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Herkogamy but not reciprocity is a better predictor of legitimate pollen transfer and fruit set in *Jasminum malabaricum,* a self-compatible species with stigma height dimorphism.

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Running title: Herkogamy but not reciprocity predicts legitimate pollen load and fruit set

Abstract

Background and aims: Reciprocity, the match between anther and stigma positions of complementary morphs, and herkogamy, the separation between anthers and stigmas within a flower, are morphological traits that define style length polymorphisms. While these traits are thought to be critical in determining legitimate pollen transfer, the consequences of individual level variation in reciprocity and herkogamy have rarely been examined. Additionally, changes in sex organ positions within a flower will affect stigma-anther separation, but will likely also alter the match of these organs with counterparts in the complementary morph. Thus, variation in herkogamy is likely related to reciprocity, but the relationship between these two fundamental traits remains unexplored.

Methods: We quantified reciprocity and herkogamy in individuals from naturally occurring populations of *Jasminum malabaricum*, a species with stigma-height dimorphism. To understand how variation in these two fundamental traits are related to legitimate pollination we examined the relationship between reciprocity and herkogamy of individuals and stigmatic pollen load, legitimate pollen fraction, and reproductive success. Additionally, we examined the relationship between herkogamy and reciprocity to understand potential consequences for avoiding self-pollen deposition and encouraging legitimate pollen deposition.

Key Results: Contrary to expectations, reciprocity was not related to total pollen load, legitimate pollen fraction or reproductive success. Herkogamy was negatively related to total stigma load, but positively related to legitimate pollen fraction and fruit set in the long-styled morph. Interestingly, we observed a negative relationship between herkogamy and reciprocity in the long-styled morph.

Conclusions: Herkogamy was more important than reciprocity in this species, and likely plays a significant role in stabilizing such polymorphic intermediates, allowing reciprocal arrangements between complementary sex-organs to arise later in the evolutionary pathways towards heterostyly. The negative relationship between herkogamy and reciprocity suggests potential trade-offs between avoidance of self-pollen deposition and promotion of legitimate pollen deposition.

Keywords: *Jasminum malabaricum* Wight., herkogamy, reciprocity**, s**tigma-height dimorphism; self-pollination, legitimate pollen deposition; fruit set; intra-population variation

Introduction

Reciprocity and herkogamy are the two defining morphological features in flowers of species with style length polymorphisms. Reciprocity is the physical match between complementary sex organ heights (Armbruster *et al.* 2017), while herkogamy is the separation of the male and female sex organs within a flower (Webb and Lloyd 1986; Opedal 2018). However, while reciprocity and herkogamy are widely assumed to play a critical role in the promotion of legitimate pollen transfer that is required to maintain style length polymorphisms, few studies have examined how variation in these traits is related to inter-morph pollen transfer and reproductive success in plants with style length polymorphisms (Cesaro *et al.* 2004; Thompson *et al.* 2012; Keller *et al.* 2014; Liu *et al.* 2016; Jacquemyn *et al.* 2018; Brys and Jacquemyn 2020).

The reciprocal arrangement of sex organs in species with style length polymorphism enables pollinators to pick up pollen from anthers on specific locations on their body from where it can be accurately deposited on stigmas of the complementary morphs (Lloyd and Webb 1992). Empirical studies in heterostylous species have focused on population level estimates of reciprocity, and these have shown that higher reciprocity is related to greater legitimate pollen transfer (Lau and Bosque 2003; Baena-Díaz *et al.* 2012), and reproductive success (Valois-Cuesta *et al.* 2011; Brys and Jacquemyn 2014; Keller *et al.* 2014; Zhou *et al.* 2015; Ferrero *et al.* 2017). While limited, studies in species with stigma-height dimorphism have also shown that population level estimates of reciprocity are positively related to reproductive success (Thompson *et al.* 2003, 2012; Stehlik *et al.* 2006). There are no studies that have examined individual level reciprocity in species with stigma-height dimorphism, and only two in species with heterostyly (Jacquemyn *et al.* 2018; Brys and Jacquemyn 2020) that show that individuals with higher reciprocity have higher proportions of legitimate pollen.

The presence of self and heteromorphic incompatibility in most species with style length polymorphism makes it difficult to tease apart the contribution of reciprocity alone to legitimate pollination and maintenance of the polymorphism. Additionally, even in the presence of physiological incompatibility, reduced male and female fitness due to pollen discounting and stigma clogging, and associated reductions in reproductive success, have been observed (Shore and Barrett 1984; Barrett and Glover 1985; Martén-Rodríguez *et al.* 2013; Keller *et al.* 2014). In self-compatible species higher legitimate pollen fractions do not guarantee greater legitimate offspring, and substantial numbers of illegitimate offspring can result despite a higher legitimate fraction of pollen (Zhou *et al.* 2015). In such cases, the role of herkogamy in ensuring more legitimate offspring becomes very important.

The function of herkogamy is to increase the fraction of legitimate pollen deposition on the stigma by reducing interference between male and female organs, and reducing self and intra-morph pollen deposition (Webb and Lloyd 1986; Barrett 2002). Herkogamy has been studied extensively in monomorphic species that do not exhibit style length polymorphisms, and results from these have shown that greater stigma-anther separation between sex organs within flowers results in higher outcrossing rates (Ennos 1981; Brunet and Eckert 1998; Takebayashi *et al.* 2006; Larrinaga *et al.* 2009; de Vos *et al.* 2012, 2018; Li *et al.* 2013). However, empirical studies in species with style length polymorphisms are few, and these show that herkogamy is related to decreased self-pollination (Nishihiro and Washitani 1998), as well as increased legitimate pollen transfer and reproductive success in heterostylous species (Liu et al. 2016). However, as with reciprocity, these studies focused on population level estimates of herkogamy while ignoring intra-population variation in herkogamy (but see Hernandez and Ornelas 2007, Keller et al. 2014). Population level estimates of herkogamy

were also observed to be associated with decreased self-pollination in species with stigma-height dimorphism (Cesaro *et al.* 2004). Again, as with reciprocity, studies on consequences of intra-population variation in herkogamy are lacking in species with stigma-height dimorphism.

