Variation in sex organ dimensions across individuals, morphs and species: consequences for reciprocity, herkogamy, and reproductive success in species with style length polymorphism

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submitted in partial fulfilment of the requirements

of the degree of

Doctor of Philosophy

by

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Dedicated to my family

CERTIFICATE

Certified that the work incorporated in the thesis entitled “Variation in sex organ dimensions across individuals, morphs and species: consequences for reciprocity, herkogamy, and reproductive success in species with style length polymorphism” submitted by Shatarupa Ganguly was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other University or institution.

Deepak Barua

Date: 05/09/2019 (Supervisor)

DECLARATION

I declare that this written submission represents my ideas in my own words and where others' ideas have been included. I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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Contents

SYNOPSIS

Title: Variation in sex organ dimensions across individuals, morphs and species: consequences for reciprocity, herkogamy, and reproductive success in species with style length polymorphism

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Chapter 1: Introduction

Style length polymorphism is a floral polymorphism characterised by the presence of two or three morphs in a population which differ in style lengths. Heterostyly is a kind of style length polymorphism in which both stigma and anther height differ between the morphs such that there is physical complementarity of anther and stigma between them. This complementarity or reciprocity increases the efficiency of pollen transfer between the morphs. Additionally, the anther and stigma within a flower in these species are spatially separated (herkogamy) to avoid the interference of male and female sexual organs within a flower, and autogamous self-pollination. Stigma-height dimorphism is another style length polymorphism where the reciprocal arrangement of anther and stigma heights between the morphs is lacking as anther positions do not differ between the morph. Although relative sex organ positions in heterostylous species have been studied for decades, broad patterns in differences between morphs across a large number of species have not been studied yet. Additionally, herkogamy and reciprocity have been studied independently, but the relationship between the two across individuals of a population has not been examined. Intra-population variation in sex organ dimensions is assumed to affect both herkogamy and reciprocity and consequently legitimate pollen transfer and fruit set. Hence, the incorporation of intra-population variation in the quantification of reciprocity between the morphs to understand reproductive success is very important. The indices which have been proposed to quantify reciprocity do not appropriately account for intra-population variation in anther and stigma heights. Moreover, quantification of reciprocity and understanding its function has always received a lot of attention while the functional consequences of change in herkogamy are relatively unexplored in species with style length polymorphism. Due to the presence of only one anther height, flowers with stigma-height dimorphism have herkogamy but, lack perfect reciprocity between morphs. Consequently, stigma-height dimorphism is an ideal study system to examine the relative importance of herkogamy and reciprocity in encouraging inter-morph pollen transfer.

Chapter 2: The relationship between herkogamy and reciprocity: the two fundamental features of species with style length polymorphism

Although relative sex organ positions in heterostylous species have been studied in a large number of species, general patterns of differences in herkogamy and reciprocity between morphs have not been studied till date. Moreover, the relationship between herkogamy and reciprocity across individuals of a morph in a population have not been studied until now. Although it is generally accepted that intra-population variation in sex organ dimensions reduces reciprocity and hence pollen transfer between morphs, the extent of it in species with style length polymorphism is not known. In this study, we extracted data on mean sex organ dimensions for more than 300 species from literature and ask questions to understand general patterns of morph-specific differences in herkogamy and reciprocity. We also extracted data on the distribution of sex organ dimensions of individuals of a population and examine the above patterns at the level of individuals. Additionally, we use this data to investigate the relationship between herkogamy and reciprocity across individuals of a morph in a population by calculating these traits for individuals. We studied the extent of intra-population variation in heterostylous species and tried to understand if it can potentially help to increase reciprocity between individuals of a population. We find that the short-styled morph has higher herkogamy in species with heterostyly. However, reciprocity was not significantly different between the morphs. Both positive and negative relationship between reciprocity and herkogamy was observed pointing towards the simultaneous optimisation of avoidance of self-pollen deposition and promotion of legitimate pollen transfer. High intra-population variation in sex organ positions was seen, and results indicated that intra-population variation could help increase reciprocity and consequently legitimate pollen transfer and fruit set in species with style length polymorphism.

Chapter 3: **Increased variation in sex organ positions across individuals can increase reciprocity and pollination success in heterostylous plant populations**

Deviation from a perfect spatial match reduces pollen transfer in heterostylous species. Reciprocity indices are used to quantify this match, but the commonly used indices fail to appropriately account for intra-population variation. In this study, we developed an index that explicitly considers intra-population variation in sexual organ positions. We used simulated populations and empirical data to understand how variation in sexual organ heights alters reciprocity in heterostylous plants. We used data from chapter 2 to determine anther and stigma positions to understand the biologically relevant ranges of mean and variation, to validate the relevance of our results. We also test the ability of the current index and other previously proposed indices to predict reproductive success in terms of fruit and seed set. When the difference in mean anther and stigma height of complementary morphs is zero or very small, increasing intra-population variation in sexual organ height resulted in a monotonous decrease in reciprocity. However, when differences in mean anther and stigma height are greater than zero, and not negligible, reciprocity initially increased with increasing variation, reached a peak, and then decreased. As the difference in mean height between the complementary anther and stigma increased, peak reciprocity is attained at higher values of intra-population variation. Comparisons with previous indices revealed important qualitative and quantitative differences in the ability to capture changes due to intra-population variation, and in determining the degree of reciprocity in heterostylous populations. The current index, along with two other previously proposed indices, showed a positive relationship with seed set. Challenging current understanding, these results suggest that increasing intra-population variation in organ heights can result in an increase in reciprocity in heterostylous populations. This might explain how heterostylous systems exhibit and tolerate high amounts of intra-population variation in organ heights. Such variation can facilitate the stabilisation and perpetuation of imperfectly reciprocal states that are in the process of evolving towards perfect heterostyly.

Chapter 4: **The reproductive ecology of *Jasminum malabaricum* Wight.: a species with stigma-height dimorphism**

Stigma-height dimorphism has an imperfect reciprocal arrangement of anther and stigma between the morphs. Due to this, pollen transfer in these species is assumed to be less efficient in these species as compared to heterostyly. Relative sex organ positions largely drive pollen transfer between morphs in these species. These sex organ positions can differ between the morphs resulting in differences in reproductive success in these species. Apart from sex organ positions, differences in spatiotemporal mate availability, pollinator availability and breeding system between the morphs also cause differences in reproductive success and hence affect the stabilization of stigma-height dimorphism. Here, we studied the reproductive ecology of four naturally occurring populations of *Jasminum malabaricum* in the context of stigma-height dimorphism. The differences in herkogamy and reciprocity between the morphs were quantified. Morph ratio, nearest neighbour morph identity, anthesis period and floral longevity were quantified as spatiotemporal mate availability. We conducted qualitative and quantitative floral visitor observations. The breeding system of the species was also determined. Herkogamy was significantly higher in the short-styled morph while reciprocity was significantly higher between the long-styled stigma and short-styled anther. No difference in spatiotemporal mate availability and floral visitation was observed between the morphs. The plants were compatible with pollen from the same individual and the same morph. The short-styled stigma should receive a higher fraction of legitimate pollen. Both the morphs had equal mating opportunities. The equal number of individuals of both the morphs also indicates equal reproductive success between the morphs. The investigation of the relative importance of herkogamy and reciprocity in promoting pollen transfer in this species was conducted as other factors affecting pollen transfer were not different between the morphs.

Chapter 5: The relative importance of herkogamy and reciprocity in promoting legitimate pollen transfer and fruit set in *Jasminum malabaricum*

The separation between the stigma and anther within a flower (herkogamy), and the relative position of stigma and anther in complementary morphs (reciprocity) is crucial in determining legitimate pollen transfer and fruit set in species with style length polymorphism. Understanding the relative importance of herkogamy and reciprocity becomes particularly important in stigma-height dimorphism, where stigmas are present at the two different heights, but anthers are at the same height in complementary morphs. This leads to imperfect reciprocity and a reduction in legitimate pollen transfer. Consequently, stigma-height dimorphism is considered to be a rare and transient intermediate state in the evolution of reciprocal heterostyly. Here we examine the relative importance of herkogamy and reciprocity in determining legitimate pollen transfer and fitness in *Jasminum malabaricum,* a self-compatible species with stigma-height dimorphism. We quantify and compare the relationship of herkogamy and reciprocity with stigma pollen load, legitimate pollen load and fruit set in naturally occurring populations from the Northern Western Ghats of Peninsular India. We observed no relationship between herkogamy and total stigma load, but as expected, we saw a positive relationship with legitimate fraction of the stigmatic pollen load, and consequently fruit set in the long-styled morph. Contrary to the expectations, we found that reciprocity was negatively related to fruit set in the short-styled morph. Finally, we observed a negative relationship between herkogamy and reciprocity in these plants. The above results indicate that herkogamy is more important than reciprocity in determining legitimate pollination and fruit set in this population. The negative relationship between herkogamy and reciprocity point towards a trade-off between avoidance of self-pollen deposition and promotion of legitimate pollen transfer. These results suggest that herkogamy can play a significant role in encouraging legitimate pollen flow and stabilizing such polymorphic intermediates, allowing reciprocity between complementary organs to arise later in the evolutionary pathways towards perfectly reciprocal heterostyly.

Chapter 6: **Conclusion**

For the first time, in this study, broad patterns in relative sex organ positions in a large number of species with style length polymorphism were explored. Morph specific differences in selection pressures were revealed in the process. Herkogamy and reciprocity could be negatively related, suggesting that there can be a trade-off between avoidance of self-pollen deposition and promotion of legitimate pollen transfer. The increase in reciprocity with an increase in intra-population variation in sex organ heights explains the persistence of a substantial amount of this variation in populations with style length polymorphism. Herkogamy was found to be relatively more important than reciprocity in *J. malabaricum* in promoting legitimate pollen transfer and fruit set. The above results are significant for understanding the maintenance and stabilization of imperfectly reciprocal character states moving towards heterostyly.

Chapter 1

Introduction

Style length polymorphism is a type of floral polymorphism in which there are two or three kinds of individuals in the population, the flowers of which differ in style length or stigma height (Barrett et al. 2000). The difference in stigma height is also associated with a difference in anther height with a reciprocal arrangement of anther and stigma positions between morphs in a form of polymorphism called heterostyly (Darwin 1877, Barrett et al. 2000). There are two types of heterostyly, namely, distyly and tristyly with the presence of two and three morphs, respectively as the name suggests (Charlesworth 1979, Ganders 1979, Barrett et al. 1996, Fornoni and Domínguez 2015). These polymorphisms are usually also associated with physiological incompatibility with pollen from the same individual or the same morph (Barrett and Cruzan 1994). Certain ancillary features like differences in pollen size, pollen exine features, stigma papillae are also present in distylous species, which can lead to a physical incompatibility. There is another kind of dimorphic style length polymorphism characterised by the presence of morphs which differ only in style length, but not in anther height, called stigma-height dimorphism (Baker 2000, Barrett et al. 2000). Stigma-height dimorphism, unlike heterostyly, is not usually associated with physiological incompatibility and ancillary features (Baker 2000). Style length polymorphisms are said to have evolved to increase the efficiency of cross-pollination. Heterostyly in general, and distyly in particular, has interested evolutionary biologists, plant reproductive ecologists, developmental biologists and geneticists since the time of Darwin (Ernst 1933, Mather 1950, Charlesworth Deborah et al. 1979, Lewis and Jones 1992, Faivre 1998, 2000, Shore et al. 2006, Sakai and Wright 2007, Hernández and Ornelas 2007, Cohen 2010, Cohen et al. 2012, Naiki 2012, de Vos et al. 2014). Heterostyly has been extensively studied and has now been documented in approximately 1500 species from 28 families, and new species with heterostyly are still being documented frequently (Jones 2012). It has been estimated that heterostyly has evolved overall at least 23 times independently in the 28 reported families (Lloyd & Webb, 1992; Barrett & Shore, 2008). However, in spite of this, many fundamental questions about heterostyly and style length polymorphism remain unanswered. In this thesis, I address some such open questions related to variation in sex organ positions across biological scales of organization from individuals, to morphs, to species. I ask how this variation is related to two defining morphological traits for species with style length polymorphism, herkogamy and reciprocity, to understand the consequences for pollen transfer and reproductive success.

The dimorphic forms of style length polymorphism have two floral morphs, the long-styled and the short-styled morph which are also known as the pin and the thrum forms respectively (Fig. 1) (Darwin 1877, Cohen 2010). In both distyly and stigma-height dimorphism, there is a vertical spatial separation between the anther and stigma within flowers of both morphs (Barrett et al. 2000). This spatial separation, called herkogamy, reduces interference between male and female sex organs, increases the precision of pollen pick up and deposition by the pollinator, and reduces autogamous self-pollen deposition (Webb and Lloyd 1986, Medrano et al. 2005, Ruan and Jiang 2006, Kulbaba and Worley 2012, Opedal 2018). In distyly, this spatial separation is also accompanied by the reciprocal arrangement of anther and stigma called reciprocity between the two complementary morphs (Fig. 1 B) (Darwin 1877, Ganders 1979, Lloyd and Webb 1992, Kohn and Barrett 1992, Barrett and Shore 2008). Due to this reciprocal arrangement, two distinct levels of organs can be seen in the flowers for both morphs. The long-styled stigma and the short-styled anther constitute the high level, whereas the short-styled stigma and the long-styled anther constitute the low level (Fig.1 B). Legitimate pollen transfer occurs between the sex organs within a level. Empirical evidence shows that the reciprocal arrangement of complementary anthers and stigmas increases the efficiency of legitimate pollen transfer in heterostylous species (Stone and Thomson 1994, Lau and Bosque 2003, Cesaro and Thompson 2004, Keller et al. 2014, Zhou et al. 2015, Costa et al. 2017). Therefore, the physical match between complementary sex organs at a level is commonly quantified to the potential for legitimate pollen transfer and hence reproductive success in these species (Richards and Koptur 1993, Eckert and Barrett 1994, Lau and Bosque 2003, Sanchez et al. 2008, Sosenski et al. 2010, Keller et al. 2012, Armbruster et al. 2017). In species with stigma-height dimorphism, the physical match between anther and stigma positions in complementary morphs is not perfect due to the presence of only one anther level (Fig. 1 A).

There are two proposed pathways to explain the evolution of heterostyly (Charlesworth and Charlesworth 1979, Lloyd and Webb 1992). The pathway proposed by Charlesworth & Charlesworth (1979) assumes that the first step towards the evolution of heterostyly is the origin of self-incompatibility in a homostylous plant population with anther and stigma present at the same height within a flower. The origin of self-incompatibility is considered essential for legitimate pollen transfer and maintenance of morph-ratios in this pathway. The pathway proposed by Lloyd & Webb (1992), on the other hand, considers a flower with stigma positioned above the anthers within a flower, also known as approach herkogamy, to be the ancestral state. This assumption was made as approach herkogamy is found to be very common in families with high occurrence of style length polymorphism. Moreover, the origin of self-incompatibility is not considered essential for the evolution of heterostyly. Both the pathways assume stigma-height dimorphism to be the intermediate character state towards the evolution of heterostyly. All the three assumptions of the pathway proposed by Lloyd & Webb (1992) viz. approach herkogamous ancestor, intermediate character state of stigma-height dimorphism and the non-essentiality of physiological incompatibility has been validated by empirical evidence (Graham and Barrett 2004, Ferrero et al. 2009, 2012). As stigma-height dimorphism does not have a perfect reciprocal arrangement of anther and stigma between the complementary morphs, it is assumed to have lower efficiency of inter-morph or legitimate pollen transfer. Hence, this character state is supposed to be transitory and evolves into the more stable distylous form (Lloyd and Webb 1992). The transitory nature of stigma-height dimorphism is also cited as the reason for its rarity in nature. However, stigma-height dimorphism is now known to be fairly common in nature and is also found in taxa with no known heterostylous species (Barrett et al. 2000). Contrary to the expectation, in the genus *Narcissus*, stigma-height dimorphism is a common character state and distyly rare (Graham and Barrett 2004). This poses the question if stigma-height dimorphism is a transitory intermediate in the pathway towards the evolution of heterostyly (Barrett et al. 2000, Simon-Porcar et al. 2014).

The relative positions of the sex organs are important determinants of efficient legitimate pollen transfer and reproductive success in these species with style length polymorphism (Kohn and Barrett 1992). Although a lot of information is available on sex organ positions in species with style length polymorphisms, general patterns across species and families are still unexplored. In this study, for the first time, the general patterns of morph and organ-level specific differences in relative sex organ positions in around 300 species with style length polymorphism was examined. I use the data extracted for relative organ position to calculate herkogamy and reciprocity, the two defining features of heterostyly (Barrett et al. 1996, 2000), and examine the relationship between the two, which still remains unexplored. The relationship between herkogamy and reciprocity can give us insights into the selection pressures, and potential constraints related the two vital mechanisms operating in flowers with style length polymorphism: the avoidance of self-pollen deposition, and the promotion of legitimate pollen transfer. One of the primary reasons that the relationship between herkogamy and reciprocity has not been examined before is because current indices used to calculate reciprocity (Richards and Koptur 1993, Lau and Bosque 2003, Sanchez et al. 2008, Armbruster et al. 2017) allow quantification for population-level estimates for reciprocity, but not for individuals.

Herkogamy within a morph can be affected by the relationship of the stigma and anther height within a flower across individuals of a population. Examination of the relationship between stigma and anther heights will allow us to understand this. In the long-styled morph when the slope of the relationship between stigma and anther heights is less than zero or between zero and one, the separation between anther and stigma height or herkogamy increases with an increase in stigma height (Fig. 2 A). However, when the slope between stigma and anther height is more than one, the separation between anther and stigma height decreases with an increase in stigma height. The situation flips in the short-styled morph as the stigma is below the anthers (Fig. 2 B).

Reciprocity of a stigma with anthers of the complementary morph is influenced by the relative distribution of complementary anther and stigma heights of a population. If the stigma heights are higher than the anther heights on an average, increasing stigma height will lower complementarity with the anthers, and therefore reduce reciprocity (Fig. 3 c & d). In a similar manner, the situation flips if stigma heights are lower than anther heights on an average and increasing stigma height under these circumstances will increase reciprocity (Fig. 3 a & b). Depending on the direction of the relationship of stigma height with herkogamy and reciprocity, a negative or positive relationship between herkogamy and reciprocity can be expected (Fig. 4). I test these expectations for the relationship between herkogamy and reciprocity across individuals of naturally occurring populations of species with style length polymorphism. These predictions are tested in seventy populations where data for individual-level organ heights were available.

Earlier reciprocity indices developed to quantify the physical match between complementary sex organs in species with style length polymorphism used mean organ positions (Richards and Koptur 1993, Eckert and Barrett 1994). We now understand that variation in sex organ heights across individuals in populations of species with style length polymorphism is common (Faivre and McDade 2001, Ferrero et al. 2011b),this variation can be substantial (Wolff and Liede-Schumann 2007, Machado et al. 2010), and this variation can significantly affect reproductive success (Faivre and McDade 2001, Ferrero et al. 2011a, 2017). However, the extent of intra-population variation in sex organ heights and its effect on reciprocity and herkogamy in species with style length polymorphism has not been comprehensively examined. Later indices recognized the importance of variation and incorporated intra-population variation in sex organ dimensions in the estimation of reciprocity (Lau and Bosque 2003, Sanchez et al. 2008, Sánchez et al. 2013, Armbruster et al. 2017).

However, these indices assume that an increase in intra-population variation in sex organ heights will always result in a decrease in reciprocity. Reciprocity can increase with an increase in intra-population variation under certain circumstances. When the difference between mean complementary sex organ heights is very low or negligible (Fig. 5 A & C), an increase in intra-population variation in sex organ heights decreases the overlap between the distributions of complementary anther and stigma heights (Fig, 5 A to C). But, when the difference between mean complementary anther and stigma heights is not zero, and reasonably high (Fig. 5 B & D), with increasing intra-population variation, the overlap between the distribution of complementary anther and stigma heights increases thereby increasing reciprocity (Fig. 5 B to D). I conducted a literature survey to understand the extent of variation in sex organ heights between individuals of a population for species with style length polymorphism. Additionally, I developed an index which appropriately accounts for intra-population variation. I used simulated heterostylous populations, and empirical data extracted data for the distribution of anther and stigma heights for more than 200 naturally occurring heterostylous populations of more than 100 species to understand if reciprocity can increase with intra-population variation under the above-mentioned circumstances. Since reciprocity indices have been used to understand and legitimate pollen transfer and reproductive success in heterostylous species, I also study the relationship between reciprocity and fruit set and seed set across more than 30 species to validate if reciprocity values calculated by indices can predict fruit set or seed set.

Empirical evidence shows that the reciprocal arrangement of anther and stigma plays an important role in legitimate pollen transfer between the morphs in heterostylous species. But, the explicit role of reciprocity in species with stigma-height dimorphism where the complementary arrangement of anthers and styles is imperfect is not clear and remains unexplored. Additionally, despite the lack of perfect reciprocity, sufficient legitimate pollen transfer has been documented in species with stigma-height dimorphism (Baker et al. 2000, Simon-Porcar et al. 2014). The function of herkogamy in increasing the fraction of legitimate pollen load in these species is not clear. Herkogamy could help to avoid stigma blocking and pollen wastage by self -pollen deposition and increase the chances of fertilization by pollen of the complementary morph (Nishihiro and Washitani 1998, Cesaro et al. 2004, Medrano et al. 2005). In the absence of perfect reciprocity, herkogamy can be more important in these species. Here, I examined the relative importance of herkogamy and reciprocity in four naturally occurring populations of *Jasminum malabaricum* Wight., a species with stigma-height dimorphism, by studying the relationship of these two traits with legitimate pollen fraction on the stigma and reproductive success. The legitimate pollen fraction on the stigma could be quantified in this species as it possesses pollen size dimorphism. Since, pollen transfer between morphs and reproductive success is also affected by spatiotemporal mate availability, pollinator availability and the breeding system; I also investigated the morph-specific differences in spatiotemporal mate availability, pollinator availability and breeding system in these populations.

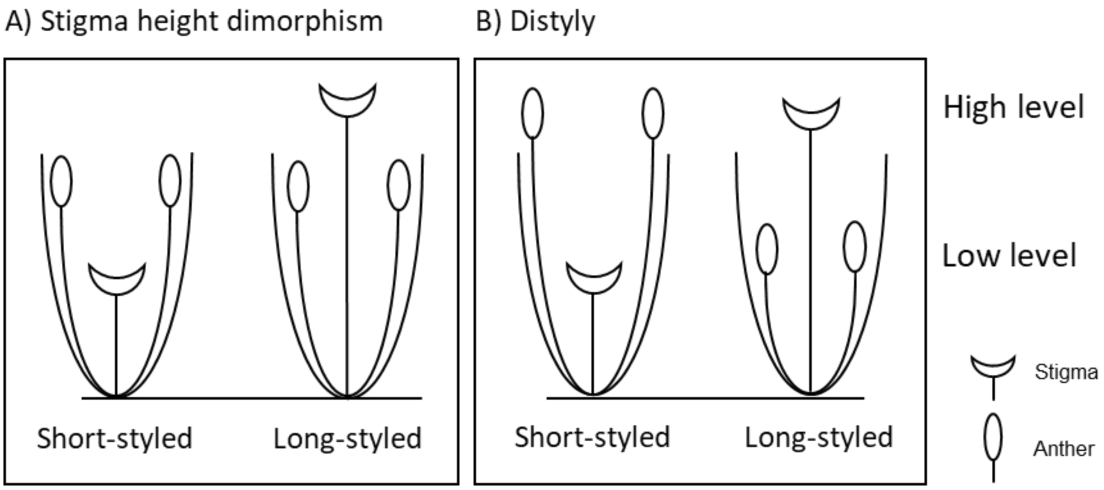

Figure 1: A schematic representation of the long- and the short-styled morphs in A) stigma-height dimorphism B) distyly. Stigma-height dimorphism has two stigma positions but only one anther position, whereas distyly has two stigma and anther positions. The stigma height of the long-styled morph and the anther height of the short-styled morph represent the high level. The stigma height of the short-styled morph and the anther height of the long-styled morph represent the low level. Legitimate pollen transfer occurs between anther and stigma of a level between complementary morphs.

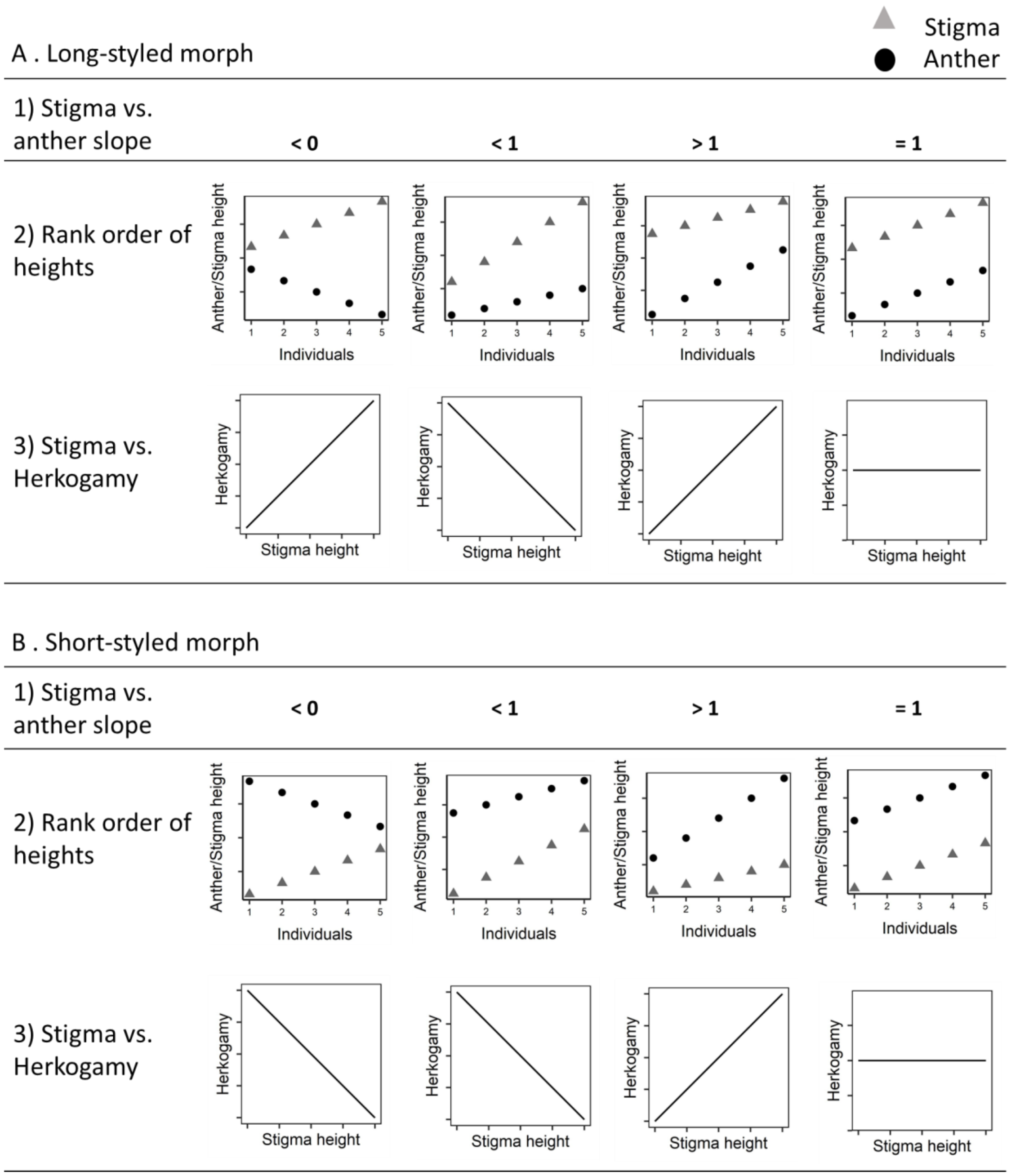

Figure 2: Schematic explaining how the slope of the relationship between anther and stigma height within a flower affects the relationship between stigma height and herkogamy. Light grey triangles represent stigma, and dark grey circles represent anther. Relationships for A) long-styled and B) short-styled morphs are shown separately. Anther and stigma heights within a flower for an individual arranged in ascending order of stigma heights are presented in A2 and B2. The resultant slope for the relationship between stigma and anther heights are shown in A1 and B1. The relationship between herkogamy and stigma height is shown in A3 and B3.

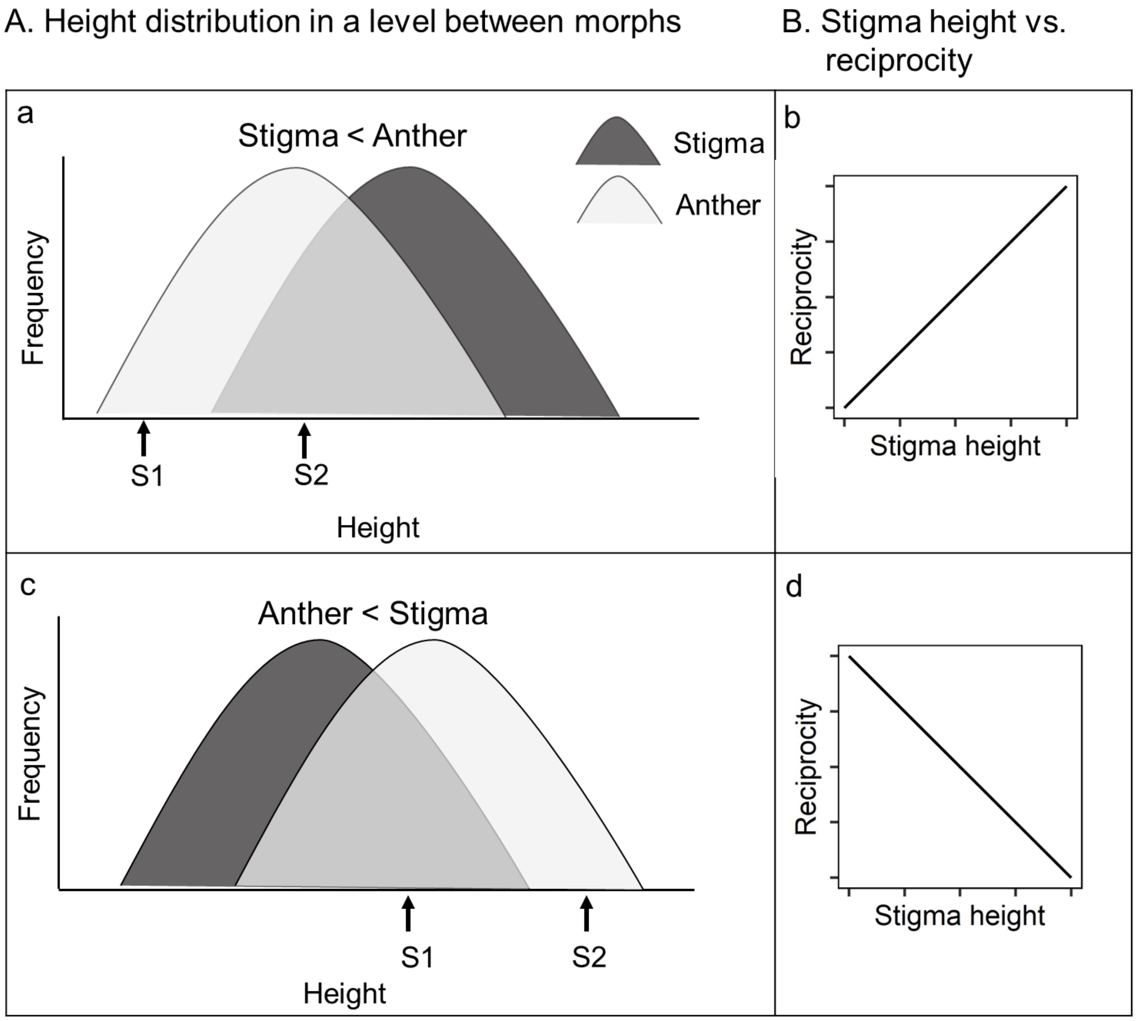

Figure 3: Schematic representation of the relationship between stigma height and reciprocity between anther and stigma of complementary morphs. X-axis is anther or stigma heights and Y-axis is frequency in (a) and (c). Light grey bell-shaped curve refers to the distribution of stigma heights, and dark grey bell-shaped curve refers to the distribution of anther heights in complementary morphs. Points S1 and S2 in (a) and (c) are two stigma heights in the distribution of stigma heights. When mean stigma height is lower than the mean anther height of the complementary morph (a), reciprocity is higher for S2 with higher stigma than S1. Hence, higher the stigma more is its reciprocity (b). When mean stigma height is higher than the mean anther height of the complementary morph (c), reciprocity is lower for S2 with higher stigma than S1. Hence, higher the stigma height, lower is its reciprocity (d).

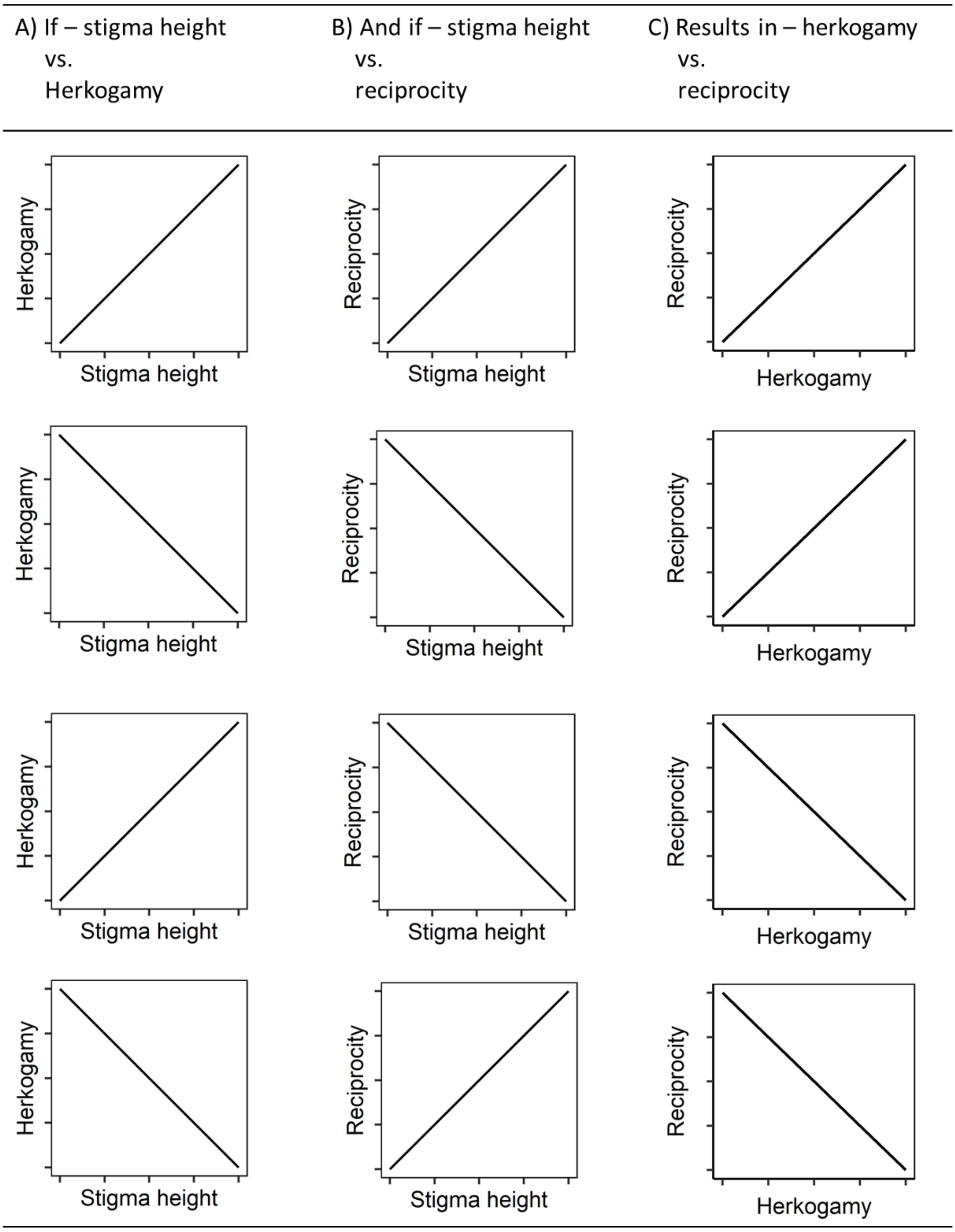

Figure 4: Schematic representation of the predicted relationship between herkogamy and reciprocity (C) as a consequence of the relationship between stigma height and herkogamy (A) and stigma height and reciprocity (B). The combination of A and B result in the relationship seen in C. The relationships between stigma height individually with herkogamy and reciprocity are explained in Fig. 2 and 3.

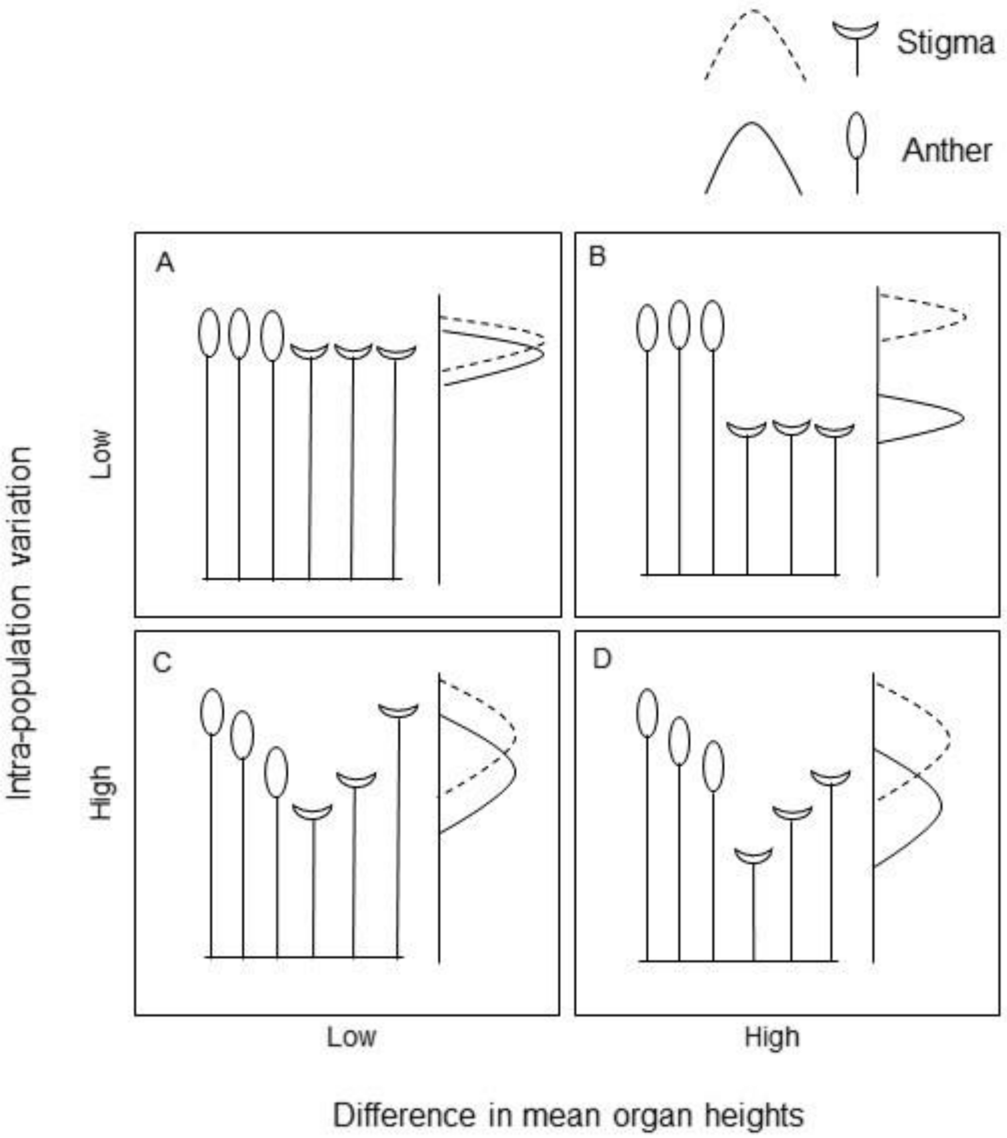

Figure 5: Schematic explaining the effect of intra-population variation in sex organ heights on reciprocity. The x-axis denotes low and high values of difference in mean complementary sex organ heights, and the y-axis denotes low and high values of intra-population variation. Each symbol of anther and stigma represents an individual anther or stigma height of the complementary morph for one level and the curves on the right denote the resulting distribution of those anther and stigma heights. Panel A to C shows the change in the overlap between the distribution of anther and stigma heights with an increase in intra-population variation when the difference in mean complementary sex organs is low. Panel B to D shows the change in the overlap between the distribution of anther and stigma heights with an increase in intra-population variation when the difference in mean complementary sex organs is high. The dotted line represents stigma height, and the solid line represents anther height.

