								
<i>Datura stramonium</i>	Infinite road: Field station	S	S	$x_\ominus - x_\delta$	0.	Additive	1.61	14.	1.	69	6	4	92.	Motten and Stone 2000		
					3			6								
					1			%								
<i>Datura stramonium</i>	Infinite road: Greenhouse	S	S	$x_\ominus - x_\delta$	0.	Additive	0.57	3.4	0.	9%	5	7	92.	Motten and Stone 2000		
					2			9								
					0			7								
<i>Gentianella campestris</i>	Kråkhättan	S	M	$x_\ominus - x_\delta$	0.	Additive	0.00	1.1	0.	8%	0	0	92.	Lennartsson et al. 2000		
					0			8								
					2			0								
<i>Ipomoea hederacea</i>	Hybrid	S	S	$x_\ominus - x_\delta$	0.	Broad		9	0.	0.2	0.12	0.17	88.	Smith and Rausher 2008		
					9			2%								
					8			7%								
<i>Ipomoea purpurea</i>	Durham County	S	M	$x_\ominus - x_\delta$	0.	Realized	0.63	39.	1.	63	6	1	92.	Chang and Rausher 1998		
					6			6								
					8			%								
<i>Ipomopsis aggregata</i>	Vera Falls	S	O	$x_\ominus - x_\delta$	0.	Additive	1.00	13.	1.	0.2	0.22	0.10	84.	Campbell 1996		
					1			09								
					4			4%								
<i>Lycopersicon</i>	Hybrid	S	M	$x_\ominus - x_\delta$	0.	Broad	0.29	48.	0.	0.6	0.64	0.21	47.	Georgiadis et		
					0.			48.								
					0.			48.								

<i>pimpinellifolium</i>	C			8	d		23	6	7%	%	%	1%	al. 2002
				4			%	0					
<i>Mimulus guttatus</i>	Indian Valley Reservoir: Meadow	S	M	$x_{\text{♀}} - x_{\text{♂}}$	0.23	Additive	15.99%	0.2					Ritland and Ritland 1996
		C		2			%	4					
<i>Mimulus guttatus</i>	Indian Valley Reservoir: Stream	S	M	$x_{\text{♀}} - x_{\text{♂}}$	0.06	Additive	8.81%	0.6					Ritland and Ritland 1996
		C		3			%	6					
<i>Mimulus guttatus</i>	Lighthouse park	S	M	$x_{\text{♀}} - x_{\text{♂}}$	0.38	Additive	4.72%	0.3					Van Kleunen and Ritland 2004
		C		8			%	8					
<i>Mimulus guttatus</i>	M13W : Dry treatment	S	M	ASD	0.34	Additive	5.83%	0.4					Ivey and Carr 2012
		C		8			%	4					
<i>Mimulus guttatus</i>	M13W : Wet treatment	S	M	ASD	0.13	Additive	2.31%	0.3					Ivey and Carr 2012
		C		5			%	3					
<i>Mimulus guttatus</i>	S	S	M	$x_{\text{♀}} - x_{\text{♂}}$	0.66	Additive	7.53%	0.6	0.2%	0.15%	0.19%	15.4%	Carr and Fenster 1994
		C		8			%	6					
<i>Mimulus guttatus</i>	T	S	M	$x_{\text{♀}} - x_{\text{♂}}$	0.96	Additive	11.06%	0.7	0.3%	0.16%	0.28%	70.8%	Carr and Fenster 1994
		C		6			%	7					
<i>Mimulus guttatus</i> × <i>nasutus</i>	Hybrid	N	N	$x_{\text{♀}} - x_{\text{♂}}$	0.17	Broader	12.59%	0.1	0.12%	0.10%	13.5%	Fishman et al. 2002	
		A	A				%	7					
<i>Mimulus micranthus</i>	301	S	S	$x_{\text{♀}} - x_{\text{♂}}$	0.01	Broader	0.03%	0.0	0.03%	0.02%	17.2%	Carr and Fenster 1994	
		C		2			%	1					
<i>Mimulus micranthus</i>	305	S	S	$x_{\text{♀}} - x_{\text{♂}}$	0.07	Broader	2.44%	0.0	0.15%	0.01%	0.14%	80.6%	Carr and Fenster 1994
		C		5			%	7					
<i>Nicotiana glauca</i>	Brazil	S	O	$x_{\text{♀}} - x_{\text{♂}}$	0.49	Additive	16.45%	0.1	0.02%	0.01%	2.8%	Bissell and Diggle 2010	
		I		5			%	1					
<i>Nicotiana glauca</i>	Brazil	S	O	$x_{\text{♀}} - x_{\text{♂}}$	0.46	Additive	23.63%	0.0	0.05%	0.04%	7.6%	Bissell and Diggle 2010	
		I		3			%	0					
<i>Polemonium brandegei</i>	Lone Tree Gulch	S	O	$x_{\text{♀}} - x_{\text{♂}}$	2.40	Additive	48.85%	0.4	0.92%	0.55%	0.60%	93.9%	Kulbaba and Worley 2008
		I		5			%	4					
<i>Raphanus raphanistrum</i>	Binghamton: Greenhouse	S	O	$x_{\text{♀}} - x_{\text{♂}}$	2.04	Additive	20.86%	0.1	1.05%	0.96%	0.42%	86.7%	Conner et al. 2003
		I		6			%	5					
<i>Raphanus raphanistrum</i>	Binghamton: Field	S	O	$x_{\text{♀}} - x_{\text{♂}}$	0.73	Additive	11.79%	0.4	0.55%	0.53%	0.12%	62.4%	Conner et al. 2003
		I		4			%	4					
<i>Saxifraga granulata</i>	Lockarp	S	M	$x_{\text{♀}} - x_{\text{♂}}$	0.26	Additive	9.32%	0.2	1.51%	1.45%	0.39%	89.8%	Andersson 1996
		C		8			%	2					