It is widely acknowledged that there can be large variation in sex organ positions among individuals in populations with style length polymorphism, and this variation can have important consequences for reciprocity, herkogamy, pollen flow, and reproductive success for these individuals (Eckert and Barrett 1994; Nishihiro *et al.* 2000; Sanchez *et al.* 2008; Sampson and Krebs 2012; Costa *et al.* 2017). Population level estimates of reciprocity and herkogamy can hide biologically important variation among individuals, and understanding intra-population variation in these traits is important as selection ultimately acts at the level of the individuals. Additionally, changes in organ positions in an individual flower have obvious implications for stigma-anther separation but are likely to also alter the match of these organs with counterparts in the complementary morph and affect reciprocity for that individual. However, the relationship between these two fundamental traits remain unexplored and the consequences for the two distinct functions in avoiding self-pollen deposition and encouraging legitimate pollen deposition remain unknown. The lack of an individual-level reciprocity measure (Jacquemyn *et al.* 2018; Brys and Jacquemyn 2020) and the focus on population-level estimates for both reciprocity and herkogamy have precluded such studies.

In this study, we examined the variation in reciprocity and herkogamy among individuals of four naturally occurring isoplethic populations of *J. malabaricum* (Ganguly and Barua 2020) with stigma-height dimorphism. We estimated reciprocity for an individual as the average mismatch between the stigma of that individual and the anthers of all other individuals of the

complementary morph. We investigated the consequences of intra-population variation in reciprocity and herkogamy for legitimate pollen transfer and reproductive success by examining the relationship of individual reciprocity and herkogamy with stigmatic pollen load and fruit set. Morph specific differences in stigmatic pollen load and reproductive success were also quantified to aid in the interpretation of the results as they can influence the above-mentioned relationships. We expected reciprocity to play an important role in legitimate pollen transfer and fruit set. However, given that our study species exhibits stigma-height dimorphism with low reciprocity and self-compatibility, we also expected herkogamy to significantly influence legitimate pollen transfer. Additionally, we examined the relationship between reciprocity and herkogamy across individuals to understand potential consequences for avoiding self-pollen deposition and encouraging legitimate pollen deposition.

Methods

Study sites and species: *Jasminum malabaricum* Wight. is a woody liana endemic to the Western Ghats of India, and Sri Lanka (Singh & Karthikeyan 2000) that exhibits stigma-height dimorphism (Ganguly 2019). This study was conducted between 2015 and 2017 on four populations of *J. malabaricum*: namely, Trimbak (19.94° N, 73.54° E), Bhimashankar (19.07° N, 73.55° E), Mulshi (18.50° N, 73.51° E) and Kaas (17.72° N, 73.81° E).

Pollen transfer and reproductive success can be influenced by morph specific differences in various factors affecting mating opportunities. In all four study populations, the short-styled morph had higher herkogamy, while reciprocity was higher between the long-styled stigmas and the short-styled anthers (Ganguly and Barua 2020). With the exception of these two traits, morphs did not differ in spatiotemporal mate availability, pollinator visitation, and did

not exhibit physiological incompatibility (Ganguly and Barua 2020). Since the mating opportunities were similar for both morphs it is unlikely to be a confounding factor in our investigations. The species exhibits pollen size dimorphism (Ganguly and Barua 2020) which is rare in species with stigma-height dimorphism.

Quantification of herkogamy and reciprocity: As reported in a previous study (Ganguly and Barua 2020), one flower from 30 individuals of each morph was collected from Bhimashankar, Mulshi and Kaas, between March and April of 2015 and 2017, and preserved in formalin acetic alcohol (FAA; 2.5%:2.5%:95%). In Trimbak, one flower was collected from 20 long-, and 19 short-styled individuals because of the small population size and limited availability of individuals. Herkogamy and reciprocity were also quantified for an additional 70 individuals for a total of 100 individuals of each morph in the Bhimashankar population. Anther and stigma heights were quantified using ImageJ (ver. 1.52a) from scanned images of dissected flowers. Since anthers were attached to the corolla tube, the distance from the base of the corolla tube to the mid-point of the anthers was measured as anther height. The distance from the base of the ovary to the mid-point of the stigma was measured as stigma height. Herkogamy was quantified for one flower per individual as the difference in anther and stigma height of a flower (Opedal 2018). As we examined the relationship of reciprocity with measures of female fitness like pollen load and fruit set, reciprocity was calculated for one flower per individual as the mean of mismatches between stigma height for that individual and anther height of every other individual of the complementary morph. These pair-wise mismatches were averaged to give an estimate of reciprocity for that individual (Ganguly and Barua 2020). We calculate mismatch, which is the inverse of reciprocity and hence higher values denote lower reciprocity. The relationship between herkogamy and reciprocity was examined using Pearson's correlation coefficient.

We use the term “mismatch” to refer to the spatial correspondence between anther and stigma heights of reciprocal morphs to describe our results. However, we use the term “reciprocity” to discuss the results to increase ease of interpretation.