Chapter 2

The relationship between herkogamy and reciprocity: the two fundamental features of species with style length polymorphism

Abstract

Reciprocity and herkogamy are considered to be one of the most important factors determining pollen transfer between the morphs and consequently reproductive success in species with style length polymorphism. Although sex organ positions in heterostylous species have been quantified in a large number of species, general patterns of differences in herkogamy and reciprocity between morphs have not been studied till date. Moreover, the relationship between herkogamy and reciprocity across individuals of a morph in a population have not been studied as reciprocity has not been quantified for an individual. Although, it is generally accepted that intra-population variation in sex organ dimensions reduces reciprocity and hence pollen transfer between morphs, the extent of it in species with style length polymorphism is not known. In this study, we extracted data on sex organ dimensions for around 300 species from the literature and ask questions to understand general patterns of morph-specific differences in herkogamy and reciprocity. The patterns were examined at a species level by extracting data on mean sex organ dimensions of a species and also for individual level by extracting data for the distribution of sex organ dimensions of individuals of a population. The distribution of anther and stigma heights of individuals of a population was also used to investigate the relationship between herkogamy and reciprocity across individuals of a morph in a population by calculating these traits for individuals. We studied the extent of intra-population variation in heterostylous species and tried to understand if it can potentially increase reciprocity between individuals of a population. As per our expectations of higher chances of self-pollen deposition, we find that the short-styled morph has higher herkogamy in species with heterostyly. However, reciprocity was not significantly different between the morphs. The mechanistic explanations for the relationship between herkogamy and reciprocity put forward in chapter 1 were validated by the patterns observed in naturally occurring populations. One of the important findings of this investigation was that herkogamy and reciprocity could be negatively related. High intra-population variation in sex organ positions was seen, and results indicated that intra-population variation could help increase reciprocity in certain circumstances. The negative relationship between herkogamy and reciprocity implies that there can be a trade-off between increasing legitimate pollen transfer and avoidance of autogamous pollen deposition. Increase in reciprocity with an increase in intra-population variation in sex organ positions can help the stabilization of imperfectly reciprocal character states evolving towards heterostyly.

Introduction

Heterostyly is a floral polymorphism characterised by the presence of two or three morphs in a population with the reciprocal arrangement of sex organs (Darwin 1877, Barrett and Shore 2008). The reciprocal arrangement of sex organs between complementary morphs increases legitimate pollen transfer between the morphs (Stone and Thomson 1994, Cesaro and Thompson 2004, Keller et al. 2014), while the separation of anther and stigma within a flower reduces interference between male and female sex organs and decreases self-pollen deposition (Webb and Lloyd 1986). Thus, the relative positions of sex organs are critical in determining efficient pollen transfer between morphs in heterostylous species (Keller et al. 2012). Heterostyly has been studied since the time of Darwin, and currently, it has been reported in over 1500 species from 28 families (Jones 2012). The extensive data available provides an excellent opportunity to ask fundamental questions about the relative sex organ position in heterostylous species in the context of functional consequences and potential constraints.

Reciprocal arrangement of anthers and stigmas between complementary morphs is a central feature of heterostyly (Keller et al. 2012). The physical match between complementary anthers and stigmas is quantified using reciprocity indices to understand legitimate pollen transfer and ultimately, reproductive success (Jacquemyn et al. 2018). Early indices estimated reciprocity as the match between population means of complementary sex organ positions (Richards and Koptur 1993, Eckert and Barrett 1994). Since then, it was recognized that intra-population variation in sex organ heights affects reciprocity (Faivre and McDade 2001), and more recent indices incorporate estimates of variation among individuals (Lau and Bosque 2003, Sanchez et al. 2008, Sánchez et al. 2013, Armbruster et al. 2017). It is reported that reciprocity can differ between morphs and across populations (Ferrero et al. 2011a). The long-styled morph usually receives higher total pollen as all pollinators can easily access the high-level stigma (Barrett and Shore 2008). With higher total pollen load, long styles might get sufficient legitimate pollen even if these stigmas are not perfectly matched to the complementary anthers. This might relax the stringent selection for perfect reciprocity and result in lower reciprocity at the higher level as compared to the lower level (Haddadchi 2013).

While herkogamy has not received as much attention relative to reciprocity, it is important and is an essential component of heterostyly (Barrett et al. 2000, Barrett 2002). Herkogamy, the separation between anther and stigma within a flower, reduces physical interference between the male and female sex organs. This helps to increase the efficiency of pollen deposition on the pollinator (Webb and Lloyd 1986). This separation is also important in reducing autogamous self-pollen deposition within flowers (Cesaro et al. 2004, Medrano et al. 2005). In short-styled individuals, while pollinators are visiting flowers, the anthers are encountered before the stigma. This makes short-styled individuals more prone to self-pollination (Luo and Widmer 2013), and based on this, it is expected that separation between the anther and stigma within flowers may be more important in the short-styled morph.

Herkogamy and reciprocity are the two central features in flowers of species with heterostyly. While both the traits increase the efficiency of legitimate pollen transfer, the mechanism by which this is achieved differs. Therefore, it is surprising that the relationship between these two traits has not been examined to date. Can individuals and species alter herkogamy and reciprocity independently of each other, or are there potential trade-offs between promoting legitimate pollen transfer and avoidance of self-pollen deposition? Examining the relations between these two important traits will allow us to explore the relative selective advantages in variation in the two traits, and also ask about the potential constraint that can limit the independent evolution of the two traits. A primary reason why this relationship has not been studied is that currently, reciprocity is estimated for populations and not at the level of individuals. Additionally, even though herkogamy is quantified at the level of individuals, most studies focus on the population level averages. It is important to understand the relationship between individual-level estimates of these traits because this is the level at which selection operates.

In this study, reciprocity for an individual is estimated for the upper and lower organ level as the average of all the pair-wise mismatches with the complementary organs in all individuals of the opposite morph (Sanchez et al. 2008). This measure is an estimate of potential legitimate pollen transfer for that individual. The relationship between herkogamy and reciprocity across individuals of a population can be predicted based on the average position of anther and stigma of the complementary morphs and on the relationship between anther and stigma heights within a flower of individuals of a population. The predictions have been clearly outlined in chapter 1 (Fig. 2, 3 and 4) explaining the circumstances under which herkogamy and reciprocity can be negatively or positively related.

Intra-population variation (IPV) in sex organ heights can reduce reciprocity between sex organs of complementary morphs and hence result in a decrease in legitimate pollen transfer and reproductive success in heterostylous species (Ferrero et al. 2011b, Keller et al. 2012, Brys and Jacquemyn 2014, Haller et al. 2014). Such variation is common, with a coefficient of variation in sex organ heights as high as 50% (Machado et al. 2010). Given that IPV has important fitness consequences, is common and can be substantial, it is important to understand its extent in heterostylous species. Additionally, it is important to understand how IPV in organ heights affects reciprocity. It is currently thought that IPV will always negatively affect reciprocity. When anthers are perfectly matched, or the difference in mean complementary anther and stigma heights is low, an increase in IPV will result in a decrease in overall reciprocity (Chapter 1, Fig. 5). However, when the difference in mean complementary anther and stigma heights is high, low IPV will result in no overlap and therefore zero or low reciprocity. In these circumstances, increasing IPV can result in higher reciprocity. Thus, increasing IPV can be beneficial, particularly when the mean difference in complementary organ heights is high.

Stigma-height dimorphism is a kind of style length polymorphism which has two morphs and differs from heterostyly in having only one anther position. In such a case, it is important to understand if stigma positions are equidistant from the single anther position making reciprocity and herkogamy equal in both the morphs or morph-specific selection pressures make reciprocity and herkogamy asymmetrical. It has been proposed that stigma-height dimorphism could have originated due to high interference between male and female organs in the long-styled morph (Yeo, 1975). The short-styled morph which invades such as population has higher herkogamy. Such conditions can cause inherent differences in sex organ positions between the morphs in stigma-height dimorphism (Barrett et al. 1996, Cesaro et al. 2004), unlike heterostyly.

In this study, an exhaustive search of the literature was conducted to identify published and unpublished work that reported sex organ dimensions in species with heterostyly and stigma-height dimorphism. These data were extracted, and relative sex organ positions, herkogamy and reciprocity in these species were calculated to ask if reciprocity was different between the upper and lower organ position in these flowers, and if herkogamy was different between the long- and short-styled morphs. Reciprocity was estimated as the mismatch between complementary sex organ positions, and this was done at two different levels of biological organization: with population mean organ heights and individual organ heights. Reciprocity was also quantified using previously proposed reciprocity indices. These estimates of reciprocity were used to test if reciprocity was lower in the high-level organs, which can compensate for the lower deposition of legitimate pollen with the higher overall pollen deposition. As with reciprocity, herkogamy was estimated with population mean organ heights, and individual-level organ heights, to test the prediction that herkogamy is greater in the short-styled morph that is more prone to self-pollination. To comprehend how labile herkogamy and reciprocity are in response to selection pressures, we investigated if relative organ positions are phylogenetically conserved. Additionally, we examined the relationship between herkogamy and reciprocity in individuals within a species. To understand the underlying mechanistic causes of such a relationship, we examined the relationship between anther and stigma heights within a flower in a morph. Finally, we estimated the extent of IPV in sex organ heights and tested if IPV increases with the difference in mean complementary sex organ heights. In all of the above questions, we asked if patterns are similar in heterostyly and stigma-height dimorphism.

Methods

An exhaustive literature search was conducted using the keywords style length polymorphism, heterostyly, distyly and stigma-height dimorphism. Initially, the ISI Web of Science and Google Scholar were used for the searches. Subsequently, searches were also conducted for references cited in the initial references identified, and also for studies that had cited these initial references found. This study was restricted to distyly and stigma-height dimorphism, the dimorphic character states in species with style length polymorphism.

Data were extracted from these references at three different levels: (a) Mean population-level sex organ heights when reported from multiple individuals from both morphs were provided; (b) Variation in sex organ heights as standard deviation or standard error, when this was estimated from at least three individuals for each morph; and, (c) Individual-level anther and stigma heights when this information was available for at least ten individuals of each morph. When provided, information for mean herkogamy for both the morphs was extracted from the references for analysis. Data were extracted from text, tables, scaled floral illustrations, and when necessary, by digitizing graphs and figures using the software PlotDigitizer. The initial searches identified approximately 850 references including botanical descriptions and Doctoral and Master's dissertations theses that had information regarding sex organ heights of species with dimorphic style length polymorphism. The final number of references used for this study after applying the selected criteria mentioned above was 137.

Flower size, which was estimated as the average of the high-level sex organs, varied considerably across the selected species. To normalize for differences in flower size, sex organ height of a species was taken as a percentage of the grand mean of all four mean sex organ heights for that species. This standardized measure of organ height was used for all further analyses.

The difference in complementary anther and stigma heights was used as a measure of reciprocity. This mismatch is inversely proportional to reciprocity and will range from zero for perfectly matched complementary stigma-anther pairs, to higher numbers as reciprocity decreases. Reciprocity was estimated for the higher and lower levels of sex organ position found in each morph. To incorporate the effect of IPV in sex organ heights on reciprocity, reciprocity was calculated for species where data were available at the individual level. Here, the mismatch for every complementary stigma-anther pair was calculated and averaged for the higher and lower levels of sex organ positions. Reciprocity was also estimated from the mean population-level data using reciprocity indices proposed by Richards & Koptur (1993), Lau & Bosque (2003) and Armbruster et al. (2017). Richards & Koptur's index was calculated as the modulus of (mean anther – mean stigma)/(mean anther + mean stigma) for sex organs of each level. Lau & Bosque's index (2003) was calculated as the percentage overlap in the distribution of anther and stigma heights of the complementary morphs. Half of the anther length was used as the bin width to create the frequency distributions as there is no overlap between anther and stigma height of complementary morphs beyond half of the anther length (Ferrero et al., 2011). Armbruster's index was calculated as the sum of the square of the difference in mean complementary sex organ heights and the variance in those organs (Armbruster et al., 2017). Like with the measure of mismatch used here, the indices proposed by Richards & Koptur (1993) and Armbruster et al. (2017) calculate the lack of match between the sex organs and are inversely related to reciprocity.

To examine the degree of separation between anther and stigma heights within a morph in these species, the difference in mean sex organ heights of a morph was calculated. Additionally, when individual-level data on sex organ heights were available, herkogamy was calculated as the difference in anther and stigma heights of an individual. These individual measures of herkogamy were averaged to get a mean herkogamy for both morphs. The coefficient of variation was calculated to understand the extent of IPV in anther and stigma heights across species.

Individual-level estimates of mismatch and herkogamy were used to examine the relationship between reciprocity and herkogamy within populations using Pearson’s correlation coefficient. To understand the underlying mechanisms, the relationships between anther and stigma heights were examined using the slope of ranged major axis (RMA) type II regression. RMA regressions were used because of different variances in the estimates of anther and stigma heights in the morphs (Legendre 1998). To categorise the slopes as less than zero, between zero and one and greater than one, the 2.5% and 97.5% (2 SD) confidence interval of the mean slopes were examined. If both upper and lower limits and the mean slope were less than zero, the slope was considered as less than zero. If both the limits and the mean were above one, the slope was taken as greater than one. Otherwise, slopes were considered to be between zero and one. Some mean slopes with very large confidence intervals which were not significant and could not be categorised as above were not included in the analysis.

To examine the relationship between the difference in mean complementary sex organ heights and coefficient of variation for a level, mean coefficient of variation for a level was calculated as the mean of the coefficient of variation for the stigma and anther height of that level.

All statistical analyses were phylogenetically corrected using the tree provided by (Smith and Brown 2018). This is the most recent and comprehensive phylogenetic tree of seed plants. For phylogenetic analyses, accepted names for the species in the extracted data were obtained from The Plant List (2013) (Version 1.1. Published on the Internet; http://www.theplantlist.org/ (accessed December 2018)**.** Species that were not present in the tree provided by Smith & Brown (2018) were excluded from the analyses. Where data for multiple populations of the same species were available, the population with the largest sample size was chosen for the analyses. As an alternative, when data for multiple populations of the same species were available, analysis with the mean value of sex organs heights for all the populations was also performed. Phylogenetically corrected statistical tests were performed using the R packages phytools (Revell 2012) and ape (Paradis et al. 2004). Pagel’s lambda was calculated to estimate the phylogenetic signal in a trait (Pagel 1999).

Phylogenetic paired t-tests were used to examine differences between the long- and short-styled morphs, or the high and low levels (Lindenfors et al. 2010). Phylogenetically independent contrasts were used for correlation analyses to examine the relationship between IPV in anther and stigma heights in complementary morphs and difference in mean complementary sex organ heights in a level (Paradis et al. 2004).

Results

From the 850 studies that were identified from the initial search, data on sex organ position for species with heterostyly and stigma-height dimorphism were extracted from 137 references. This represented a total of 677 populations from 356 species (Table 1) and included representatives from 25 of the 28 families (Naiki 2012) in which heterostyly has been documented (Fig. 1). A lower number of species that were recognized by the most recently published tree (Smith and Brown 2018) were used for the phylogenetically corrected analysis (Table 1, Table S1). The range of flower sizes examined in this study (Fig. 1) was representative of the flower sizes reported for heterostylous species (Ganders, 1979).

As expected, the mean sex organ positions showed a high variation in distyly and stigma-height dimorphism. Comparison of the four sex organ positions in distyly clearly shows two levels and the expected reciprocal positions of complementary sex organs (Fig. 2 A). The separation between mean anther and stigma heights of each morph was found to be similar between the morphs. Species with stigma-height dimorphism also showed two stigma levels but only one anther level (Fig. 2 B). However, the two anther positions were not perfectly matched, and the short-styled morph had higher mean anther height. This led to higher separation between mean anther and stigma of the short-styled morph and also higher reciprocity between the mean anther and stigma of the complementary morphs of the high level.

In contrast to the expectation that reciprocity might be higher in the lower level, there was no difference in reciprocity in both species with distyly or stigma-height dimorphism. This was consistent when examining the difference in mean anther and mean stigma height across complementary morphs (Table 2, Fig. 3 A & C), and also for individual-level mismatch estimates (Table 2, Fig. 3 B & D). In comparing reciprocity in the high and low levels using previously proposed indices it was seen that the index proposed by Armbruster et al. (2017) was consistent with the above results and did not show any significant differences in species with distyly or stigma-height dimorphism (Table 3). The results from the other indices were not consistent with each other or with expectations. The indices proposed by Richards & Koptur (1993), and its modified form, which incorporates individual-level variation, counter-intuitively showed higher reciprocity in the higher levels for species with heterostyly. Similarly, the index proposed by Lau and Basque (2003) counter-intuitively showed higher reciprocity in the higher levels for species with stigma-height dimorphism.

As expected, mean herkogamy was significantly higher in the short-styled morph in species with distyly (Table 4 A, Fig. 4 B), but surprisingly, was not different between morphs in species with stigma-height dimorphism (Table 4 B, Fig. 4 D). At the level of population means there was no difference between morphs in stigmas and anthers separation in species with distyly or stigma-height dimorphism (Table 4, Fig 4 A & C).

Both measures of complementarity, the difference in anther and stigma heights between opposite morphs measured at the population level, and at the individual level, had low but significant values for Pagel's ƛ indicating weak phylogenetic signal in the lower level in species with distyly, but no significant signal for the higher organ level (Table 5A). Similar results were observed for species with stigma-height dimorphism for the population-level measure of reciprocity with a weak phylogenetic signal in the lower level, but no significant signal for the higher organ level. However, in contrast to species with distyly, there was a strong phylogenetic signal for both low and high organ levels for mean mismatch, the individual level measure of reciprocity. In examining the separation between anthers and stigmas within a flower, for the measures at the population level means for both morphs, there was a weak but significant phylogenetic signal for species with distyly and no signal for species with stigma-height dimorphism (Table 5B). For stigma-anther separation measured for individuals, i.e. herkogamy, there was a strong phylogenetic signal for both morphs in distylous species, and in species with stigma-height dimorphism.

Both positive and negative relationship between herkogamy and reciprocity were observed in distyly and stigma-height dimorphism (Table 6). There was no significant difference in the number of positive and negative relationship in the long-styled (χ^2 test: $p = 0.41$, $n = 71$ populations) and short-styled (χ^2 test $p = 0.14$, $n = 77$ populations) morphs in distylous populations. On the other hand, stigma-height dimorphism had significantly higher number of populations with a positive relationship in both long-styled (χ^2 test: $p < 0.001$, $n = 22$ populations) and short-styled (χ^2 test: $p < 0.001$, $n = 19$ populations) populations.

The slope of the type II RMA regression between anther and stigma were mostly positive as expected, with a few negative values. Mean stigma height was found to be higher than the mean complementary anther height in most cases in the high level in distyly (Table 7). Mean stigma was lower than the mean complementary anther heights in most cases in the low level in distyly. In stigma-height dimorphism mean stigma height was higher than the anther height in most cases in the high level and was always lower than the anther height in the low level as expected (Table 8). The ensuing relationship between herkogamy and reciprocity based on the combinations of the relative position of mean stigma and anther in the complementary morphs and the slope of the relationship between anther and stigma within a morph matched the predictions as discussed in Chapter 1 (Fig. 2, 3 and 4).

The range of intra-population variation (IPV) was high in all the four sex organ heights in both the morphs with coefficient of variation reaching up to 50% in both distyly and stigma-height dimorphism (Fig. 5). The 10th and 90th percentile of the distribution of coefficient of variation across the species were 4.86 and 15.27 for the long-styled stigma height, 4.06 and 21.00 for the long-styled anther height, 4.42 and 17.27 for the short-styled stigma height and 5.72 and 25.19 for the short-styled anther height. The mean coefficient of variation of the high level was positively related to the difference in mean complementary sex organ heights in both distyly and stigma-height dimorphism as expected (Table 9 A & B). Counterintuitively, the mean coefficient of variation of the low level was negatively related to the difference in mean complementary sex organ heights of the low level in distyly and not significantly related in stigma-height dimorphism (Table 9 A & B).

Discussion

One of the most important novel findings of this study is that herkogamy and reciprocity can be negatively related across individuals of a population, which suggests a trade-off between avoidance of self-pollen deposition and promotion of legitimate pollen transfer. Short-styled morph had higher herkogamy in distylous species as expected but, contrary to the expectation, no difference in reciprocity was found between the two levels in distylous species. This could lead to differences in the illegitimate fraction of pollen deposited on the stigma. High IPV in sex organ dimensions was seen in species with style length polymorphism. Mean IPV was positively related to the difference in mean complementary sex organ heights, suggesting that IPV can help increase reciprocity between individuals of complementary morphs and is not always detrimental to the efficiency of pollen transfer. Herkogamy will be less amenable to changes in response to environmental selection pressures as compared to reciprocity as it showed higher phylogenetic signal than reciprocity.

Morph specific differences in herkogamy have been reported before in species with style length polymorphism, and examples of higher herkogamy have been documented for both the morphs (Thompson et al. 1998, Chen and Zhang 2010). However, this comparative study of relative sex organ positions of 296 species revealed that the short-styled morph exhibits higher herkogamy more commonly. The higher chance of self-pollen deposition in the short-styled morph can account for this phenomenon (Webb and Lloyd 1986). The higher herkogamy can lead to lower illegitimate pollen deposition in the short-styled morph (Nishihiro and Washitani 1998, Cesaro et al. 2004).

Contrary to the expectation, reciprocity was not found to be significantly different between the levels. This could mean symmetry in reciprocity between the levels or that higher reciprocity can be found at either level. Empirical evidence suggests that all three possibilities exist in nature (Ferrero et al. 2011a, Haddadchi 2013, Santos-Gally et al. 2015, Jacquemyn et al. 2018).

As herkogamy influences outcrossing rates by reducing self-pollen deposition, it changes within and across populations in response to selection pressures like inbreeding depression and reproductive assurance (Eckert, Ozimec, Herlihy, Griffin, & Routley, 2009). This implies that the relationship between anther and stigma within a flower for outcrossing species is amenable to changes in response to selection pressures. Nevertheless, it is also known that flowers are highly integrated modules due to genetic correlations and developmental constraints (Berg 1960, Armbruster et al. 1999, Ferrero et al. 2011b). The latter is what we see in our results, i.e., mean herkogamy was found to be phylogenetically conserved in both the morphs in this study. This indicates that stigma-anther relationships within a flower are conserved in a taxon. Previous studies have shown that stigma and anther positions can show phylogenetic signal but less as compared to perianth traits (Alcantara and Lohmann 2011). The relative sex organ positions between complementary morphs were not found to be phylogenetically conserved. This indicates that the relative sex organ positions between the morphs can be more labile than herkogamy and is likely to change more easily in response to selection pressures like changes in the pollinator type (Ferrero et al. 2011a). Further studies are required to understand how constraints to independently changing the dimensions of the male and female sex organs within a flower (pleiotropy) affect the response to a selection pressure that selectively demands changes in relative sex organ positions between complementary morphs in one level.

The variation in herkogamy and its relationship with reciprocity has not been explored to date. It is important to study the variation of these two traits across individuals within a population as selection works at the level of an individual. Moreover, the consequences of differences across populations become more difficult to understand as differences in a myriad of other environmental factors come into play (Takebayashi et al. 2006). The relationship between herkogamy and reciprocity can result due to the constraints on the modification of anther and stigma heights independently (Herrera et al. 2002, Brock and Weinig 2007) and can also be shaped by selection to optimise the functional role of the two traits in heterostylous species. In this study, both positive and negative relationship between herkogamy and reciprocity were observed. The relationships conform to the expectations laid out in chapter 1 (Fig. 2, 3 and 4) based on the difference in mean complementary sex organ heights and the slope of the relationship between anther and stigma heights across the individuals of a population. The later likely resulting from the nature of the pleiotropic relationship between anther and stigma heights. Mean stigma position was both higher and lower than the mean anther position in distylous species. But the slope of the relationship between stigma and anther was primarily observed to be between zero and one indicating that the changes in herkogamy are mostly brought about by changes in stigma position, a pattern which has been recorded in an earlier study (Herlihy and Eckert 2007). In such a situation, changes in herkogamy will likely influence the pollen deposition on the stigma more than the pollen removal from anthers (Barrett, 2002).

When the relationship between herkogamy and reciprocity is positive, plants can increase their capability to avoid self-pollen deposition while also increasing their chances of legitimate pollen transfer. But, when this relationship is negative, there will be a trade-off between the two functions. Pleiotropy between anther and stigma heights can be modified due to selection pressures like pollinators (Kulbaba and Worley 2012), which in turn can modify the relationship between herkogamy and reciprocity. This likely explains the lack of a relationship or negative relationships between anther and stigma heights in some rare populations in this study. The relationship between herkogamy and reciprocity can affect the response of plant populations to different ecological scenarios. When plant populations are not pollen limited, but there is high inbreeding depression, herkogamy becomes relatively more important (Ushimaru and Nakata 2002). Alternatively, when populations are pollen limited and need reproductive assurance (Ashman et al. 2004), reciprocity becomes more important. The presence of high inbreeding depression and the lack of reproductive assurance together makes both equally important.

Floral morphological traits are assumed to harbour less variation than vegetative traits (Berg 1960, Armbruster et al. 1999). Although it is acknowledged that IPV in floral morphological traits is common and substantial (Faivre and McDade 2001, Ferrero et al. 2011b), its extent, especially in sex organ dimensions, is not completely understood. This study shows that floral sex organs also harbour high IPV, which can sometimes be comparable to or higher than the IPV within vegetative traits (Hansen et al. 2007). Contrary to the current understanding, which states that variation in sex organ heights in a population reduces reciprocity and hence legitimate pollen transfer (Armbruster et al. 2017), sufficient legitimate pollen transfer happens, and isoplethic populations are maintained even in the presence of high variation. One of the causes behind this phenomenon could be that IPV can increase reciprocity and hence legitimate pollen transfer and fruit set under the circumstances explained earlier (Chapter 1 Fig. 5). This is supported by the positive relationship between the difference in mean complementary sex organ heights and mean coefficient variation of the high level. Although The negative relationship in the low level is not entirely explicable, it points towards morph-specific selection pressures.

Although we had predicted differences in relative sex organ positions between distyly and stigma-height dimorphism, no such patterns were observed. This could mean that the anther position in species with stigma-height dimorphism lies symmetrically between the two stigma positions. This is contrary to what we see in the distribution of mean sex organs heights across species with this character state. The anther position in the short-styled morph was qualitatively higher than the anther position of the long-styled morph. The reason for the inconclusive results could also be the low sample size for species with style length polymorphism which, in turn, is a reflection of the lack of studies done on this character state.

In this study, the general patterns of relative sex organ positions common to a large number of species with style length polymorphism have been investigated for the first time. It helped us gain important insights into the selective forces and constraints shaping the relative sex organ positions in species with style length polymorphism. Moreover, the relationship between herkogamy and reciprocity at the level of an individual was revealed. The predictions explaining the mechanism of how the direction of this relationship is determined were validated, emphasizing the role of the constraints shaping the association between anther and stigma height within a flower. This result coupled with the information that herkogamy is more phylogenetically conserved in these species could help us predict the response of plant populations in various ecological scenarios demanding the avoidance of interference of male and female sex organs and self-pollination or promotion of legitimate pollen transfer. Furthermore, the positive relationship between IPV and the difference in mean sex organ heights could indicate the role of IPV in increasing reciprocity between individuals of a population, thereby increasing legitimate pollen transfer and fruit set in these species. This could be especially important for the stabilization of style length polymorphism in species with imperfect reciprocity moving towards heterostyly.

Table 1: Details of data extraction for species with distyly and stigma-height dimorphism. Species mean refers to the number of species or populations for which dimensions of mean sex organ heights were available. Individual distribution refers to the number of species or populations for which distribution of anther and stigma heights for the individuals of a population were available. Data for multiple populations were extracted for a species as evident for the populations and species rows. Species recognized denotes the number of species in the extracted data that were recognized by the phylogenetic tree of seed plants provided by Smith & Brown (2018) which was used for phylogenetic correction of the statistical analyses.

	Distyly		**Stigma-height dimorphism**	
	Species mean	**Individual distribution**	**Species mean**	**Individual distribution**
Populations	622	236	55	26
Species	326	115	30	16
Species recognized	296	111	27	15

Table 2: Difference in reciprocity between the high and low levels. The *P*-value for the phylogenetically corrected paired t-test is presented separately for A) distyly and B) stigma-height dimorphism. The mean stigma – mean anther across has been derived from mean positions of anther and stigma heights across anther and stigma heights of the complementary morphs. Mean mismatch was calculated as the mean of all pairwise mismatch values for anther and stigma of every individual of the complementary morphs calculated for each level separately Number of species analysed in each category is denoted by *n*.

Character state	**Trait**	***n***	***P*-value**
A) Distyly	(Mean Stigma - Mean Anther) across	296	0.45
	Mean Mismatch	111	0.39
B) Stigma-height dimorphism	(Mean Stigma - Mean Anther) across	31	0.62
	Mean Mismatch	15	0.98

Table 3: Difference in reciprocity between the high and low levels as per reciprocity indices. *P*-value for the phylogenetically corrected paired t-test is presented separately for A) distyly and B) stigma-height dimorphism. Richards & Koptur (1993) was quantified using mean sex organ heights. Modified Richards & Koptur (as per Sanchez, Ferrero, & Navarro, 2008) and Lau & Bosque (2003) were calculated using the distribution of anther and stigma heights of individuals of a population. Armbruster et al. (2017) was calculated using mean and the variance in the sex organ heights of a population.

Character state	**Index**	***n***	***P*-value**
A) Distyly	Richards & Koptur (1993)	296	0.001
	Richards & Koptur modified	111	0.029
	Lau & Bosque (2003)	111	0.482
	Armbruster et al. (2017)	231	0.989
B) Stigma-height dimorphism	Richards & Koptur (1993)	27	0.441
	Richards & Koptur modified	15	0.250
	Lau & Bosque (2003)	15	0.019
	Armbruster et al. (2017)	24	0.971

Table 4: Difference in stigma-anther separation within a morph compared between the long- and short-styled morphs. The *P*-value for the phylogenetically corrected paired t-test is presented separately for A) distyly and B) stigma-height dimorphism. The mean stigma – mean anther within has been derived from mean positions of anther and stigma heights for a morph. Herkogamy is the mean of the difference in anther and stigma height of flower across individuals of a morph. The number of species analysed in each category is denoted by *n*.

Character state	**Trait**	***n***	***P*-value**
A) Distyly	(Mean Stigma - Mean Anther) within	296	0.110
	Mean herkogamy	224	0.006
B) Stigma height dimorphism	(Mean Stigma - Mean Anther) within	27	0.935
	Mean herkogamy	19	0.872

Table 5: Phylogenetic signal calculated using Pagel's lambda and its *P*-value calculated for A) reciprocity and B) herkogamy in L: long-styled and S: short-styled morphs and high and low levels for distyly and stigma-height dimorphism. Mean stigma – Mean Anther in reciprocity denotes the difference in mean complementary sex organ heights, whereas in herkogamy it is the separation between mean anther and stigma height within a morph. Mean mismatch was calculated as the mean of all pairwise mismatch values for anther and stigma of every individual of the complementary morphs calculated for each level separately. Herkogamy is the mean of the difference in anther and stigma height of flower across individuals of a morph. The number of species analysed in each category is denoted by *n*.

Trait	***n***	**λ High/Long**	**λ Low/Short**
A) Reciprocity			
Distyly			
(Mean Stigma - Mean Anther) across	296	ns	**0.29***
Mean Mismatch	111	ns	**0.23***
Stigma-height dimorphism			
(Mean Stigma - Mean Anther) across	27	ns	**0.37***
Mean Mismatch	15	**0.99***	**0.84***
B) Herkogamy			
Distyly			
(Mean Stigma - Mean Anther) within	296	**0.46***	**0.33***
Mean herkogamy	231	**0.74***	**0.67***
Stigma-height dimorphism			
(Mean Stigma - Mean Anther) within	27	ns	ns
Mean herkogamy	19	**0.91***	**0.92***

Table 6: Relationship between herkogamy and reciprocity in A) distyly and B) stigma-height dimorphism for the long- and the short-styled morph. Negative and positive denote negative or positive Pearson's correlation coefficient between the two traits. The numbers denote the number of populations with the direction of the relationship followed by the bolded number after a comma, which is the number of populations with correlation coefficients with $P < 0.05$.

Character state	Morph	Negative	Positive
A) Distyly	Long-styled	32, **23**	39, **32**
	Short-styled	32, **17**	45, **26**
B) Stigma-height dimorphism	Long-styled	1, **0**	21, **19**
	Short-styled	1, **0**	18, **10**

Table 7: Relationship between herkogamy and mismatch: H vs M presented for distyly. Slope denotes the slope of type II ranged major axis regression between anther and stigma heights of individuals of a morph. Sample size *n* for each kind of slope has been provided for the long-styled: L and the short-styled: S morph. S and A represent anther and stigma, respectively. Mean S > Mean A denotes that mean complementary stigma height is higher than anther height. Similarly, Mean S < Mean A denotes that mean complementary stigma height is lower than the anther height. The numbers in the H vs M positive and H vs M negative columns denote how many populations show a positive or negative Pearson's correlation coefficient between herkogamy and mismatch followed by how many of them were significant with $P < 0.05$ in brackets. The cells with the expected direction of the relationship between herkogamy and mismatch have been shaded grey. Refer to Fig. 2, 3 and 4 in chapter 1 and the introduction of this chapter for more details.

			Mean S > Mean A			**Mean S < Mean A**	
Morph	**Slope**	***n***	**H vs M positive**	**H vs M negative**	***n***	**H vs M positive**	**H vs M negative**
Long-styled	<0	3	3 (2)	0 (0)	3	0 (0)	3 (2)
	0 - 1	33	30 (28)	3 (0)	23	4 (0)	19 (12)
	>1	0	-	-	0	-	-
Short-styled	<0	1	0 (0)	1 (1)	2	2 (2)	0 (0)
	0 - 1	15	2 (0)	13 (7)	36	30 (10)	6 (1)
	>1	1	1 (0)	0 (0)	5	2 (1)	3 (0)

Table 8: Relationship between herkogamy and mismatch: H vs M presented for stigma-height dimorphism. Slope denotes the slope of type II ranged major axis regression between anther and stigma heights of individuals of a morph. Sample size *n* for each kind of slope has been provided for the long-styled: L and the short-styled: S morph. S and A represent anther and stigma, respectively. Mean S > Mean A denotes that mean complementary stigma height is higher than anther height. Similarly, Mean S < Mean A denotes that mean complementary stigma height is lower than the anther height. The numbers in the H vs M positive and H vs M negative columns denote how many populations show a positive or negative Pearson's correlation coefficient between herkogamy and mismatch followed by how many of them were significant with *P* < 0.05 in brackets. The cells with the expected direction of the relationship between herkogamy and mismatch have been shaded grey. Refer to Fig. 2,3 and 4 in chapter 1 and the introduction of this chapter for more details.

			Mean S > Mean A			**Mean S > Mean A**	
Morph	**Slope**	***n***	**H vs M positive**	**H vs M negative**	***n***	**H vs M positive**	**H vs M negative**
Long-styled	<0	0	-	-	0	-	-
	0 - 1	16	16 (15)	0 (0)	1	0 (0)	1 (0)
	>1	1	1 (0)	0 (0)	0	-	-
Short-styled	<0	0	-	-	1	1 (1)	0 (0)
	0 - 1	0	-	-	12	11 (6)	1 (0)
	>1	0	-	-	1	0 (0)	1 (0)

Table 9: Phylogenetically corrected Pearson’s correlation coefficient *r* between the difference in mean complementary sex organ heights and mean coefficient of variation of that level. Values have been presented for A) distyly and B) stigma-height dimorphism for the high and low level separately. The sample size was 231 species for distyly and 24 species for stigma-height dimorphism.

Character state	**Level**	***r***	***P*-value**
A) Distyly	High	0.35	< 0.001
	Low	-0.25	< 0.001
B) Stigma-height dimorphism	High	0.79	< 0.001
	Low	-0.03	0.869

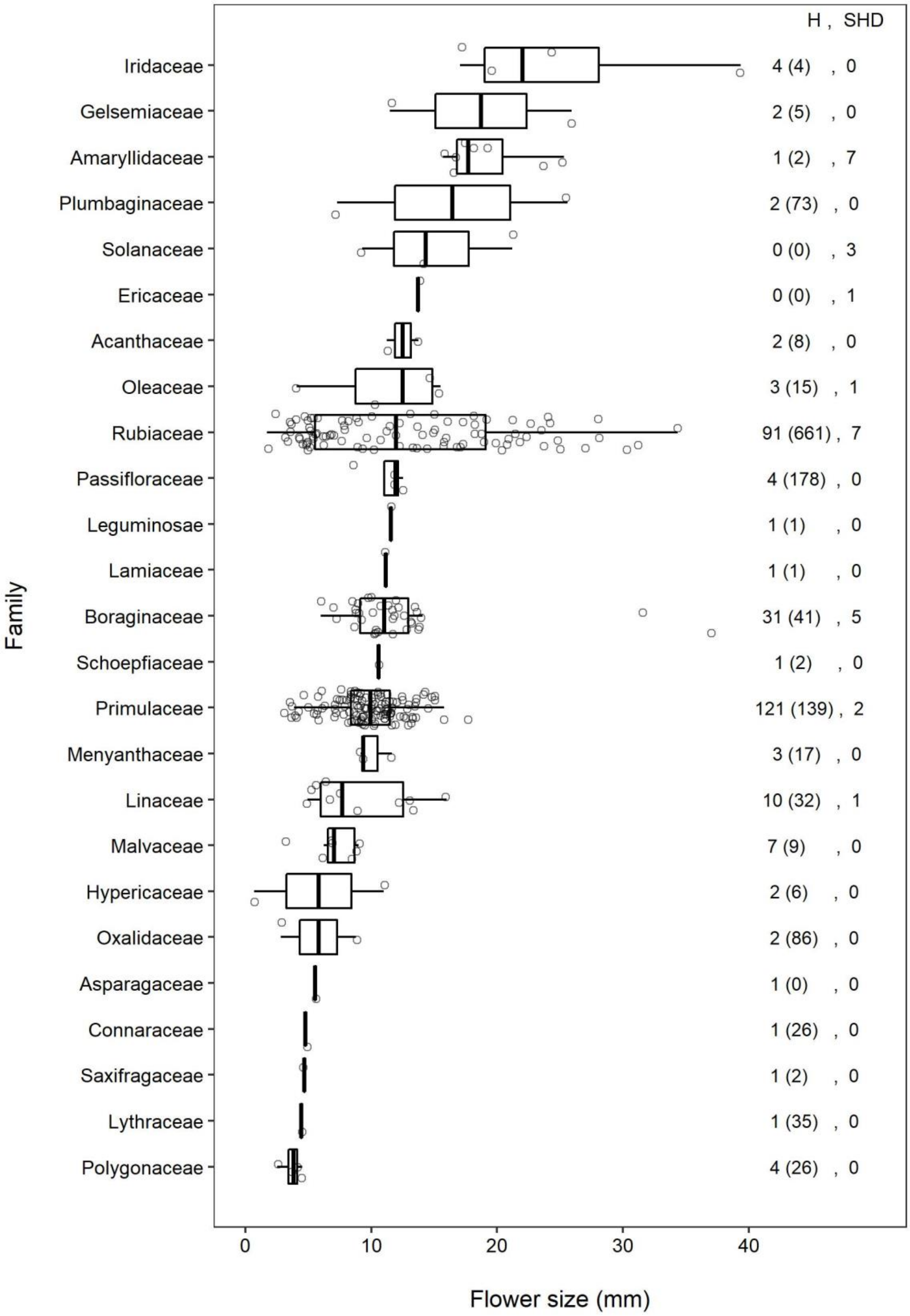

Figure 1. The taxonomic distribution of the 296 distylous species and 27 species with stigma-height dimorphism of the current dataset across different families. Values represent the number of distylous species in that family in the current dataset followed by the reported

number of heterostylous species in that family (after Naiki, 2012) and the number of species with stigma-height dimorphism in the current dataset after a comma. The points represent the mean of the high-level sex organs as a proxy for flower size. The families have been arranged by the median of the flower size (represented by the mean of high-level organs) found in each family. Three species in the family Boraginaceae and one species in Rubiaceae have been reported to have distyly, and stigma-height dimorphism in different populations, and hence have been added to distyly as well as stigma-height dimorphism in the graph. Three flower sizes above 44 mm have been removed from the Family Rubiaceae to help in better visualization of the data. 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.