					0				6					
<i>Solanum carolinense</i>	Landfil l	S I	O	$x_{\text{♀}} - x_{\text{♂}}$	0. 4	Add itive	0.65	4.6 4%	0. 6	0.6 0%	0.49 %	0.22 %	95. 2%	Elle 1998
					3				5					
<i>Solanum carolinense</i>	Old Field	S I	O	$x_{\text{♀}} - x_{\text{♂}}$	0. 4	Add itive	0.60	8.5 5%	0. 6	0.5 6%	0.38 %	0.37 %	88. 4%	Elle 1998
					5				0					
<i>Solanum carolinense</i>	Sheep Pasture	S I	O	$x_{\text{♀}} - x_{\text{♂}}$	0. 5	Add itive	1.30	7.1 0%	1. 3	1.0 7%	0.86 %	0.43 %	94. 7%	Elle 1998
					0				0					
<i>Turnera ulmifolia</i>	A20	S C	S	$x_{\text{♀}} - x_{\text{♂}}$	0. 5	Broa d	2.41	17. 71	2. 5	0.5 0%	0.29 %	0.23 %	99. 7%	Shore and Barrett 1990
					7			%	0					
Median					0. 3		0.55	9.0 7%	0. 6	0.5 0%	0.32 %	0.20 %	85. 5%	
					8				0					

\*The Tulum and Tovar populations of *Dalechampia scandens* belong to two distinct, but undescribed species (Bolstad et al. 2014).

Table 2. Median evolvabilities ( $e$ ) per trait group, given as percentages (i.e.  $\times 100$ ). Medians are shown for all data combined (additive and total genetic variances), and for additive genetic variances analyzed separately.

Trait group	All data		Additive genetic variances	
	n	$e$ (95% CI)	n	$e$ (95% CI)
Flower size	57	0.44% (0.36%, 0.64%)	40	0.38% (0.32%, 0.55%)
Male organs	25	0.42% (0.19%, 0.49%)	19	0.43% (0.24%, 0.58%)
Female organs	32	0.50% (0.29%, 0.73%)	23	0.58% (0.38%, 0.83%)
Herkogamy (ratio)	34	9.07% (6.76%, 13.09%)	21	9.32% (4.70%, 14.08%)
Herkogamy (signed-ratio)	20	0.42% (0.22%, 0.67%)	12	0.45% (0.20%, 0.99%)

Table 3. Directional selection gradients on herkogamy in natural populations.  $\beta_{\sigma}$  is variance-standardized selection gradients,  $\beta_{\mu}$  is mean-standardized selection gradients given as percentages (i.e.  $\times 100$ ) of mean absolute herkogamy.