Quantification of stigma pollen load: We quantified stigma pollen load for the same individuals from the Bhimashankar (March 2016) and Kaas (April 2017) populations for which floral morphometry was quantified. Flowers of *J. malabaricum* open between 7 p.m. to 9 p.m. and last for approximately 33 h (Ganguly and Barua 2020). Post 3 p.m. on the day after anthesis, the flowers wilt and no pollinator activity is seen on these flowers (Ganguly and Barua 2020). Hence, to allow for maximal pollen deposition, flowers were collected between 2 and 3 p.m. the day after anthesis and preserved in formalin acetic alcohol (FAA; 2.5%:2.5%:95%). The quantification was done for 30 long- and 29 short-styled individuals for Bhimashankar, and 32 long-, and 30 short-styled individuals for Kaas. Stigmas were washed in 70% ethanol and softened in 4N NaOH for 12 h (Kearns and Inouye 1993). Subsequently, they were washed in distilled water and passed through serial concentrations of ethanol from 10% to 100% in steps of 15% to wash the oil from the pollen surface. The stigmas were then resuspended in a series of ethanol dilutions of glycerol with increasing concentration of glycerol from 10% to 90% in steps of 20%, squashed with a coverslip, and washed and mounted in 100% glycerol.

To quantify the total number of pollen deposited on a stigma, images of stigma squashes for two flowers per individual were taken at 40x magnification. An exhaustive pollen count was performed for all the stigmas. Legitimate pollen fraction was estimated for one stigma for each individual. Ten images were taken at 400x magnification for each stigma, and this accounted for greater than 15% of the total pollen on that stigma. Both the pollen count and

measurement of pollen size were performed using ImageJ. As the equatorial and polar diameters were strongly correlated, only the equatorial diameter of the pollen was used as a measure of pollen size. The pollen were discriminated based on differences in diameter between the morphs using the estimates of pollen size obtained previously. The short-styled morph produced larger pollen but pollen production was not significantly different between morphs (Ganguly and Barua 2020). The diameter of the long-styled pollen ranged between 34 µm to 55 µm and the short-styled pollen between 47 µm to 73 µm (Ganguly and Barua 2020). Approximately 35% of the pollen were in the range from 47 µm to 55 µm, which was common to both morphs. The ratio of the frequency of pollen grains extracted from anthers of the long- and short-styled morph in each size class of 1 µm was used to estimate the probability of occurrence of pollen from each morph in this overlapping size range. The pollen from the stigma which belonged to these size classes were distributed among the morphs based on this probability of occurrence. The legitimate pollen fraction was calculated as the percentage of the number of legitimate pollen identified in the total number of pollen sampled. Differences between morphs in natural total stigma pollen load and the legitimate pollen fraction were examined using a non-parametric Mann-Whitney U test as the values were not normally distributed.

<u>Autogamous pollen deposition:</u> To investigate the function of herkogamy in decreasing self-pollination, three mature buds were tagged and emasculated for five individuals of each morph to quantify autogamous self-pollination in March 2017 in individuals from the Bhimashankar population. Another three buds on the same individuals were tagged as controls to account for individual differences in pollen deposition. Flowers were collected between 2 p.m. and 3 p.m. on the day after anthesis. The total pollen load and the legitimate fraction of pollen were quantified as described above. Wilcoxon’s signed-rank test was used

to examine differences in total pollen load and the legitimate fraction between the control and the emasculated flowers from the same individual. This non-parametric test was chosen as the variables contained extreme values and so that control and treatment comparisons within the same individual could be made.

Quantification of fruit set: Given the time-, and labour-intensive nature of this work, fruit set was quantified in March 2015 and 2016 in only one of the four study populations (Bhimashankar). This was done for the same individuals for which we had quantified herkogamy and reciprocity and estimated stigma pollen load. This included 30 long- and 29 short-styled individuals in 2015, and 29 individuals of each morph in 2016. For both morphs, 20 mature buds were tagged per individual in the peak flowering season and followed till the fruits matured. The percentage of total mature fruits formed from the 20 buds was taken as the final fruit set. Differences in natural fruit set between the two morphs were examined using a non-parametric Mann-Whitney U test as the values obtained were not normally distributed. The relationships of herkogamy and reciprocity with the natural total pollen load, the legitimate fraction of stigmatic pollen load and the fruit set were examined using Spearman's rank correlation coefficient as most of the examined variables were counts and percentages, and had extreme values and skewed distributions.

Results

There was substantial intra-population variation in estimates of mismatch and herkogamy between individuals in the populations (Fig. 1, Table 1). The long-styled morph exhibited a much larger range and higher coefficient of variation in mismatch as compared to the short-styled morph in all four study populations (Table 1). Similarly, the long-styled morph

exhibited a larger range and higher coefficient of variation in herkogamy as compared to the short-styled morph (Table 1).

There was high variation between individuals in natural total stigma pollen load, with counts ranging from 13 to 5015 in the long-styled, and 38 to 1394 in the short-styled morph in the Bhimashankar population, and from 6 to 1542 in the long-styled and 1 to 1350 in the short-styled morph (Fig. 2 a, c) in the Kaas population. Although long-styled morphs had higher mean stigma pollen load, no significant differences were detected between the two morphs in either of the two populations examined (Fig. 2 a, c; Mann-Whitney U test: Bhimashankar p = 0.41 and Kaas p = 0.11; n = 30 and 29 individuals for Bhimashankar, and 32 and 30 individuals for Kaas for the long- and short-styled morphs). The legitimate pollen fraction was significantly higher in the short- than in the long-styled morph for both populations (Fig 2 b, d; Mann-Whitney U test: Bhimashankar $p < 0.001$ and Kaas $p < 0.001$; n for long-styled and short-styled morph was 30 and 29 individuals for Bhimashankar, and 32 and 30 individuals for Kaas).