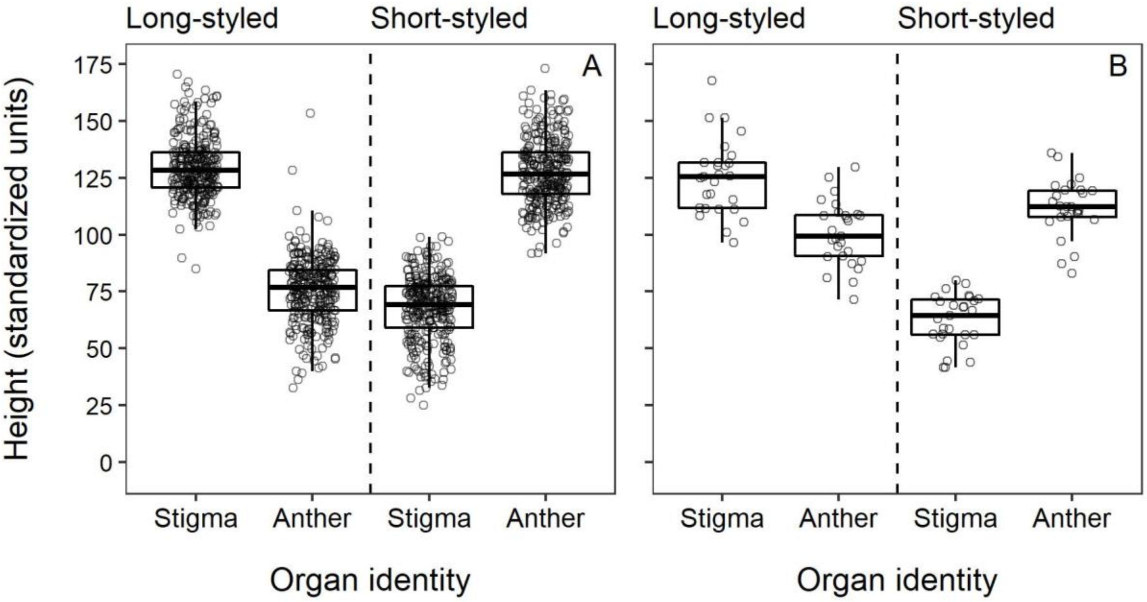

Figure 2: Position of mean anther and stigma heights in the two morphs standardized using the grand mean of all four mean sex organ heights for A) distyly and B) stigma-height dimorphism. Note the perfect match between anther and stigma heights of complementary morphs in distyly which is absent in stigma-height dimorphism where the anthers of both the morphs are approximately at the same position. 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.

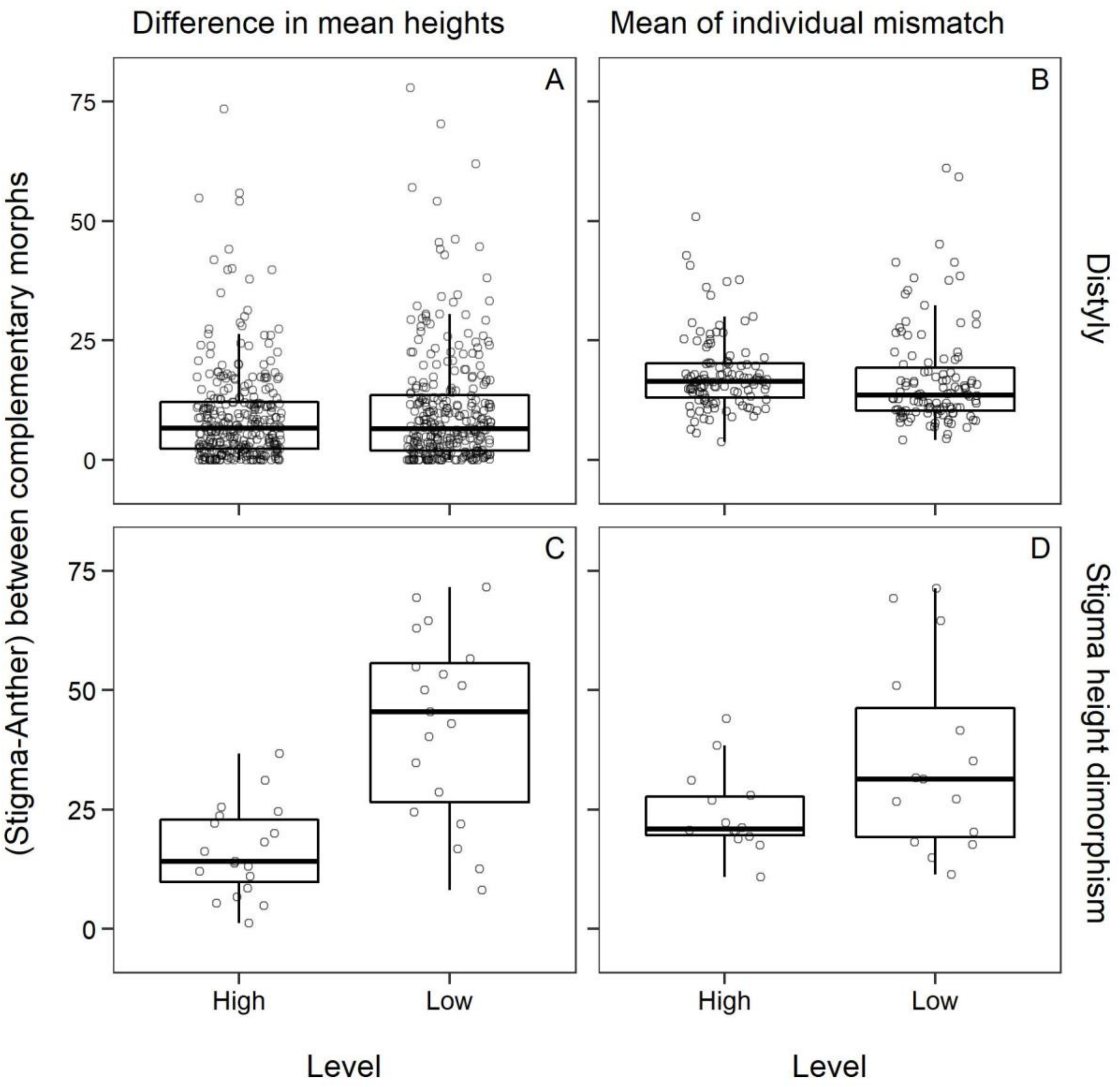

Figure 3. The difference in mean complementary anther and stigma heights and mean of pairwise mismatch between all anther and stigma heights of complementary morphs standardized using the grand mean of all four mean sex organ heights in the high and low level for A & B) distyly and C & D) stigma-height dimorphism. Note the low mismatch between anther and stigma heights of complementary morphs in distyly as compared to stigma-height dimorphism where the anthers of both the morphs are approximately at the same position. 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.

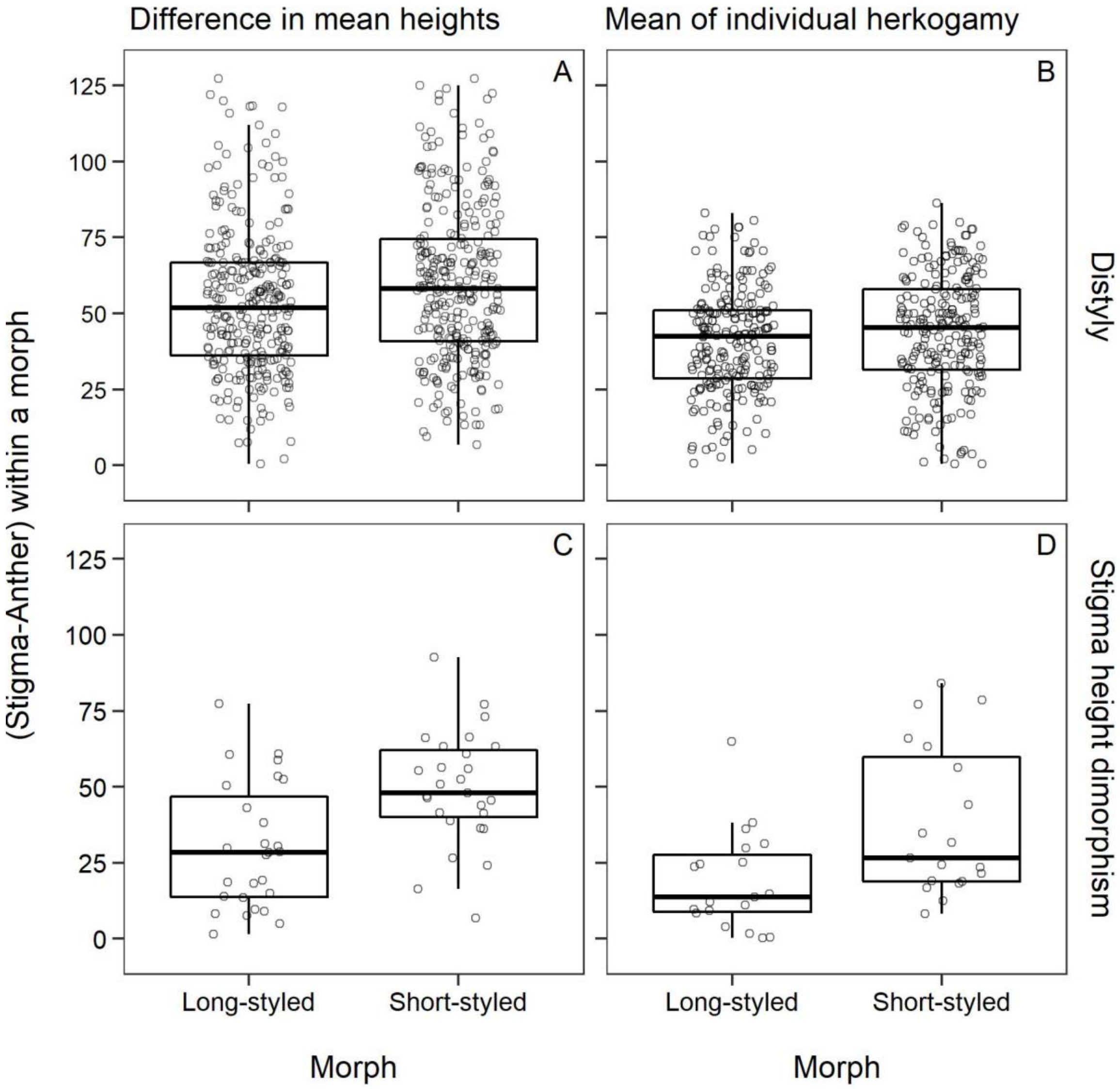

Figure 4. Difference in mean anther and stigma heights of a morph denoting the position of the lower level organ and mean of herkogamy of flowers across individuals standardized using the grand mean of all four mean sex organ heights in the long- and the short-styled morph for A & B) distyly and C & D) stigma-height dimorphism. 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.

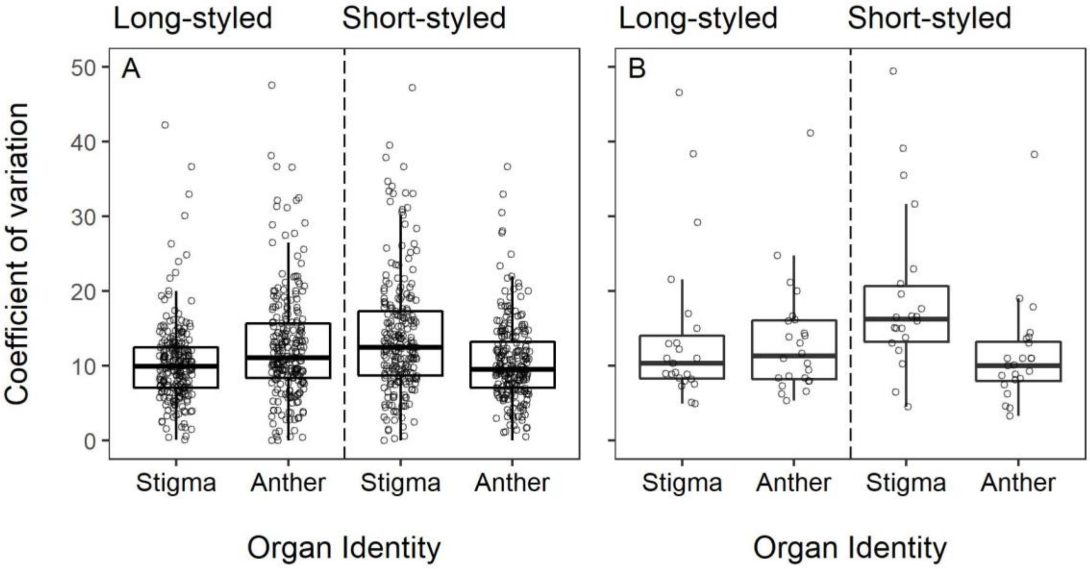

Figure 5: Coefficient of variation calculated for anther and stigma heights of both the morphs in A) distyly and B) stigma-height dimorphism. The bold line in the boxplots represents the median, the ends of the box represent the first and the third quartiles and the whiskers represent 1.5 times the interquartile range.

Chapter 3

Increased variation in sex organ positions across individuals can increase reciprocity and pollination success in heterostylous populations

Abstract

Deviation from a perfect spatial match reduces pollen transfer in heterostylous species. Reciprocity indices are used to quantify this match, but the commonly used indices fail to appropriately account for intra-population variation. In this study, we developed an index that explicitly considers intra-population variation in sexual organ positions. We used simulated populations and empirical data to understand how variation in sexual organ heights alters reciprocity in heterostylous plants. We used data from chapter 2 to determine anther and stigma positions to understand the biologically relevant ranges of mean and variation, to validate the relevance of our results. We also test the ability of the current index and other previously proposed indices to predict reproductive success in terms of fruit and seed set. When the difference in mean anther and stigma height of complementary morphs is zero or very small, increasing intra-population variation in sexual organ height resulted in a monotonous decrease in reciprocity. However, when differences in mean anther and stigma height are greater than zero, and not negligible, reciprocity initially increased with increasing variation, reached a peak, and then decreased. As the difference in mean height between the complementary anther and stigma increased, peak reciprocity is attained at higher values of intra-population variation. Comparisons with previous indices revealed important qualitative and quantitative differences in the ability to capture changes due to intra-population variation, and in determining the degree of reciprocity in heterostylous populations. The current index, along with two other previously proposed indices, showed a positive relationship with seed set. Challenging current understanding, these results suggest that increasing intra-population variation in organ heights can result in an increase in reciprocity in heterostylous populations. This might explain how heterostylous systems exhibit and tolerate high amounts of intra-population variation in organ heights. Such variation can facilitate the stabilisation and perpetuation of imperfectly reciprocal states that are in the process of evolving towards perfect heterostyly.

Introduction

The reciprocal arrangement of anthers and stigmas in complementary floral morphs is central to our understanding of the function and evolution of heterostyly (Darwin 1877, Lloyd and Webb 1992). Perfectly reciprocal positions ensure that pollen from anthers of a morph is deposited on the pollinator's body to match the position of the stigma in the complementary morph, and deviation from this reduces the precision of pollen transfer (Stone and Thomson 1994, Armbruster et al. 2009, Keller et al. 2012, Brys and Jacquemyn 2014, Liu et al. 2016). This spatial match between anthers and stigmas is commonly quantified using a reciprocity index and used as a proxy for pollen transfer and reproductive success in heterostylous populations (Richards and Koptur 1993, Eckert and Barrett 1994, Sanchez et al. 2008, Sosenski et al. 2010, Keller et al. 2012, Armbruster et al. 2017). The commonly used reciprocity indices incorporate mean anther and stigma positions but fail to appropriately account for intra-population variation in organ heights. In this study, we developed an index to estimate reciprocity that explicitly considers variation in sex organ positions among individuals.

Distylous species have individuals of long-styled (approach herkogamous), and a short-styled (reverse herkogamous) morph and individuals of each morph have flowers with sex organs at the higher and lower levels (Chapter 1 Fig. 1B). Legitimate pollen transfer takes place between anthers and stigmas of the complementary morphs at the same level. Increased differences in the heights of sex organs of the same level in complementary morphs of a population result in a reduction in reciprocity (Richards and Koptur 1993). In addition to differences in mean organ height, variation in heights across individuals in the population can also result in a loss of reciprocity. Such Intra-population variation in sex organ position has been widely reported and is likely common in heterostylous plants (Eckert and Barrett 1994, Pailler and Thompson 1997, Faivre and McDade 2001, Sanchez et al. 2008, Ferrero et al. 2009). The extent of such variation can be substantial, with some reports of coefficient of variation in sex organ heights that are greater than 50% (Wolff and Liede-Schumann 2007, Brys et al. 2008, Chen 2009, Machado et al. 2010). The intra-population variation will result in mismatches between complementary stigmas and anthers, a reduction in pollen transfer between morphs, and decreased reproductive success (Ferrero *et al.,* 2011; Keller *et al.,* 2012; Brys and Jacquemyn 2014; Haller *et al.,* 2014).

The earlier indices developed to quantify reciprocity focused on mean organ heights, and neglected intra-population variation (Richards and Koptur 1993). Eckert and Barrett (1994) developed a precision index based on the coefficient of variation in sex organ heights to account for intra-population variation, but provide no explanation regarding how the precision index in combination with the reciprocity index can be used to compare different populations or species. Later indices recognised the importance of intra-population variation in sex organ positions and attempted to incorporate this variation into estimates of reciprocity (Lau and Bosque 2003, Sanchez et al. 2008, Armbruster et al. 2017). However, the manner in which this was done differed, and the interpretation of these remained problematic.

A common problem with previous indices, including how they incorporate estimates of variation, was the lack of biological justification for most mathematical operations, and this limited interpretation of the estimates of reciprocity. The index proposed by Lau & Bosque calculates reciprocity as the percentage overlap between the frequency distributions of complementary anther and stigma heights of individuals, by categorising the heights into bins using a predetermined bin size. The estimates of reciprocity are very sensitive to the choice of bin sizes used for the frequency distributions of the sex organ heights. This choice of bin size is arbitrary and has no apparent biological significance. Similarly, the calculation of Sanchez's index requires multiplication of the spatial reciprocity estimates with the standard deviation in organ heights, but the biological rationale for this is not explained (Sanchez et al. 2008, Sánchez et al. 2013).

Higher intra-population variation in traits is often associated with decreased fitness due to deviation from an optimal trait value. However, higher intra-population variation can be beneficial for a population under certain circumstances (Crean and Marshall 2009), and this has also been documented for sex-organ positioning in plants (Dai et al. 2016). In heterostylous plants, for an individual with anthers of a particular height, the potential matches for successful pollen transfer are represented by the range of stigmas heights in individuals of the complementary morph in the population and are not just dependent on the mean or modal complementary stigma height. When the mean heights of the complementary sex organs are perfectly matched, increasing intra-population variation in sex organ height, will result in a decrease in reciprocity (chapter 1: Fig. 5). However, when there is some mismatch in mean complementary sex organ heights, lack of intra-population variation in sex organ heights will result in no overlap in complementary anthers and stigmas, and reciprocity will be zero. In such situations, with some increase intra-population variation in sex organ heights, we would expect to see an increase in reciprocity, as ultimately, the tallest stigmas will be overlap with the shortest anthers.

The reciprocity index developed in this study quantifies mismatch in vertical heights of complementary anthers and stigmas in distylous plant populations. For every combination of anther and complementary stigma, we assign a pollen transfer success value that is inversely related to mismatch. Thus, pollen transfer success equals one for a perfect match and decreases to zero with increasing mismatch between the stigma and anther. We quantify reciprocity as the average pollen transfer success for all pair-wise combinations of anthers and stigmas. This differs from previous indices in that we convert the spatial match in anther-stigma pairs to a pollen transfer success between sex organs, a measure that reciprocity inherently tries to capture.

We examined how the index developed in this study behaved with simulated plant populations that varied in mean complementary sex organ heights and intra-population variation in heights. Using these same simulated populations, we compared the current index to the previously proposed indices. As described earlier (chapter 2: Methods), we had extracted data on mean sex organ heights for 296 species and the standard deviation in sex organ heights for 231 species. We referred to this data set to understand the biologically relevant ranges of mean and intra-population variation in anther and stigma positions and used them in the simulations. We also compared the current and previous indices using extracted data on the distribution of anther and stigma heights of naturally occurring heterostylous populations. This data set was represented by 210 populations of 119 species from 14 families, which showed similar biologically relevant combinations of mean and intra-population variation in sex organ positions seen in the 296 species [Appendix: Table S2; Appendix references]. While reciprocity indices are commonly used to understand potential reproductive success in heterostylous plants, the relationship between proposed indices and measures of reproductive success have not been tested. To do this, we examined the relationship between the current and previous indices to reproductive success using data on fruit set (56 populations, 38 species) and seed set (34 populations, 27 species) in heterostylous plants.

Methods

Calculation of the Reciprocity index: To quantify reciprocity, we calculated the mismatch for every combination of complementary anther and stigma in the population. Mismatch, *MM* was defined as the modulus of the difference in vertical position of the anther and stigma, and this was calculated separately for the lower and higher organ levels.

$$MM_{ij} = \left| AHL_i - SHS_j \right| \text{ (Low level)}$$

$$MM_{ij} = \left| AHS_i - SHL_j \right| \text{ (High level)}$$

Where *AHL* is the anther height of the long-styled morph; *SHS* is the stigma height of the short-styled morph; *AHS* is the anther height of the short-styled morph; *SHL* is the stigma height of the long-styled morph for the *i*th and *j*th individual of the long-, or the short-styled morph.

We expected pollen transfer success to decrease with an increasing mismatch. Given the lack of empirical information regarding how the positioning of anthers and stigmas might translate to pollen transfer between complementary sex organs, we initially used a concave-shaped decreasing function to convert each value of mismatch to a pollen transfer success value, R_{ij}.

$$R_{ij} = e^{\left(-4 * \frac{MM_{ij}}{0.5 * anther\ length}\right)}$$

Where R_{ij} is pollen transfer success, and MM_{ij} the mismatch between the *i*th anther and *j*th stigma of complementary morphs. Values for R_{ij} ranged from one for a perfectly matched stigma-anther pair, to zero when the mismatch is greater than half of the anther length, i.e. the stigma no longer overlapped with the anther (Ferrero et al. 2011b). In a later section, we examined the consequences of relaxing these assumptions and using different decreasing functions.

The average pollen transfer success for all possible combinations of anthers and stigmas was calculated separately for both high and low-level for a population.

$$R_{level} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{m} R_{ij}}{n * m}$$

Here level refers to either high or low level of a distylous system and *n* and *m* are the numbers of individuals of the short-styled and long-styled morph, respectively.

Sex organ positions in natural heterostylous populations: We used the dataset compiled on sex organ heights in distylous species to understand (a) relative positions of the lower and higher-level sex organs; (b) differences in mean complementary sex organ positions; and, (c) intra-population variation in sex organ heights. This information was used to determine the relative position of sex organ heights in the simulated flowers and the range of sex organ heights and intra-population variation that we explored in our simulated populations. For the calculation of the indices using the distribution of anther and stigma heights in a population, we used studies that reported data for more than 10 individuals per morph. We considered data for populations independently, i.e. different populations of the same species were treated as independent data points for further analysis. When available, we also extracted information from these studies on anther length for the study species, and fruit set and/or seed set for the study populations.

Explorations with simulated populations: We used simulated populations of heterostylous plants to examine how our measure of reciprocity changed with mean sex organ heights and increasing intra-population variation. The relative positions for the higher-level anther and stigma in the simulated individuals were set at 100, and the low-level decided based on the empirical data obtained from the naturally occurring heterostylous populations. For the simulations, to ensure that the relative positions of the sex organs in the two levels stayed the same when we increased the difference in mean sex organ heights, we distributed the difference equally amongst the mean anther and stigma heights of the complementary sex organs at that level.

We created normal distributions of anther and stigma heights for 200 individuals with 100 individuals of each morph. To examine how sex organ height alters reciprocity, we estimated reciprocity across a range of intra-population variation in heights, and for a range of mean height differences between complementary sex organs. The lower and upper bounds of the range for both intra-population variation and height differences were determined from the empirical data obtained from the naturally occurring heterostylous populations. We assumed that the intra-population variation and difference in mean complementary sex organs heights were similar for both high and low levels. We used the average value of the estimated reciprocity from 100 simulation runs for each combination of the difference in mean sex organ heights, and intra-population variation examined.

Comparison of pollen transfer success functions: To understand if the results were robust to different pollen transfer success functions, we compared three decreasing functions: (a) convex; (b) linear; and (c) concave (Fig. 1). Additionally, we also examined a uniform function where the pollen transfer success was independent of mismatch (one when mismatch was less than half anther length and was zero when mismatch was greater than half anther length). These functions represent a gradient of increasing penalty with respect to pollen transfer success with increasing mismatch in the following order: uniform < convex < linear < concave.

Comparison of indices with simulated populations: Using the simulated populations, the current index was compared to four other commonly used reciprocity indices: a) R & K - Richards & Koptur (1993); b) L & B - Lau & Bosque (2003); c) SAN - Sanchez *et al.* (2013); and d) A - Armbruster *et al.* (2017). Richards & Koptur’s index was modified as suggested by Sanchez *et al.* (2008) to incorporate intra-population variation. We used a subset of difference in mean complementary sex organ heights of the simulated populations representing high and low values, to compare the indices.

Comparison of indices using empirical data: We used the individual level empirical data obtained from naturally occurring heterostylous populations to quantify and compare the estimates of reciprocity using the current and previous indices. The empirical data allowed us to compare the indices for biologically relevant combinations of sex organ heights and intra-population variation. The reciprocity for each population was calculated separately for the higher and lower sex organ level, except for the Sanchez *et al.* (2013) index that estimates reciprocity for the population as a whole. For all comparisons with the Sanchez *et al.* (2013) index, we use the average of both organ levels’ estimates from the other indices. We categorised the high and low level of each population based on the value of difference in mean complementary sex organ heights expressed as the percentage of the mean of high-level sex organs into four categories: 0% - 5%; 5% - 10%; 10% - 15%; and, 15% - 20%. For each category, we examined the change in reciprocity in the populations with an increase in intra-population variation in sex organ heights for the high and low level of all the indices separately. To maintain parity and compare with the normally distributed populations of the simulations, we checked for normality of the distribution of anther and stigma heights of the extracted data.

Do reciprocity indices predict reproductive success? Finally, we examined the relationship between estimates of reciprocity and reproductive success. Fruit and seed set data were used as a measure of reproductive success and were used as percentage values per flower, and per ovule per flower, respectively. Spearman’s rank correlation coefficients were used to examine the relationship between the reciprocity estimates from the current and previous indices, and fruit or seed set for the upper and lower sex-organ levels separately. For eight populations, fruit set for the short morph was zero, i.e. they were functionally dioecious, and these populations were excluded from the analysis.

Results

Sex organ positions in natural heterostylous populations: The relative position of the lower sex organ was at approximately 60% of the height of the high level. For the long-, and short-styled morphs this was 58.92% ± 15.62, and 54.45% ± 16.54 respectively (*n* = 296 species). The differences in mean complementary sex organ heights were similar for the higher and lower organ levels, at 9.14% ± 9.57 and 9.82% ± 10.70 (*n* = 296 species) respectively. These values are presented as a percentage of the average of mean organ heights for the higher level. Intra-population variation in sex organ heights was higher for the high-level sex organs and were 12.11 ± 10.13, and 12.15 ± 9.46, for stigma height in the long-styled morph, and anther height in the short-styled morph, respectively (*n* = 231 species). Intra-population variation in the lower level organs were 7.34 ± 5.15, and 7.76 ± 7.22 for stigma height short-styled morph and anther height in the long-styled morph respectively. Relative anther length was approximately 20% (18.21 ± 8.05, *n* = 89 populations/ 53 species) of the mean height of the higher-level organs.

We fixed the lower sex organ position at 60% of the higher sex organ positions in our simulated flowers based on the empirical ranges of organ positions. Similarly, we decided the range of intra-population variation in sex organ heights and height difference in mean complementary sex organs to explore as 0-24, and 0-22, respectively.

Reciprocity as a function of intra-population variation and difference in mean sex organ heights: For the higher organ level, when the difference between mean complementary anther and stigma was low, the index showed a monotonous decrease in reciprocity with an increase in intra-population variation (Fig. 2 inset). However, when the difference in mean sex organ heights was three or greater, reciprocity was initially low or zero at low intra-population variation, but increased as intra-population variation increased, reached a peak and then decreased (Fig. 2). The intra-population variation at which peak reciprocity was observed was higher for higher values of difference in mean organ heights. Reciprocity for a given value of intra-population variation decreased with an increase in the difference in mean height of reciprocal sex organs, and this decrease is non-linear. Hence, as the difference in mean heights of sex organs increased the difference in reciprocity between them decreased. The results for the lower sex organ level is mathematically identical. The peak value of the index is observed at values within the range of difference in mean sex organ heights and intra-population variation as reported in naturally occurring heterostylous plant populations.

Comparison of pollen transfer success functions: The results for how reciprocity changed with intra-population variation in sex organ heights and difference in mean complementary sex organ heights were similar for the other pollen transfer success functions examined (Fig. 3). The absolute values of reciprocity differed and were highest for the uniform function and lowest for the concave function.

Comparison of indices with simulated populations: We observed stark differences in the values of reciprocity obtained from the different indices, and in how these changed with intra-population variation in sex organ heights, and the difference in mean complementary sex organs (Fig. 4). When the difference in mean complementary sex organ heights is high, we expect reciprocity to be low. Additionally, increasing intra-population variation should result in an increase in reciprocity (refer to Chapter 1: Introduction). Both of the above were seen for the current index and for Lau and Bosque’s index (Fig. 4B), but not the other three indices. The current index differed from Lau and Bosque’s index in that it showed a unimodal peak in reciprocity. For the Lau and Bosque’s index, reciprocity continued to increase with increasing intra-population variation and saturates at a particular value of intra-population variation (Fig. 4 B).

The reciprocity indices proposed by Sanchez, Armbruster and the modified Richards & Koptur's index always decreased monotonously with increasing intra-population variation (Fig. 4 A, C & D). The index proposed by Sanchez *et al.* (2008,2013) reaches negative values at very high values of difference in mean organ heights and intra-population variation (Fig. 4 C). The modified Richards & Koptur's index is very insensitive to changes in both intra-population variation and difference in mean sex organ heights, and the values indicate high reciprocity throughout (Fig. 4 A).

<u>Comparison of indices using empirical data</u>: Shapiro-Wilk's test for normality showed that out of the 236 populations, 194 and 189 populations for anther and stigma height respectively of the short-styled morph while 198 and 203 populations for anther and stigma height respectively of the long-styled morph were normally distributed. The reciprocity indices behaved in the same way as in the simulated populations across high and low values of difference in mean complementary sex organ heights (Appendix Table S2). The current index showed a monotonous decline in reciprocity with an increase in intra-population variation when the difference in mean complementary sex organ height is low (Fig. 5 Mdiff = 0-5). But, when the difference in mean complementary sex organ height is high (Fig. 5 Mdiff > 5) reciprocity increases with an increase in intra-population variation reaches a peak and then decreases. The indices proposed by Richards & Koptur (1993) modified to include intra-population variation and the index proposed by Sanchez, Ferrero, & Navarro (2008, 2013) always decrease with an increase in intra-population variation (Fig. 5). As seen in the simulations, the index proposed by Sanchez, Ferrero, & Navarro (2008, 2013) shows high values of reciprocity even when difference in mean complementary sex organs is high (above 10%), and intra-population variation is negligible denoting no overlap between the distribution of anther and stigma heights of the complementary morphs. Lau & Bosque (2003) decreases with an increase in intra-population variation when the difference in mean complementary sex organ heights is low (Fig. 5). When the difference in mean complementary sex organ heights is high, it increases with an increase in intra-population variation. Armbruster et al. (2017) shows very little difference in reciprocity with an increase in intra-population variation in all the categories of difference in mean complementary sex organ heights (Fig. 5).

Do reciprocity indices predict reproductive success? Data were extracted for fruit set from 56 populations (38 species) and for seed set from 34 populations (27 species). None of the indices showed a significant correlation with fruit set in both the levels (Table 1). Significant positive correlations were seen between index values and seed set for the current index, Sanchez et al.'s index and the Armbruster et al.'s index in both the levels (Table 1). Modified Richards & Koptur's index showed a significant positive correlation for seed set in the higher level only.

Discussion

The results from the simulated populations demonstrated that when the difference in mean anther and stigma height of complementary morphs is zero or very small, increasing intra-population variation in sex organ heights resulted in a monotonous decrease in reciprocity as quantified using the index developed in this study. However, when the difference in mean anther and stigma height is greater than zero, and not negligible, reciprocity initially increased with increasing variation, reached a peak and then decreased. These changes in reciprocity as a function of changing intra-population variation and difference in mean sex organ heights are not captured by other indices. Additionally, a comparison of the current index with previous indices using empirical individual-level data revealed important qualitative and quantitative differences in the estimates of reciprocity in naturally occurring heterostylous populations. These results challenge our current understanding that increasing variation in sex organ heights should always result in a decrease in reciprocity.

The primary use of reciprocity indices has been for understanding the functional consequences of variation in sex organ position for efficient legitimate pollen transfer between complementary morphs of heterostylous species (Pailler and Thompson 1997, Faivre and McDade 2001, Ferrero et al. 2009, Consolaro et al. 2011). These indices have been utilized for studying plant-pollinator interactions, pollen flow, fruit set, and the general reproductive biology of heterostylous plant populations (Brys et al. 2008, Ferrero et al. 2011a, Santos-Gally et al. 2013). The use of reciprocity indices in such studies has been applied to the management and conservation of species (Meeus et al. 2011, Casazza et al. 2013, Aronne et al. 2014, Chen et al. 2014). Reciprocity indices have contributed significantly to our understanding of the evolution of sexual systems and floral polymorphisms (Sosenski et al. 2010, Baena-Díaz et al. 2012, Yuan et al. 2017). Measures of reciprocity between species have been used to assess the potential for hybridization, and conversely the reproductive isolation between species (Zhu et al. 2009, Keller et al. 2012, 2016, Huang 2015). Finally, quantitative estimates of sex organ positions and measures of reciprocity have also been used to define the floral polymorphic status of populations (Dulberger 1973, Richards and Koptur 1993, Baker 2000), and as a taxonomic tool (Eiten 1963, Selvi 1998, Esteves and Vicentini 2013). Thus, reciprocity indices that quantitatively estimate this important morphological adaptation of complementary spatial positioning of anthers and stigmas are widely used in studying heterostylous plants.

We found in chapter 2 (results section) that intra-population variation in sex organ heights and differences in mean heights between complementary sex organs can often be substantial in heterostylous populations. Thus, it is important to understand how combinations of intra-population variation and difference in mean sex organ heights might alter reciprocity. The commonly used reciprocity indices, Richard and Koptur's and Sanchez's indices, and an index recently proposed by Armbruster failed to capture the loss of reciprocity that should result from increased differences in mean sex organ heights. This was captured by Lau and Bosque's index and the current index proposed in this study, as was the expected increase in reciprocity with increasing intra-population variation at high difference in mean sex organ heights of complementary morphs. However, counter-intuitively, Lau and Bosque's index continued increasing with increasing intra-population variation and saturated at some value of intra-population variation, whereas the current index reached a maximum reciprocity and then decreased. This would be expected as with high intra-population variation overlap between complementary sex organs is surpassed by mismatches.

How mismatches ultimately translate to the amount of pollen transferred between complementary sex organs will depend on pick-up from the anthers, deposition on the pollinator body, redistribution during flight, and ultimately deposition onto the stigma, but empirical evidence for these are very limited. Studies have shown that spatial distribution of pollen on the body, or proboscis of long-tongued pollinators (Levin and Berube 1972, Courtney et al. 1982, Washitan et al. 1994, Harder and Wilson 1998, Keller et al. 2014) can be specific for pollen from different morphs and therefore likely important for the reciprocal transfer to complementary sex organs. However, these and other studies also show substantial variability in the precision of pick up and deposition of pollen, and redistribution on the pollinator body during flight post pick-up, and this weakens the case for effective, legitimate pollen transfer in heterostylous species. Given this lack of empirical evidence, we assumed a simple concave decreasing function for converting values of mismatch to a pollen transfer probability. We showed that relaxing this assumption, and testing other decreasing functions did not change the important qualitative nature of our results. However, this highlights the urgent need to better understand patterns of pollen pick-up and deposition with respect to spatial distribution on the pollinator.

As would be expected from the disparate behaviour of the different indices in the results from the simulated populations, we found the reciprocity indices yielded very different measures for naturally occurring plant populations. With the exception of Lau and Bosque (2003), the other indices consistently overestimated reciprocity in natural plant populations in comparison to the current index. This is likely because the other indices were not sensitive to decreases in reciprocity that should result from increased differences in mean sex organ heights. As such, the current index was more sensitive and better at discriminating between species.

The current index and the indices proposed by Sanchez et al. (2008) and Armbruster *et al.* (2017) showed a significant positive correlation with seed set values of both the levels. This is an important result of this study, and it explains the functional implications of reciprocity indices. As mentioned before, reciprocity indices are proposed to predict the efficiency of legitimate pollen transfer and consequently, fitness. This study shows that reciprocity indices can predict the fitness of a population in terms of seed set, as demonstrated by the analysis using the current index. Nonetheless, it is also important to understand that fruit set and seed set are influenced by a myriad of factors like pollen limitation, resource limitation, resource allocation, self-incompatibility etc. (Charlesworth 1989) This is perhaps the reason we find no significant relationship between index values and fruit set.

The reciprocity index proposed here exhibited markedly different quantitative and qualitative behaviour from previous indices as a response to increasing intra-population variation in sex organ heights and difference in mean complementary sex organ heights. Challenging current understanding, the results using this reciprocity index suggest that increasing intra-population variation in sex organ heights can result in an increase in reciprocity in heterostylous populations. This might explain how heterostylous systems exhibit, and tolerate high amounts of intra-population variation in sex organ heights. Such variation can facilitate the stabilisation and perpetuation of imperfectly reciprocal states that are in the process of evolving towards perfect heterostyly.

Table 1. Spearman's correlation coefficients for relationships between reciprocity index values and measures of reproductive success (seed set and fruit set). High and low refer to the fruit set and seed set for the higher and lower sex-organ levels, respectively. Sample size: n = 56 populations for long-styled and n = 47 populations for short-styled morphs for fruit set; and n = 34 populations for seed set. Significant correlations at $p < 0.05$ are denoted in bold and marked with *.