Species	Family	Population (Year)	Treatment	Mean (mm)	SD (mm)	$\beta_{\sigma}$	$\beta_{\mu}$	Reference
<i>Dalechampia scandens</i>	Euphorbiaceae	La Mancha	Open	2.00	1.06	-	-	Pérez-Barrales et al. 2013
						0.0	12.7	
<i>Dalechampia schottii</i>	Euphorbiaceae	Puerto Morelos	Open	1.97	1.29	-	-	Bolstad et al. 2010
						0.2	33.2	
<i>Ipomoea wolcottiana</i>	Convolvulaceae	Chamela (1991)	Open	2.11	1.92	0.2	32.6	Parra-Tabla and Bullock 2005
<i>Ipomoea wolcottiana</i>	Convolvulaceae	Chamela (1992)	Open	1.62	1.63	0.1	16.4	Parra-Tabla and Bullock 2005
<i>Mimulus guttatus</i>	Phrymaceae	Guenoc	Open	1.00	0.80	0.0	1.0	Fenster and Ritland 1994
						1		
<i>Mimulus guttatus</i>	Phrymaceae	Hough spring	Open	0.80	0.70	0.0	3.7	Fenster and Ritland 1994
						3		
<i>Mimulus guttatus</i>	Phrymaceae		Pollinators excluded			-		Fishman and Willis 2008
						0.3	6	
<i>Mimulus guttatus</i>	Phrymaceae		Open			0.0		Fishman and Willis 2008
						4		
<i>Mimulus guttatus</i>	Phrymaceae		Supplemental pollination			0.0		Fishman and Willis 2008
						3		
<i>Mimulus guttatus</i>	Phrymaceae	Fly Creek - NY	Open	1.50	0.61	0.0	9.8	Murren et al. 2009
						4		
<i>Mimulus guttatus</i>	Phrymaceae	Guenoc - G	Open	1.50	0.99	-	-	Murren et al. 2009
						0.1	15.0	
						7		
<i>Mimulus guttatus</i>	Phrymaceae	St. John - NB	Open	1.90	0.49	0.0	7.8	Murren et al. 2009
						2		
<i>Mimulus guttatus</i>	Phrymaceae	Tullock - T	Open	1.30	0.73	0.1	18.0	Murren et al. 2009
						0	0	
<i>Mimulus guttatus</i>	Phrymaceae	Cone Peak (1989)	Open			0.0		Willis 1996
						6		
<i>Mimulus guttatus</i>	Phrymaceae	Cone Peak (1990)	Open			0.2		Willis 1996
						0		
<i>Mimulus guttatus</i>	Phrymaceae	Iron Mountain (1989)	Open			0.2		Willis 1996
						6		
<i>Mimulus guttatus</i>	Phrymaceae	Iron Mountain (1990)	Open			-		Willis 1996
						0.1	8	
<i>Mimulus guttatus</i>	Phrymaceae	Lighthouse park	Open	2.85	1.17	0.0	0.5	van Kleunen and Ritland 2004
						0		
<i>Mimulus guttatus</i> $\times$ <i>nasutus</i>	Phrymaceae	Tullock	Open	0.80	0.90	-	-	Fenster and Ritland 1994
						0.2	28.6	
						0		

<i>Mimulus luteus</i>	Phrymaceae	Pollinators	5.52	1.39	-	-	Carvalho et al. 2010
		excluded			0.3	128	
					3	.8	
<i>Mimulus luteus</i>	Phrymaceae	Open	5.21	1.39	0.0	24	Carvalho et al. 2010
					7	8	
					0.0	3.7	
					3		
					0.0	16.	
					8	1	