In both morphs, emasculation resulted in a decrease in the total pollen deposition (Fig. 3 a; Wilcoxon signed-rank test: long-styled p = 0.04 and short-styled p = 0.04; 3 flowers per treatment for 5 individuals of each morph). In the short-styled morphs emasculation did not affect the legitimate pollen fraction (Fig. 3 b; Wilcoxon signed-rank test: p = 0.34). In contrast, in the long-styled morphs emasculation resulted in an increase in the legitimate pollen fraction (Wilcoxon signed-rank test: p = 0.04). Fruit set was not significantly different between the long-, and short-styled morphs, and this was consistent across the two years in which we examined this in individuals from the Bhimashankar population (Fig. 4; Mann-

Whitney U test: year 2015 $p = 0.52$, $n = 30$ long- and 29 short-styled individuals; year 2016 $p = 0.80$, $n = 29$ individuals of each morph).

Mismatch was not related to total pollen deposition or the legitimate pollen fraction. Counter-intuitively, mismatch was positively related to fruit set in the short-styled morph in Bhimashankar (Table 2 a). Herkogamy, on the other hand, was positively related to disassortative pollen load and fruit set in the long-styled morph, but not in the short-styled morph in Bhimashankar (Table 2 a). Herkogamy was negatively related to total pollen load in the long-styled morph, but not related to pollen deposition in the short-styled morph in Kaas (Table 2 b).

Herkogamy and mismatch showed a significant positive correlation in the long-styled morph in all study populations (Fig. 5 a-d). However, such a positive relationship between herkogamy and mismatch was seen in only one of the four study populations in the short-styled morph (Fig. 5 e-h). Similar results were observed when examining this relationship in a larger number of individuals from the Bhimashankar population with a significant positive relationship between herkogamy and mismatch in the long-, but not the short-styled morph (Supplementary Information Fig. S1 a, b).

Discussion

Surprisingly, individual level variation in reciprocity was not related to legitimate pollen fraction or fruit set. In contrast, individuals of the long-styled morph with higher herkogamy had a higher fraction of legitimate pollen and greater fruit set. Thus, herkogamy may be relatively more important than reciprocity in increasing legitimate pollen deposition and reproductive success in *Jasminum malabaricum*, a species with stigma height dimorphism

that lacks self-, and heteromorphic incompatibility. Additionally, we report here for the first time a negative relationship between reciprocity and herkogamy, the two defining traits of style length polymorphism. This suggests a potential trade-off between increasing legitimate pollen deposition and avoidance of self-pollen deposition.

Intra-population variation in anther and stigma heights is expected to reduce reciprocity and hence legitimate pollen transfer and reproductive success in species with style length polymorphism (Sanchez *et al.* 2008; Armbruster *et al.* 2017). We observed large variation in anther and stigma positions, and in the resultant reciprocity in individuals of all populations of our study species. However, this observed variation in reciprocity was not associated with any negative consequences and was unrelated to the legitimate fraction of pollen load or fruit set. Previous studies have shown that higher herkogamy but not reciprocity was related to higher legitimate pollen transfer and reproductive success (Keller *et al.* 2014). Additionally, legitimate pollen transfer in some species with style length polymorphism is likely to be primarily governed by effective long-tongued pollinators rather than sex organ positions (Simón-Porcar *et al.* 2014).

The higher fraction of legitimate pollen received by the short-styled morph is likely due to reduced self-pollen deposition as a consequence of higher herkogamy in this morph (Liu *et al.* 2016). High legitimate pollen transfer has also been associated with efficient long-tongued pollinators (Haddadchi 2013; Simón-Porcar *et al.* 2014) that are capable of reaching the short-styled stigma. However, the higher legitimate pollen fractions observed in the short-styled morph did not translate into higher fruit set. As both morphs lack physiological incompatibility, similar total pollen loads resulted in equal fruit set between morphs. This is congruent with an observation from a previous study, where despite much higher legitimate

pollen deposition, a considerable number of illegitimate offspring are formed due to the absence of self- and heteromorphic incompatibility (Zhou *et al.* 2015).

In the long-styled morph, emasculation resulted in a decrease in total pollen load and an increase in the legitimate pollen fraction. Thus, reduced self pollen deposition with increasing herkogamy is likely to result in the negative relationship between herkogamy and total pollen load, and the positive relationship between herkogamy and the legitimate pollen fraction that was observed in the long-styled morph. The higher fruit set associated with higher herkogamy in the same morph could be attributed to lower mechanical interference between the male and female sex organs. The lack of any change in the legitimate pollen fraction with emasculation in the short-styled morph implies that the higher herkogamy observed in this morph was sufficient to avoid self-pollen deposition. This was also reported in *Persicaria japonica* where emasculation had no effect on the deposition of incompatible pollen (Nishihiro and Washitani 1998). This likely explains the absence of a relationship between herkogamy and reproductive success in the short-styled morph, where due to the higher herkogamy observed in this morph minor changes in herkogamy did not influence self-pollen deposition or fruit set. Similar results were reported in *Primula* spp., where the legitimate pollen fraction increased with herkogamy in the long-, but not the short-styled morph, and the "threshold" effect as mentioned above was offered as a possible explanation (Keller *et al.* 2014).