	Seed set		**Fruit set**	
Reciprocity index	**High**	**Low**	**High**	**Low**
Current index	**0.40****	**0.34***	-0.06	-0.08
Richards & Koptur (1994)	**0.39***	0.21	-0.20	0.02
Sanchez *et al.* (2013)	**0.35***	**0.35***	-0.06	-0.05
Lau & Bosque (2003)	0.07	0.14	-0.01	-0.03
Armbruster *et al.* (2017)	**0.58***	**0.65***	-0.07	-0.10

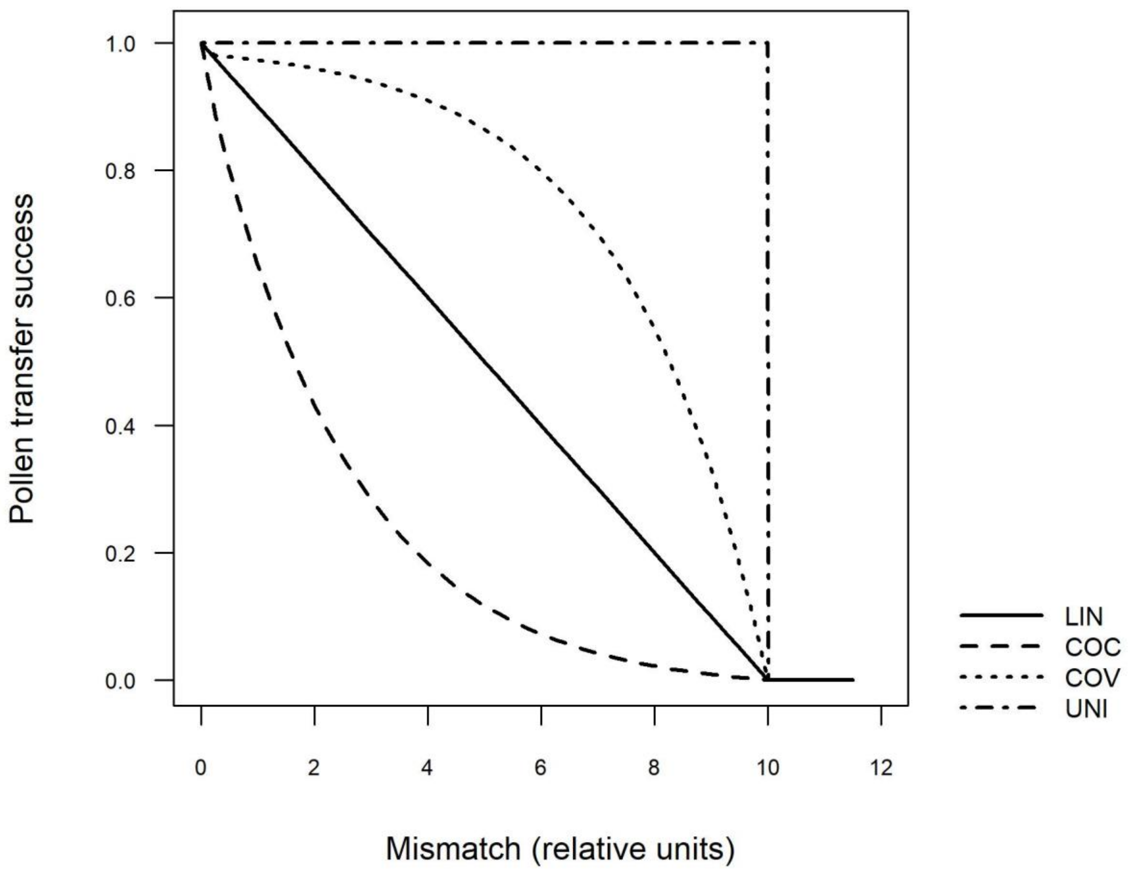

Figure 1: The different pollen transfer functions examined: COC - concave decreasing; LIN - linear decreasing; COV - Convex decreasing; and, UNI - Uniform step function. This function allowed mismatch between complementary organ heights to be converted to pollen transfer success estimates. We assumed that there was a critical value of mismatch beyond which there was no pollen transfer for all functions examined.

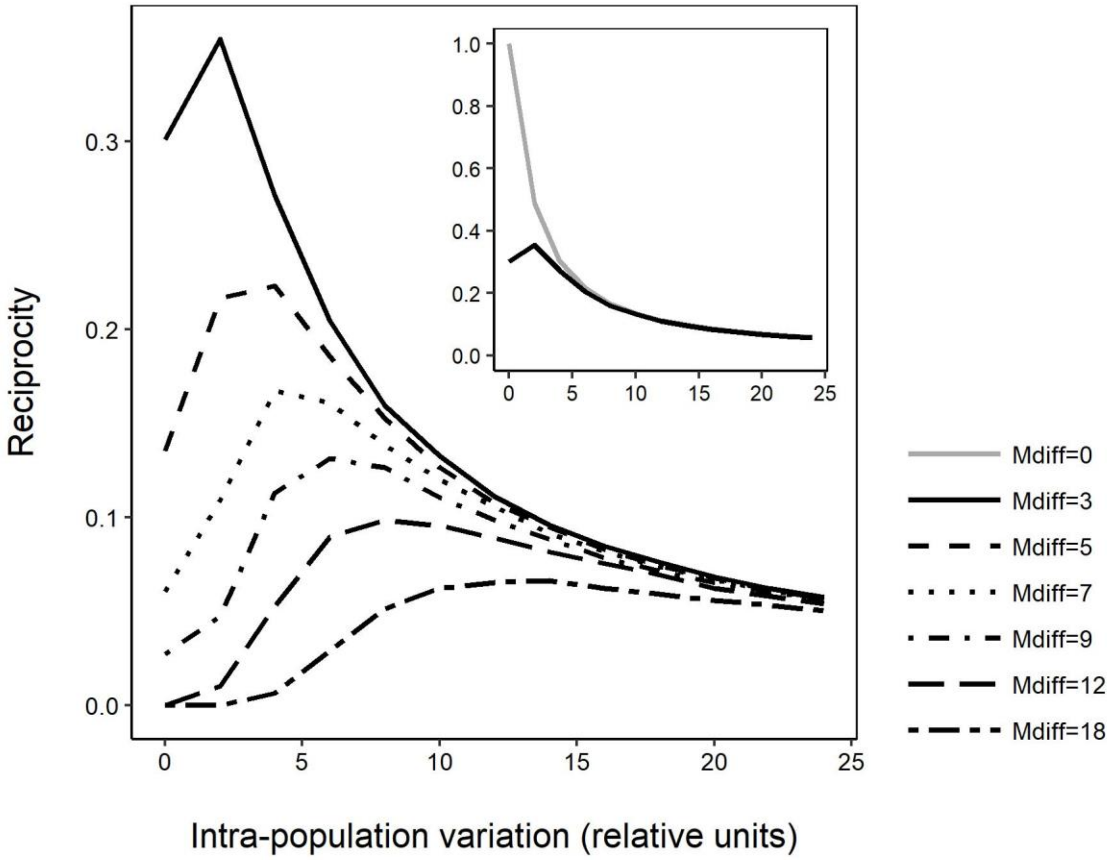

Figure 2: Reciprocity estimates for the simulated populations as a function of increasing intra-population variation in anther and stigma heights, for a range of values for mean difference in sex organ heights (Mdiff; shown by the different curves). The inset shows reciprocity estimates for lower values of mean difference in sex organ heights (Mdiff = 0, Mdiff = 3)

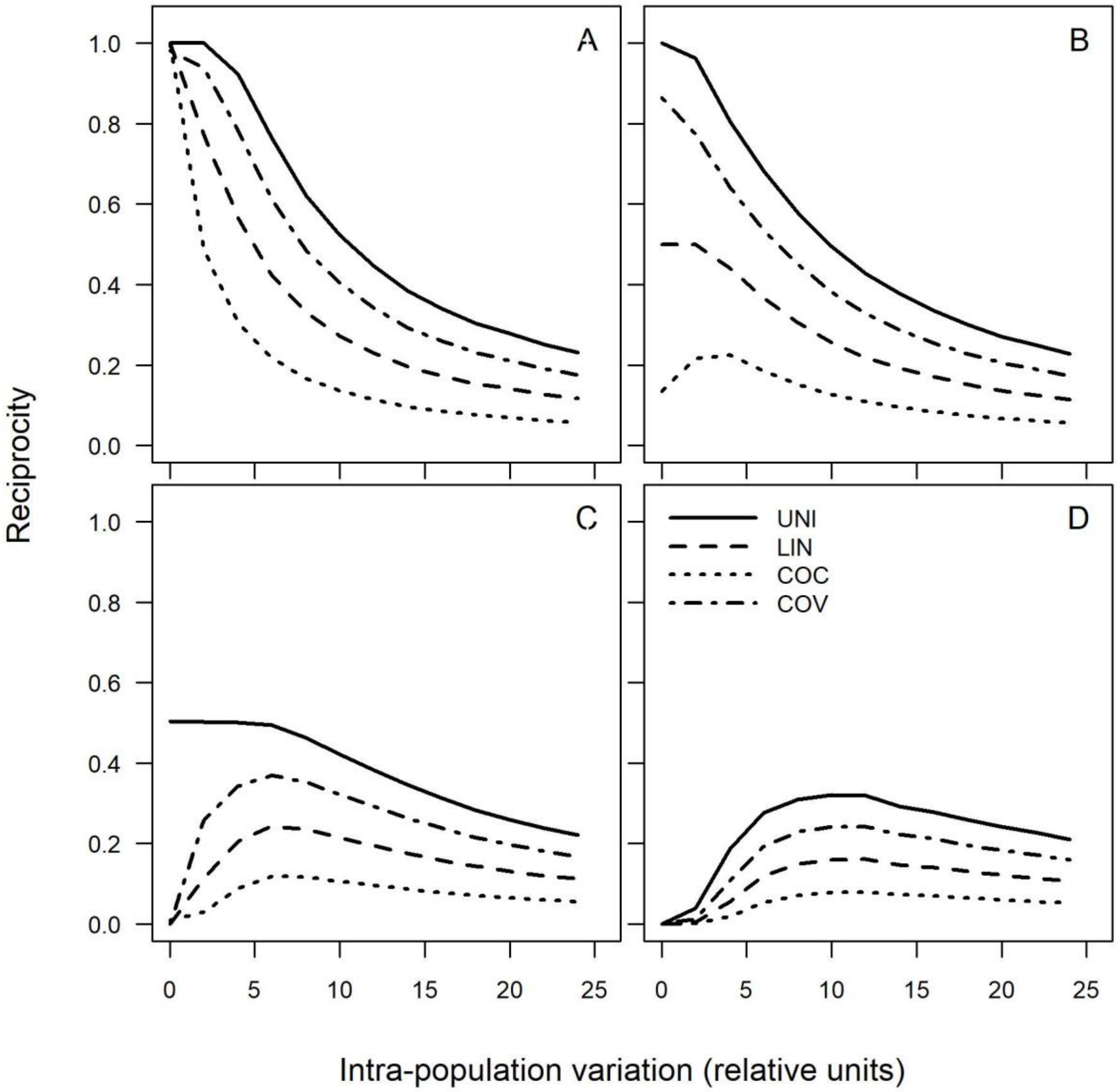

Figure 3: Comparison of the pollen transfer probability functions. Reciprocity estimates as a function of increasing intra-population variation in anther and stigma heights for the simulated populations. The panels represent increasing mean difference in sex organ heights (Mdiff): A) Mdiff = 0: B) Mdiff = 5; b) Mdiff = 10; b) Mdiff = 15. The four decreasing the pollen transfer probability functions examined: COC - concave decreasing; LIN - linear decreasing; COV - convex decreasing; and, UNI - Uniform step function.

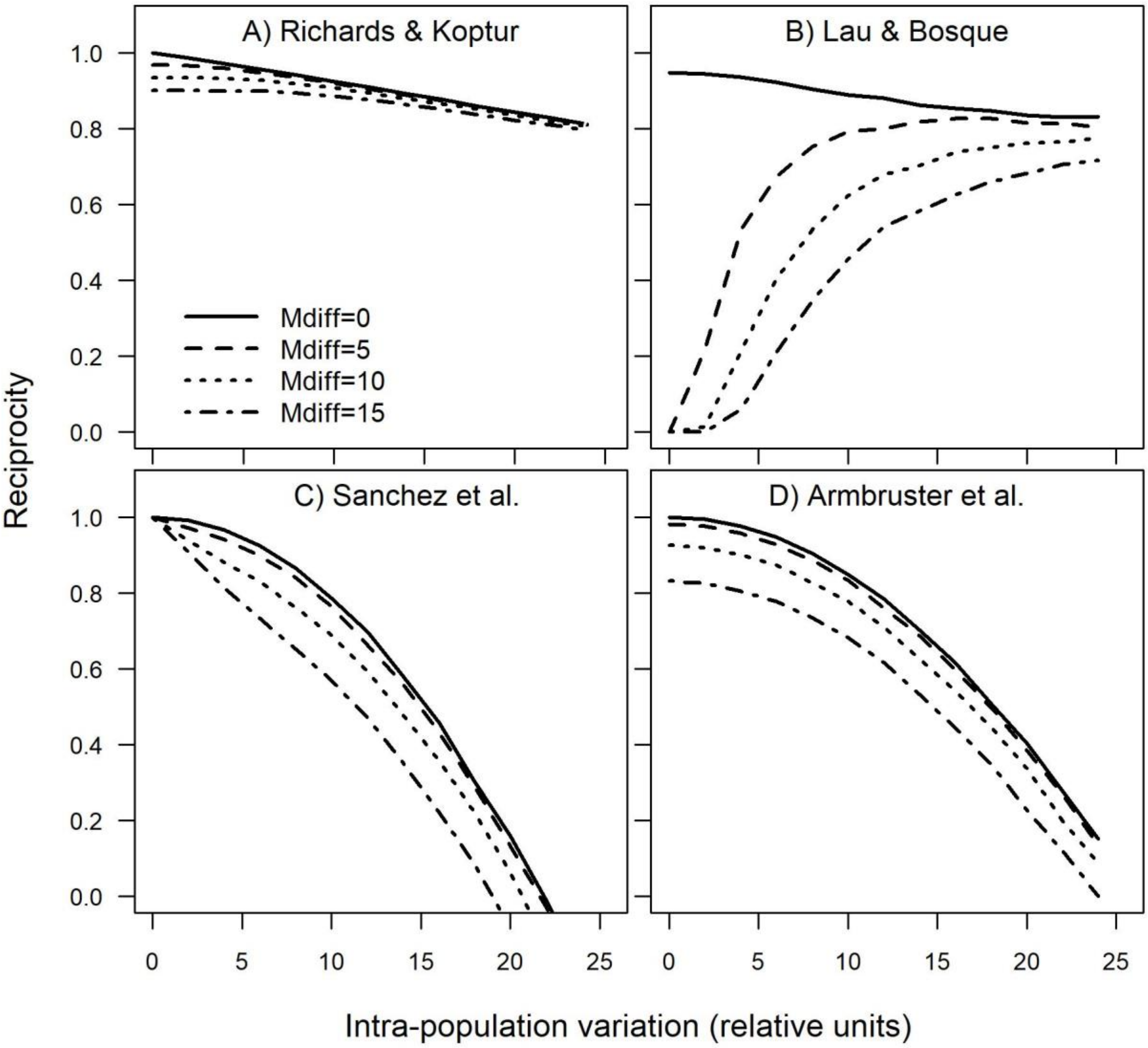

Figure 4: Reciprocity estimates for the simulated populations as a function of increasing intra-population variation in anther and stigma heights, for a range of values for mean difference in sex organ heights (Mdiff; shown by the different curves) for indices proposed by: A) Richards & Koptur modified (as in Sanchez et al. (2008)); B) Lau & Bosque (2003); C) Sanchez et al. (2013); and, D) Armbruster et al. (2017). Armruster et al. (2017)'s index values do not have a lower and upper bound unlike other indices and have been scaled with the highest value seen in the data set to compare them to other indices.

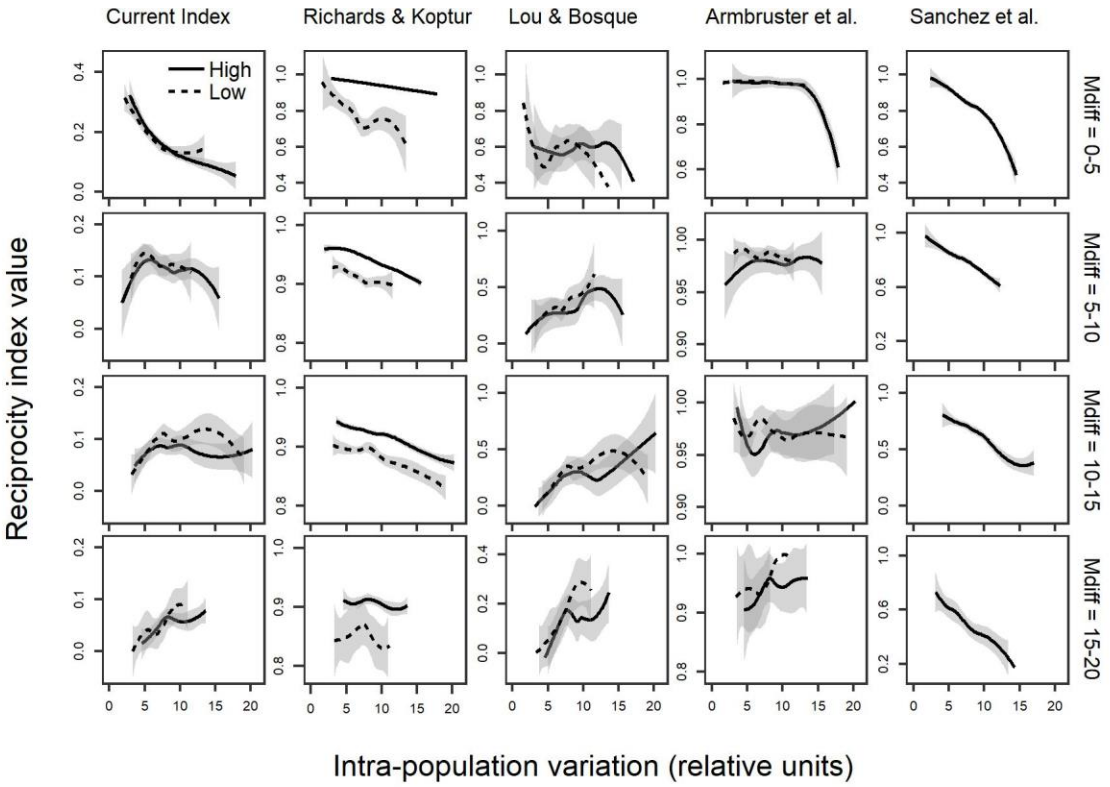

Figure 5: Comparison of the current index with previous indices over a range of intra-population variation using naturally occurring heterostylous populations. Each row in this graph denotes a range of difference in mean complementary sex organ height - Mdiff = 0-5: difference in mean sex organ heights = 0-5; Mdiff = 5-10: difference in mean sex organ heights = 5-10; Mdiff = 10-15: difference in mean sex organ heights = 10-15 and Mdiff = 15-20: difference in mean sex organ heights = 15-20. Each column represents the value of a particular index. The solid and dotted lines represent high and low levels, respectively. The index proposed by Sanchez et al. (2008) is a composite value for the two levels, and hence only one solid line representing the value is shown for the index.

Chapter 4

The reproductive ecology of *Jasminum malabaricum* Wight.: a species with stigma-height dimorphism

Abstract

Studies of floral polymorphisms have focused on heterostyly, while stigma-height dimorphism has received considerably less attention. Species with stigma-height dimorphism exhibit herkogamy within flowers, but do not have perfect reciprocity in anther and stigma positions between morphs, and offer an excellent system to examine the relative importance of these two important traits. In this study, we examined variation in floral morphology, spatiotemporal mate availability, pollinator visitation and breeding system between morphs, and among populations of *Jasminum malabaricum*, a species with putative stigma-height dimorphism. The positions of anthers and stigmas were characteristic of stigma-height dimorphism, the first report of this floral polymorphism in this genus. All populations had high herkogamy but low reciprocity, and while herkogamy did not differ among populations, reciprocity decreased from the peripheral to interior populations. Consistent with expectations of higher self-pollen deposition on stigmas, herkogamy was higher in the short-styled morph. Reciprocity was higher between the long-styled stigma and short-styled anthers. Morphs differed in most floral traits, and exhibited pollen size dimorphism, which is rare in these species. We observed no differences in spatiotemporal mate availability or pollinator visitation between morphs, and both morphs did not exhibit physiological incompatibility. All study populations were isoplethic, implying equal fitness for both morphs. Higher herkogamy in *J. malabaricum*, likely results in lower self-pollen deposition and compensates for the lower reciprocity observed in short-styled morphs. These results emphasize the importance of herkogamy as compared to reciprocity in species with stigma-height dimorphism.

Introduction

Floral polymorphisms have been extensively studied since Darwin (Darwin 1877, Cohen 2010), but the primary focus has been heterostyly, a style length polymorphism characterised by the reciprocal positioning of stigma and anther in complementary floral morphs (Barrett and Shore 2008). Stigma-height dimorphism, another form of style length polymorphism, is distinct from heterostyly in that the morphs differ in stigma, but not anther heights (Barrett 1992, Baker et al. 2000a, 2000b). This arrangement results in an imperfect reciprocal positioning of anthers and stigmas in complementary morphs, which likely reduces pollen transfer efficiency and reproductive success (Baker et al. 2000a). Therefore, this polymorphism is assumed to be unstable and transient in nature, and an intermediate step in the pathway to the evolution of perfectly reciprocal heterostyly (Charlesworth and Charlesworth 1979, Lloyd and Webb 1992). Stigma-height dimorphism has received significantly less attention and has been studied in only around 28 species (Baker et al. 2000a, 2000b, Thompson et al. 2003, Li et al. 2010, Liu et al. 2012, Perez-Barrales et al. 2014).

Two key floral traits seen in species with style length polymorphism are the separation of anthers and stigmas within a flower and the reciprocal positioning of anthers and stigmas in complementary morphs. Herkogamy, the separation of anthers and stigmas within a flower, reduce autogamous pollination and lowers physical interference between the male and female sex organs (Webb and Lloyd 1986, Kulbaba and Worley 2012). The reciprocal spatial positions of anthers and stigmas in complementary morphs increases the inter-morph or legitimate pollen transfer. This reciprocity results in pollen from anthers of a morph being picked up on parts of the pollinator body from where it can be accurately deposited on the stigma of flowers of the complimentary morph (Barrett and Shore 2008). Species with stigma-height dimorphism exhibit herkogamy within flowers, but do not have perfect reciprocity in anther and stigma positions between morphs, and thus, offer an excellent system to test the relative importance of herkogamy and reciprocity.

Preliminary examinations indicated that *Jasminum malabaricum* from the Western Ghats Range in peninsular India exhibited style length polymorphism in the form of stigma-height dimorphism. A primary objective of this study was to quantify sex organ positions in multiple individuals from multiple populations to confirm stigma-height dimorphism in this species. It is recognized that variation in anther and stigma heights can be substantial and that such variation has important functional consequences (Ferrero et al. 2011, Armbruster et al. 2017). Inferences based on restricted sampling and on mean morphologies may lead to incorrect conclusions and may overlook meaningful variation in this trait (Eckert and Barrett 1994). It is, therefore, important to examine multiple individuals and populations to infer the type of polymorphism present.

In species with style length polymorphism, the likelihood of self-pollination is thought to be higher in flowers of the short-styled morph with reverse herkogamy. Here, while probing flowers, pollinators encounter the longer anthers at the higher level before the stigma at the lower level. This will likely result in the deposition of self-pollen on the stigma (Webb and Lloyd 1986, Luo and Widmer 2013). On the other hand, total pollen deposition is reported to be greater for the long-styled morph with approach herkogamy (Barrett and Shore 2008, Liu et al. 2016, Jacquemyn et al. 2018, Wu et al. 2018). While autogamous pollination may not be a problem for the long-styled morph, selection for high reciprocity may be relaxed in the high level due to the high absolute number of pollen deposited on the long-styled stigma. Thus, different selection pressures may be important for determining anther and stigma positions in the morphs. Herkogamy is likely to be important in the short-styled morph to minimize self-pollination but may not be as important in the long-styled morph where the relative importance of reciprocity may be greater. Such differences in herkogamy and reciprocity can result in differences in pollen transfer and reproductive success between morphs (Kálmán et al. 2007).

Besides herkogamy and reciprocity, spatiotemporal mate availability can influence reproductive success in species with style length polymorphisms (Kitamoto et al. 2006, 2008, Stehlik et al. 2006, Brys and Jacquemyn 2010). The morph ratio of a population determines the overall availability of mates (Endels et al. 2002, Stehlik et al. 2006, Brys et al. 2007, Meeus et al. 2011). At a smaller spatial scale, the chances of legitimate pollen transfer increases when the nearest neighbour is of the complementary morph (Ishihama et al. 2003, Faife-Cabrera et al. 2015). The temporal availability of flowers of the different morphs over the season, and during the day will also affect mate availability (Okayama et al. 2003, Elzinga et al. 2007). Synchronous flowering between morphs can increase legitimate pollen transfer between morphs and result in greater reproductive success (Hirao and Kudo 2008, Faria and Araujo 2016).

Pollinator visitation and effective pollination will also influence pollen transfer and reproductive success of species with style length polymorphism. Long-tongued pollinators that can reach the short stigma and anther can effectively pollinate both morphs (Simon-Porcar et al. 2014). However, short-tongued pollinators may not be able to effectively pollinate the short-styled morphs, and this may result in an asymmetry in fitness between morphs (Santos-Gally et al. 2013). The abundance of effective pollinators can vary across species distributions, particularly at the range extremes where pollinator abundances may be lower (Pérez-Barrales et al. 2009). The lack of effective pollinators can result in differential fitness between the morphs, lead to changes in the distribution of stigma and anther heights in the population, and in extreme cases leads to the loss of a morph from the population (Pérez-Barrales and Arroyo 2010).

Although physiological incompatibility is considered to be a defining feature of species with heterostyly (Barrett and Shore 2008), an increasing number of studies have shown that many heterostylous species do not possess this trait (Ferrero et al. 2012). In contrast, for species with stigma-height dimorphism, the absence of physiological incompatibility is more common (Simon-Porcar et al. 2014). Given the lack of physiological incompatibility in species with stigma-height dimorphism, herkogamy might play an important role in avoiding self-pollination. Additionally, differences in the degree of self-incompatibility between the morphs can cause disparate reproductive successes (Manicacci and Barrett 1996).

The genus *Jasminum* belonging to Oleaceae has approximately 200 species with a tropical distribution (Green and Miller 2009). India harbours around 47 species, of which 16 are endemic (Jeyarani et al. 2018). Style length polymorphism has been reported in this genus with most of these documented as distyly (Ganders 1979, Green 1991, 1997, Thompson and Dommee 2000, Olesen et al. 2003, Naiki 2012). Stigma-height dimorphism has not been reported in this genus.

In this study, floral dimensions were quantified in multiple individuals from four populations of *J. malabaricum* Wight. The populations included one population in the northern limits of the species and others with relatively more interior locations. The two characteristic traits of species with style length polymorphism, i.e. herkogamy and reciprocity, were estimated to examine the difference between morphs and populations. In one focal population additional examinations were carried out that included: 1) examination of pollen dimorphism as an associated ancillary feature, and pollen-ovule ratios; 2) Quantification of morph ratio, the nearest neighbour morph identity, flowering and fruiting phenology, anthesis and longevity of flowers to understand spatiotemporal mate availability; and, 3) pollinator visitation observations and 4) the determination of the breeding system.

Methods

Study site: The study was conducted between 2015 and 2017 in four populations of *J. malabaricum* in the state of Maharashtra (India) in the northern extremes of the Western Ghats Range in peninsular India (Fig. 1 A and B). The four populations were located in Trimbak (19.9374° N, 73.5364° E), Bhimashankar Wildlife Reserve (19.0732° N, 73.5535° E), Mulshi (18.5011° N, 73.5138° E) and Kaas (17.7150° N, 73.8125° E) (Fig. 1 A and B). The Trimbak population is located at the northern limits of the distribution of this species with the other populations being more interior (Singh and Karthikeyan 2000).

Study species: *J. malabaricum* Wight. (Fig. 2A) is a woody liana endemic to the moist deciduous forests of the Western Ghats of India and Sri Lanka (Singh and Karthikeyan 2000). It is found in open habitats as a woody climber on other trees and shrubs. The sweet-smelling white flowers are borne in an open cymose-paniculate inflorescence (Green 2003). The ovoid fruits which are approximately 1 cm long and 0.6 cm wide (Singh and Karthikeyan 2000) are green when immature and purplish-black when ripe.

Floral morphometry: One flower from 30 plants of each morph was collected between March and April of 2015 and 2017, and preserved in formalin acetic alcohol (FAA; 2.5%:2.5%:95%) for later analysis. In Trimbak, because of the small population size, one flower was collected from 19 long- and 20 short-styled individuals. The diameter of the flower (free corolla lobes) was measured using a digital vernier callipers (Mitutoyo Absolute digimatic). Corolla tube length, anther height and stigma height were quantified using ImageJ (ver. 1.52a) from the scanned images of dissected flowers (Fig. 2B). Since anthers were attached to the corolla tube, anther height was measured as the distance from the base of the corolla tube to the mid-point of the anthers. Stigma height was measured from the base of the ovary to the mid-point of the stigma. Herkogamy was calculated as the mean of anther-stigma separation of all individuals of each morph for all the populations. To understand reciprocity, the mean mismatch between all combinations of anther and stigma heights of the complementary morphs was calculated for each population (Sanchez et al. 2008). This estimate of average mismatch in complementary organ positions is inversely proportional to reciprocity. Factorial two-way ANOVA with morph (two levels: long-styled and short-styled) and population (four levels: Trimbak, Bhimashankar, Mulshi and Kaas) as fixed factors was performed to examine variation in morphological traits.

Pollen size dimorphism and the pollen-ovule ratio: Mature buds were collected from 29 individuals of each morph from the Bhimashankar population and preserved in FAA (2.5%:2.5%:95%). Pollen was extracted from one bud per individual, suspended in 1:1 lactic acid and glycerol, and the total number estimated using a haemocytometer (Kearns and Inouye 1993, Webb 1994). To quantify pollen size, the extracted pollen were washed with 70% ethyl alcohol and mounted in lactic acid and glycerol (Kearns and Inouye 1993). The equatorial diameter of 30 pollen per individual was quantified using 400x magnified images and ImageJ (ver. 1.52a). A mixed model nested ANOVA with morph (two levels: long-styled and short-styled) as a fixed factor and individuals (29 levels: 1 to 29 individuals) as a random factor nested within morphs was conducted to examine pollen size differences between morphs.

Spatial mate availability: In all populations, morph identity, and GPS coordinates were recorded of all individuals within 10 m on either side of a pre-determined path (Fig. 1C to F). At least 60 individuals were sampled in each site except in Trimbak due to unavailability of sufficient individuals. The population level mate availability was quantified as the morph ratio. A chi-square test was performed to test if the estimated morph ratio was significantly different from1:1. To understand local mate availability, the cartesian coordinates of the individuals' positions obtained from latitude and longitude information was used to determine the morph identity of the nearest neighbour. Further, a segregation index (Levin 1974) was used to quantify the spatial affinity between morphs. The index values range from -1, indicating high affinity between the morphs, to zero indicating a random distribution of morphs, to +1 indicating that the morphs are segregated in space. A chi-square test was performed to establish if the observed number of long-long, long-short, short-short and short-long pairs of neighbours is significantly different from 1:1:1:1.

Temporal mate availability: Flowering phenology of the two morphs was examined to understand temporal mate availability at a seasonal scale, and anthesis and floral longevity determined to understand temporal mate availability at a diurnal scale. Fruiting and flowering phenology were recorded as flower or fruit counts for individuals. When flowers or fruits were less than 100, the total number was counted. When flowers or fruits were greater than 100, they were counted for a quarter of the canopy and multiplied by four to estimate the total number in the individual. Observations were conducted fortnightly during the flowering and fruiting season, from February to July, in 2016 for 50 individuals of each morph in the Bhimashankar population. To control for differences in flowering and fruiting resulting from size-related differences between individuals, the flowering and fruiting counts were normalized by the maximum count observed for each individual. To determine anthesis and floral longevity, ten buds in fifteen individuals of each morph were tagged and observed four times a day at 0600-0800 hrs,1000-1200 hrs, 1400-1600 hrs and 1830-2030 hrs. The individuals were followed until all the flowers looked dry and were regarded as unreceptive on visual inspection. A Mann-Whitney U test was performed to compare the floral longevity of the long and the short-styled morphs as the data were not distributed normally.

Flower visitation observation: To identify the major potential pollinators for each morph, diurnal and nocturnal flower visitation surveys were conducted from 18th to 20th March and 22nd to 25th March 2016 on individuals from Bhimashankar. For diurnal visitors, surveys were conducted on six individuals of each morph in the following three time periods: 0830-1100 hrs, 1300-1530 hrs, 1530-1800 hrs. For nocturnal visitors, surveys were conducted on three individuals of each morph in the following time periods: 1900-2030 hrs, 0000-0130 hrs and 0500-0630 hrs. This resulted in a total of 36 hours of observation. Most bees and a few butterflies were identified to the level of species, but other pollinators to the level of Order and Family.

Quantitative flower visitor observations were performed from 7th to 12th April 2017 on six and three individuals of both morphs, for diurnal and crepuscular visitors, respectively. Two time periods, 0830-1030 hours and 1030-1230 hours were chosen for the diurnal observations, based on information from the surveys, to maximize visitor diversity and numbers. Similarly, the two time periods selected for the crepuscular surveys were 0545-0615 hours and 1845-1915 hours. Focal individuals of similar size with roughly an equal number of flowers were chosen for each morph. For each focal individual, at each sampling period, five two-minute scans were performed for a total of 10 minutes during which the identity and the number of flower visitors were recorded (Aslan et al. 2016). It was assumed that the visitors stayed on the plant for the duration of the two-minute scan. Hence, the number of visitors recorded in each scan was multiplied by two minutes to get the time spent by a visitor on the focal plant per scan. The cumulative time for each visitor over all five scans and for all four observation periods was calculated for each focal plant, and final time spent averaged across all individuals of each of the two morphs. This represents a total of 360 and 180 minutes of observations for each morph for the diurnal and crepuscular observations, respectively. At the end of the observations, three flowers per individual were picked, and the number of resident pollinators like beetles and thrips counted. Wilcoxon's signed-rank test, a non-parametric matched samples test, was performed to test for differences in the visitation time between the long- and the short-styled morph.

Breeding system: The breeding system of the *J. malabaricum* was determined in the Bhimashankar population in 2016 by hand pollinating tagged emasculated flowers by pollen from the opposite morph, another individual of the same morph and the same individual. Ten flowers of each treatment in ten individuals of both morphs were followed until they set fruit. As the fruit set data set was not normally distributed with many zero values, Friedman's test was performed. It is a non-parametric substitute of one-way repeated measures ANOVA. The test was done separately for both the morphs to examine the differences between the fruit sets of the three treatments.

Results

The distribution of anther and stigma in all four populations of *J. malabaricum* was characteristic of stigma-height dimorphism. There were two distinct levels for the stigma, but only one level for the anthers in the two morphs (Fig. 2 and Fig. 3). With the exception of flower diameter, all other key morphological traits examined differed between the long- and short-styled morphs (Table 1 and Table 2). Corolla tube length was higher in the short-styled morphs, and as expected stigma height was higher in the long-styled morphs. However, the magnitude of the difference between the morphs was dependent on the population. While anther heights appeared continuous (Fig. 3), this was significantly higher for the short-styled morph. Consistent with expectations that avoiding self-pollination may be more important in the short-styled morph, the stigma-anther separation was higher in the short-styled morph. Mismatch between complementary stigma and style of the two morphs, which is the inverse of reciprocity was also higher in the short-styled morph. None of the key morphological traits quantified differed between population, with the exception of mismatch, which showed an increasing trend going from the northern populations in the range extremes to the more interior populations in the south. The morphs and populations did not differ in any other perianth traits except corolla tube length (Table 3 and Table 4).

J. malabaricum exhibited pollen size dimorphism with larger pollen in the short-styled morph (Fig. 4). Both morphs had two ovules per flower. Pollen-ovule ratio was higher in the long-styled morph (9236 ± 3004) than in the short-styled morph (7672 ± 3032), but this was not statistically significant (*p* = 0.103, Mann-Whitney U test, sample size for long-styled/short-styled morph = 1 bud/ 29 individuals).

The populations were isoplethic, and the morph ratios in all four populations were not significantly different from 1:1 (Table 5). The results from Levin's segregation index indicated that the morphs were segregated in space in Trimbak and Bhimashankar,and had a slight affinity for like morphs in Mulshi and Kaas (Table 5). However, the chi-square goodness of fit test for equal ratios of the four combinations (long-long, long-short, short-short and short-long) of neighbour-pairs was not significantly different from 1:1:1:1 except in the Mulshi population.

For both the morphs, flowering started in the third week of February and lasted till the end of April, with peak flowering in mid-March (Fig. 5). Fruiting started in the end of April and showed a flat peak in the month of May. All the fruits were dispersed by the beginning of July (Fig. 5). The peak time of fruiting was slightly earlier for the short-styled morph than the long-styled morph. However, there were no significant differences between the morphs in the flowering or fruiting scores. In both the morphs, flowers opened late in the evening between 1900 hours and 2100 hours and lasted for a little over a day (Table 6). The longevity of flowers was not significantly different between the morphs (*p* =0.44, Mann-Whitney U test, *n* = 15 individuals of each morph)

The qualitative visitor surveys showed that 0500-0630 hrs, 0830-1100 hrs and 1900-2030 hrs are the peak timings of visitation with almost no visitation at other times during the day. The plants were visited by social and solitary bees, wasps, butterflies, hawkmoths, hoverflies and flies during the day and primarily by hawkmoths and other moths in the evening. Bees were the most frequent visitors to the flowers during the day (Table 7). Among the bees, the solitary bee *Amegilla* sp. was the most frequent visitor followed by *Apis florea, Trigona* sp., *Apis cerana, Apis dorsata* and *Xylocopa* sp. Lepidopteran visitors like the hawkmoth and butterflies like *Papilio polytes, Papilio polymnestor,* were also common. Hawkmoths were the most frequent visitors during the crepuscular periods. Resident pollinators like beetles (2-3 mm) and thrips were commonly found in the flowers. There were no significant differences in visitation between the two morphs (Wilcoxon matched pairs test, $p = 0.42$, number of individuals for long- and short-styled morphs = 6).

The hand-pollinated individuals set fruit with pollen from the same individual, with pollen from the same morph but different individuals, and also with pollen from the opposite morph indicating that the species is compatible for all the three kinds of pollen (Fig. 6, Friedman's test long-styled morph $p = 0.88$, short-styled morph $p = 0.17$; number of individuals long-styled morph = 9, short-styled morph = 7). There was no fruit set in all the three pollination treatments in one individual of the long-styled morph and three individuals of the short-styled morph, and these were therefore excluded from the analysis. The results did not change when those individuals were included in the analysis.

Discussion

Stigma-height dimorphism is reported here for the first time in the genus *Jasminum.* In addition to the key floral morphological traits examined, the two defining traits in species with style length polymorphism, herkogamy and reciprocity, showed morph-specific differences in *J. malabaricum*. Herkogamy was higher in the short-styled, and reciprocity higher in the long-styled morph. While there were no differences between populations in herkogamy and the other floral traits examined, reciprocity was significantly different across the four populations examined. There were no differences in spatiotemporal mate availability and pollinator visitation for the morphs, and as has been commonly reported in species with stigma-height dimorphism (Thompson et al. 2012), physiological incompatibility was absent.

The distribution of anther and stigma across multiple individuals of the four populations of *J. malabaricum* clearly showed two distinct levels for stigmas, but only one level for anthers in the two morphs. This is characteristic of stigma-height dimorphism, and distinct from distyly which has also been documented in this genus for *J. fruticans* and *J. odoratissimum* (Dommée et al. 1992, Olesen et al. 2003). While there are no unambiguous reports of stigma-height dimorphism in Jasminum, descriptions of style and anther positions in previous publications suggest that this may be fairly common, e.g. in *J. angustifolium* (Raju 1988), *J.*

dispermum, J. quinatum, J. kitchingii, *J. beesianum, J. grandiflorum*, and *J. polyanthum* (Green 1997). There are also reports of approach herkogamy in the genus, e.g. *J. dallachii*, *J. mesnyi, J. nudiflorum* (Green 2001), *J. domatiigerum* subsp. australis (Harris and McDonald 2000) and *J. jenniae* (Harris and Holmes 1999). The presence of all three character states: approach herkogamy, stigma-height dimorphism and distyly (Green and Miller 2009), makes *Jasminum* an excellent genus to examine the evolution of heterostyly.