### Figure legends

Fig. 1. Simple floral architecture (a) with a positive correlation (b) between pistil length ( $x_{\text{♀}}$ ) and stamen length ( $x_{\text{♂}}$ ). Here, herkogamy can be measured as  $x_{\text{♀}} - x_{\text{♂}}$  (c, d), which corresponds to the phenotypic direction perpendicular to the one-to-one slope between the lengths of the pistil and the stamens (along the black arrow in b). Average  $x_{\text{♀}} - x_{\text{♂}}$  corresponds to the distance between the population mean and the one-to-one slope. Vertical dashed lines indicate mean herkogamy on a signed ratio scale (d), and on a ratio scale (i.e. the mean of the absolute values; e). The conditional evolvability of herkogamy while either the female or male organ is under stabilizing selection is illustrated by the grey arrows.

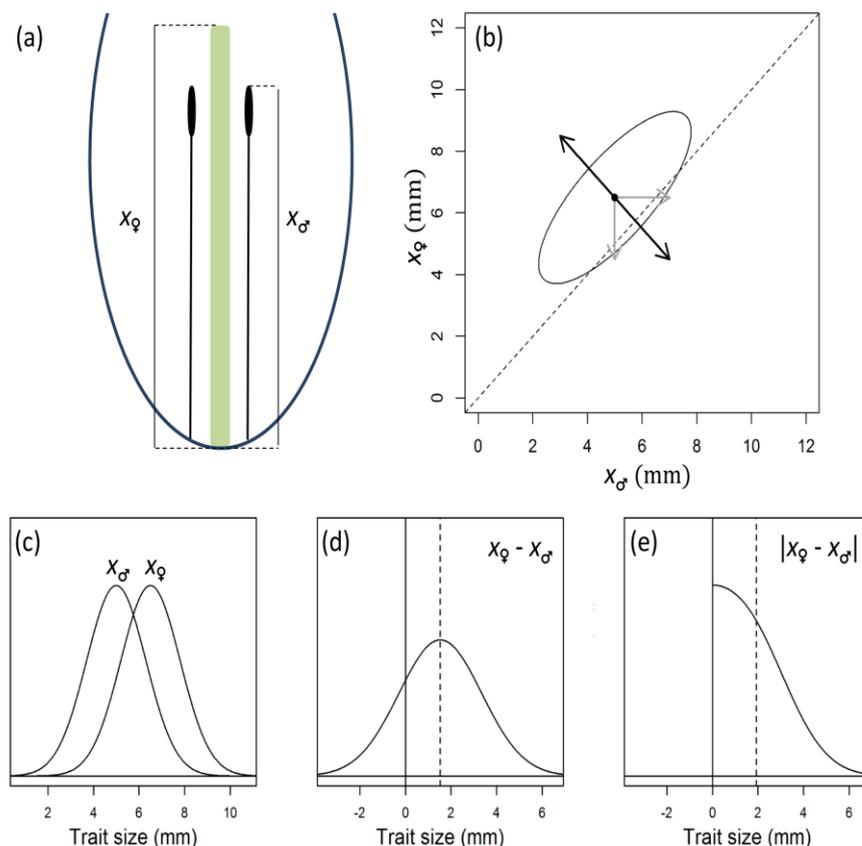


Fig. 2. Relationship between selection strength and evolutionary half-time (i.e. the number of generations before the trait is halved or doubled) at different levels of evolvability. Selection strength is the strength of selection relative to selection on fitness as a trait, 100% indicating selection as strong as selection on fitness. Lines represent evolvabilities of 0.1%, 1%, 10% and 100%, which are interpretable as the expected response in percentage of the trait mean to an episode of unit strength selection (100%).