Reciprocity was negatively related to herkogamy in individuals of the long-styled morph. This relationship suggests a potential trade-off between maximizing legitimate pollen transfer and avoiding self-pollination and will be useful in providing insights into the response of these species under different ecological scenarios. For example, in situations of pollen

limitation due to the scarcity of mate and pollinators, and an absence of intra-morph incompatibility or inbreeding depression (Baker *et al.* 2000), reproductive assurance is important. If the relationship between reciprocity and herkogamy is negative, the individuals with high reciprocity and low herkogamy will be selected for as they would have the highest reproductive assurance in the population. A positive relationship can be maladaptive in such a situation. Hence, such relationships can help us understand the change in the distribution of anther and stigma heights in a population under different ecological scenarios. There was no relationship between reciprocity and herkogamy in individuals of the short-styled morph with the exception of the Mulshi population where, similar to the long-styled individuals, a negative relationship was observed. The difference in the relationship between reciprocity and herkogamy between morphs and among populations indicates the effect of morph specific selection pressures and differences in local environmental factors among populations (Santos-Gally *et al.* 2013). The short-styled morph does not show a relationship even at a higher sample size implying that the lack of a relationship is unlikely due to insufficient sample sizes.

Several studies have highlighted the functional significance of sex organ positions in species with style length polymorphism (Faivre and McDade 2001; Sosenski *et al.* 2010; V. Ferrero *et al.* 2011; Victoria Ferrero *et al.* 2011; Keller *et al.* 2012; Yuan *et al.* 2017), but the emphasis of these has primarily been on reciprocity while herkogamy has been relatively understudied (but see Nishihiro and Washitani 1998, Keller et al. 2014, Liu et al. 2016, Faife-Cabrera et al. 2016, Barranco et al. 2019). Moreover, although it has been acknowledged that intra-population variation in sex organ positions affects legitimate pollen transfer and reproductive success (Eckert and Barrett 1994; Lau and Bosque 2003; Sanchez *et al.* 2008; Armbruster *et al.* 2017), studies examining this are rare. This study reveals that

herkogamy can be more important than reciprocity in promoting legitimate pollen transfer and fruit set. The result highlights the functional role of herkogamy and has especially important implications for the maintenance of imperfectly reciprocal intermediates in the pathway towards heterostyly. The results also suggest that intra-population variation in herkogamy and reciprocity might not affect legitimate pollen transfer and reproductive success across individuals and is perhaps the reason why species with style length polymorphisms display high variation in sex organ positions. Examination of the intra-population variation in reciprocity and herkogamy also allowed us to investigate the relationship between the two which revealed a trade-off between self-pollination and legitimate pollination. Further studies on the causes and consequences of intra-population variation in reciprocity and herkogamy can substantially increase our understanding of the coevolution of the dimensions of floral parts with pollinators.

Author contributions

SG and DB conceived the idea. SG conducted field studies to collect the data. SG analysed the data. SG and DB wrote the manuscript.

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Tables

Table 1: Range (Min-Max) and coefficient of variation (CV) of individual mismatch and herkogamy observed in the four study populations a) Trimbak, b) Bhimashankar, c) Mulshi and d) Kaas. L and S stand for long- and short-styled morph respectively. The number of individuals sampled for the long- and the short-styled morph for the populations are 20 and 19 for Trimbak; 30 and 30 for Bhimashankar, Mulshi, and Kaas.

		Mismatch		**Herkogamy**	
Population	**Morph**	**Min-Max (mm)**	**CV %**	**Min-Max (mm)**	**CV %**
a) Trimbak	L	1.25, 9.27	38.70	2.47, 9.49	23.90
	S	4.39, 6.47	10.80	5.91, 11.30	17.70
b) Bhimashankar	L	1.74, 7.80	36.70	2.70, 11.40	25.20
	S	4.22, 7.02	12.30	2.61, 12.50	22.90
c) Mulshi	L	1.27, 8.80	41.10	3.53, 11.40	22.20
	S	4.24, 6.92	12.00	5.56, 11.50	16.80
d) Kaas	L	2.10, 9.34	38.50	2.00, 11.30	29.60
	S	4.93, 7.42	10.50	6.51, 13.10	15.80

Table 2: Relationship of herkogamy and reciprocity to total stigma pollen load, legitimate pollen fraction and fruit set in the Long-styled (L) and short-styled (S) morph in a) Bhimashankar, and b) Kaas. Fruit set was estimated only for the Bhimashankar population. $P < 0.05$ and $P < 0.1$ are denoted by * and ᴪ, respectively, next to the Spearman's rank-order correlation coefficients presented. For the Bhimashankar population $n = 30$ long-styled and 29 short-styled individuals for total pollen load and legitimate pollen fraction, and $n = 29$ individuals for each morph for fruit set. For the Kaas population $n = 32$ long-styled and 30 short-styled individuals.