With the exception of floral diameter, all other floral traits and dimensions examined differed between morphs. Morph specific differences in floral traits is common in heterostylous species (Cohen 2010), and have also been previously reported in species with stigma-height dimorphism (Barrett et al. 1996, Liu et al. 2012). The floral traits and dimensions examined did not differ between populations, with the exception of mismatch, the inverse of reciprocity. Herkogamy, the separation of organs within a flower, was higher in the short-styled morph, and reciprocity between complementary organs higher in the long-styled morph. There are few reports of morph-specific differences in herkogamy and reciprocity in species with stigma-height dimorphism (Baker 2000, Santos-Gally et al. 2015). These results suggest that herkogamy may be more important in the short-styled morph to reduce interference between the male and female sex organs and decrease self-pollination (Webb and Lloyd 1986). Herkogamy may not be as important in the long-styled morph where the relative importance of reciprocity and the ability to differentiate between legitimate and illegitimate pollen may be more important. That herkogamy was not different between populations, but reciprocity was, implies that maintaining herkogamy may be vital in these species, with important fitness consequences. Contrary to our understanding, reciprocity might be relatively less important, more labile and responsive to changing local selection pressures.

In addition to the floral traits examined, morphs also differed in the pollen size, and this is rare in species with stigma-height dimorphism (Simon-Porcar et al. 2014). The absence of pollen size dimorphism prevents easy identification of the morph identity of the pollen, and this has limited the ability to understand legitimate pollen transfer in species with stigma-height dimorphism. The one study that investigated pollen transfer in stigma-height dimorphism was able to do so with the use of artificial flowers (Stone and Thomson 1994). The presence of pollen size dimorphism implies that *J. malabaricum* and possibly other species in this genus could be used to further our understanding of pollen transfer in species with stigma-height dimorphism.

All four study populations were isoplethic, implying that the long- and the short-styled morphs had equal fitness in all four study populations. In addition to the equal morph ratios at the level of the populations, morphs were randomly distributed in space, had synchronous flowering phenology through the season, and similar anthesis periods and floral longevity. Together these patterns in space and time suggest equal and high mate availability for both morphs that should increase the chance of legitimate inter-morph pollen transfer between morphs.

Flowers of both morphs were visited by both long- and short-tongued pollinators. The large hawkmoths and butterflies that visited flowers are likely to have proboscises long enough to facilitate efficient legitimate pollen transfer in both morphs. The most frequent diurnal visitor was a solitary bee species belonging to the genus *Amegilla*, but these had proboscis lengths of 5.54 ± 0.70 mm (*n* = 6), and this is not long enough to reach the stigma of the short-styled morph. Other bees that were also common diurnal visitors included *Apis florea, A. cerana*, and *A.dorsata*. All of these species have tongues that are not long enough to efficiently pollinate both morphs (Cushman, 2007). The large numbers of short-tongued pollinators imply that legitimate pollination may be lower in the short-styled morph (Simon-Porcar et al. 2014) as short-tongued pollinators can effectively pollinate the long-, but not the short-styled morphs (Santos-Gally et al. 2013). However, understanding the consequences of legitimate and illegitimate pollen transfer for reproductive success requires knowledge of intra-morph incompatibility. *J. malabaricum* did not exhibit intra-morph incompatibility, and thus illegitimate pollen transfer can also result in fruit set. The isoplethic nature of the populations indicate equal fitness for both morphs and suggest that potential asymmetries in legitimate pollen transfer between morphs do not translate to fruit set or reproductive success.

The documentation of stigma-height dimorphism in this species is a first for the genus *Jasminum*. Overall these results add to the very few species where the reproductive biology of stigma-height dimorphism has been studied (Baker 2000, Baker et al. 2000a, Li et al. 2010, Sánchez et al. 2010, Ferrero et al. 2011, 2017, Liu et al. 2012). The presence of species with all the intermediate character states in the pathway to heterostyly provides an excellent opportunity for further studies on the evolution of heterostyly in this genus. The morph-specific differences in herkogamy and reciprocity highlight the importance of anther-stigma separation within flowers in the short-styled flowers, and the reciprocity in complementary stigma and anther between flowers in the long-styled morphs. While recognized to be a crucial trait with important functional consequences, herkogamy has received considerably less attention and is overshadowed by studies that have focused on reciprocity. These results show that it is particularly important in species with stigma-height dimorphism where reciprocity between complementary anthers and stigmas is not perfect. Quantification of legitimate pollen transfer enabled by pollen size dimorphism and estimation of fruit set will help better understand the relative importance of herkogamy and reciprocity in promoting legitimate pollen transfer and reproductive success of species with stigma-height dimorphism.

Table 1: Key floral morphological traits in the long- (L) and short-styled (S) morphs of the four study populations of *Jasminum malabaricum*. All values are in mm, and represent mean ± SE. For the Bhimashankar, Mulshi and Kaas populations, 30 individuals for each morph were examined. For the Trimbak populations 19 and 20 individuals for the long- and short-styled morphs, respectively, were examined. The stigma-anther separation was calculated as the mean of the difference between anther and stigma heights for each morph. Mismatch represents the inverse of reciprocity and was estimated as the mean of all pairwise mismatch values between complementary anther and stigma heights.

		Trimbak	**Bhimshankar**	**Mulshi**	**Kaas**
Flower diameter	L	31.52 ± 1.14	33.76 ± 1.02	33.62 ± 0.88	33.35 ± 0.68
	S	30.97 ± 1.75	32.59 ± 0.87	35.35 ± 0.83	32.67 ± 0.74
Corolla tube length	L	15.48 ± 0.44	16.23 ± 0.37	15.83 ± 0.39	15.57 ± 0.68
	S	17.93 ± 0.44	18.53 ± 0.49	18.16 ± 0.33	18.57 ± 0.30
Anther height	L	10.44 ± 0.25	10.66 ± 0.28	10.42 ± 0.26	11.10 ± 0.25
	S	13.64 ± 0.36	13.94 ± 0.39	13.61 ± 0.23	14.16 ± 0.28
Stigma height	L	17.70 ± 0.40	18.13 ± 0.40	18.56 ± 0.34	19.23 ± 0.49
	S	4.98 ± 0.13	5.00 ± 0.13	5.26 ± 0.35	4.72 ± 0.12
Stigma-Anther separation	L	7.27 ± 0.39	7.47 ± 0.34	8.14 ± 0.28	8.13 ± 0.43
	S	8.66 ± 0.36	8.94 ± 0.37	8.60 ± 0.32	9.44 ± 0.27
Mismatch	L	4.22 ± 0.31	4.48 ± 0.44	4.95 ± 0.25	5.54 ± 0.45
	S	5.44 ± 0.11	5.65 ± 0.47	5.47 ± 0.14	6.35 ± 0.17

Table 2: Variation in the key floral morphological traits examined - results from a two-way ANOVA (*F*-statistics) testing for differences between the four study populations and the long- and short-styled morphs. For the Bhimashankar, Mulshi and Kass populations, 30 individuals for each morph were examined. For the Trimbak populations 19 and 20 individuals for the long- and short-styled morphs, respectively, were examined. Asterisk represents $P < 0.05$.

	Flower diameter	**Corolla tube length**	**Anther height**	**Stigma height**	**Stigma-Anther separation**	**Mismatch**
Population	2.60	0.83	1.86	1.25	2.13	5.36*
Morph	0.19	63.48*	236.39*	3502.39*	24.58*	30.79*
Morph x Population	0.62	0.10	0.09	2.93*	0.25	0.13

Table 3: Additional floral morphological traits in the long- (L) and short-styled (S) morphs of the four study populations of *Jasminum malabaricum*. All values are in mm and represent mean ± SE. For the Bhimashankar, Mulshi and Kaas populations, 30 individuals for each morph were examined. For the Trimbak populations 19 and 20 individuals for the long- and short-styled morphs, respectively, were examined. Stigma exertion was measured as the vertical length of the stigma that protruded from the corolla tube in the long-styled morph.

Traits		**Trimbak**	**Bhimshankar**	**Mulshi**	**Kaas**
Number of petals	L	7.30 ± 0.28	7.37 ± 0.20	7.50 ± 0.14	7.27 ± 0.21
	S	7.55 ± 0.24	7.30 ± 0.17	7.38 ± 0.23	7.06 ± 0.20
Petal lobe length	L	15.09 ± 0.55	16.16 ± 0.50	16.04 ± 0.44	15.78 ± 0.31
	S	15.28 ± 0.43	15.84 ± 0.40	16.53 ± 0.40	15.37 ± 0.36
Anther length	L	4.09 ± 0.13	3.96 ± 0.10	4.08 ± 0.10	4.25 ± 0.12
	S	4.90 ± 0.14	4.67 ± 0.15	4.55 ± 0.11	4.65 ± 0.10
Ovary length	L	1.32 ± 0.04	1.47 ± 0.04	1.35 ± 0.03	1.33 ± 0.04
	S	1.38 ± 0.05	1.47 ± 0.05	1.48 ± 0.06	1.36 ± 0.05
Ovary diameter	L	1.32 ± 0.04	1.35 ± 0.03	1.35 ± 0.03	1.37 ± 0.03
	S	1.38 ± 0.05	1.43 ± 0.05	1.47 ± 0.09	1.37 ± 0.05
Stigma length	L	2.78 ± 0.13	3.11 ± 0.09	2.96 ± 0.14	2.87 ± 0.13
	S	2.44 ± 0.16	2.34 ± 0.09	2.32 ± 0.10	2.47 ± 0.10
Stigma exsertion	L	3.08 ± 0.47	2.95 ± 0.22	3.38 ± 0.38	4.01 ± 0.37
	S	-	-	-	-

Table 4: Variation in the additional floral morphological traits examined - results from a two-way ANOVA (*F*-statistics) testing for differences between the four study populations and the long- and short-styled morphs. For the Bhimashankar, Mulshi and Kass populations, 30 individuals for each morph were examined. For the Trimbak populations 19 and 20 individuals for the long- and short-styled morphs, respectively, were examined. Asterisk represents $P < 0.05$.

Effect	**Petal number**	**Petal lobe length**	**Anther length**	**Ovary length**	**Ovary size**	**Stigma length**	**Stigma exsertion**
Population (P)	0.81	2.43	1.17	4.09*	0.35	0.95	1.52
Morph (M)	0.25	0.04	54.85*	1.69	3.53	34.34*	-
P x M	0.86	0.84	1.17	0.92	0.60	0.95	-

Table 5: The morph ratio and segregation index for the four study populations of *Jasminum malabaricum*. For morph ratios, chi-square goodness of fit test was conducted for the expected 1:1 long-styled: short-styled ratio. Levin's index for segregation was calculated based on the morph identity of the nearest neighbour (Levin 1974). For this, chi-square goodness of fit test was conducted for the expected equal frequencies of the nearest neighbour pairs, i.e. long-long, long-short, short-short and short-long. For the chi-square results, ns denotes not significant.

Population	**n**	**Morph ratio LS:SS**	**Segregation index**
Trimbak	40	1.22 : 1, ns	0.15, ns
Bhimashankar	60	1.14 : 1, ns	0.20, ns
Mulshi	60	0.90 : 1, ns	-0.23, *P* = 0.06
Kaas	60	0.76 : 1, ns	-0.10, ns

Table 6: Anthesis period and floral longevity to understand temporal mate availability for the long- and short-styled morphs in the Bhimashankar population of *Jasminum malabaricum*. The start and stop of anthesis period were calculated as the 97.5% confidence interval; the number of individuals examined for long-styled = 14; short-styled = 13. Floral longevity is represented as mean ± SE; the number of individuals examined for each morph = 15.

Morph	**Anthesis**	**Longevity**
Long-styled	1904 hrs - 2112 hrs	34.08 ± 2.97 h
Short-styled	1853 hrs - 2125 hrs	32.81 ± 2.44 h

Table 7: Floral visitors in *Jasminum malabaricum* from the Bhimashankar population. A) Visitation times for the different categories of potential pollinators was determined as the mean ± SE of the cumulative time spent on flowers for six individuals of each morph. Visitors are organized by orders, and also by size. B) Resident visitors (beetles and thrips) were quantified as the number present in three flowers for six individuals of each morph. The time period of visitation is denoted by C for crepuscular, D for diurnal, and R for resident. When a visitor was not recorded visiting a morph, it has been marked as Absent.

Order	**Visitor**	**Period**	**Long-styled**	**Short-styled**
A) Cumulative time on flowers (min)				
Lepidoptera	Moths small (size < 3 cm)	C	1.33 ± 1.19	1.33 ± 1.40
	Unidentified moth			
	Moths large (> 3cm)	C	5.33 ± 3.84	4.33 ± 3.61
	Unidentified moth			
	Sphingidae (Hawkmoth)			
	Butterfly small (size < 3 cm)	D	Absent	0.33 ± 0.33
	Unidentified butterfly			
	Butterfly large (> 3cm)	D	2.67 ± 1.11	1.00 ± 0.68
	Unidentified butterfly			
	Papilio polytes			
	Papilio polymnestor			
	Catopsilia pomona			
	Euploea core			
Hymenoptera	*Amegilla sp.*	D	11.00 ± 5.67	6.00 ± 1.93
	Apis cerana	D	0.33 ± 0.33	Absent
	Apis dorsata	D	0.33 ± 0.33	5.33 ± 3.82
	Apis florea	D	5.33 ± 3.08	3.67 ± 1.40
	Trigona sp.	D	3.33 ± 1.60	5.00 ± 2.18
	Xylocopa sp.	D	0.33 ± 0.33	Absent
	Apocrita (Wasp)	D	0.67 ± 0.42	0.67 ± 0.42
Diptera	Syrphidae (Hoverfly)	D	0.33 ± 0.33	0.67 ± 0.42
	Unidentified fly (1cm)	D	1.33 ± 0.42	Absent
B) Counts of floral visitor in flowers				
Coleoptera	Unidentified beetles (2-3 mm)	R	0.50 ± 0.20	0.56 ± 0.23
Thysanoptera	Thrips	R	1.17 ± 0.40	1.33 ± 0.55

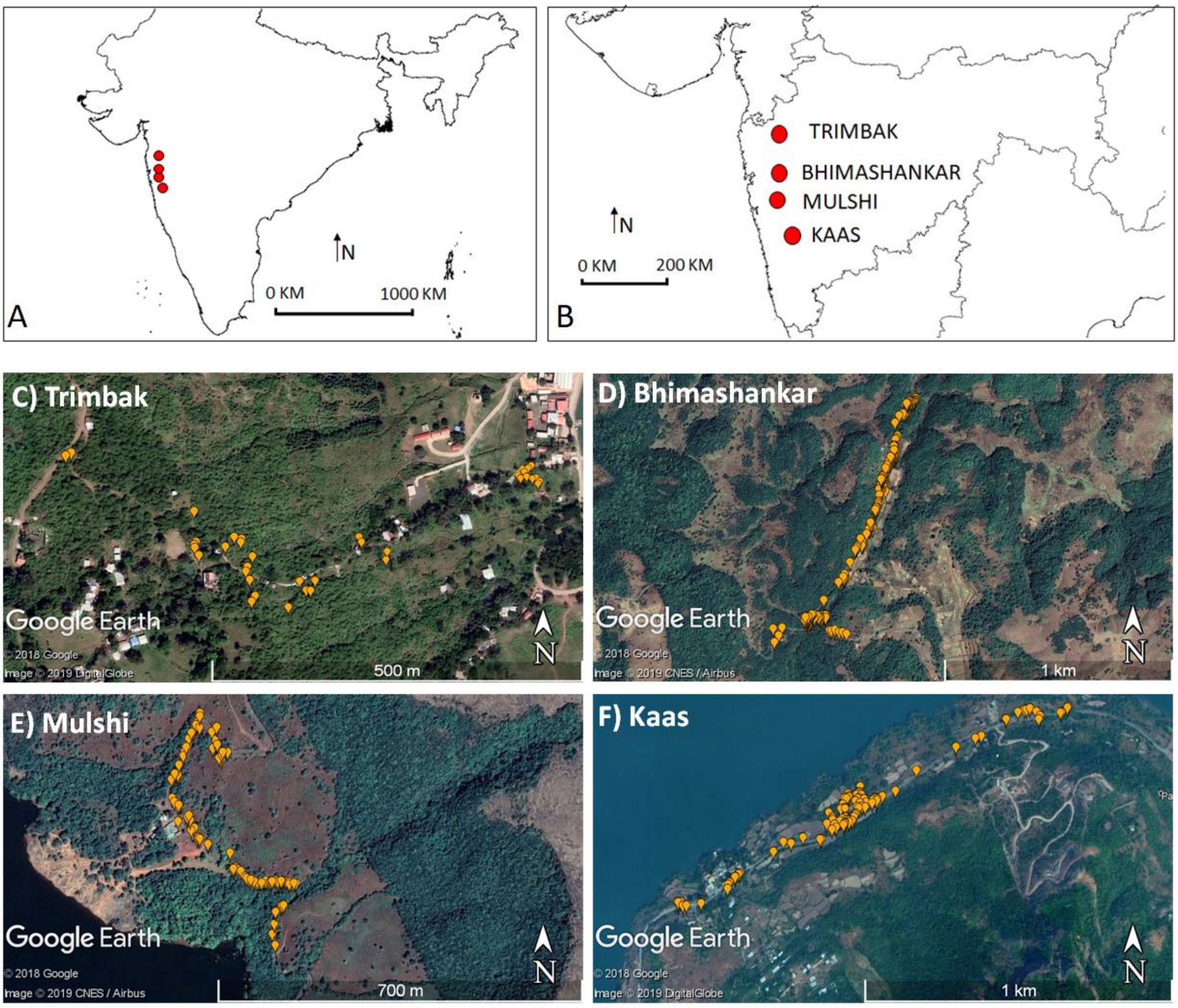

Figure 1: The four study populations of *Jasminum malabaricum* shown in a map of: A) peninsular India; and B) the state of Maharashtra. The spatial distribution of the individuals (marked by the yellow tags) used to estimate the population morph ratio and the nearest neighbour morph identity in the four populations: C) Trimbak; D) Bhimashankar; E) Mulshi; and, F) Kaas.

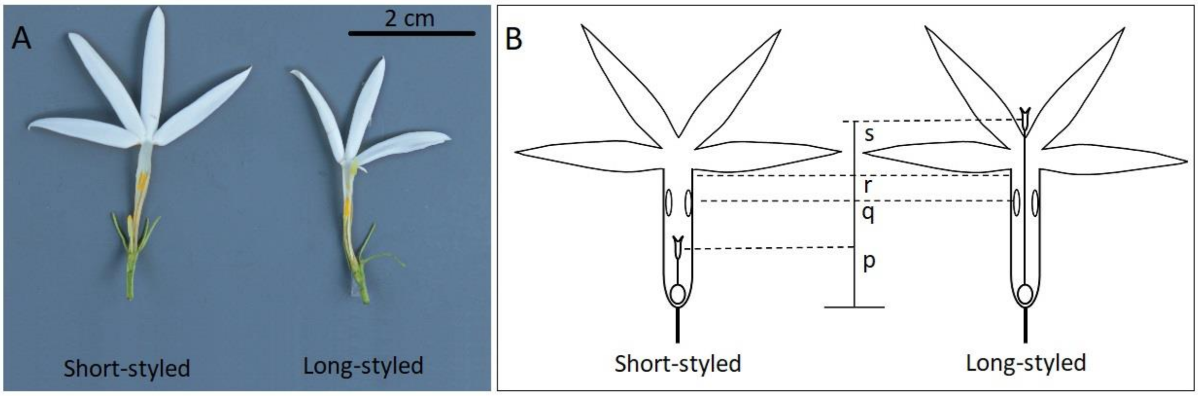

Figure 2: A) Partially dissected flowers of the short- and long-styled morphs of *Jasminum malabaricum*. B) Schematic representation of the short- and long-styled morphs to illustrate the key floral organ dimensions quantified. All heights were measured from the base of the flower represented by the solid horizontal line; p – stigma height of the short-styled flower , q – anther height, r– corolla tube length and s – stigma height of the long-styled flower. Stigma-anther separation and mismatch were derived from the measured anther height and stigma height.

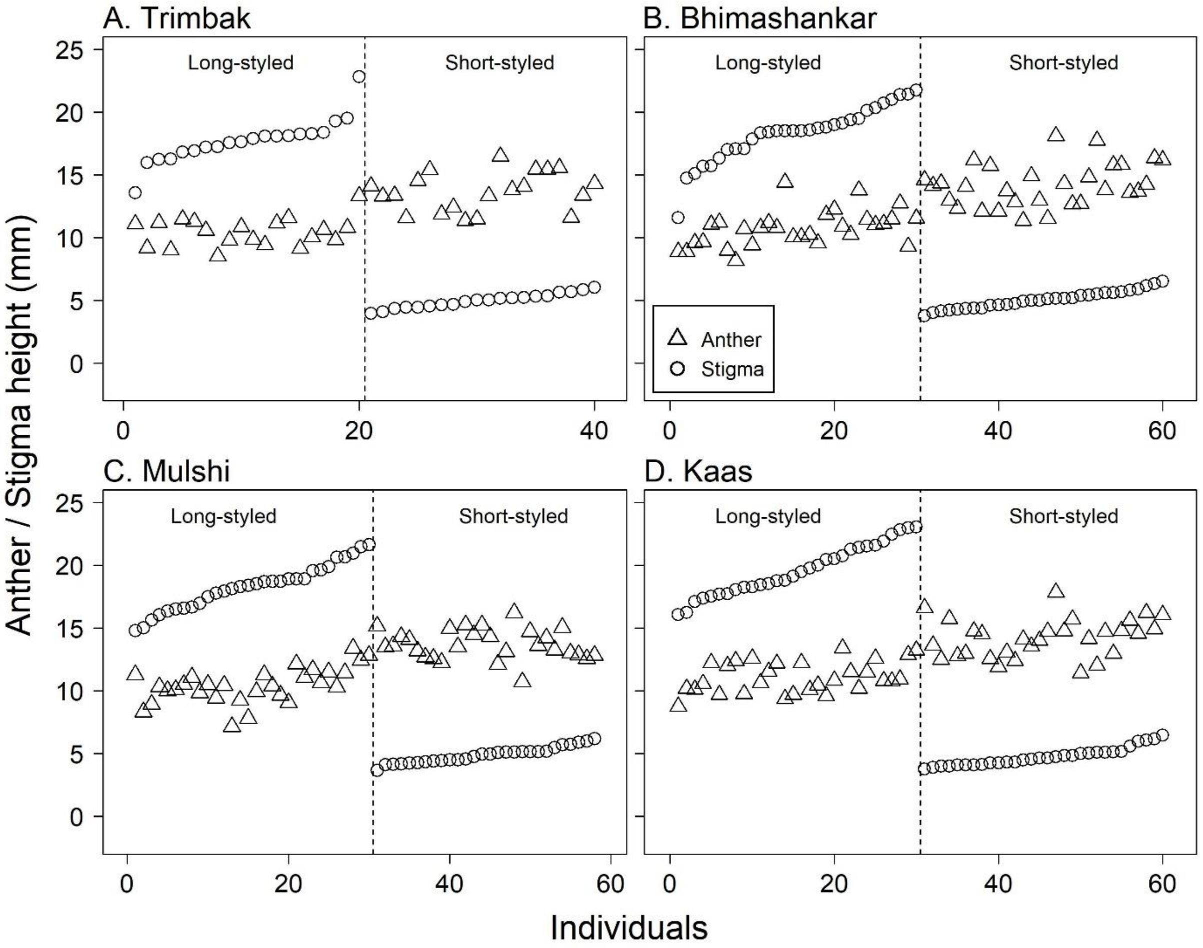

Figure 3: The distribution of anthers (triangles) and stigmas (circles) in individuals from the four study populations of *Jasminum malabaricum*. Individuals are arranged in ascending order of stigma heights within the long- and short-styled morphs. For the Bhimashankar, Mulshi and Kass populations, 30 individuals for each morph were examined. For the Trimbak populations 19 and 20 individuals for the long- and short-styled morphs, respectively, were examined.

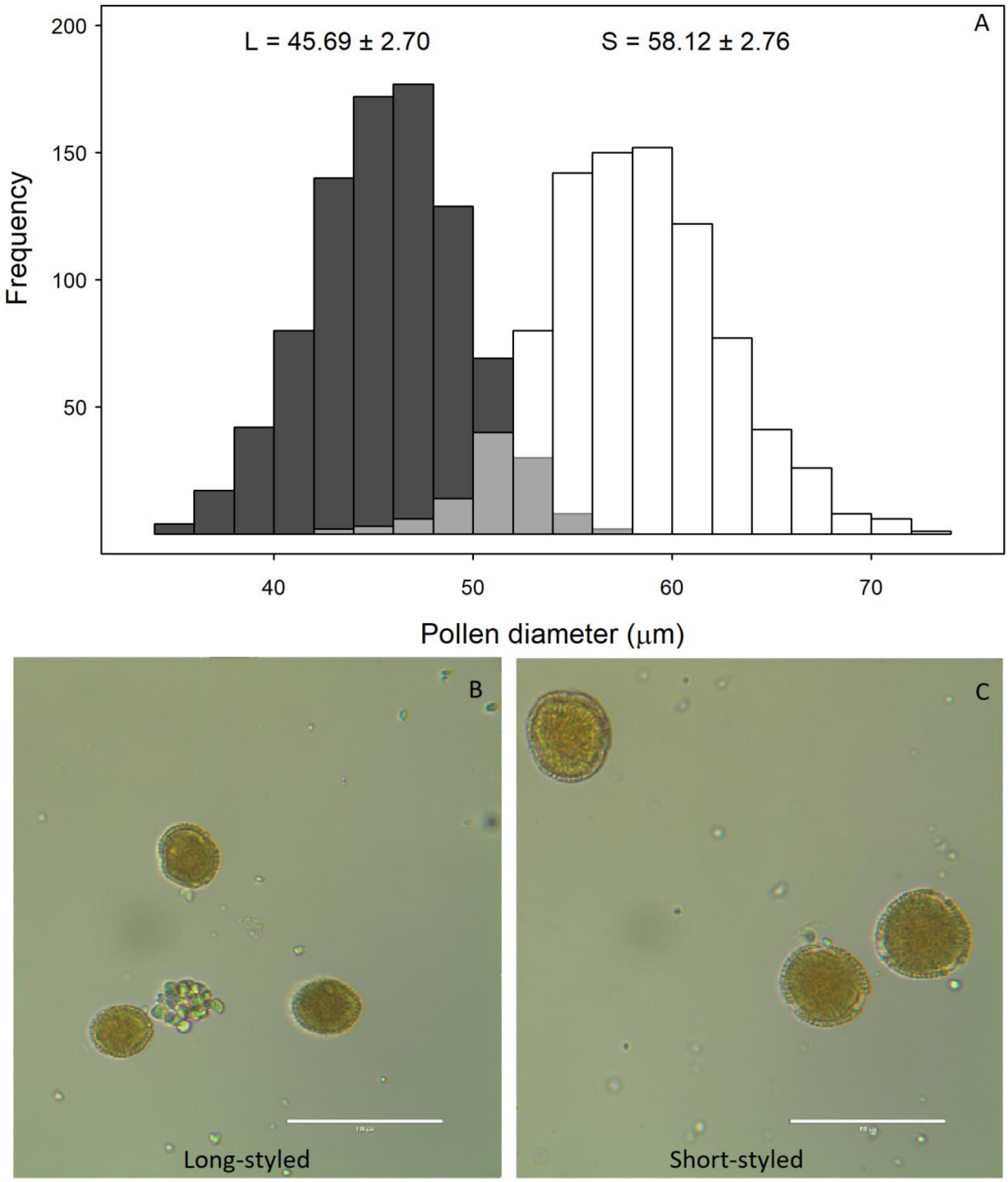

Figure 4: Pollen size in *Jasminum malabaricum*. A) Distribution of pollen sizes in the long- (L, dark grey bars) and short-styled (S, open bars) morphs from the Bhimashankar population (sample size for each morph = 30 pollen/29 individuals). The lighter shade of grey denotes the pollen size seen in both morphs. B) and C) Representative images of long-styled and short-styled pollen taken at 400x magnification. The scale bar denotes 100µm.

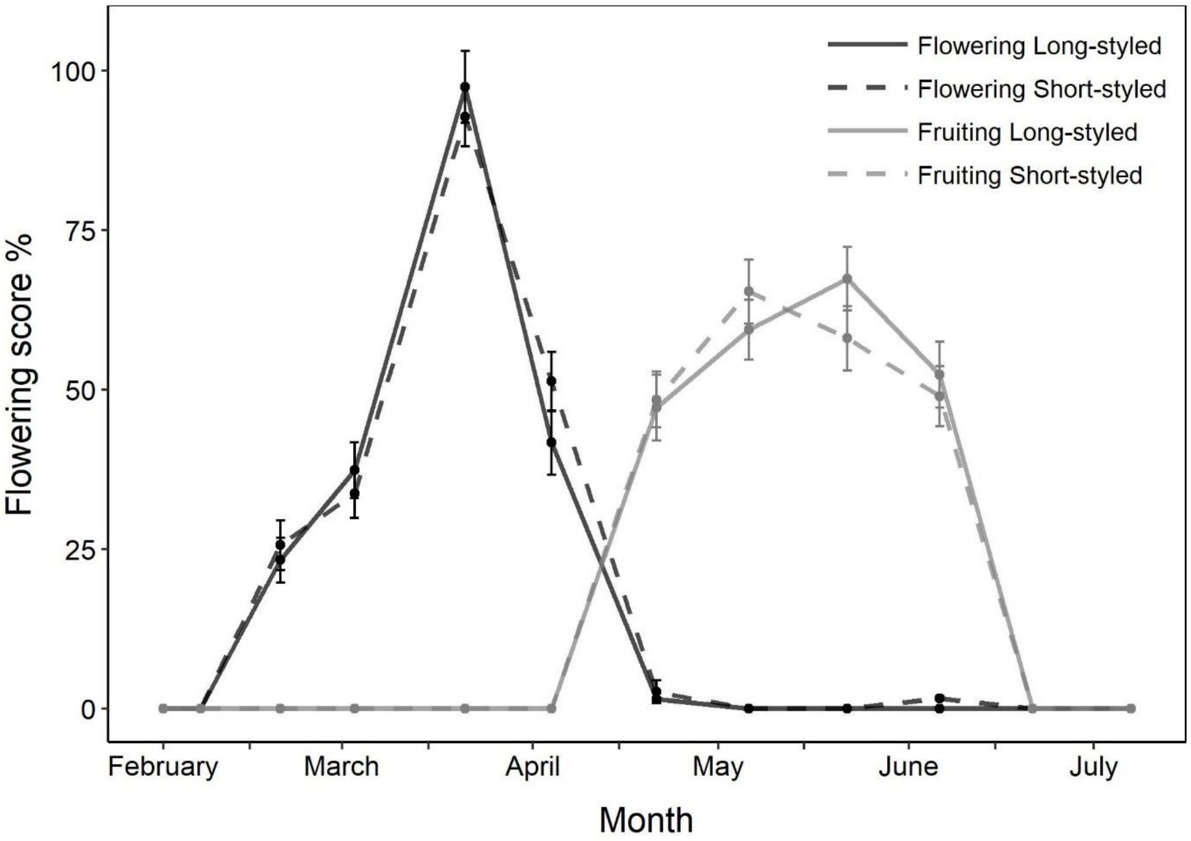

Figure 5: Flowering and fruiting phenology of *Jasminum malabaricum*. Flowering (black lines) and fruiting (grey lines) scores of the long-styled (solid lines) and short-styled (dashed lines) morphs from the Bhimashankar population in 2016. Observations were made fortnightly for 50 individuals of each morph. Error bars denote SEM.

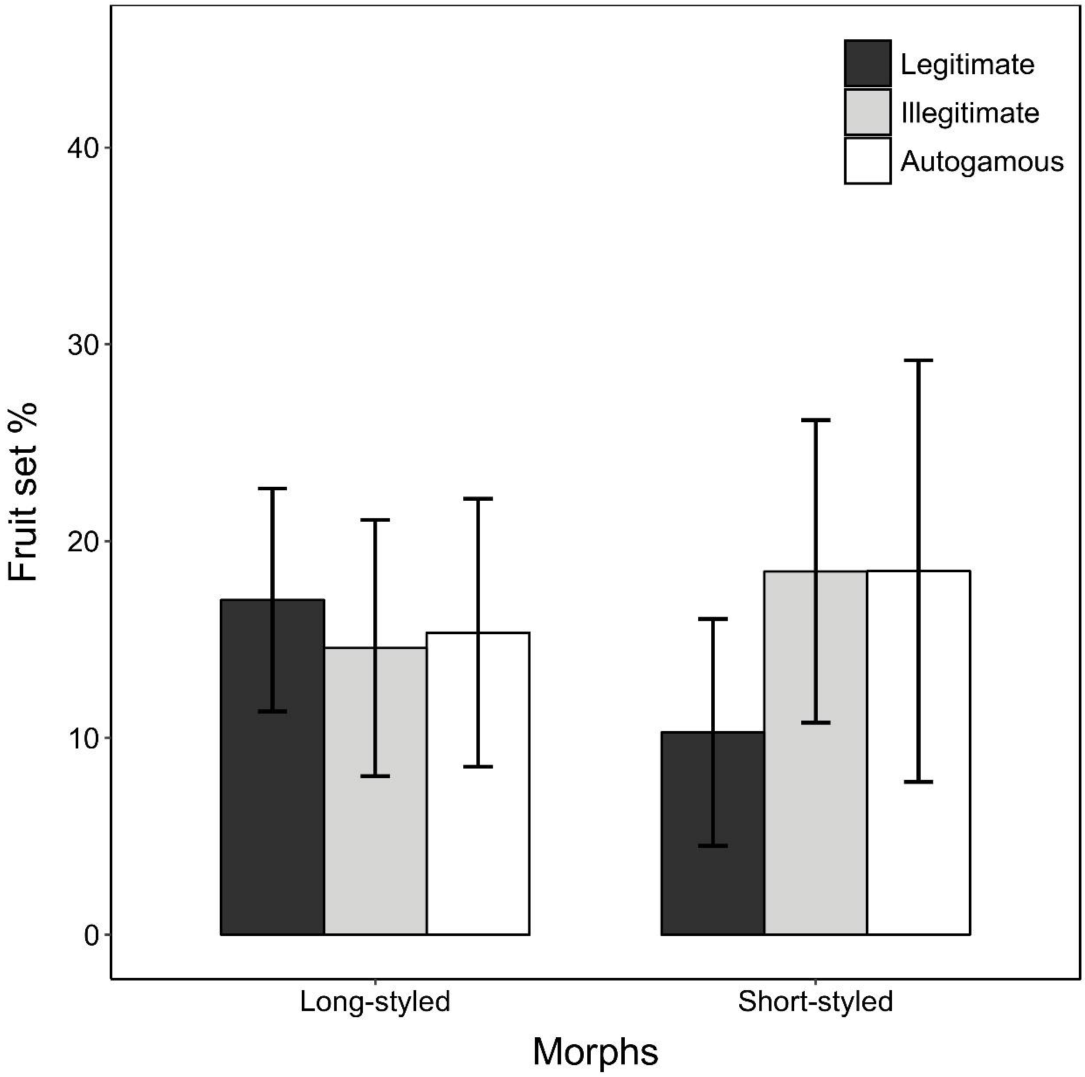

Figure 6: Inter-morph, intra-morph and intra-individual compatibility in *Jasminum malabaricum*. Fruit set was quantified as the percentage of fruits formed out of ten flowers for each of the three hand-pollination treatments in the long- and short-styled morphs in the Bhimashankar population. Error bars denote SEM; number of individuals for long-styled - 9; short-styled - 7. No significant difference was observed between the legitimate, illegitimate and autogamous fruit set in both the morphs.

Chapter 5

The relative importance of herkogamy and reciprocity in promoting legitimate pollen transfer and fruit set in *Jasminum malabaricum*

Abstract

Herkogamy and reciprocity are crucial in determining legitimate pollen transfer and fruit set in species with style length polymorphism. Understanding how these two floral features affect legitimate pollen transfer becomes particularly important in stigma-height dimorphism, where herkogamy is present but reciprocity is imperfect. Here we examine the relative importance of herkogamy and reciprocity in determining legitimate pollen transfer and fitness in *Jasminum malabaricum*, a self-compatible stigma-height dimorphic species. We quantify and compare the relationship of herkogamy and reciprocity of every individual with stigma pollen load, legitimate pollen load and fruit set in naturally occurring populations from the Northern Western Ghats of Peninsular India. As expected according to the predictions in chapter 1, we observed a negative relationship between herkogamy and reciprocity in these plants. We observed no relationship between herkogamy and total stigma load, but as expected, we saw a positive relationship with legitimate fraction of the stigmatic pollen load, and consequently fruit set in the long-styled morph. Contrary to the expectations, we found that reciprocity was negatively related to fruit set in the short-styled morph. The negative relationship between herkogamy and reciprocity points towards a trade-off between avoidance of self-pollen deposition and promotion of legitimate pollen transfer. Herkogamy was more important than reciprocity in determining legitimate pollination and fruit set in this species. This suggests that herkogamy can play a significant role in encouraging legitimate pollen flow and stabilizing such polymorphic intermediates, allowing reciprocity between complementary organs to arise later in the evolutionary pathways towards perfectly reciprocal heterostyly.

Introduction

It is surprising that the relationship between reciprocity and herkogamy, the two defining morphological features in flowers of species with style length polymorphism (Fornoni and Domínguez 2015) remains unexplored. Reciprocity between complementary sex organs increases legitimate pollen transfer between morphs (Zhou et al. 2015), while herkogamy, the separation of the male and female sex organs within a flower, primarily acts to reduce self-pollen deposition (Nishihiro and Washitani 1998). Given the fundamental, but distinct roles of reciprocity and herkogamy (Cohen 2010), it is important to understand the relationship between the two, and the causes and consequences any such relationship. This is particularly true for species with stigma-height dimorphism where herkogamy is present, but the reciprocal arrangement of complementary anther and stigma is not perfect (Lloyd and Webb 1992, Baker 2000).

The reciprocal arrangement of sex organs in species with style length polymorphism increases legitimate pollen transfer efficiency by enabling pollinators to pick up pollen from anthers on specific locations on their body from where it can be accurately deposited on stigmas of complementary morphs (Stone and Thomson 1994, Lau and Bosque 2003, Keller et al. 2014, Costa et al. 2017). This match between anthers and stigmas is quantified to calculate reciprocity indices which are widely used to understand legitimate pollen transfer and reproductive success (Richards and Koptur 1993, Eckert and Barrett 1994, Lau and Bosque 2003, Sanchez et al. 2008, Armbruster et al. 2017). Empirical studies in heterostylous species have shown that higher population level reciprocity is related to greater legitimate pollen transfer (Keller et al. 2012, Baena-Díaz et al. 2012, Jacquemyn et al. 2018) and reproductive success (Valois-Cuesta et al. 2011, Zhou et al. 2015, Ferrero et al. 2017). While limited, a few studies in species with stigma-height dimorphism have also shown that reciprocity is positively related to disassortative pollination (Cesaro and Thompson 2004), and reproductive success (Thompson et al. 2012).

Herkogamy, the separation of anthers and stigmas within a flower, reduces interference between male and female organs and results in reduced self-pollen deposition and more efficient pollen pick up by pollinators (Webb and Lloyd 1986, Barrett 2002). Herkogamy has been studied extensively in monomorphic populations, and these have shown that it is positively related to legitimate pollen transfer and outcrossing rates (Ennos 1981, Brunet and Eckert 1998, de Vos et al. 2012, 2018, Li et al. 2013). Empirical studies in species with style length polymorphism are few, but these show that like in monomorphic species, herkogamy is related to increased legitimate pollen transfer and reproductive success in heterostylous species (Keller et al. 2014, Liu et al. 2016), and decreased self-pollination in species with stigma-height dimorphism (Nishihiro and Washitani 1998, Cesaro et al. 2004).

There can be large variation in organ heights among individuals in species with style length polymorphism, and these differences have important consequences for reciprocity, pollen flow, fruit set and reproductive success for these individuals (Eckert and Barrett 1994, Sanchez et al. 2008, Ferrero et al. 2011, Sampson and Krebs 2012). However, the current reciprocity indices used are calculated at the level of the population (Sanchez et al. 2008), and for morphs (Richards and Koptur 1993, Lau and Bosque 2003, Armbruster et al. 2017). Understanding intra-population variation in reciprocity is important as selection ultimately acts at the level of the individuals, and because populations level estimates might hide biologically important variation. While herkogamy is quantified at the level of individual flowers, like with reciprocity, it is mostly studied as a mean population-level measure ignoring intra-population variation (Kulbaba and Worley 2012). The lack of an individual-level reciprocity measure and the focus on population-level estimates for both reciprocity and herkogamy has precluded the examination of the relationship between these two important traits in species with style length polymorphism. Examining this relationship in individuals of a population will allow us to ask if there is a trade-off between avoiding self-pollen deposition and promoting legitimate pollen deposition.