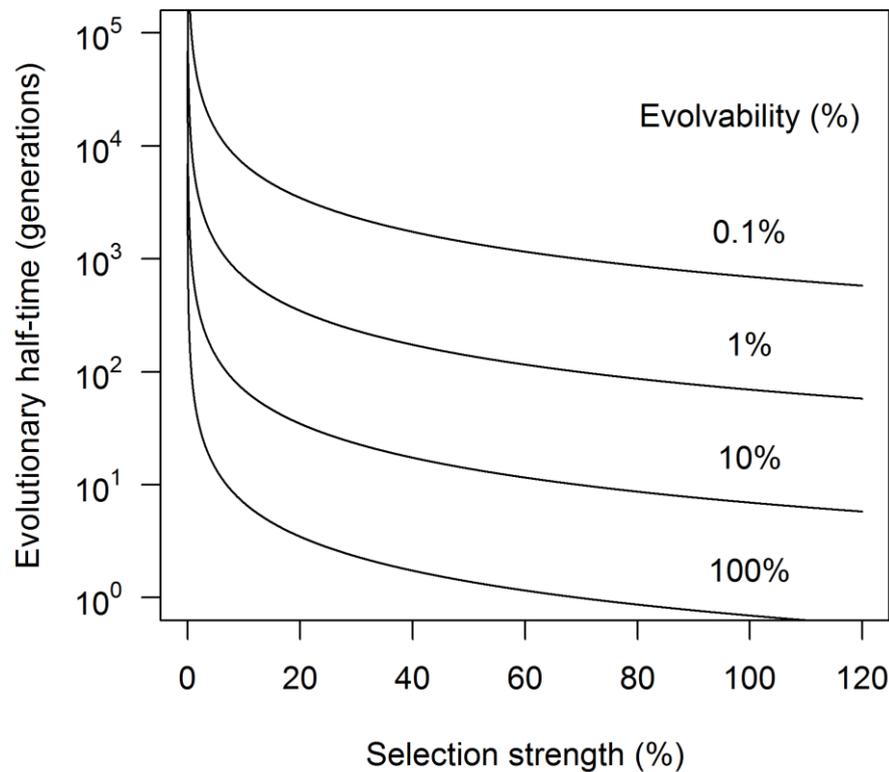


Fig. 3. Relationship between absolute mean signed-ratio scale herkogamy ( $x_{\text{♀}} - x_{\text{♂}}$ ) and mean ratio-scale herkogamy ( $|x_{\text{♀}} - x_{\text{♂}}|$ ) at different levels of variance in signed-ratio scale herkogamy ( $\sigma^2 = \text{Var}[x_{\text{♀}} - x_{\text{♂}}]$ ). Grey dots represent population means for a sample of studies listed in Table 1.

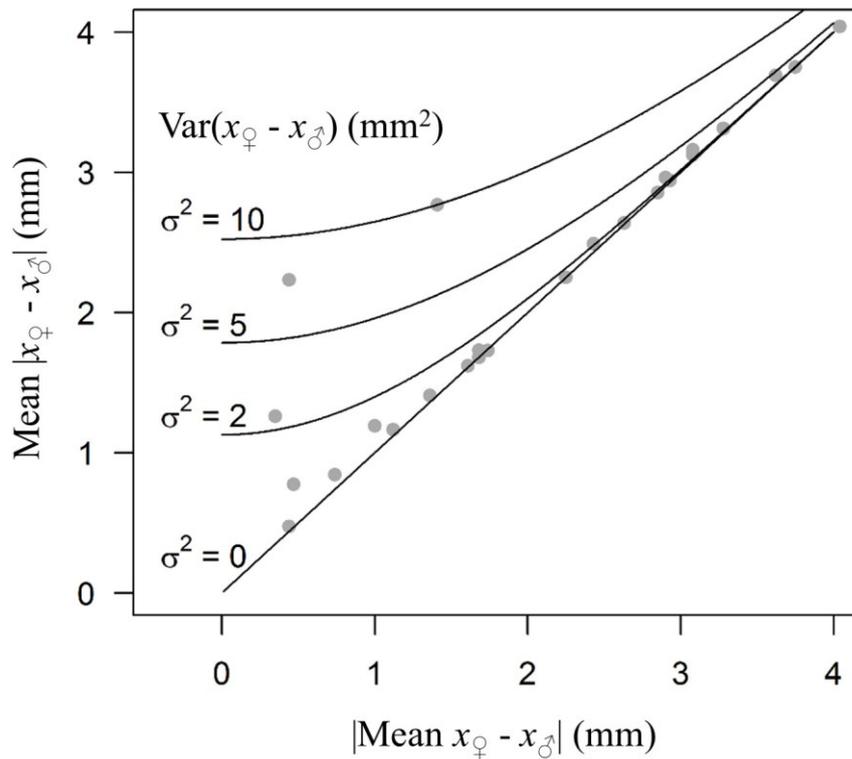


Fig. 4. Median evolvabilities with 95% confidence intervals for species with different mating systems. SC = self-compatible species, SI = self-incompatible species, S = predominantly selfing species, M = mixed-mating species, O = predominantly outcrossing species.