Population	**Trait**	**Morph**	**Total pollen load**	**Legitimate fraction**	**Fruit set**
a) Bhimashankar	Mismatch	L	0.14	0.28	0.18
		S	0.03	-0.23	0.33ᴪ
	Herkogamy	L	0.06	0.45*	0.39*
		S	-0.05	-0.20	0.06
b) Kaas	Mismatch	L	-0.19	0.04	
		S	0.19	-0.18	
	Herkogamy	L	-0.33ᴪ	0.13	
		S	-0.27	-0.06	

Figures

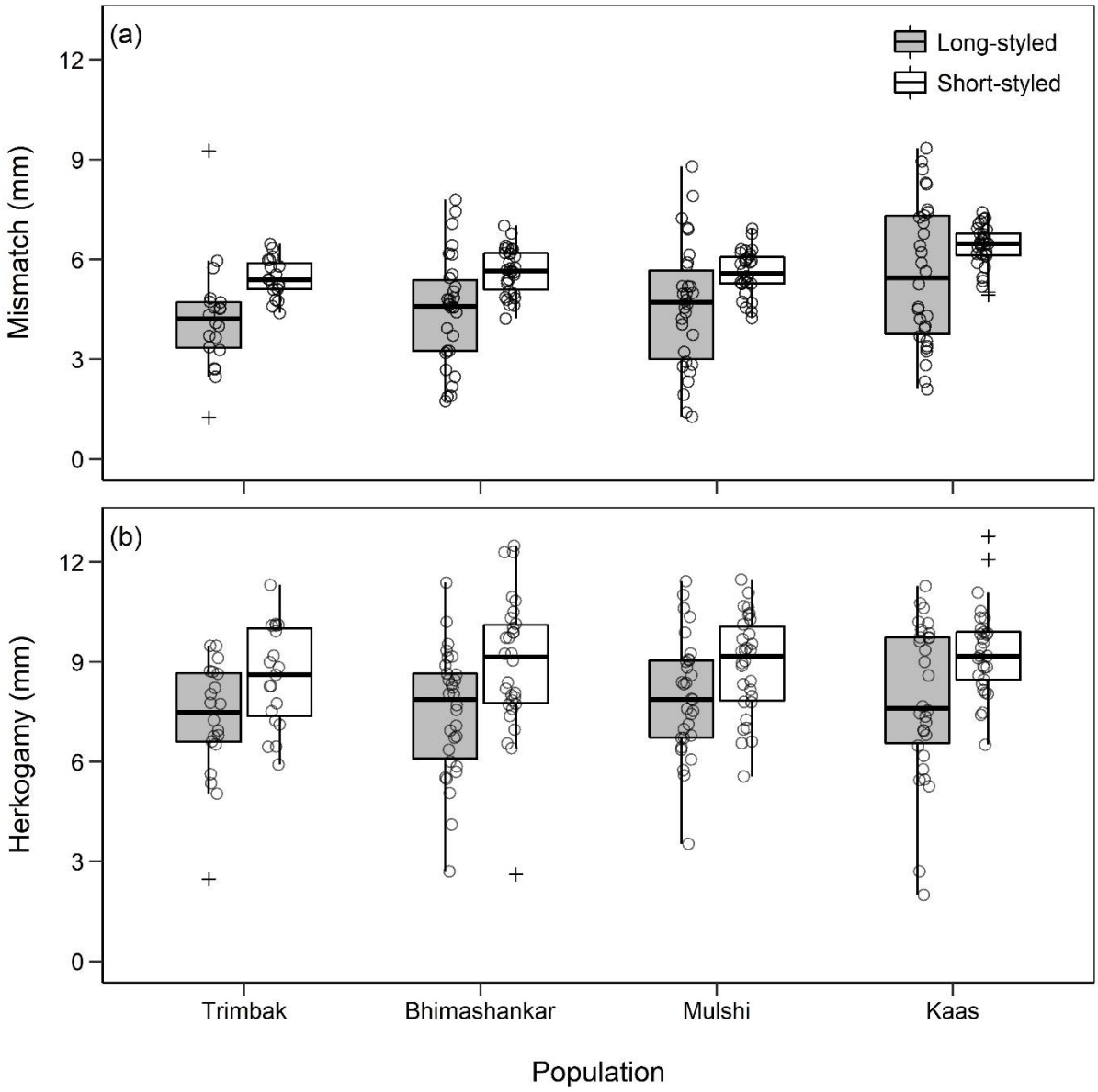

Figure 1: (a) Mismatch and (b) herkogamy in the long-styled (dark grey boxes) and short-styled (open boxes) morphs of the four study populations. The number of individuals sampled for the long- and the short-styled morph for the populations are 20 and 19 for Trimbak; 30 and 30 for Bhimashankar, Mulshi, and Kaas. Outliers are denoted by +. The bold line in the boxplots represents the median, the ends of the box represent the first and the third quartiles and the whiskers represent 1.5 times the interquartile range.