Understanding the underlying mechanism that results in this relationship is important to identify potential constraints that may determine whether selection can act independently on anther and stigma heights. When mean population stigma height is higher than mean anther height in complementary morphs, higher stigma height of long-styled individuals will result in greater herkogamy (assuming lesser change in anther height across individuals), but lower reciprocity (chapter 1 Fig. 2, 3 and 4). Thus, we may expect that under these circumstances, herkogamy will be negatively related to reciprocity. In a short-styled individual, increasing stigma height will reduce both herkogamy and reciprocity, and we might expect a positive relationship between herkogamy and reciprocity as stigma heights move closer to the higher level anthers within the flower, but further from the population level mean anther heights of the complimentary morphs. The opposite will be true if mean population stigma height is lower than mean anther height in the complementary morph. Under these conditions, we would expect positive relationships for the short-, and negative relationships for the long-style morph between herkogamy and reciprocity. The above predictions assume that changes in anther heights are less than that in stigma. If changes in anther heights are equal to that in stigma, herkogamy will remain constant and will not be related to changes in reciprocity. Finally, if changes in anther height are greater than that in stigma, the predicted changes in herkogamy will be in the opposite direction.

In this study, herkogamy and reciprocity were quantified for multiple individuals from four naturally occurring populations of *Jasminum malabaricum*, a species with stigma-height dimorphism. Reciprocity was quantified at the individual level using a recently developed index. The individual-level measures of reciprocity and herkogamy allowed examination of variation across morphs and population, and importantly, the relationship between the reciprocity and herkogamy in individuals from the study populations. The morph- and population-level estimates of reciprocity are compared with previously proposed indices (Richards and Koptur 1993, Lau and Bosque 2003, Sanchez et al. 2008, Armbruster et al. 2017).

In an effort to understand the possible underlying causes for the relationship between reciprocity and herkogamy, the relationship between anther and stigma heights within flowers were examined. As seen in other species with stigma-height dimorphism, mean stigma height in the long-styled morph is greater than mean anther height in the complementary short-styled morph in *J. malabaricum*. In contrast, mean stigma height in the short-styled morph is smaller than mean anther height in the complementary long-styled morph. Under these circumstances, if anther heights are positively related to stigma heights with a slope less than one, we would expect a negative relationship between herkogamy and reciprocity in the long- and positive relationship in the short-styled morph.

To examine the consequence of variation in herkogamy and reciprocity on pollen transfer, total stigma pollen load and the legitimate fraction was quantified for two populations. Legitimate pollen fraction has not been reported before in species with stigma-height dimorphism. To understand the fraction of illegitimate pollen that is a result of self-pollination within the flower, autogamous pollen load was quantified in one of the study populations. The long-styled morph is expected to have higher total pollen deposition as the stigma is easily accessible to all pollinators. The short-styled morph, on the other hand, is known to receive lower total pollen but with a higher legitimate fraction as it can be reached only by long-tongued pollinators. Finally, to understand the consequences of variation in reciprocity and herkogamy for pollen transfer and reproductive success, the relationship between individual-level measures of this reciprocity and herkogamy, with total pollen load and legitimate fraction of stigmatic pollen load was studied in Bhimashankar and Kaas populations. The relationship between herkogamy and reciprocity with fruit set was also studied in the Bhimashankar population.

Methods

Study sites and species: The study was conducted in 2016 and 2017 in four *Jasminum malabaricum* Wight. populations as described in chapter 4, i.e. Trimbak, Bhimashankar, Mulshi and Kaas in the Northern Western Ghats range of Maharashtra, India. *J. malabaricum* is a woody liana with stigma-height dimorphism found in open habitats on other trees and shrubs. Previous results showed that there are no differences in spatiotemporal mate availability or pollinator visitation for the morphs and that this species does not have physiological incompatibility.

Quantification of herkogamy and reciprocity: Herkogamy and mismatch were calculated from anther and stigma height measurements for both morphs from the four populations as described previously. The number of individuals sampled was: 30 each for the long- and short-styled morphs for Bhimashankar and Mulshi; 32 long- and 31 short-styled for Kaas; and, 20 long- and 19 short-styled for Trimbak. Herkogamy and reciprocity were also quantified for an additional 70 individuals for a total of 100 individuals of each morph in the Bhimashankar population.

Herkogamy was quantified for one flower per individual as the difference in anther and stigma height of a flower (Opedal 2018). Reciprocity was calculated for one flower per individual using the reciprocity index proposed previously. Briefly, the mismatch between complementary stigma-anther pairs is quantified and converted to an estimate of pollen transfer success. It is assumed that pollen transfer decreases with increasing mismatch, ranging from one for a perfect match, ultimately to zero beyond some critical value of mismatch. It is assumed that there is no pollen transfer beyond this value. A convex decreasing function was used such that the rate of decrease in pollen transfer increased with mismatch. Earlier results showed that concave, linear and convex decreasing functions yielded qualitatively similar results. The critical value of mismatch beyond which pollen transfer success was assumed to be zero was set at 7 mm. This value falls in the range of anther lengths found in the *J. malabaricum* populations examined and was selected to maximize the range of reciprocity values obtained and the ability to distinguish between individuals. The mismatch between stigma height for an individual and anther height of every individual of the complementary morph was quantified. These pair-wise mismatches were converted to pollen transfer success values as described above and averaged to give an estimate of reciprocity for that individual. To compare the current index to previously proposed indices, reciprocity was also calculated for the morphs and populations using previous indices proposed by Richards & Koptur (1993) which was modified to incorporate intra-population variation in anther and stigma heights (by Sanchez et al. 2008), Lau & Bosque (2003), Sanchez et al. (2008) and Armbruster et al. (2017). To better understand the relationship between herkogamy and reciprocity, we quantified the slope of the relationship between anther and stigma heights of a flower across individuals, which will affect the change in herkogamy with a change in stigma height.

Factorial two-way ANOVA with morph (long-styled and short-styled) and population (Trimbak, Bhimashankar, Mulshi and Kaas) as fixed factors was performed to examine variation in herkogamy and reciprocity. The relationship between herkogamy and reciprocity was examined using Pearson's correlation coefficient. The relationship between anther and stigma heights was studied using the slope of ranged major axis type II regression as both anther and stigma heights were expected to contain error and the stigmas of the two morphs have different variances due to size differences (Legendre 1998).

Quantification of stigma pollen load: As the quantification of pollen load is labour intensive, it was only possible to do this for the Bhimashankar (March 2016) and Kaas (April 2017) populations in the same individuals for which floral morphometry was quantified. 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%). After 3 p.m. the flowers wilt and no pollinator activity is seen on these flowers. Quantification was done for 29 individuals of each morph for Bhimashankar, and 32 long- and 31 short-styled individuals for Kaas. Stigmas were washed in 70% ethanol and softened in 4N NaOH for twelve hours (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 glycerol:ethanol concentrations from 10% to 100% in steps of 15%, squashed with a coverslip and mounted in 100% glycerol.

To count the total number of pollen deposited on a stigma, images of stigma squashes for two stigmas per individual were taken at 40x magnification. An exhaustive pollen count was performed for all the stigmas using ImageJ. 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. The equatorial diameter of the pollen was measured using ImageJ. The pollen were discriminated based on differences in diameter between the morphs using the estimates of pollen obtained previously (Chapter 4, Fig. 4). The diameter of the long-styled pollen ranged between 34µm to 55 µm and the short-styled pollen between 47µm to 73 µm. Approximately 35% of the pollen were in the range from 47µm to 55µm that is common to both the morphs. The ratio of the frequency of pollen of anthers from the long- and short-styled morph was used to estimate the probability of occurrence of pollen from each morph in these size classes. 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 found in the total number of pollen sampled. Differences in natural total stigma pollen load and the legitimate pollen fraction between the two morphs were examined using a non-parametric Mann-Whitney U test as the values were not normally distributed.

Autogamous pollen transfer: Three mature buds were tagged and emasculated for five individuals of each morph to quantify autogamous self-pollen deposition. 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 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 the Bhimashankar population in 29 individuals of each morph. For both morphs, 20 mature buds were tagged per individual in the peak flowering season and followed till 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 natural total pollen load, legitimate fraction and the fruit set were examined using Spearman's rank correlation coefficient as most of the examined variables had extreme values and skewed distributions.

Results

Estimates of reciprocity were significantly different among populations and decreased from Trimbak, the northernmost population, to Kaas, the most southern population examined (Table 1A, Fig. 1A). Reciprocity was higher in the long-styled than in the short-styled morph. The lack of a significant interaction indicated that differences between morphs were consistent across populations (Table 1A). Unlike reciprocity, herkogamy was not different across populations (Table 1B, Fig. 1B). While different in the two morphs, in contrast to reciprocity, herkogamy was higher in the short-, than in the long-styled morph. In addition to the differences observed between populations and morphs above, there was a large variation in both estimates of reciprocity and herkogamy between individuals in the populations (Fig. 1).

In comparing the estimates of reciprocity obtained from the index used in this study to previously proposed indices, it was seen that the qualitative differences between morphs and populations were consistent with the index proposed by Armbruster et al. (2017) (Table 2). Note that the index proposed by Armbruster et al. (2017) calculates imprecision in pollen transfer, which is the opposite of reciprocity. Therefore, unlike the other indices, higher values of the index represent lower reciprocity. The differences observed between morphs while using the current index were consistent with the reciprocity index proposed by Richards & Koptur (1993), but the latter was not able to distinguish between the four populations. The index proposed by Sanchez et al. (2008) was problematic as it resulted in negative values and the index by Lau & Bosque (2003) resulted in zero values for reciprocity.

Herkogamy and reciprocity showed a significant negative correlation in the long-styled morph in all study populations (Fig. 2 A-D). However, such a negative relationship between herkogamy and reciprocity was seen in only one of the four study populations in the short-styled morph (Fig. 2 E-H). Similar results were observed when examining this relationship in a larger number of individuals from the Bhimashankar population with a significant negative relationship between herkogamy and reciprocity in the long-, but not the short-styled morph (Fig. 3 A, B).

The two factors that affect this relationship are the relative average height of anther and stigma of the complementary morphs, which affects reciprocity, and the slope of the relationship between anther and stigma heights within a flower which affects herkogamy (chapter 1 Fig. 2, 3 and 4). Like in other species with stigma-height dimorphism, mean stigma height of the long-styled morph was higher than its complementary anther, and mean stigma height of the short-styled morph was lower in than its complementary anther. In the long-styled morph of all populations except for Trimbak, anther height was positively related to stigma height within flowers, and the slope of this relationship was between zero and one (Fig. 4 A-D). No significant relationship was observed between stigma and anther heights in the short-styled morph (Fig. 4 E-H). Examining the relationship in a larger number of individuals from the Bhimashankar population (n=100 for each morph) showed that both the morphs had significant positive correlations between stigma and anther heights (Fig 3 C, D). The slope of this relationship between stigma and anther heights was between zero and one for the long-styled morphs and was greater than one for the short-styled morph.

Although long-styled morphs had higher stigma pollen load, no significant differences were detected between the two morphs in either of the two populations examined (Fig. 5 A, C; Mann-Whitney U test: Bhimashankar $p = 0.41$ and Kaas $p = 0.11$; n for long- and short-styled morph was 29 and 29 for Bhimashankar, and 32 and 31 for Kaas). There was high variation between individuals in 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. 5 A, C) in the Kaas population. Legitimate pollen fraction was significantly higher in short- than in long-styled morph for both populations (Fig 5 B, D; Mann-Whitney U test: Bhimashankar $p < 0.001$ and Kaas $p < 0.001$; n for long-styled and short-styled morph was 29 and 29 for Bhimashankar, and 32 and 31 for Kaas).

As was seen in the previous results with unmanipulated flowers, the short-styled morph had lower total pollen load but higher legitimate pollen fraction (Fig. 6A, B). In both morphs, emasculation resulted in a decrease in total pollen deposition (Fig. 6A; Wilcoxon signed-rank test: long-styled $p = 0.04$ and short-styled $p = 0.04$; 3 flowers per treatment for 5 individuals of both the morphs). In the short-styled morphs emasculation did not affect legitimate pollination (Fig. 6B; Wilcoxon signed-rank test: $p = 0.34$). In contrast, in the long-styled morphs emasculation resulted in an increase in legitimate pollination (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. 7; Mann-Whitney U test: 2015 $p = 0.41$; 2016 $p = 0.80$; $n = 29$ individuals for both morphs and both years).

Herkogamy was positively related to disassortative pollen load and fruit set in the long-styled morph, but not to total or disassortative pollen load, or fruit set in the short-styled morph in Bhimashankar (Table 3 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 Kass (Table 3 B). Reciprocity, on the other hand, was not related to total pollen deposition or legitimate fraction. Counter-intuitively, Reciprocity was negatively related to fruit set in the short-styled morph in Bhimashankar (Table 3 A).

Discussion

Reported here for the first time is a negative relationship between reciprocity and herkogamy, the two defining traits of style length polymorphism, in long-styled individuals of *Jasminum malabaricum*. Consistent with this negative relationship, the short-styled morph had lower reciprocity and higher herkogamy, while the long-styled morph had greater reciprocity and lower herkogamy. Surprisingly, reciprocity was not positively related to legitimate pollen transfer or fruit set. Higher herkogamy, on the other hand, was positively related to higher legitimate pollen transfer and fruit set under some circumstances. These results suggest that there is a trade-off between increasing legitimate pollen transfer between complementary morphs and avoidance of self-pollen deposition within a flower. Species may not be able to increase both at the same time, and in *J. malabaricum* herkogamy may be relatively more important than reciprocity.

Reciprocity was higher in the long- than in the short-styled morph, and this has been previously reported for species with stigma-height dimorphism (Santos-Gally et al. 2013). The result was contrary to the expectation that the long-styled morph might have a relaxed selection for perfect match due to higher pollen deposition and hence exhibit lower reciprocity (Haddadchi 2013). This suggests that increasing legitimate pollen transfer efficiency by having the pollinator pick up pollen on parts of its body to match the position of the long stigma is more important in the long- than in the short-styled morph of this species. Herkogamy was higher in the short-styled morph, consistent with the expectations that greater separation may be necessary for this morph to avoid self-pollen deposition (Cesaro et al. 2004, Haddadchi 2013). Greater herkogamy in the short-styled morph in species with stigma-height dimorphism has been commonly reported and has been cited as a reason for the origin and persistence of the short-styled morph in the genus *Narcissus* (Yeo 1975, Barrett et al. 1996, Liu et al. 2012, Ferrero et al. 2017).

Reciprocity was negatively related to herkogamy in individuals of the long-styled morph. In long-styled flowers of species with stigma-height dimorphism, mean stigma height is higher than the mean complementary anther height. Together with the observation that changes in anther heights were proportional to, but lower than the changes in stigma heights within flowers, the above suggests that increases in herkogamy are related to reduced reciprocity. Anthers are attached to the corolla tube in this species, and increasing anther height might require changes in corolla length, which may be developmentally constrained (Wessinger and Hileman 2016). Additionally, changes in corolla lengths may come at large fitness costs because of long term co-evolution with pollinators (Anderson and Johnson 2008).

There was no relationship between herkogamy and reciprocity in individuals of the short-styled morph with the exception of the Mulshi population where, like in the long-styled individuals, a negative relationship between herkogamy and reciprocity was observed. In the Bhimashankar population, there was no detectable relationship between herkogamy and reciprocity even when 100 short-styled individuals were examined. Thus, the lack of a relationship in the short-styled morphs is unlikely to be due to insufficient sample sizes. This lack of a significant relationship between anther and stigma heights within flowers of the short-styled morph is consistent with the observations of no relationship between herkogamy and reciprocity. If organ heights can change independently of each other within short-styled flowers, reciprocity may be able to change independently from herkogamy. Even when stigma heights are related to anther heights, if they change at the same rate, i.e. the slope of organ height relationships equals one, then herkogamy remains the same, and reciprocity may be independent of herkogamy. The Mulshi population showed a negative but non-significant relationship between anther and stigma heights within flowers in the short-styled morph and a corresponding negative relationship between herkogamy and reciprocity. These results are interesting as they suggest that synergistic pleiotropic relationships between sexual organs which are present in a large number of species (Smith 2015) can be lost in a morph and retained in another due to morph-specific selective pressures. The difference in the relationship between anthers and stigmas across populations also shows that these pressures can differ across populations due to local environmental factors (Santos-Gally et al. 2013).

The short-styled morph of *J. malabaricum* received significantly higher fractions of legitimate pollen as is commonly seen in heterostylous species (Liu et al. 2016, Wu et al. 2018). This could be because of the higher herkogamy and reduced self-pollen deposition. This can also arise because the short stigma can only be efficiently pollinated by long-tongued pollinators, which are very efficient pollinators (Haddadchi 2013, Simon-Porcar et al. 2014). However, the observed higher legitimate pollen load did not translate into higher fruit set. As both morphs are self-compatible, high fruit set can also be achieved due to fertilization by pollen from the same morph, and the large absolute number of pollen deposited on the long-styled morph may compensate for the low fraction of legitimate pollen. Actual rates of legitimate mating and estimation of inbreeding depression is needed to understand this better. The high herkogamy in the short-styled morph was enough to avoid autogamous self-pollen deposition in the morph as implied by the lack of change in legitimate fraction with emasculations (Liu et al. 2016). The decrease in total pollen load in both the morphs can be attributed to a significant reduction in self-pollen deposition. These results further highlight the effectiveness of herkogamy in avoiding self-pollen deposition in this species.

Higher herkogamy was related to higher legitimate pollen fraction and fruit set in the long-styled morph, and this could have been achieved by reducing self-pollen deposition. The positive relationship with fruit set also points towards high interference in male and female sex organs and potential inbreeding depression in the long-styled morph. This is also seen in other species with stigma-height dimorphism, where herkogamy increases fruit set by avoiding ovule sterility due to self-pollen deposition (Cesaro et al. 2004). Herkogamy was negatively related to total pollen load, and this may be because of reduced self-pollen deposition, consistent with the reduction in total pollen load seen in the emasculated flowers. The absence of a relationship between herkogamy and reproductive success in the short-styled morph can be a consequence of the presence of very high herkogamy in this morph, and thus minor changes in herkogamy do not influence pollen deposition or fruit set. Surprisingly, reciprocity was not related to any of the measures of pollen deposition or reproductive success, expect counterintuitively, negatively related to fruit set in the short-styled morph. While the likely causes of this are not clear, overall these results point towards the greater relative importance of herkogamy than of reciprocity in this species with stigma-height dimorphism.

That herkogamy was more important than reciprocity in promoting legitimate pollen transfer and fruit set has important implications for the stabilization and maintenance of imperfectly reciprocal intermediates. This will help increase legitimate, as compared to than illegitimate pollen transfer, and these results highlight the relative importance of herkogamy in influencing legitimate pollen. There is currently a large emphasis on reciprocity as the primary trait that promotes legitimate pollen transfer (Stone and Thomson 1994, Keller et al. 2014, Costa et al. 2017). However, there is evidence of sufficient legitimate pollen transfer in species with imperfect reciprocity like stigma-height dimorphism (Simón-Porcar et al. 2015).

This negative relationship observed in the long-styled morphs highlights the trade-off between maximizing legitimate pollen transfer and avoiding self-pollination and will be useful in providing insights into maximizing fitness under different ecological scenarios. For instance, if there is adequate legitimate pollen deposition due to high mate and pollinator availability, but there is also high inbreeding depression, herkogamy becomes relatively more important than reciprocity. In situations of pollen limitation due to the scarcity of mate and pollinators and absence of intra-morph incompatibility or inbreeding depression (Baker et al. 2000), reproductive assurance is important, and hence reciprocity may become relatively more important than herkogamy. Ultimately, mate and pollinator availability, intra-morph incompatibility and inbreeding depression (Van Rossum et al. 2006, Meeus et al. 2011), will determine the selective pressures that will influence the relative importance of reciprocity and herkogamy.

Table 1: Variation in herkogamy and reciprocity. Results from a two-way ANOVA testing for differences between the four study populations and the long- and short-styled morphs. For the Bhimashankar, Mulshi and Kaas populations, 30 individuals for each morph were examined. For the Trimbak populations 19 and 20 individuals for the long- and short-styled morphs, respectively, were examined.

Trait	**Effect**	***F*-statistics**	***P* value**
A) Reciprocity	Population	4.241	0.006
	Morph	34.825	< 0.001
	Morph x Population	0.105	0.957
B) Herkogamy	Population	2.13	0.097
	Morph	24.58	< 0.001
	Morph x Population	0.25	0.864

Table 2: Comparison of the reciprocity index (RI) used in this study with previously proposed indices: R & K (modified from Richards and Koptur, 1993); L & B (Lau & Bosque, 2003) ; SAN (Sanchez, Ferrero, & Navarro, 2008); and, ARM (Armbruster et al., 2017). Reciprocity was calculated for the stigma of each morph to estimate the complementarity to the anthers of the opposite morph for the long-styled morph (L), short-styled morph (S), or for the entire population (P). The index proposed by Sanchez, Ferrero, & Navarro (2008) is a composite reciprocity value for the population that does not allow us to examine reciprocity for the two morphs independently.

Populations	**Level**	**RI**	**R & K**	**L & B**	**SAN**	**ARM**
A) Trimbak	L	0.65	0.86	0.00	-	22.81
	S	0.42	0.65	0.00	-	31.15
	P	0.54	0.76	0.00	0.14	26.98
B) Bhimashankar	L	0.59	0.85	0.13	-	27.01
	S	0.42	0.64	0.00	-	34.77
	P	0.51	0.75	0.07	-0.07	30.89
C) Mulshi	L	0.58	0.86	0.03	-	27.23
	S	0.41	0.64	0.00	-	33.75
	P	0.50	0.75	0.02	0.03	30.49
D) Kaas	L	0.47	0.84	0.06	-	35.56
	S	0.29	0.60	0.00	-	43.00
	P	0.38	0.72	0.03	-0.13	39.28

Table 3: 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 Ψ, respectively, next to the Spearman's rank-order correlation coefficients presented. The number of individuals sampled for the long- and short-styled morph for the two populations is 29 and 29 for Bhimashankar, and 32 and 31for Kaas.

Population	**Trait**	**Morph**	**Total pollen load**	**Legitimate fraction**	**Fruit set**
A) Bhimashankar	Reciprocity	L	-0.06	-0.20	-0.13
		S	-0.04	0.14	-0.39*
	Herkogamy	L	0.11	0.43*	0.42*
		S	-0.11	-0.17	0.01
B) Kaas	Reciprocity	L	0.19	-0.03	
		S	-0.18	0.18	
	Herkogamy	L	-0.34Ψ	0.14	
		S	-0.27	-0.04	

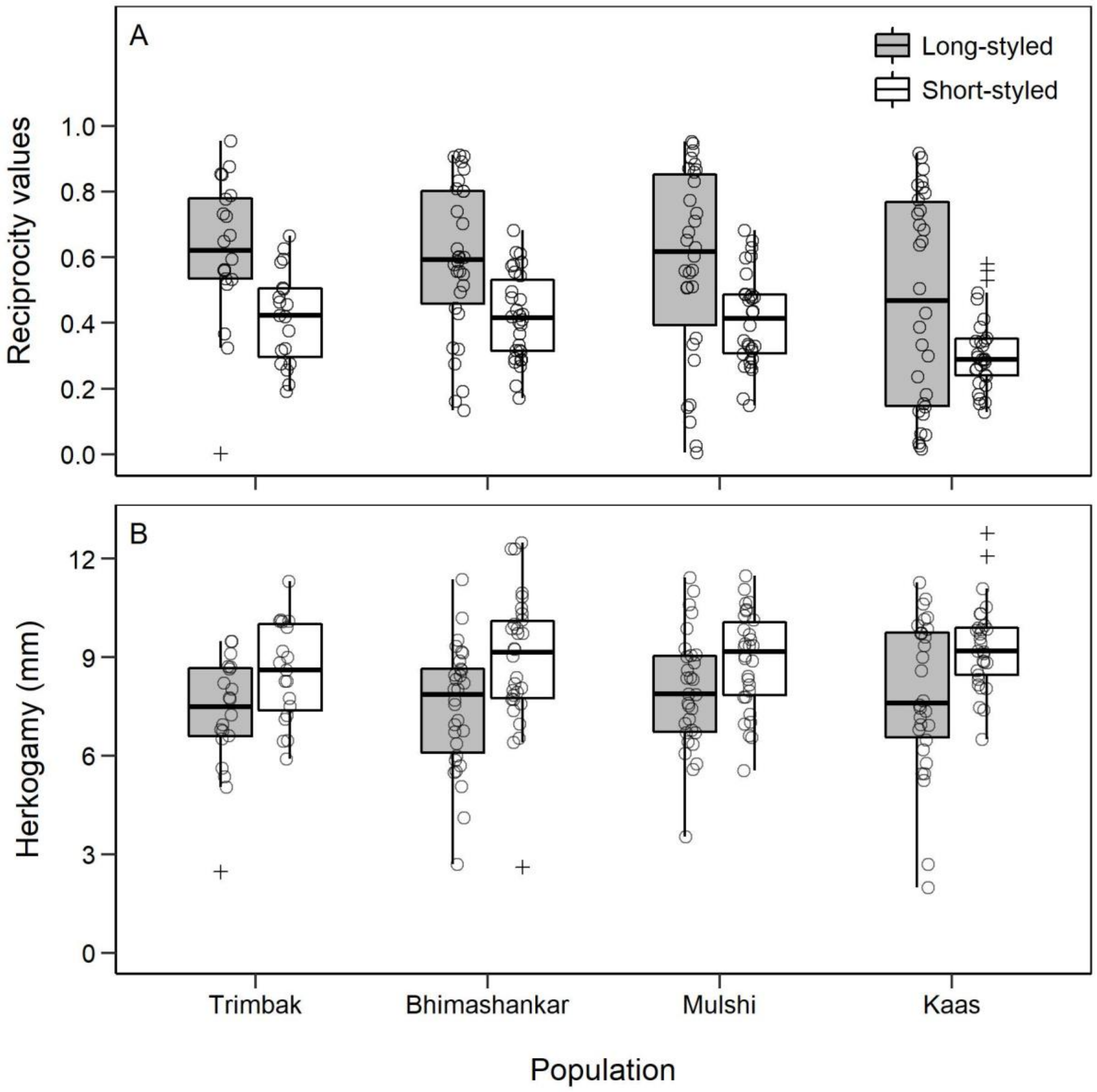

Figure 1: A) Reciprocity and B) herkogamy in the long-styled (Dark grey boxes) and short-styled (Open boxes) morphs of the four study populations. The number of individuals sampled for the long- and the short-styled morph for the populations are 20 and 19 for Trimbak; 30 and 30 for Bhimashankar, Mulshi, 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.

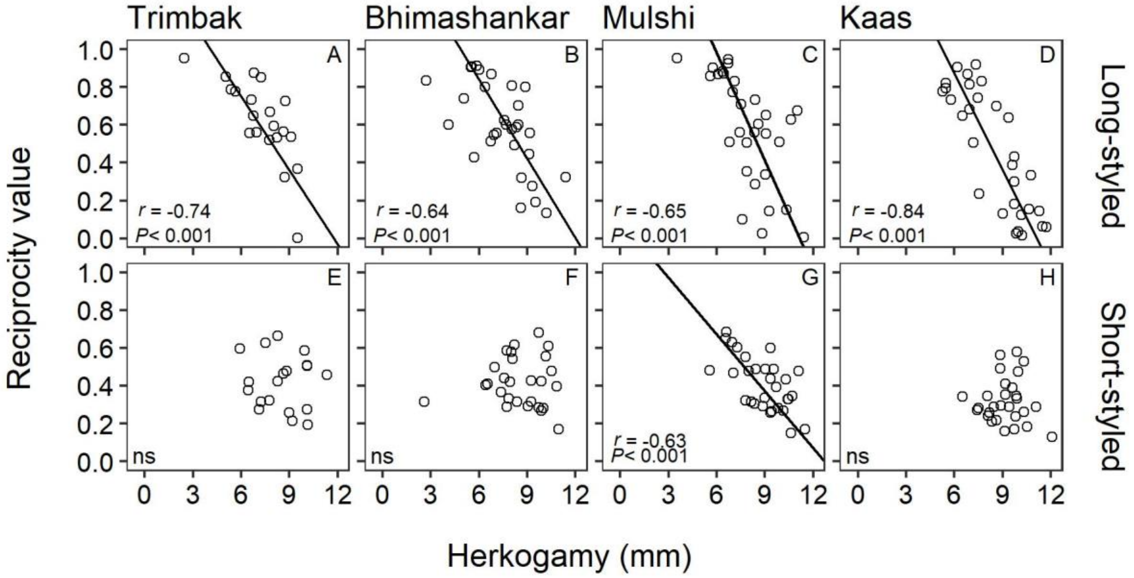

Figure 2: Relationship between herkogamy and reciprocity in the two organ levels (long-styled morph: A, B, C, D; short-styled morph: E, F, G, H) in the four study populations. Pearson's correlation coefficient (*r*), and the corresponding *P*-value are shown where $P < 0.05$, and ‘ns’ denotes not significant. 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.

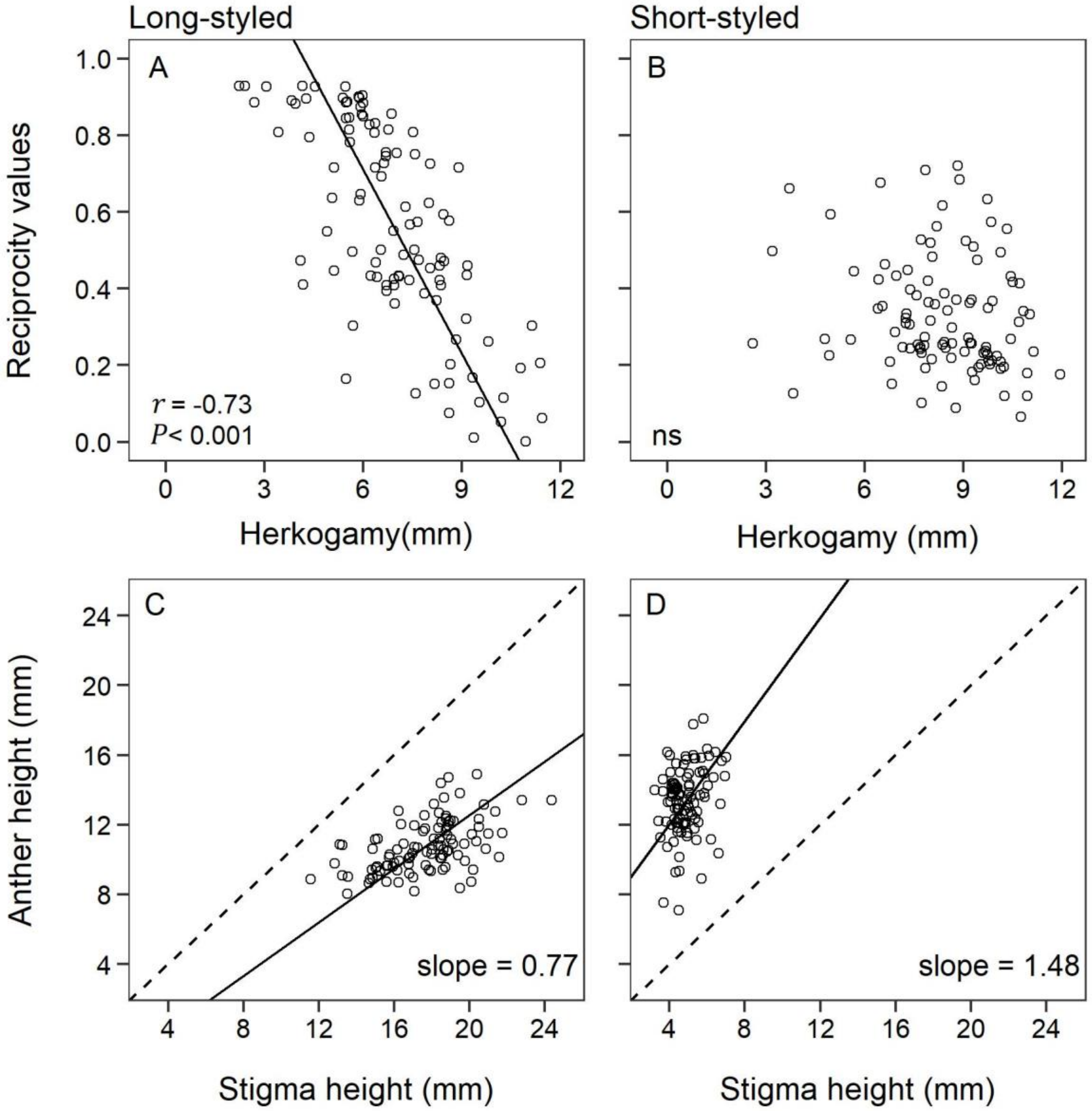

Figure 3: Relationship between herkogamy and reciprocity in the A) long-styled morph and B) short-styled morph for individuals from Bhimashankar. Pearson's correlation coefficient (*r*) and the corresponding *P*-value are shown where $P < 0.05$, and ‘ns’ denotes not significant. Relationship between stigma and anther height in C) long-styled, and D) short-styled morphs from Bhimashankar. The slope for type II regression is shown where $P < 0.05$. The dotted line is a reference line for slope = 1. A total of 100 individuals each were sampled for the long- and the short-styled morph.

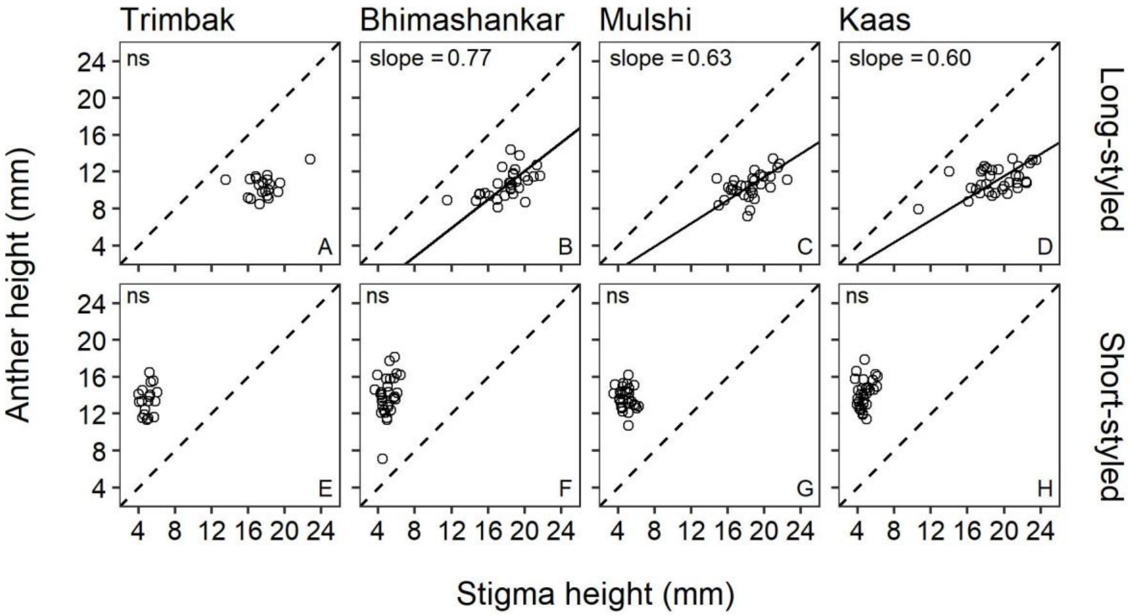

Figure 4: Relationship between stigma and anther height in the long-styled (A, B, C, D) and short-styled (E, F, G, H) morphs from the four study populations. The slope for type II regression is provided for significant relationships where $P < 0.05$, and 'ns' denotes not significant. The dotted line is a reference line for slope = 1. 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.

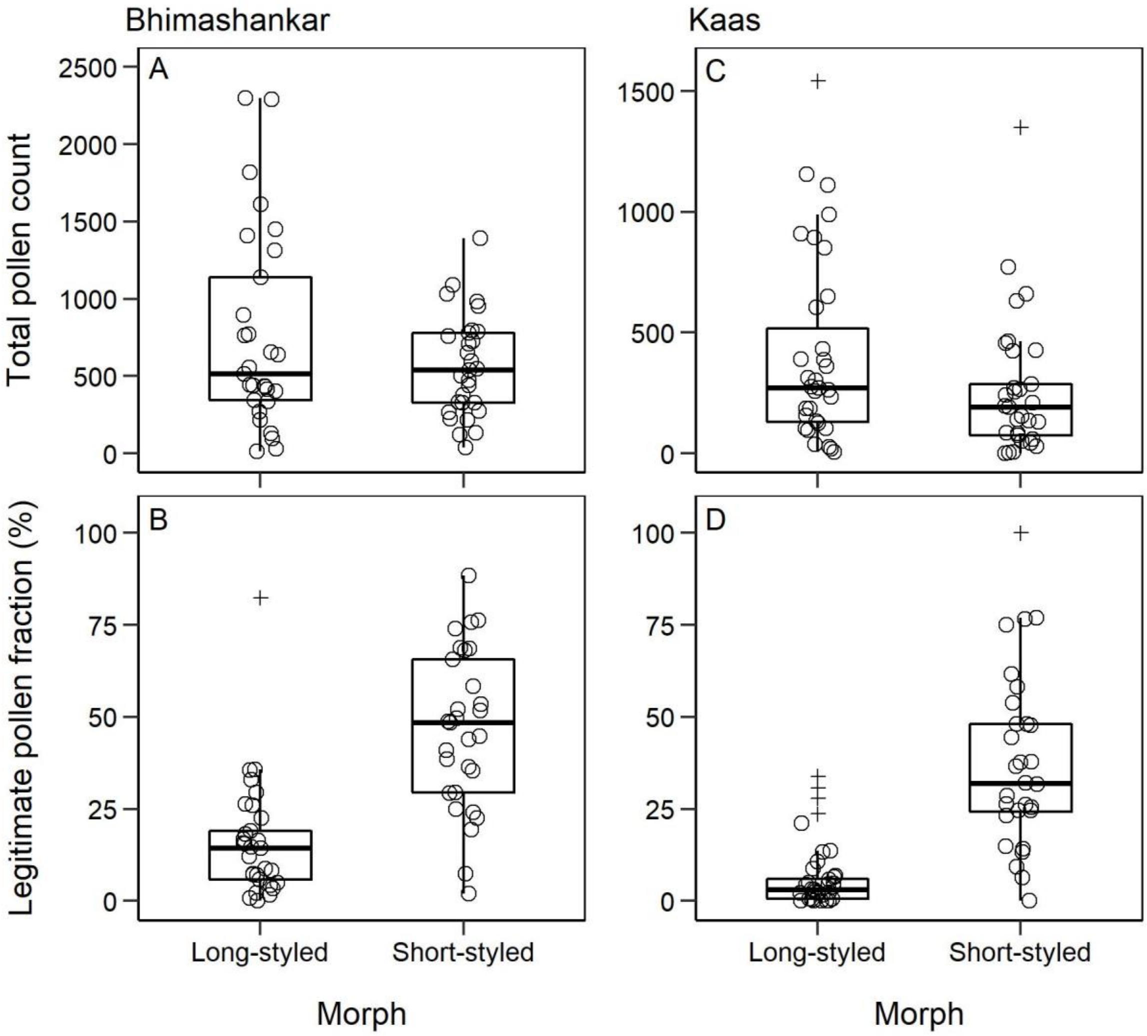

Figure 5: The total stigma pollen load (A, C) and the legitimate pollen fraction (B, D) in the Bhimashankar and Kaas populations. The number of individuals sampled for the long- and short-styled morph is 29 and 29 for Bhimashankar, and 32 and 31 for Kaas. One extreme outlier value of greater than 5000 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.