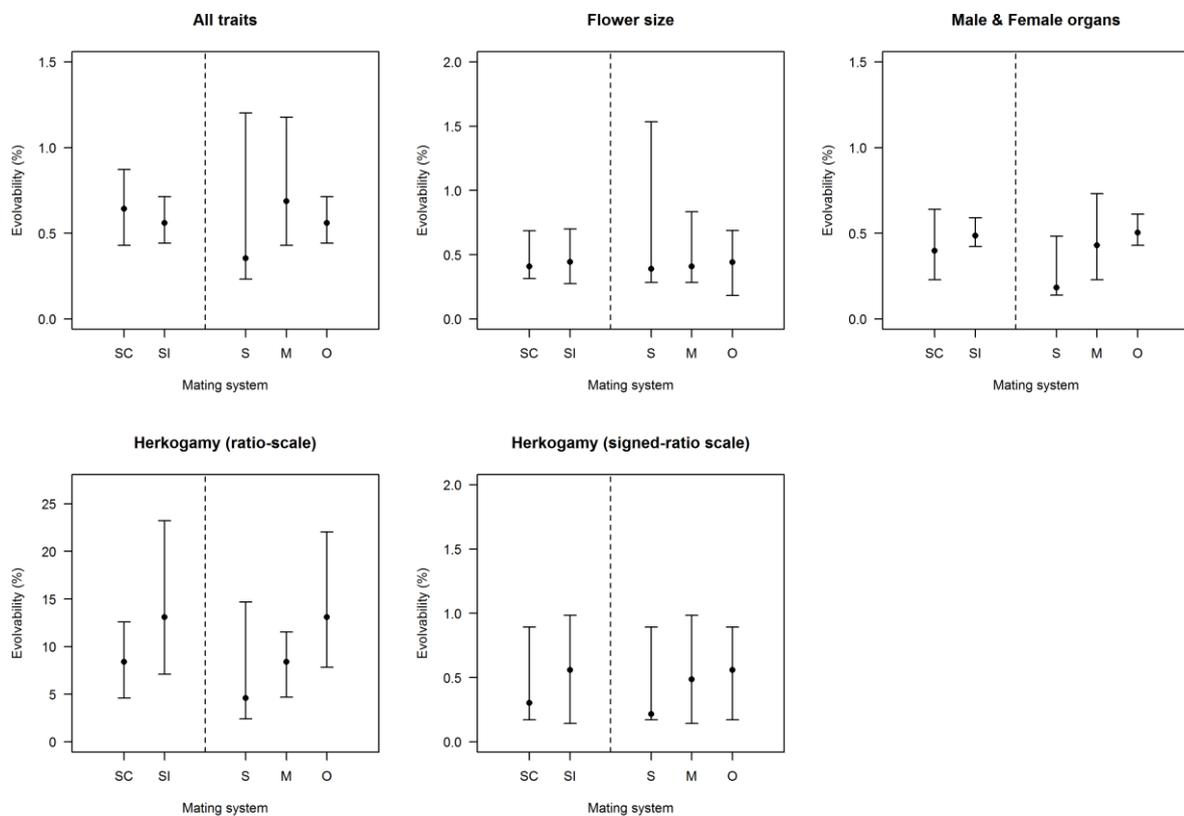


Fig. 5. Relationship between evolvability and outcrossing rate for different trait groups. The correlations (on arithmetic scale) are weak or absent for all traits combined ( $r^2 = 0\%$ ), flower size ( $r^2 = 1.8\%$ ), male and female organs ( $5.5\%$ ), ratio-scale herkogamy ( $r^2 = 2.4\%$ ), and signed-ratio scale herkogamy ( $r^2 = 13.3\%$ ).