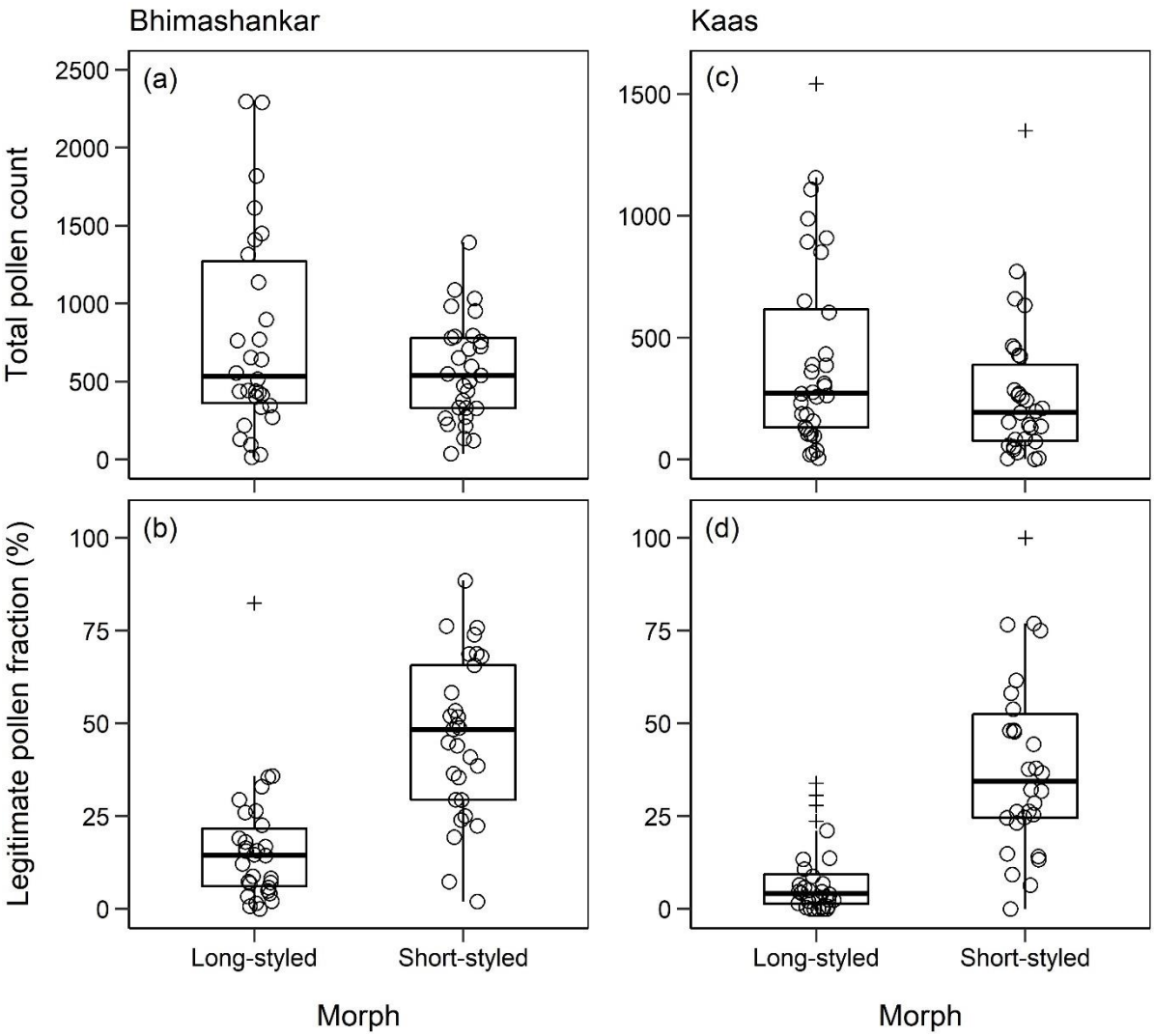

Figure 2: The total stigma pollen load (a, c) and the legitimate pollen fraction (b, d) in the Bhimashankar and Kaas populations. The number of individuals sampled for the long- and short-styled morph is 30 and 29 for Bhimashankar, and 32 and 30 for Kaas. One extreme outlier value of greater than 5000 for total pollen load in a long-styled individual from the Bhimashankar population has been removed from the plot for better visualization of the differences between the morphs. The bold line in the boxplots represents the median, the ends of the box represent the first and the third quartiles and the whiskers represent 1.5 times the interquartile range.

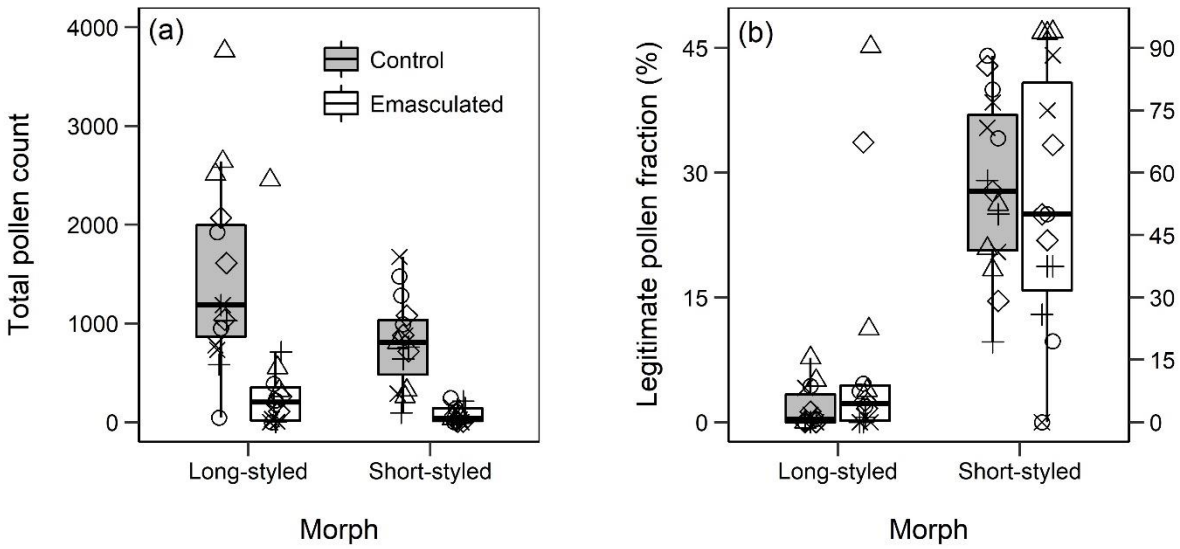

Figure 3: (a) The total pollen load, and (b) the legitimate pollen fraction in control (non-emasculated, grey boxes) and emasculated flowers (open boxes) to determine autogamous pollen deposition. One point from the legitimate pollen fraction for the emasculated group of long-styled morph (80%) has been removed from this plot for better visualization of the differences between the control and emasculation treatments. Data are from three flowers from five individuals (represented by the different symbols). The bold line in the boxplots represents the median, the ends of the box represent the first and the third quartiles and the whiskers represent 1.5 times the interquartile range.