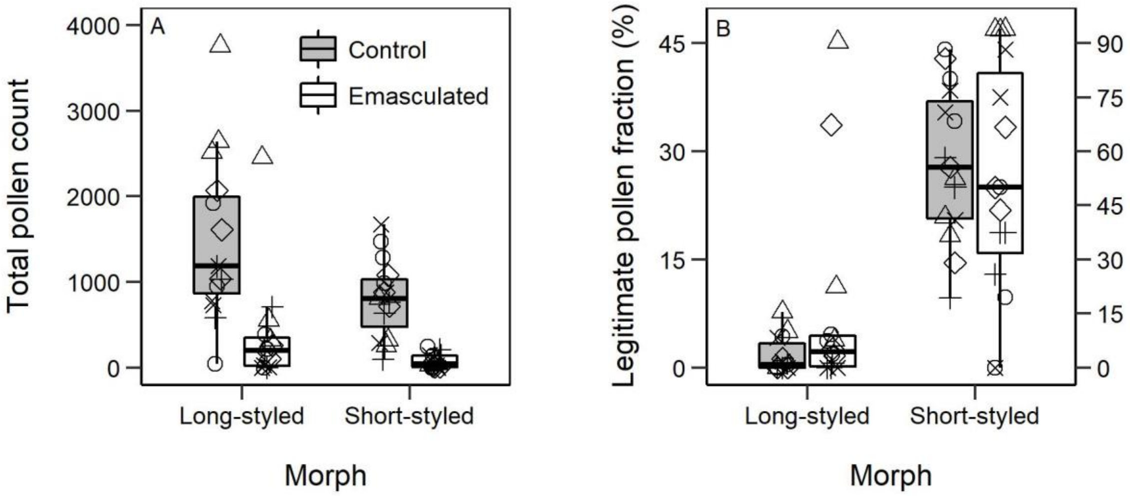

Figure 6: A) The total pollen load ,and B) the legitimate pollen fraction in control (non-emasculated, grey boxes) and emasculated flowers (open boxes) to determine autogamous pollen deposition. One point from the legitimate pollen fraction for the emasculated group of long-styled morph (80%) has been 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.

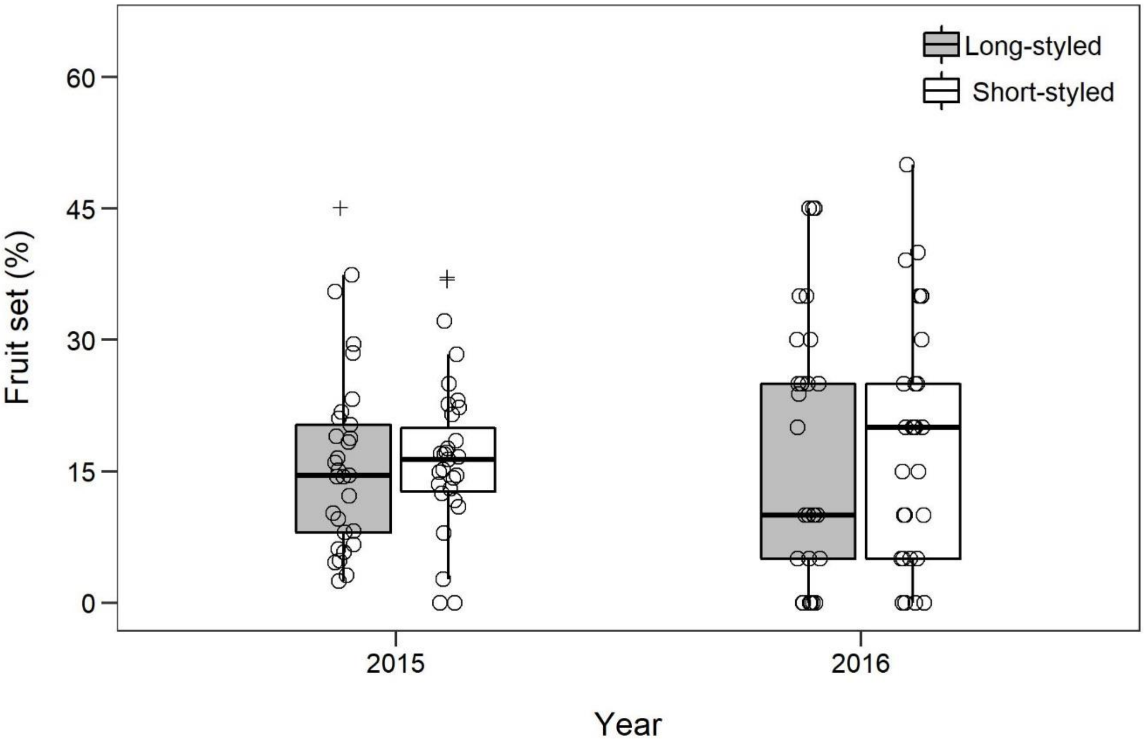

Figure 7: Natural fruit set in long- and short-styled individuals from the Bhimashankar population measured as the percentage fruit formed per flower in 2015 (grey boxes) and 2016 (open boxes). The number of individuals sampled for the long- and the short-styled morph are 29 and 29 respectively. The 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.

Chapter 6

Conclusions

In this study, the information on sex organ positions for a large number of species with style length polymorphism was compiled to view broad patterns in relative sex organ positions. The short-styled morph had significantly higher herkogamy in distylous species as per the expectations of a higher chance of self-pollen deposition in this morph. In opposition to the expectations, reciprocity was not significantly different between the levels. Herkogamy showed a stronger phylogenetic signal than reciprocity, suggesting that the former can be less responsive to selection pressures. Herkogamy and mismatch (the opposite of reciprocity) were found to be both negatively and positively related across individuals of a morph in a population. This has implications for the optimisation of the functions of the two traits in these species. Substantial intra-population variation in sex organ dimensions was observed in species with style length polymorphism, which has consequences for reciprocity between individuals of the opposite morphs. The index developed in this study appropriately incorporates intra-population variation in sex organ dimensions and captures the predicted pattern of change in reciprocity with an increase in intra-population variation, which is not done by previously proposed indices. The index showed the capability to predict fitness in terms of seed set as well. The current index was also helpful in calculating reciprocity for individuals for the empirical study on the naturally occurring populations of *Jasminum malabaricum*, a species with stigma-height dimorphism. This allowed us to examine the relationship of herkogamy and reciprocity to reproductive success across individuals of both the morphs. In this species, herkogamy showed a positive relationship with legitimate pollen fraction of the stigmatic pollen and fruit set while reciprocity showed no relationship except a counterintuitive negative relationship with fruit set in the short-styled morph.

Floral traits, especially sex organs, are assumed to have low intra-population variation as the latter decreases the match between flowers and also between flowers and pollinators (Berg 1960, Armbruster et al. 1999, Hansen et al. 2007, Pérez-Barrales et al. 2007). In opposition to the expectations based on the current understanding, substantial intra-population variation in sex organ dimensions was observed. The index developed in this study showed that when the difference in mean complementary sex organ heights is high, the increase in reciprocity can occur with an increase in intra-population variation in sex organ dimensions. This pattern was observed in the simulated as well as naturally occurring populations of distylous species. The result was also corroborated by the positive relationship between the difference in mean complementary sex organ heights and intra-population variation in the high level in species exhibiting distyly and stigma-height dimorphism. The relationship was found to be much stronger in species with stigma-height dimorphism. This emphasises the adaptive benefit of high intra-population variation in sex organs in species with style length polymorphism, particularly in species with the imperfect reciprocal arrangement of sex organs. It also explains the persistence of the substantial intra-population variation in sex organs in these species. This is the first study which tries to understand the ability of reciprocity indices to predict reproductive success. The developed index successfully predicts seed set across species. Although the indices proposed by Sanchez et al. (2008) and Armbruster et al. (2017) also showed the same pattern, they do not capture the change in reciprocity with an increase in intra-population variation correctly.

The short-styled morph had significantly higher herkogamy in distylous species. This is in accordance with the prediction that the short-styled morph has higher chances of self-pollen deposition as the pollinator already brushes past the anthers before reaching the stigma of the flower (Webb and Lloyd 1986). The higher herkogamy can reduce illegitimate pollen deposition on the short-styled stigma (Nishihiro and Washitani 1998, Cesaro et al. 2004). The pattern also supports the hypothesis of the invasion of the short-styled morph into the long-styled population as the former had lower interference of male and female sex organs due to high herkogamy (Yeo 1975). Herkogamy also showed higher phylogenetic signal than reciprocity, which indicates that it will be less pliable than reciprocity in response to selection pressures. Unlike herkogamy, reciprocity was not different between the levels. This can happen when reciprocity is similar in the two levels or when higher reciprocity in either level is equally common. In the empirical study, the four naturally occurring populations with stigma-height dimorphism showed significantly higher herkogamy in the short-styled morph, surprisingly conforming to the results of the larger study on heterostylous species (Chapter 2). Additionally, reciprocity was found to be higher in the higher level, which is again not seen with regards to stigma-height dimorphism in the larger study. The lack of any significant difference between levels and morphs in species with stigma-height dimorphism in the larger study could be the result of low sample size. Although reciprocity was higher in the high-level in *J. malabaricum*, the short-styled morph had a significantly higher legitimate fraction of pollen deposited. Moreover, the emasculated flowers only of the short-styled morph showed no difference in legitimate pollen fraction of the stigmatic pollen load. Both these results confirm that herkogamy effectively reduces self-pollen deposition in short-styled, but not in the long-styled morph (Liu et al. 2016). This highlights the importance of (a) herkogamy in increasing the efficiency of legitimate pollen transfer, and (b) morph-specific differences in selection pressures modulating the degree of herkogamy in species with style length polymorphism.

Reciprocity and herkogamy increase the efficiency of pollen transfer by different mechanisms. Herkogamy reduces the interference between male and female sex organs and self-pollen deposition, thereby reducing pollen wastage and stigma clogging (Barrett 2002). Reciprocity increases the precision of the pick-up and deposition of pollen by the pollinator (Barrett 2002). The relationship between reciprocity and herkogamy could be explored in this study, as reciprocity was calculated for an individual. A negative relationship between the two points towards a trade-off between these two functions. A positive relationship denotes that the two functions can be augmented simultaneously. The relationship is partly influenced by the relationship between anther and stigma within a flower. Floral parts are known to be highly integrated (Smith 2015), and any change in the relationship between herkogamy and reciprocity will require a change in the direction or magnitude of the relationship between stigma and anther height within a flower. As found in almost all the species with stigma-height dimorphism in the larger study (Chapter 2: Table 8), the naturally occurring populations of *J. malabaricum* also show a negative relationship between herkogamy and reciprocity in the long-styled morph. However, no relationship was seen in the short-styled morph in three out of the four populations. This also shows that morph-specific differences in herkogamy and reciprocity and their relationship can exist due to different selection pressures acting on them (Kálmán et al. 2007).

The functional importance of herkogamy in species with style length polymorphism has rarely been highlighted (but see Nishihiro and Washitani 1998, Cesaro et al. 2004, Liu et al. 2016). On the other hand, the importance of reciprocity in increasing legitimate pollen transfer and fruit set has been studied extensively in distylous species (Ferrero et al. 2011, Keller et al. 2012, Jacquemyn et al. 2018). Still, the role of reciprocity in increasing legitimate pollen transfer in species with stigma-height dimorphism, which has imperfectly reciprocal sex organs between complementary morphs, is not properly understood (but see Thompson et al. 2012). Moreover, the effect of change in reciprocity on reproductive success should be studied at the level of an individual as selection works at the level of an individual. Additionally, comparison of the effect of an increase in reciprocity on pollen transfer across individuals of a population as opposed to between populations, controls for the environmental differences that might occur between the populations. The index developed in this study allows the calculation of reciprocity for individuals which previous indices cannot calculate. Herkogamy was found to be positively related to the fraction of legitimate pollen on the stigma and consequently also to the fruit set. Reciprocity was not related to legitimate pollen fraction of the stigmatic pollen load and was surprisingly negatively related to fruit set. Herkogamy was also found to be efficient in reducing self-pollen deposition in the short-styled morph, as mentioned earlier. All the above results indicate that herkogamy is more important in *J. malabaricum,* which exhibits stigma-height dimorphism. This emphasizes the importance of herkogamy in the stabilization of style length polymorphism in general and imperfectly reciprocal character states moving towards heterostyly in particular.

Reciprocity and herkogamy are both important to understand legitimate pollen transfer. The effect of herkogamy on legitimate pollen transfer was not examined using the developed index. This is because reciprocity and herkogamy affect legitimate pollen transfer by different mechanisms, and it is difficult to have an equivalent currency for both to combine them in an index. Additionally, the effect of the degree of herkogamy on the chances of self-pollen deposition and illegitimate reciprocity with other individuals of the same morph was also not explored. Similar to the previous issue, self-pollen deposition and intra-morph pollen deposition cannot be combined in an index as they are affected by traits like self- and intra-morph physiological incompatibility. For example, in *Narcissus assoanus*, the plants are incompatible to the pollen from the same individual but compatible with the pollen from other individuals of the same morph (Baker et al. 2000). Increase in illegitimate reciprocity with an increase in intra-population variation in sex organ heights was also not examined here. As mentioned earlier, this is because the effect of illegitimate reciprocity is contingent on physiological incompatibility. The maximum value of mismatch at which pollen transfer success was considered to be zero was assumed to be the length of the anther for all species. The lack of information on the distribution of pollen on the body of the pollinator and its consequences on the deposition of pollen on the stigma is the primary reason for making a general assumption (but see Washitani et al. 1994, Adler 2005). The lack of studies on the subject can be attributed to the difficult nature of the process of quantification of the amount of pollen on the body of a pollinator under natural conditions. The long- and the short-styled morphs can also be visited by different pollinators, which leads to disparate pollen transfer between the levels. Although such information was not taken into account during this study, these differences can be incorporated in the proposed index, unlike any of the previously proposed indices. As the value of reciprocity is calculated separately for the high and the low level, separate pollen transfer functions and the upper threshold of mismatch at which pollen transfer success falls to zero, can be assumed during the calculations to account for differences in pollinators.

The difference in herkogamy in the two morphs indicates morph-specific selection pressures. Such general patterns over a large number of species can help reveal the actual cause of the origin and persistence of the short-styled morph in a population and can help in better comprehension of the causes of the evolution of style length polymorphism. More studies along these lines can elucidate if the primary cause of the origin of a second morph was to increase the efficiency of pollen transfer or to decrease the interference between male and female sexual organs and self-pollen deposition. Although a lot of research on relative sex organ positions has been done on distylous species, stigma-height dimorphism has not been studied as widely. This study suggests that the stabilization of stigma-height dimorphism by facilitating legitimate pollen transfer can happen by a different mechanism as compared to distyly, which makes it a very interesting study system. The genus *Jasminum* with all the three character-states viz. approach herkogamy, stigma-height dimorphism and distyly and also pollen size dimorphism presents a great opportunity for both ecological and evolutionary studies. Further empirical work on *J. malabaricum* continuing from this study can involve quantification of actual inbreeding depression and rate of legitimate mating to better understand the lack of difference in fruit set despite seeing differences in legitimate pollen fraction of the stigmatic pollen load. This study also provides a fresh perspective on the effects of intrapopulation variation in sex organ dimensions on pollen transfer. It paves the way for more exploration of the benefits of high intra-population variation in sex organ heights. Further studies on its role in the stabilization and maintenance of style length polymorphism, especially when the arrangement of complementary sex organs is not reciprocal, could be a significant step in this direction.

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Appendix Table S1: Mean and SD of stigma height, anther height and herkogamy (in mm) of the species used in chapter 1. The character states included are distyly (D) or stigma-height dimorphism (SHD). Sample size is denoted by *n*. Data type denotes whether the species was included in the analysis for mean sex organ positions (M) or both mean and distribution of sex organ positions for individuals of a population (M, I). Reference denotes the serial number for the study from which data was extracted as per the Appendix References.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Abeliophyllum distichum	D	L	30	4.29	0.17	2.99	0.12	-	-	M	(1)
		S	30	2.08	0.12	3.85	0.56	-	-		
Amsinckia douglasiana	D	L	10	13.40	0.94	5.30	0.48	-	-	M	(2)
		S	10	5.60	0.44	14.20	0.86	-	-		
Amsinckia grandiflora	D	L	10	13.90	-	4.11	-	-	-	M	(2)
		S	10	3.32	-	11.92	-	-	-		
Amsinckia vernicosa	D	L	18	14.00	0.81	6.40	0.38	-	-	M	(2)
		S	18	6.90	0.57	14.20	0.93	-	-		
Anchusa officinalis	SHD	L	76	7.80	0.70	6.00	0.50	-	-	M	(3)
		S	47	4.60	0.30	6.90	0.60	-	-		
Arcytophyllum aristatum	D	L	12	5.40	0.50	3.80	0.20	1.70	0.50	M, I	(4)
		S	12	4.20	0.20	5.50	0.50	1.30	0.40		
Arcytophyllum capitatum	D	L	17	7.50	1.00	3.40	0.50	4.10	1.20	M, I	(4)
		S	16	3.60	0.60	6.30	0.70	2.70	0.90		
Arcytophyllum ciliolatum	D	L	12	4.60	0.50	2.60	0.30	2.00	0.40	M, I	(4)
		S	12	2.30	0.40	4.80	0.70	2.50	0.60		
Arcytophyllum filiforme	D	L	10	5.30	1.00	4.00	0.20	1.30	0.60	M, I	(4)
		S	10	3.40	0.60	4.70	0.90	1.30	0.80		

Table S1 continued.

Species	Character state	Morph	*n*	Stigma	SD	Anther	SD	Herkogamy	SD	Data type	Reference
Arcytophyllum lavarum	D	L	104	5.00	0.80	3.50	0.70	-	-	M, I	(5)
		S	100	2.70	0.50	5.60	0.80	-	-		
Arcytophyllum rivetii	D	L	11	5.60	0.80	3.50	0.60	2.00	0.60	M, I	(4)
		S	10	3.00	0.40	5.30	0.60	2.20	0.60		
Arcytophyllum setosum	D	L	10	8.10	0.80	5.40	0.60	2.80	0.80	M	(4)
		S	10	4.20	0.80	7.40	0.80	3.20	0.70		
Arcytophyllum thymifolium	D	L	11	6.70	0.60	3.10	0.10	3.50	0.50	M	(4)
		S	10	3.40	0.80	6.40	0.70	3.00	0.80		
Arcytophyllum vernicosum	D	L	14	5.10	0.50	2.80	0.40	2.30	0.50	M	(4)
		S	14	3.20	0.40	5.00	0.90	1.80	1.10		
Arnebia szechenyi	D	L	20	13.72	1.30	8.91	0.72	-	-	M	(6)
		S	20	7.17	0.54	13.57	1.03	-	-		
Bouvardia ternifolia	D	L	25	27.50	2.60	20.70	2.50	6.60	1.00	M, I	(7)
		S	25	21.00	2.10	28.40	2.70	7.40	2.30		
Carapichea ipecacuanha	D	L	31	7.20	0.80	3.90	0.40	3.21	0.49	M, I	(8)
		S	33	3.20	0.50	6.40	0.70	3.11	0.55		
Chassalia corallioides	D	L	25	13.18	1.23	10.79	1.39	3.45	1.06	M, I	(9)
		S	25	7.61	1.46	14.00	1.60	9.35	1.82		
Chlorogalum angustifolium	D	L	70	7.67	1.17	7.32	0.28	-	-	M	(10)
		S	35	3.55	0.53	6.87	0.64	-	-		
Cordia curassavica	D	L	21	7.09	0.44	5.56	0.51	1.53	0.59	M, I	(11)
		S	8	4.74	0.52	6.84	0.60	2.10	0.69		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Cordia dodecandra	D	L	8	38.60	2.70	27.90	1.60	7.90	2.50	M	(12)
		S	10	26.10	3.20	35.31	4.00	5.70	3.70		
Cordia macrocephala	D	L	22	13.69	1.35	9.15	1.13	4.54	1.16	M, I	(11)
		S	17	11.27	0.94	12.37	1.12	1.12	0.71		
Cordia nodosa	D	L	14	8.90	0.30	6.00	0.40	2.80	0.30	M, I	(11)
		S	7	7.00	0.60	8.40	0.80	1.30	0.60		
Cordia panamensis	D	L	12	6.85	0.85	3.79	0.51	3.06	0.74	M, I	(11)
		S	8	3.80	0.30	5.16	0.48	1.37	0.43		
Cordia sebestena	D	L	9	31.30	2.70	25.80	2.40	6.00	1.90	M	(12)
		S	11	25.30	2.20	31.90	2.10	5.30	2.40		
Coussarea platyphylla	SHD	L	50	43.18	9.31	37.76	7.99	9.73	7.22	M, I	(13)
		S	47	28.31	3.42	45.24	6.29	17.03	5.92		
Cratoxylum formosum	D	L	3	10.40	-	8.20	-	-	-	M	(14)
		S	3	4.40	-	11.60	-	-	-		
Cryptantha flavoculata	D	L	34	9.96	0.70	5.48	0.52	-	-	M	(15)
		S	34	5.57	0.52	7.79	0.64	-	-		
Damnacanthus macrophyllus	D	L	11	11.68	1.43	8.83	0.99	2.85	0.75	M, I	(16)
		S	11	7.50	0.89	10.63	1.04	3.13	0.75		
Damnacanthus major	D	L	10	13.48	1.65	7.57	1.20	5.92	1.71	M, I	(16)
		S	12	8.56	1.35	11.05	1.04	2.49	1.06		
Declieuxia fruticosa	D	L	84	5.46	0.71	3.22	0.43	2.20	0.56	M, I	(17)
		S	90	3.53	0.59	5.53	0.81	2.01	0.79		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Dionysia aretioides	D	L	2	14.50	-	7.75	-	6.75	-	M	(18)
		S	2	7.75	-	15.50	-	7.75	-		
Dionysia bryoides	D	L	2	11.00	-	4.75	-	6.25	-	M	(18)
		S	2	4.75	-	9.50	-	4.75	-		
Dionysia hissarica	D	L	2	14.00	-	7.00	-	7.00	-	M	(18)
		S	2	7.00	-	14.00	-	7.00	-		
Dionysia lurorum	D	L	2	13.00	-	6.00	-	7.00	-	M	(18)
		S	2	4.75	-	14.00	-	9.25	-		
Dionysia tapetodes	D	L	2	11.75	-	7.75	-	4.00	-	M	(18)
		S	2	4.75	-	11.50	-	6.75	-		
Fagopyrum esculentum	D	L	25	2.58	0.15	1.73	0.10	-	-	M	(19)
		S	25	1.41	0.10	2.51	0.15	-	-		
Faramea occidentalis	D	L	50	13.35	1.51	9.48	1.25	3.87	0.85	M, I	(20)
		S	50	6.78	1.09	12.94	1.11	6.17	1.66		
Faramea suerrensis	D	L	21	7.32	0.42	5.27	0.55	2.05	0.63	M, I	(21)
		S	11	2.96	0.27	8.48	0.76	5.52	0.65		
Gaertnera vaginata	D	L	25	22.12	1.57	13.69	1.38	8.43	1.55	M, I	(22)
		S	25	11.68	1.33	18.41	2.01	6.78	1.92		
Galianthe peruviana	D	L	50	3.80	0.57	2.17	0.42	1.61	2.4	M	(17)
		S	98	2.13	0.68	3.34	0.62	1.28	1.04		
Galianthe valerianoides	D	L	72	5.03	0.57	3.46	0.46	1.57	0.57	M	(17)
		S	38	2.76	0.52	4.94	0.85	2.17	0.63		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Gelsemium elegans	D	L	10	12	-	5	-	-	-	M	(23)
		S	10	7	-	11	-	-	-		
Gelsemium sempervirens	D	L	50	28.13	3.846	14.7	2.326	13.43	4.651	M	(17)
		S	98	15.87	3.6339	23.73	3.041	8.24	4.598		
Glandora diffusa	D	L	54	11.39	1.2529	7.11	1.067	0.71	-	M, I	(24, 25)
		S	46	7.36	0.9568	12.1	1.331	0.25	-		
Glandora diffusa	SHD	L	44	10.05	1.26	7.05	0.96	3.01	0.76	M, I	(26, 27)
		S	54	5.73	0.76	10.15	1.59	4.42	1.44		
Glandora moroccana	D	L	51	14.01	1.401	7.96	0.876	1.29	-	M, I	(24, 25)
		S	49	7.47	1.1205	12.72	1.526	0.49	-		
Glandora moroccana	SHD	L	51	14.02	1.41	7.97	0.87	6.05	1.13	M, I	(26, 27)
		S	49	7.44	1.07	12.68	1.49	5.25	1.08		
Glandora nitida	D	L	34	12.14	1.21	6.41	1.03	0.61	-	M	(24)
		S	40	5.82	0.81	11.53	1.84	0.59	-		
Glandora oleifolia	D	L	27	13.85	1.39	8.01	0.72	0.37	-	M, I	(24, 25)
		S	37	7.43	1.49	13.48	1.62	0.59	-		
Glandora oleifolia	SHD	L	30	13.84	1.67	8.21	0.94	5.59	1.78	M, I	(26, 27)
		S	38	7.56	1.56	13.39	1.68	5.84	1.86		
Glandora prostrata	SHD	L	49	11.18	1.23	6.72	1.34	1.16	-	M, I	(24, 25)
		S	50	6.03	0.90	10.02	1.90	0.69	-		
Glandora prostrata subsp. lusitanica	D	L	44	11.03	1.32	7.00	1.40	1.78	-	M	(24)
		S	52	5.86	1.11	9.25	1.67	1.14	-		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Glandora rosmarinifolia	D	L	56	13.83	1.52	7.56	0.91	1.04	-	M, I	(24, 25)
		S	40	8.04	1.53	12.79	1.92	0.48	-		
Goniolimon italicum	D	L	27	7.63	0.49	6.16	0.56	1.47	0.41	M, I	(28)
		S	20	6.09	0.50	6.98	0.40	0.89	0.43		
Guettarda platypoda	D	L	10	16.79	2.20	15.62	1.69	-	-	M	(29)
		S	10	11.11	1.96	17.90	2.49	-	-		
Guettarda platypoda	SHD	L	10	10.21	8.80	9.48	3.90	-	-	M, I	(30)
		S	10	6.02	8.40	9.67	3.70	-	-		
Guettarda scabra	D	L	38	16.90	3.30	14.00	2.50	2.00	2.00	M	(31)
		S	53	13.50	2.20	17.60	3.10	4.10	0.70		
Guettarda speciosa	D	L	74	32.45	3.40	27.00	3.56	2.01	1.29	M	(32)
		S	42	18.77	2.60	36.21	5.26	15.40	3.69		
Houstonia caerulea	D	L	380	5.95	1.96	3.16	0.92	-	-	M, I	(33, 34)
		S	400	3.79	1.25	6.62	2.18	-	-		
Houstonia longifolia	D	L	47	5.53	0.46	2.21	0.20	3.30	0.38	M, I	(34)
		S	42	2.34	0.29	4.65	0.43	2.32	0.35		
Houstonia procumbens	D	L	94	8.53	0.96	5.89	0.51	2.65	0.85	M, I	(34)
		S	81	5.34	0.76	9.13	0.75	3.83	0.90		
Jasminum fruticans	D	L	34	11.81	1.29	6.31	0.83	5.50	1.12	M, I	(35)
		S	33	6.18	1.04	8.85	0.95	2.67	1.05		
Jasminum malabaricum	SHD	L	100	17.65	2.29	10.78	1.49	7.58	2.01	M, I	This study
		S	100	4.85	0.80	13.36	1.93	9.17	1.70		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Jasminum odoratissimum	D	L	27	13.70	3.60	11.90	1.60	3.90	0.10	M	(36)
		S	27	8.60	1.10	15.60	1.10	5.00	1.80		
Jepsonia parryi	D	L	27	4.80	-	2.30	-	-	-	M	(37)
		S	27	2.40	-	4.60	-	-	-		
Kalmiopsis fragrans	SHD	L	41	12.75	1.32	9.45	1.36	3.39	1.57	M, I	(38)
		S	41	6.36	0.88	14.71	1.10	8.35	1.35		
Leptodermis lanceolata	D	L	27	15.00	1.00	8.00	1.00	-	-	M	(39)
		S	29	8.00	1.00	15.00	1.00	-	-		
Linum aretioides	D	L	30	5.03	0.09	2.27	0.07	-	-	M, I	(40)
		S	30	3.07	0.04	6.05	0.07	-	-		
Linum campanulatum	D	L	23	13.44	1.18	10.04	1.26	3.40	0.86	M, I	(35)
		S	23	8.28	1.01	13.48	1.04	5.20	1.09		
Linum grandiflorum	SHD	L	73	11.86	1.08	12.74	1.01	-	-	M, I	(41, 42)
		S	73	8.11	0.83	14.27	1.16	-	-		
Linum narbonense	D	L	15	5.01	0.11	3.28	0.03	-	-	M	(43)
		S	15	3.11	0.08	4.84	0.16	-	-		
Linum perenne	D	L	15	5.51	0.01	3.44	0.04	-	-	M	(43)
		S	15	3.11	0.09	4.84	0.16	-	-		
Linum pubescens	D	L	84	13.30	-	7.80	-	-	-	M	(44)
		S	80	8.20	-	10.80	-	-	-		
Linum suffruticosum	D	L	32	6.31	0.92	4.19	0.55	2.12	0.90	M, I	(45)
		S	32	3.64	0.89	6.79	0.67	3.14	0.30		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Linum tenue	D	L	34	6.09	0.45	4.35	0.22	1.75	0.39	M, I	(45)
		S	34	2.47	0.39	6.79	0.39	4.32	0.37		
Linum tenuifolium	D	L	67	7.61	0.93	5.87	0.72	1.74	0.56	M, I	(46)
		S	59	5.81	0.79	7.76	0.78	1.95	0.55		
Linum thracicum	D	L	15	8.70	0.04	5.13	0.03	-	-	M	(43)
		S	15	3.74	0.01	8.94	0.10	-	-		
Lithodora fruticosa	D	L	53	9.53	1.24	7.25	0.94	1.79	-	M	(26)
		S	47	4.71	0.99	7.74	0.85	2.54	-		
Lithodora fruticosa	SHD	L	53	9.53	1.24	7.25	0.94	1.79	-	M, I	(24, 27)
		S	44	4.71	0.99	7.74	0.85	2.54	-		
Lithodora hispidula	D	L	44	9.34	1.21	6.45	0.77	0.47	-	M	(24)
		S	56	6.48	0.91	8.87	1.06	0.03	-		
Lithodora hispidula	SHD	L	51	10.77	1.83	6.64	0.93	0.99	-	M	(24)
		S	49	5.61	0.90	9.78	0.98	1.03	-		
Lithodora hispidula subsp. versicolor	D	L	41	9.34	1.21	6.45	0.77	0.47	-	M	(24)
		S	59	6.48	0.91	8.87	1.06	0.03	-		
Lithodora zahnii	D	L	15	12.40	1.86	9.30	1.40	1.30	-	M	(26)
		S	42	7.54	1.21	11.10	1.44	1.76	-		
Lithodora zahnii	SHD	L	17	13.81	2.07	9.28	1.48	2.48	-	M	(24)
		S	33	7.53	1.13	11.33	1.25	1.75	-		
Lithospermum cobrense	D	L	50	11.49	1.38	4.86	0.62	-	-	M	(47)
		S	50	4.19	0.50	11.19	1.12	-	-		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Luculia pinceana	D	L	66	30.75	2.03	22.74	1.46	3.98	1.38	M, I	(48)
		S	60	19.80	2.71	29.75	3.64	4.40	1.16		
Melochia nudiflora	D	L	27	9.56	0.55	4.83	0.30	4.73	0.54	M, I	(49)
		S	11	5.34	0.46	8.47	0.71	3.14	0.42		
Melochia parvifolia	D	L	43	9.00	0.95	4.68	0.42	-	-	M	(50)
		S	41	5.04	0.42	7.99	0.75	-	-		
Melochia pyramidata	D	L	24	3.01	0.51	2.07	0.36	-	-	M, I	(49, 50)
		S	30	1.84	0.28	3.35	0.60	-	-		
Melochia savannarum	D	L	46	9.28	0.79	4.76	0.92	4.52	0.54	M, I	(49)
		S	54	5.48	0.66	8.49	1.24	3.01	0.65		
Melochia tomentosa	D	L	39	7.58	1.32	4.38	0.69	-	-	M, I	(49, 50)
		S	23	4.22	0.58	6.52	1.05	-	-		
Melochia villosa	D	L	18	7.46	0.59	4.16	0.36	-	-	M, I	(49, 50)
		S	24	5.18	0.47	6.27	0.67	-	-		
Menyanthes trifoliata	D	L	23	12.60	1.49	6.70	0.96	5.90	0.96	M, I	(51)
		S	17	8.70	1.15	10.70	1.61	2.10	0.95		
Mitchella repens	D	L	10	13.56	-	9.60	-	-	-	M	(52)
		S	10	10.21	-	16.57	-	-	-		
Mussaenda decipiens	D	L	25	24.90	1.48	15.06	1.14	9.84	0.91	M, I	(53)
		S	20	15.19	1.19	20.80	1.69	5.61	1.67		
Mussaenda divaricata	D	L	25	24.36	0.94	17.89	0.73	6.47	0.80	M, I	(53)
		S	25	11.21	0.66	23.47	0.75	12.26	0.51		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Mussaenda erosa	D	L	25	23.12	0.88	13.47	0.54	9.65	0.95	M, I	(53)
		S	25	9.75	1.03	19.42	1.28	9.67	0.91		
Mussaenda hainanensis	D	L	16	27.84	1.48	15.10	0.78	12.74	0.98	M, I	(53)
		S	19	7.16	0.54	20.30	1.69	13.14	1.49		
Mussaenda hirsutula	D	L	20	23.36	1.20	16.18	1.39	7.17	0.94	M, I	(53)
		S	17	12.63	0.58	19.73	1.21	7.10	0.81		
Mussaenda kwangsiensis	D	L	12	20.59	3.17	12.73	1.94	7.86	1.57	M, I	(53)
		S	26	6.42	1.19	21.17	3.54	14.74	3.22		
Mussaenda kwangtungensis	D	L	25	25.86	1.24	17.95	0.67	7.91	1.17	M, I	(53)
		S	25	6.95	0.48	23.53	2.51	16.58	2.25		
Mussaenda lancipetala	D	L	40	23.72	2.42	13.75	1.32	9.97	1.70	M, I	(53)
		S	38	13.94	1.58	21.13	2.10	7.19	1.41		
Mussaenda macrophylla	D	L	25	20.73	1.33	11.55	0.73	9.18	1.46	M, I	(53)
		S	25	15.95	1.76	19.03	1.78	3.08	1.31		
Mussaenda mollissima	D	L	15	26.03	3.63	19.62	3.50	6.42	1.52	M, I	(53)
		S	14	15.89	1.73	22.59	2.04	6.70	2.14		
Mussaenda multinervis	D	L	25	25.51	1.87	14.44	0.89	11.07	1.80	M, I	(53)
		S	25	16.65	0.79	17.92	0.62	1.27	0.44		
Mussaenda parviflora	D	L	32	12.40	1.18	6.46	1.01	6.02	0.79	M, I	(54)
		S	22	5.76	0.75	8.28	0.98	2.51	1.11		
Mussaenda pingbianensis	D	L	25	25.37	1.51	18.01	1.40	7.36	0.81	M, I	(53)
		S	25	6.60	0.31	24.70	1.36	18.10	1.13		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Mussaenda pubescens	D	L	25	11.20	1.34	8.14	0.66	3.06	1.17	M, I	(53)
		S	25	4.63	0.55	10.63	0.65	6.00	0.84		
Mussaenda pubescens	SHD	L	26	13.71	5.25	10.70	2.65	3.95	1.12	M	(55)
		S	26	4.54	2.24	14.64	1.99	9.17	6.02		
Narcissus albimarginatus	D	L	45	25.53	1.87	16.78	1.40	-	-	M, I	(56)
		S	52	9.59	1.50	25.11	2.07	-	-		
Narcissus assoanus	SHD	L	275	24.53	2.18	22.63	2.34	0.84	1.61	M, I	(57, 58)
		S	53	13.15	2.18	23.15	2.34	4.91	1.61		
Narcissus broussonetii	SHD	L	128	37.55	3.67	29.33	3.54	7.89	3.96	M, I	(59)
		S	128	24.63	3.35	31.40	2.74	13.20	3.58		
Narcissus calcicola	SHD	L	81	17.84	1.41	15.11	1.76	1.20	1.30	M	(58)
		S	80	8.00	1.41	15.97	1.75	3.47	1.30		
Narcissus cuatrecasasii	SHD	L	68	15.67	1.28	16.39	1.08	0.25	0.73	M	(58)
		S	54	8.47	1.28	17.26	1.08	2.64	0.72		
Narcissus dubius	SHD	L	450	15.62	1.72	15.42	1.23	0.08	-	M	(57)
		S	272	9.38	2.97	15.79	2.82	2.66	-		
Narcissus gaditanus	SHD	L	52	18.62	1.56	15.95	1.51	1.64	1.19	M	(58)
		S	51	8.10	1.59	16.24	1.51	3.16	1.19		
Narcissus papyraceus	SHD	L	33	18.06	1.60	20.39	1.71	0.03	1.06	M, I	(58, 60)
		S	30	9.61	1.60	20.47	1.70	3.24	1.06		
Narcissus rupicola	SHD	L	27	15.91	1.21	20.63	1.78	1.51	1.27	M	(58)
		S	75	9.21	1.20	20.10	1.78	5.22	1.27		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Nivenia argentea	D	L	166	25.48	2.02	20.91	1.65	-	-	M, I	(61)
		S	120	15.41	2.18	23.14	1.90	-	-		
Nivenia binata	D	L	39	18.05	1.76	13.73	1.62	4.29	0.97	M	(61)
		S	15	12.03	1.60	16.05	1.12	4.18	0.75		
Nivenia corymbosa	D	L	102	19.46	2.20	12.24	1.28	7.67	1.42	M, I	(61)
		S	104	10.68	1.27	19.89	1.84	8.93	1.16		
Nivenia inaequalis	D	L	158	41.38	2.95	35.25	4.74	6.95	2.00	M, I	(61)
		S	138	30.90	2.80	37.28	3.84	6.75	2.94		
Nymphoides geminata	D	L	20	10.26	0.54	5.86	0.22	2.61	0.45	M	(62)
		S	20	5.88	0.27	8.07	0.76	2.19	0.58		
Nymphoides montana	D	L	20	10.43	0.54	5.88	0.45	-	-	M, I	(63)
		S	20	5.08	0.40	8.31	0.58	-	-		
Oldenlandia salzmannii	D	L	80	5.79	0.47	1.79	0.29	-	-	M	(64)
		S	80	3.74	0.41	4.44	0.62	-	-		
Oldenlandia umbellata	D	L	20	2.50	-	0.84	-	-	-	M	(65)
		S	20	0.80	-	2.56	-	-	-		
Ophiorrhiza japonica	D	L	37	13.29	0.82	8.02	0.78	5.27	0.54	M, I	(66)
		S	40	8.84	0.87	13.78	0.99	4.93	0.98		
Ophiorrhiza napoensis	D	L	32	26.60	2.10	19.27	1.84	-	-	M	(67)
		S	32	19.59	1.31	27.39	1.49	-	-		
Ophiorrhiza radicans	D	L	20	3.00	0.38	2.99	0.11	-	-	M	(68)
		S	20	1.45	0.07	3.67	0.23	-	-		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Oplonia armata	D	L	18	13.99	-	9.68	-	-	-	M	(23)
		S	13	8.53	-	13.55	-	-	-		
Oplonia nannophylla	D	L	20	11.45	1.30	7.56	1.06	3.74	0.76	M, I	(69)
		S	20	7.09	1.20	11.02	1.41	3.93	0.78		
Oxalis rosea	D	L	6	9.18	-	9.01	-	-	-	M	(70)
		S	6	6.01	-	8.41	-	-	-		
Pagamea coriacea	SHD	L	35	4.11	1.20	4.80	0.80	-	-	M	(71)
		S	35	2.82	1.00	3.07	0.40	-	-		
Palicourea coriacea	D	L	106	9.21	1.49	7.81	1.39	1.82	0.66	M	(17, 72)
		S	118	8.07	1.34	9.83	1.53	1.97	0.67		
Palicourea crocea	D	L	47	17.63	1.73	13.02	1.24	4.61	1.13	M	(73)
		S	40	12.38	1.11	18.81	0.91	6.43	1.79		
Palicourea croceoides	D	L	46	14.84	1.58	11.27	1.14	3.59	1.36	M, I	(13)
		S	44	9.61	2.25	13.76	1.73	4.16	1.40		
Palicourea demissa	D	L	30	17.10	1.10	13.30	0.82	0.10	0.02	M	(74)
		S	30	13.40	0.77	19.60	1.26	0.20	0.03		
Palicourea marcgravii	D	L	77	18.48	1.70	13.66	1.49	4.80	1.39	M	(17)
		S	130	14.00	1.86	19.91	3.03	5.90	2.60		
Palicourea officinalis	D	L	224	12.31	1.50	9.62	1.33	2.88	1.05	M	(17)
		S	174	8.30	1.27	11.88	1.74	3.54	1.52		
Palicourea padifolia	D	L	677	15.91	1.20	11.48	1.08	4.77	0.97	M, I	(75, 76)
		S	743	8.38	1.10	15.65	1.03	6.23	1.13		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Palicourea petiolaris	D	L	18	12.60	1.50	7.40	0.70	-	-	M	(77)
		S	20	6.40	0.50	13.90	0.60	-	-		
Palicourea rigida	D	L	33	15.43	1.76	12.15	1.30	3.28	1.54	M, I	(17, 78)
		S	33	10.78	1.74	16.29	2.63	5.51	2.83		
Palicourea tetragona	D	L	60	53.70	3.49	43.40	2.79	10.30	2.94	M, I	(79, 80)
		S	60	45.60	3.10	54.00	2.48	8.40	2.63		
Pemphis acidula	D	L	60	4.42	-	2.66	-	-	-	M	(81)
		S	60	1.68	-	4.51	-	-	-		
Pentanisia angustifolia	D	L	26	21.26	2.45	16.76	2.19	5.48	1.37	M, I	(82)
		S	24	15.46	1.52	20.06	2.20	4.73	1.01		
Pentanisia prunelloides	D	L	26	19.36	1.89	14.66	1.48	4.49	1.00	M, I	(82)
		S	24	13.86	1.27	17.96	1.57	4.98	2.00		
Persicaria chinensis	D	L	25	4.00	-	3.00	-	-	-	M	(83)
		S	25	2.50	-	4.00	-	-	-		
Persicaria odorata	D	L	10	3.50	0.02	2.20	0.01	-	-	M	(84)
		S	10	1.66	0.01	3.90	0.02	-	-		
Persicaria wugongshanensis	D	L	20	4.33	0.19	2.23	0.06	1.99	0.12	M	(85)
		S	20	2.18	0.07	4.73	0.08	2.52	0.09		
Piriqueta cistoides subsp. caroliniana	D	L	6	8.52	1.03	4.90	0.61	3.62	1.03	M	(86)
		S	4	4.67	0.52	8.50	1.30	3.83	0.93		
Plumbago auriculata	D	L	28	27.90	4.20	20.00	0.00	-	-	M, I	(87)
		S	24	20.00	0.00	23.30	4.80	-	-		

Table S1 continued.