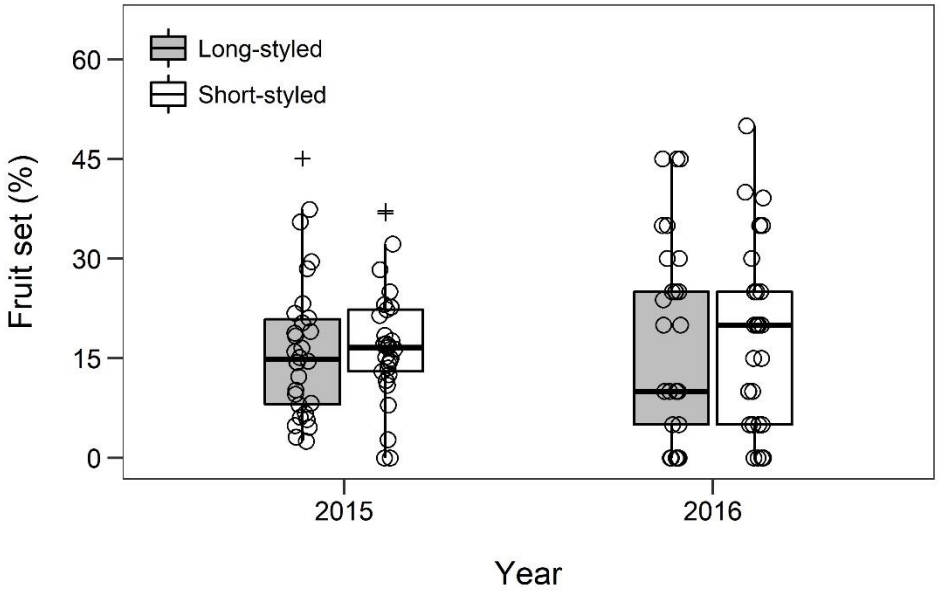

Figure 4: Natural fruit set in long- (grey boxes) and short-styled (open boxes) individuals from the Bhimashankar population measured as the percentage fruit formed per flower in 2015 and 2016. The number of individuals sampled for the long- and the short-styled morphs are 30 and 29 respectively in 2015, and 29 individuals of each morph in 2016. The plus sign represents outliers. The bold line in the boxplots represents the median, the ends of the box represent the first and the third quartiles and the whiskers represent 1.5 times the interquartile range.

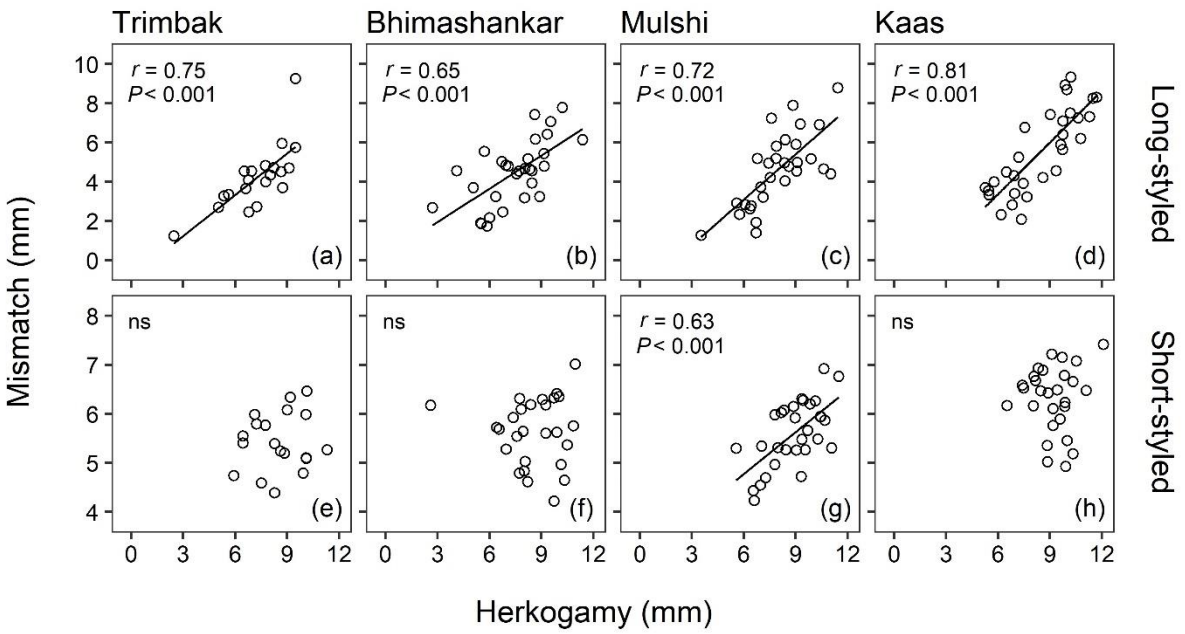

Figure 5: Relationship between herkogamy and mismatch in the two morphs (long-styled morph: a,b,c,d; short-styled morph: e,f,g,h) in the four study populations. Pearson's correlation coefficient (*r*), and the corresponding *P*-value are shown where $P < 0.05$, and 'ns' denotes not significant. The number of individuals sampled for the long- and the short-styled morph for the populations are 20 and 19 for Trimbak; 30 and 30 for Bhimashankar, Mulshi, and Kaas.