Species	Character state	Morph	*n*	Stigma	SD	Anther	SD	Herkogamy	SD	Data type	Reference
Primula algida	D	L	2	4.00	-	2.75	-	1.25	-	M	(18)
		S	5	4.00	1.41	7.10	2.86	3.10	1.47		
Primula aliciae	D	L	2	9.50	-	3.50	-	6.00	-	M	(18)
		S	2	3.50	-	9.50	-	6.00	-		
Primula allionii	D	L	40	8.25	1.16	3.51	0.62	-	-	M, I	(88, 89)
		S	39	3.17	0.56	9.92	1.46	-	-		
Primula alpicola	D	L	17	9.76	1.42	4.76	0.69	5.00	1.90	M	(18)
		S	17	3.88	0.84	11.62	1.36	7.74	1.19		
Primula amethystina	D	L	2	4.00	-	2.50	-	1.50	-	M	(18)
		S	2	1.20	-	4.50	-	3.30	-		
Primula anisodora	D	L	30	7.28	0.56	4.04	0.52	-	-	M	(90)
		S	30	3.27	0.36	8.09	0.60	-	-		
Primula aromatica	D	L	2	11.50	-	3.00	-	8.50	-	M	(18)
		S	2	2.00	-	9.00	-	7.00	-		
Primula asarifolia	D	L	2	13.00	-	4.25	-	8.75	-	M	(18)
		S	2	4.00	-	12.00	-	8.00	-		
Primula aurantiaca	D	L	27	10.67	1.12	6.94	0.81	3.72	0.67	M	(18)
		S	27	6.98	1.15	11.94	1.14	4.96	1.22		
Primula auriculata	D	L	5	8.60	0.42	6.50	0.50	2.10	0.22	M	(18)
		S	5	5.20	0.45	10.20	0.45	5.00	-		
Primula barbicalyx	D	L	5	13.00	2.92	3.70	0.67	9.30	2.33	M	(18)
		S	5	3.10	1.02	13.40	4.08	10.30	3.09		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula beesiana	D	L	30	10.26	0.84	6.63	0.68	-	-	M	(90)
		S	30	6.13	0.52	10.76	1.12	-	-		
Primula bella	D	L	2	8.00	-	2.00	-	6.00	-	M	(18)
		S	2	2.00	-	8.00	-	6.00	-		
Primula bellidifolia	D	L	22	7.66	1.43	4.05	1.30	3.61	0.62	M	(18)
		S	31	3.61	0.91	7.61	1.31	4.00	1.47		
Primula blattariformis	D	L	7	11.93	1.10	6.71	1.22	5.21	2.02	M	(18)
		S	2	4.00	-	10.00	-	6.00	-		
Primula blinii	D	L	2	9.00	-	2.00	-	7.00	-	M	(18)
		S	2	2.00	-	9.00	-	7.00	-		
Primula boreiocalliantha	D	L	2	11.50	-	4.50	-	7.00	-	M	(18)
		S	2	3.00	-	12.50	-	9.50	-		
Primula boveana	SHD	L	22	18.17	0.92	16.61	0.89	1.56	0.73	M	(91)
		S	9	13.04	0.59	17.38	0.57	4.34	0.47		
Primula bracteata	D	L	2	11.75	-	10.50	-	1.25	-	M	(18)
		S	2	3.00	-	7.50	-	4.50	-		
Primula bracteosa	D	L	2	12.50	-	6.25	-	6.25	-	M	(18)
		S	2	8.50	-	10.00	-	1.50	-		
Primula bulleyana	D	L	30	10.88	0.84	6.09	0.64	-	-	M	(90)
		S	30	5.60	0.80	12.10	0.88	-	-		
Primula calderiana	D	L	2	14.00	-	6.00	-	8.00	-	M	(18)
		S	2	6.50	-	11.00	-	4.50	-		

Table S1 continued.

Species	Character state	Morph	*n*	Stigma	SD	Anther	SD	Herkogamy	SD	Data type	Reference
Primula calliantha	D	L	2	13.00	-	6.50	-	6.50	-	M	(18)
		S	2	3.00	-	13.00	-	10.00	-		
Primula capitata	D	L	12	6.17	1.19	4.00	1.30	2.17	0.78	M	(18)
		S	12	3.25	1.08	8.13	1.19	4.88	0.57		
Primula caveana	D	L	2	11.00	-	3.75	-	7.25	-	M	(18)
		S	2	5.50	-	7.50	-	2.00	-		
Primula cernua	D	L	7	9.36	0.99	2.86	0.63	6.50	1.15	M	(18)
		S	7	3.21	0.99	9.07	1.17	5.86	1.44		
Primula chapaensis	D	L	2	8.00	-	5.00	-	3.00	-	M	(18)
		S	2	4.00	-	9.50	-	5.50	-		
Primula chionantha	D	L	2	12.00	-	4.50	-	7.50	-	M	(18)
		S	2	3.00	-	11.00	-	8.00	-		
Primula chungensis	D	L	23	11.46	1.04	7.72	0.72	3.70	0.67	M, I	(18, 92)
		S	14	6.67	0.72	11.36	1.36	4.71	0.87		
Primula cortusoides	D	L	10	7.55	0.64	4.70	0.35	2.85	0.53	M	(18)
		S	10	4.40	0.39	9.60	0.46	5.20	0.59		
Primula darialica	D	L	8	6.38	0.35	4.50	0.27	1.88	0.23	M	(18)
		S	5	4.50	0.00	6.60	0.22	2.10	0.22		
Primula davisii	D	L	7	10.00	1.06	5.70	0.93	-	-	M	(93)
		S	8	6.20	1.27	11.00	1.56	-	-		
Primula deflexa	D	L	13	10.35	1.64	3.85	0.69	6.50	2.17	M	(18)
		S	13	3.31	0.63	9.88	2.08	6.58	2.26		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula denticulata	D	L	50	9.00	1.00	5.00	1.00	-	-	M	(39)
		S	50	5.00	1.00	10.00	1.00	-	-		
Primula dryadifolia	D	L	2	7.75	-	4.00	-	3.75	-	M	(18)
		S	2	4.00	-	7.75	-	3.75	-		
Primula edelbergii	D	L	17	12.10	1.24	7.40	1.65	-	-	M	(93)
		S	20	6.40	1.34	12.00	1.79	-	-		
Primula efarinosa	D	L	5	7.60	1.02	4.40	0.96	3.20	0.27	M	(18)
		S	5	2.60	1.47	6.70	1.04	4.10	1.39		
Primula elatior	D	L	50	12.40	1.10	7.50	0.80	-	-	M	(94)
		S	50	6.90	0.90	13.60	1.30	-	-		
Primula elliptica	D	L	3	9.00	0.87	4.17	0.29	4.83	0.76	M	(18)
		S	3	4.00	0.50	9.83	0.29	5.83	0.29		
Primula erratica	D	L	11	5.86	0.23	3.59	0.92	2.27	0.96	M	(18)
		S	5	2.90	0.82	6.20	0.27	3.30	0.67		
Primula exscapa	D	L	2	13.00	-	4.00	-	9.00	-	M	(18)
		S	2	3.50	-	10.00	-	6.50	-		
Primula farinosa	D	L	7	4.57	1.13	3.43	0.67	1.29	0.49	M	(18)
		S	7	2.84	1.12	5.29	1.47	2.44	1.93		
Primula fasciculata	D	L	13	7.50	1.17	5.31	1.07	2.19	0.75	M	(18)
		S	12	4.63	0.80	7.54	1.30	2.92	0.93		
Primula firmipes	D	L	25	10.28	0.72	3.96	0.75	6.32	0.92	M	(18)
		S	18	3.78	1.14	10.25	1.22	6.47	0.96		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula flaccida	D	L	17	10.38	1.24	4.18	0.92	6.21	1.13	M	(18)
		S	17	3.97	1.01	10.88	1.10	6.91	1.09		
Primula floribunda	D	L	42	10.60	1.61	5.82	1.09	4.77	1.59	M	(18)
		S	33	5.82	1.50	12.05	2.01	6.23	2.79		
Primula forbesii	D	L	2	3.00	-	1.50	-	1.50	-	M	(18)
		S	2	1.00	-	3.00	-	2.00	-		
Primula forrestii	D	L	2	9.00	-	5.50	-	3.50	-	M	(18)
		S	2	2.50	-	9.00	-	6.50	-		
Primula gaubaeana	D	L	7	12.30	0.93	7.60	1.46	-	-	M	(93)
		S	15	7.00	1.55	12.60	1.74	-	-		
Primula gemmifera	D	L	10	11.20	1.40	7.05	1.48	4.15	0.94	M	(18)
		S	10	6.45	1.14	12.05	1.48	5.60	1.22		
Primula geraniifolia	D	L	16	11.16	1.31	4.59	1.21	6.56	2.10	M	(18)
		S	13	4.27	1.24	11.15	2.42	6.88	2.78		
Primula glabra	D	L	7	3.79	0.76	2.64	0.63	1.14	0.63	M	(18)
		S	7	2.29	0.39	3.93	0.79	1.64	0.48		
Primula glomerata	D	L	2	8.25	-	2.00	-	6.25	-	M	(18)
		S	2	2.00	-	8.25	-	6.25	-		
Primula heucherifolia	D	L	5	13.10	1.14	3.40	1.29	9.70	1.96	M	(18)
		S	5	3.60	0.55	12.70	2.28	9.10	1.82		
Primula hidakana	D	L	75	10.00	1.00	6.37	0.61	-	-	M	(95)
		S	75	5.31	0.61	11.07	0.89	-	-		

Table S1 continued.

Species	Character state	Morph	*n*	Stigma	SD	Anther	SD	Herkogamy	SD	Data type	Reference
Primula involucrata	D	L	26	11.10	1.41	5.96	1.23	5.13	1.20	M	(18)
		S	26	5.85	1.13	11.71	1.01	5.87	1.06		
Primula latisecta	D	L	2	11.50	-	2.00	-	9.50	-	M	(18)
		S	2	2.50	-	8.50	-	6.00	-		
Primula littledalei	D	L	2	8.50	-	2.50	-	6.00	-	M	(18)
		S	2	2.50	-	8.50	-	6.00	-		
Primula luteola	D	L	5	13.00	0.61	8.10	0.22	4.90	0.65	M	(18)
		S	6	4.75	0.52	12.25	0.99	7.50	0.95		
Primula malacoides	D	L	20	5.73	1.37	3.63	1.13	2.10	0.80	M	(18)
		S	20	3.70	1.40	5.68	1.09	1.98	0.97		
Primula malvacea	D	L	7	10.43	2.26	5.57	0.73	4.86	2.15	M	(18)
		S	2	7.50	-	10.00	-	2.50	-		
Primula marginata	D	L	13	9.03	1.50	2.79	0.59	5.67	1.20	M, I	(96)
		S	13	3.43	0.62	9.11	1.41	5.34	1.32		
Primula maximowiczii	D	L	2	11.00	-	4.50	-	6.50	-	M	(18)
		S	2	3.50	-	14.50	-	11.00	-		
Primula membranifolia	D	L	5	9.30	2.80	3.70	1.15	5.60	1.71	M	(18)
		S	5	3.70	1.15	9.00	2.52	5.30	1.44		
Primula merrilliana	D	L	20	7.10	2.99	4.45	1.43	-	-	M, I	(97)
		S	20	4.34	1.48	7.33	1.61	-	-		
Primula minor	D	L	2	10.00	-	5.00	-	5.00	-	M	(18)
		S	2	3.75	-	10.50	-	6.75	-		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula mistassinica	D	L	297	3.72	0.38	1.93	0.24	1.20	0.33	M, I	(98)
		S	295	1.99	0.26	3.30	0.40	0.65	0.38		
Primula modesta	D	L	5	5.20	0.27	4.00	0.00	1.20	0.27	M	(18)
		S	5	3.90	0.22	6.10	0.22	2.20	0.27		
Primula mollis	D	L	5	10.00	1.22	5.70	0.45	4.30	0.84	M	(18)
		S	2	6.00	-	9.00	-	3.00	-		
Primula moupinensis	D	L	2	13.50	-	6.00	-	7.50	-	M	(18)
		S	2	5.50	-	7.75	-	2.25	-		
Primula muscarioides	D	L	17	8.26	1.80	3.94	0.79	4.32	2.24	M	(18)
		S	15	3.40	1.61	8.63	0.77	5.23	1.55		
Primula nivalis	D	L	2	9.50	-	3.50	-	6.00	-	M	(18)
		S	2	2.00	-	9.50	-	7.50	-		
Primula nutans	D	L	8	9.69	1.41	6.31	1.56	3.38	1.83	M	(18)
		S	8	5.94	0.98	8.63	1.75	2.69	0.92		
Primula obconica	D	L	10	11.50	1.85	3.85	1.06	7.19	1.65	M	(18)
		S	9	4.05	0.96	11.61	1.96	7.17	1.71		
Primula odontocalyx	D	L	2	9.50	-	4.75	-	4.75	-	M	(18)
		S	2	7.50	-	8.50	-	1.00	-		
Primula orbicularis	D	L	2	8.75	-	4.50	-	4.25	-	M	(18)
		S	2	4.50	-	8.75	-	4.25	-		
Primula oreodoxa	D	L	111	9.68	0.78	5.73	0.53	3.95	0.94	M, I	(53)
		S	117	5.78	0.56	9.70	0.84	3.92	0.77		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula ovalifolia	D	L	2	9.00	-	4.50	-	4.50	-	M	(18)
		S	2	4.25	-	11.00	-	6.75	-		
Primula palinuri	D	L	30	13.30	1.16	7.50	1.08	5.80	1.69	M	(99)
		S	30	7.40	1.07	13.60	1.90	6.20	2.30		
Primula partschiana	D	L	2	11.00	-	5.50	-	5.50	-	M	(18)
		S	2	5.50	-	11.00	-	5.50	-		
Primula petiolaris	D	L	10	14.00	1.00	8.00	1.00	-	-	M	(39)
		S	10	8.00	1.00	15.00	2.00	-	-		
Primula pinnatifida	D	L	12	8.25	0.66	3.04	0.62	5.21	0.50	M	(18)
		S	7	2.43	0.35	8.29	0.81	5.86	0.63		
Primula poissonii	D	L	60	7.85	0.58	3.83	0.30	4.02	0.56	M	(18)
		S	60	3.24	0.33	8.11	0.67	4.87	0.63		
Primula polyneura	D	L	33	13.53	1.47	8.24	1.23	5.29	1.08	M	(18)
		S	33	7.43	1.36	13.67	1.57	6.24	0.87		
Primula primulina	D	L	2	4.00	-	2.00	-	2.00	-	M	(18)
		S	2	2.00	-	4.00	-	2.00	-		
Primula prolifera	D	L	46	9.53	1.46	4.27	0.70	5.29	1.39	M	(18)
		S	37	4.12	0.45	11.31	1.88	7.19	1.88		
Primula pulchella	D	L	5	9.90	1.43	4.10	1.95	5.80	2.46	M	(18)
		S	3	3.33	2.31	10.00	2.00	6.67	3.06		
Primula pulverulenta	D	L	17	13.65	1.09	9.29	1.02	4.35	0.49	M	(18)
		S	22	8.32	1.28	14.59	1.27	6.27	0.53		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula pumilio	D	L	2	4.50	-	2.25	-	2.25	-	M	(18)
		S	2	2.25	-	4.50	-	2.25	-		
Primula pycnoloba	D	L	2	12.00	-	7.00	-	5.00	-	M	(18)
		S	2	9.50	-	18.00	-	8.50	-		
Primula reidii	SHD	L	7	9.57	1.17	6.86	1.11	2.71	0.39	M	(18)
		S	2	4.00	-	8.00	-	4.00	-		
Primula reticulata	D	L	22	12.57	1.68	7.48	2.16	5.09	1.43	M	(18)
		S	10	3.95	1.04	11.20	1.06	7.25	1.40		
Primula rotundifolia	D	L	2	11.00	-	5.50	-	5.50	-	M	(18)
		S	2	5.50	-	11.00	-	5.50	-		
Primula rugosa	D	L	2	7.00	-	3.50	-	3.50	-	M	(18)
		S	2	3.50	-	7.00	-	3.50	-		
Primula rupestris	D	L	2	9.25	-	6.50	-	2.75	-	M	(18)
		S	2	5.25	-	8.50	-	3.25	-		
Primula saturata	D	L	2	13.00	-	7.00	-	6.00	-	M	(18)
		S	2	5.00	-	8.50	-	3.50	-		
Primula secundiflora	D	L	60	11.31	0.76	5.07	0.44	6.23	0.72	M	(100)
		S	60	4.73	0.46	11.58	0.79	6.84	0.80		
Primula serratifolia	D	L	23	11.17	1.00	6.09	1.14	5.08	1.16	M	(18)
		S	21	5.48	1.17	11.29	1.35	5.81	0.86		
Primula sertulum	D	L	2	7.50	-	2.00	-	5.50	-	M	(18)
		S	2	2.00	-	7.50	-	5.50	-		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula sieboldii	D	L	8	12.01	0.30	9.56	0.32	-	-	M	(101)
		S	18	8.31	0.33	14.29	0.58	-	-		
Primula sinensis	D	L	2	13.50	-	6.75	-	6.75	-	M	(18)
		S	2	6.75	-	13.50	-	6.75	-		
Primula sinolisteri	D	L	5	11.20	1.64	4.50	0.71	6.70	0.97	M	(18)
		S	5	4.20	1.10	11.00	2.74	6.80	1.64		
Primula sinomollis	D	L	2	5.00	-	3.00	-	2.00	-	M	(18)
		S	2	4.75	-	6.50	-	1.75	-		
Primula sonchifolia	D	L	2	10.50	-	4.75	-	5.75	-	M	(18)
		S	2	6.00	-	11.00	-	5.00	-		
Primula soongii	D	L	2	13.00	-	6.50	-	6.50	-	M	(18)
		S	2	7.50	-	14.00	-	6.50	-		
Primula souliei	D	L	2	6.50	-	2.50	-	4.00	-	M	(18)
		S	2	1.00	-	6.00	-	5.00	-		
Primula takedana	D	L	75	9.20	1.04	5.22	0.79	-	-	M	(95)
		S	75	4.26	0.58	8.47	0.99	-	-		
Primula tenuiloba	D	L	2	3.50	-	2.00	-	1.50	-	M	(18)
		S	2	2.00	-	3.50	-	1.50	-		
Primula tschuktschorum	D	L	29	7.50	0.70	4.61	0.81	-	-	M	(102)
		S	18	4.92	0.55	6.90	0.47	-	-		
Primula veris	D	L	199	15.57	1.16	8.79	0.88	6.54	1.37	M, I	(103, 104)
		S	192	8.94	0.86	14.82	1.18	7.22	1.48		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Primula vialii	D	L	10	8.05	0.86	3.40	1.24	4.65	1.16	M	(18)
		S	15	3.27	1.13	8.63	0.64	5.37	1.20		
Primula violacea	D	L	3	8.67	0.29	4.67	0.29	4.00	-	M	(18)
		S	3	2.83	0.29	9.83	0.29	7.00	-		
Primula vulgaris	D	L	186	15.89	1.27	9.33	1.05	6.81	1.15	M	(103)
		S	188	8.53	0.76	15.73	1.45	5.88	1.18		
Primula walshii	D	L	2	3.75	-	1.75	-	2.00	-	M	(18)
		S	2	1.75	-	3.75	-	2.00	-		
Primula waltonii	D	L	24	10.15	1.16	4.35	0.83	5.79	1.34	M	(18)
		S	25	4.30	0.94	11.80	1.24	7.50	0.69		
Primula watsonii	D	L	5	9.20	1.30	5.30	1.15	3.90	0.65	M	(18)
		S	2	4.25	-	8.50	-	4.25	-		
Primula wigramiana	D	L	7	11.64	0.63	6.07	0.67	5.57	1.02	M	(18)
		S	3	5.17	0.58	10.50	1.00	5.33	0.58		
Primula yunnanensis	D	L	8	8.81	0.65	3.19	0.88	5.63	1.25	M	(18)
		S	8	3.00	1.00	8.75	1.00	5.94	1.29		
Psychotria asiatica	D	L	11	3.30	0.22	2.70	0.32	0.52	0.26	M, I	(105)
		S	18	2.14	0.28	3.60	0.29	1.34	0.36		
Psychotria birotula	D	L	23	4.50	0.16	2.70	0.14	-	-	M	(106)
		S	26	2.70	0.12	4.40	0.12	-	-		
Psychotria boninensis	D	L	20	3.70	0.40	2.40	0.30	1.39	0.30	M, I	(107)
		S	21	1.90	0.30	4.20	0.50	2.20	0.43		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Psychotria brachypoda	D	L	31	15.54	0.40	11.11	1.15	-	-	M	(108)
		S	37	10.17	0.98	14.59	1.58	-	-		
Psychotria capitata	D	L	73	9.92	1.04	6.42	0.88	3.46	0.77	M, I	(17, 109)
		S	46	5.84	0.84	9.42	1.07	3.60	0.97		
Psychotria carthagenensis	D	L	173	4.89	0.55	3.12	0.46	1.78	0.57	M, I	(17, 110)
		S	12	3.10	0.52	4.67	1.09	1.57	0.90		
Psychotria cephalophora	D	L	10	4.90	0.48	3.30	0.32	-	-	M, I	(111)
		S	10	2.70	0.32	5.00	0.38	-	-		
Psychotria colorata	D	L	38	16.68	1.60	11.45	1.15	5.23	1.44	M, I	(112)
		S	37	11.43	1.16	16.66	1.62	5.23	1.46		
Psychotria conjugens	D	L	8	7.30	0.42	4.70	0.63	2.70	0.59	M	(113)
		S	16	4.20	0.42	7.02	0.64	2.86	0.68		
Psychotria deflexa	D	L	40	6.11	0.75	3.90	0.47	2.21	0.55	M, I	(109, 114)
		S	40	3.25	0.41	6.48	0.64	3.22	0.68		
Psychotria elata	D	L	30	1.82	-	0.70	-	-	-	M	(115)
		S	22	0.89	-	1.59	-	-	-		
Psychotria gracilenta	D	L	36	5.91	0.86	4.34	0.92	1.57	0.67	M, I	(112)
		S	40	3.08	0.94	5.48	0.80	2.40	0.78		
Psychotria hastisepala	D	L	15	10.80	1.47	9.99	1.47	2.18	0.87	M	(113)
		S	16	8.57	0.52	12.87	1.21	4.14	0.79		
Psychotria hoffmannseggiana	D	L	43	4.60	0.60	3.00	0.50	1.73	0.81	M, I	(109)
		S	57	2.50	0.30	4.60	0.60	1.97	0.71		

Table S1 continued.

Species	Character state	Morph	*n*	Stigma	SD	Anther	SD	Herkogamy	SD	Data type	Reference
Psychotria homalosperma	D	L	36	24.15	2.54	17.53	2.27	6.62	1.95	M, I	(116)
		S	39	11.78	2.21	22.49	3.53	5.56	2.58		
Psychotria jasminoides	D	L	26	11.70	0.53	7.60	0.52	-	-	M, I	(106)
		S	23	7.80	0.30	11.40	0.61	-	-		
Psychotria leiocarpa	D	L	45	7.85	0.69	5.38	0.57	2.47	0.53	M	(117)
		S	45	5.38	0.41	7.79	0.59	2.40	0.59		
Psychotria limonensis	SHD	L	-	3.50	-	2.00	-	-	-	M	(112)
		S	-	2.20	-	3.50	-	-	-		
Psychotria mapourioides	D	L	73	8.61	1.12	6.27	1.06	2.33	0.75	M	(17)
		S	77	5.84	0.74	9.10	1.30	3.26	1.01		
Psychotria nemorosa	D	L	24	9.20	0.29	7.10	0.20	-	-	M	(106)
		S	21	5.90	0.16	8.60	0.12	-	-		
Psychotria nervosa	D	L	30	55.40	0.88	29.40	0.66	26.03	0.92	M, I	(118)
		S	30	30.80	0.93	49.30	0.99	21.23	2.25		
Psychotria olgae	SHD	L	-	6.00	-	3.50	-	-	-	M	(112)
		S	-	3.00	-	4.00	-	-	-		
Psychotria orosiana	SHD	L	-	6.50	-	3.50	-	-	-	M	(112)
		S	-	2.00	-	3.50	-	-	-		
Psychotria poeppigiana	D	L	40	15.87	1.84	11.54	1.09	-	-	M, I	(21, 119)
		S	40	7.80	1.22	15.94	1.92	-	-		
Psychotria racemosa	D	L	3	5.16	0.74	3.64	0.51	1.51	-	M	(17)
		S	3	3.01	0.14	6.18	0.56	3.17	-		

Table S1 continued.

Species	Character state	Morph	*n*	Stigma	SD	Anther	SD	Herkogamy	SD	Data type	Reference
Psychotria serpens	D	L	19	4.20	0.51	2.50	0.24	-	-	M, I	(120)
		S	24	2.20	0.22	4.50	0.38	-	-		
Psychotria spectabilis	D	L	30	30.30	2.20	22.50	0.12	-	-	M	(121)
		S	30	23.80	0.90	32.20	1.00	-	-		
Psychotria suerrensis	D	L	14	17.11	2.05	10.01	1.07	7.11	1.33	M, I	(21)
		S	15	9.05	0.79	15.27	1.28	6.22	1.05		
Psychotria suterella	D	L	8	16.98	0.25	12.83	0.17	5.39	0.18	M, I	(122)
		S	8	12.05	0.18	17.43	0.30	4.14	0.15		
Psychotria tenuinervis	D	L	69	8.10	1.01	5.80	0.59	-	-	M	(123)
		S	69	5.60	0.54	8.40	0.76	-	-		
Pulmonaria affinis	D	L	9	11.76	1.36	6.69	0.98	5.07	0.75	M, I	(124)
		S	8	5.52	0.42	11.97	1.43	6.45	1.20		
Pulmonaria angustifolia	D	L	10	8.82	1.11	4.58	0.39	4.24	1.07	M, I	(124)
		S	10	3.74	0.73	8.91	0.57	5.17	0.92		
Pulmonaria collina	D	L	10	9.71	1.04	4.71	0.48	5.00	1.23	M, I	(124)
		S	11	5.20	0.44	9.94	0.89	4.74	1.10		
Pulmonaria longifolia	D	L	28	11.74	1.31	5.86	0.83	5.88	1.19	M, I	(35)
		S	20	5.67	0.62	11.62	1.02	5.95	0.98		
Pulmonaria mollis	D	L	10	10.76	1.40	5.31	0.32	5.45	1.35	M, I	(124)
		S	10	5.07	1.04	10.32	1.15	5.25	1.12		
Pulmonaria montana	D	L	8	9.48	0.71	5.14	0.30	4.35	0.82	M, I	(124)
		S	10	5.25	0.66	9.85	0.67	4.60	0.67		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Pulmonaria obscura	D	L	30	10.50	1.10	6.20	0.55	4.30	1.10	M, I	(124, 125)
		S	30	6.00	0.55	10.90	0.55	5.00	0.55		
Pulmonaria officinalis	D	L	102	10.42	1.03	5.33	0.50	5.09	0.97	M, I	(126)
		S	74	5.92	0.65	10.55	0.86	4.62	0.87		
Pulmonaria saccharata	D	L	10	12.64	1.07	5.71	0.75	6.94	1.14	M, I	(124)
		S	10	5.76	1.17	11.90	1.14	6.14	1.35		
Quinchamalium chilense	D	L	50	9.85	1.05	8.50	0.72	-	-	M	(127)
		S	50	6.62	0.38	11.32	1.01	-	-		
Reinwardtia indica	D	L	26	16.00	1.00	9.00	1.00	-	-	M	(39)
		S	26	8.00	1.00	16.00	1.00	-	-		
Rourea induta	D	L	32	4.66	0.41	4.19	0.58	-	-	M	(128)
		S	32	2.09	0.20	4.85	0.44	-	-		
Rudgea sessilis	D	L	26	7.80	1.11	5.55	0.50	2.34	1.28	M	(113)
		S	25	5.05	0.90	7.85	0.47	2.92	1.16		
Sabicea cinerea	D	L	10	19.60	0.34	14.80	0.44	-	-	M	(129)
		S	10	14.60	0.27	18.00	0.37	-	-		
Salvia brandegeei	D	L	50	11.01	0.71	6.85	0.64	3.87	0.52	M, I	(130)
		S	50	8.00	0.71	11.27	0.78	3.52	0.57		
Sarcotheca celebica	D	L	37	2.86	0.19	1.58	0.13	-	-	M	(131)
		S	39	1.34	0.10	2.80	0.19	-	-		
Schizomussaenda henryi	D	L	25	30.07	2.02	23.17	1.79	6.90	1.31	M, I	(53)
		S	25	23.14	1.92	25.95	1.57	2.81	1.01		

Table S1 continued.

Species	**Character state**	**Morph**	***n***	**Stigma**	**SD**	**Anther**	**SD**	**Herkogamy**	**SD**	**Data type**	**Reference**
Solanum melongena	SHD	L	100	18.20	8.46	10.90	1.20	-	-	M	(132)
		S	100	8.50	5.36	10.47	1.15	-	-		
Solanum torvum	SHD	L	13	10.08	0.74	8.93	0.65	1.15	0.69	M, I	(133)
		S	17	3.40	1.33	8.53	0.39	5.10	1.43		
Solanum wrightii	SHD	L	14	23.86	1.17	18.73	1.17	5.13	1.44	M, I	(133)
		S	14	7.64	1.75	18.52	0.80	10.88	2.23		
Spermadictyon suaveolens	D	L	30	18.00	1.00	12.00	1.00	-	-	M	(39)
		S	30	12.00	1.00	18.00	1.00	-	-		
Turnera scabra	D	L	30	11.80	1.10	8.40	0.80	1.30	1.00	M, I	(134)
		S	30	5.80	0.80	13.30	1.00	4.20	0.90		
Turnera subulata	D	L	30	11.80	0.90	6.50	0.60	2.40	0.90	M, I	(134)
		S	30	5.70	0.70	12.00	0.90	2.90	0.60		
Turnera ulmifolia	D	L	30	11.30	1.00	7.70	0.80	1.70	0.70	M, I	(134)
		S	30	5.90	0.70	12.70	0.90	3.70	0.90		
Tylosema esculentum	D	L	24	10.90	1.42	5.00	0.72	-	-	M	(135)
		S	24	6.50	0.51	12.20	0.68	-	-		
Vismia guianensis	D	L	10	0.70	0.03	0.41	0.03	0.29	0.05	M, I	(136)
		S	10	0.40	0.03	0.70	0.03	0.29	0.03		
Waltheria ovata	D	L	11	7.48	0.53	5.00	0.00	4.41	0.55	M	(137)
		S	10	4.28	0.26	5.00	0.00	2.32	0.43		

Appendix Table S2: Comparison of the proposed index (COC- concave-shaped pollen transfer probability function) with modified Richards & Koptur - R & K (Sanchez et al, 2008), Sanchez - SAN (Sanchez et al, 2013), Lou & Bosque – L & B (Lu & Bosque, 2003) & Armbruster – ARM (Armbruster et al., 2017) using extracted individual level empirical data. Sanchez's index represents a combined value for both the levels of organ positions in heterostylous species and is compared to an average value of index of both the high and low levels for all other indices. Fruit-set and seed-set are represented as percentage per flower and per ovule, respectively. Reference denotes the serial number for the study from which data was extracted as per the Appendix References.

		Reciprocity index values					**Reproductive success**		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Arcytophyllum aristatum	High	0.22	0.96	-	1.00	0.00	-	53.80	(4)
	Low	0.15	0.94	-	0.33	0.00	-	79.10	
	Average	0.19	0.95	0.91	0.67	0.00	-	-	
Arcytophyllum capitatum	High	0.10	0.91	-	0.56	0.03	-	43.80	(4)
	Low	0.18	0.92	-	0.82	0.01	-	50.00	
	Average	0.14	0.91	0.59	0.69	0.02	-	-	
Arcytophyllum ciliolatum	High	0.12	0.92	-	0.75	0.01	-	58.00	(4)
	Low	0.16	0.90	-	0.92	0.00	-	65.10	
	Average	0.14	0.91	0.72	0.83	0.01	-	-	
Arcytophyllum filiforme	High	0.07	0.87	-	0.70	0.02	-	50.50	(4)
	Low	0.13	0.87	-	0.60	0.01	-	69.30	
	Average	0.10	0.87	0.41	0.65	0.02	-	-	
Arcytophyllum lavarum	High	0.23	0.94	-	0.82	0.01	-	88.00	(4)
	Low	0.18	0.86	-	0.60	0.01	-	90.10	
	Average	0.21	0.90	0.67	0.71	0.01	-	-	
Arcytophyllum macbridei	High	0.12	0.92	-	0.64	0.03	-	50.20	(4)
	Low	0.19	0.93	-	0.53	0.01	-	65.00	
	Average	0.15	0.93	0.76	0.58	0.02	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Arcytophyllum macbridei	High	0.04	0.91	-	0.40	0.03	-	54.10	(4)
	Low	0.26	0.93	-	0.87	0.01	-	54.20	
	Average	0.15	0.92	0.75	0.63	0.02	-	-	
Arcytophyllum rivetii	High	0.15	0.93	-	0.90	0.01	-	85.70	(4)
	Low	0.19	0.91	-	0.73	0.01	-	89.30	
	Average	0.17	0.92	0.70	0.81	0.01	-	-	
Arcytophyllum setosum	High	0.09	0.93	-	0.60	0.02	-	82.80	(4)
	Low	0.10	0.86	-	0.40	0.02	-	84.50	
	Average	0.09	0.89	0.66	0.50	0.02	-	-	
Arcytophyllum thymifolium	High	0.11	0.92	-	0.57	0.01	-	82.30	(4)
	Low	0.15	0.91	-	0.71	0.01	-	97.70	
	Average	0.13	0.91	0.70	0.64	0.01	-	-	
Arcytophyllum vernicosum	High	0.12	0.92	-	0.71	0.01	-	62.60	(4)
	Low	0.15	0.91	-	0.58	0.01	-	85.80	
	Average	0.14	0.91	0.69	0.65	0.01	-	-	
Bouvardia ternifolia	High	0.14	0.95	-	0.12	15.33	-	-	(7)
	Low	0.16	0.94	-	0.12	10.90	-	-	
	Average	0.15	0.94	0.86	0.12	13.11	-	-	
Carapichea ipecacuanha	High	0.11	0.93	-	0.55	1.10	25.00	-	(8)
	Low	0.19	0.91	-	0.28	0.47	16.70	-	
	Average	0.15	0.92	0.79	0.42	0.79	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Carapichea ipecacuanha	High	0.18	0.95	-	0.48	0.96	-	-	(8)
	Low	0.15	0.91	-	0.42	0.62	-	-	
	Average	0.16	0.93	0.82	0.45	0.79	-	-	
Carapichea ipecacuanha	High	0.12	0.92	-	0.61	1.57	-	-	(8)
	Low	0.17	0.90	-	0.52	0.69	-	-	
	Average	0.15	0.91	0.72	0.56	1.13	-	-	
Chassalia corallioides	High	0.09	0.93	-	0.22	7.55	58.60	-	(9)
	Low	0.01	0.77	-	0.05	29.83	0.00	-	
	Average	0.05	0.85	0.46	0.13	18.69	-	-	
Chassalia corallioides	High	0.07	0.90	-	0.14	15.12	45.90	-	(9)
	Low	0.01	0.68	-	0.00	42.63	0.00	-	
	Average	0.04	0.79	-0.02	0.07	28.87	-	-	
Chassalia corallioides	High	0.10	0.94	-	0.33	4.09	36.60	-	(9)
	Low	0.04	0.82	-	0.08	14.02	0.00	-	
	Average	0.07	0.88	0.57	0.21	9.05	-	-	
Chassalia corallioides	High	0.11	0.94	-	0.32	5.24	28.30	-	(9)
	Low	0.02	0.81	-	0.04	19.38	0.00	-	
	Average	0.07	0.87	0.53	0.18	12.31	-	-	
Chassalia corallioides	High	0.09	0.94	-	0.20	8.45	29.90	-	(9)
	Low	0.01	0.82	-	0.03	21.77	0.00	-	
	Average	0.05	0.88	0.64	0.12	15.11	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Cordia alba	High	0.08	0.92	-	0.10	3.41	23.00	-	(11)
	Low	0.08	0.92	-	0.14	1.16	21.00	-	
	Average	0.08	0.92	0.76	0.12	2.29	-	-	
Cordia inermis	High	0.08	0.92	-	0.50	0.72	27.00	-	(11)
	Low	0.14	0.90	-	0.13	0.37	1.00	-	
	Average	0.11	0.91	0.70	0.32	0.55	-	-	
Cordia macrocephala	High	0.09	0.93	-	0.14	4.84	32.00	-	(11)
	Low	0.06	0.89	-	0.18	6.67	30.00	-	
	Average	0.08	0.91	0.73	0.16	5.75	-	-	
Cordia nodosa	High	0.06	0.90	-	0.14	1.50	21.00	-	(11)
	Low	0.07	0.89	-	0.29	0.81	1.00	-	
	Average	0.06	0.89	0.56	0.21	1.15	-	-	
Cordia panamensis	High	0.02	0.86	-	0.00	3.79	21.00	-	(11)
	Low	0.17	0.94	-	0.25	0.35	0.00	-	
	Average	0.09	0.90	0.55	0.13	2.07	-	-	
Coussarea croceoides	High	0.21	0.93	-	0.43	6.67	-	-	(13)
	Low	0.18	0.88	-	0.43	9.13	-	-	
	Average	0.19	0.90	0.67	0.43	7.90	-	-	
Coussarea platyphylla	High	0.04	0.89	-	0.04	130.44	85.00	-	(13)
	Low	0.03	0.83	-	0.04	164.84	90.00	-	
	Average	0.03	0.86	0.37	0.04	147.64	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Damnacanthus macrophyllus	High	0.08	0.93	-	0.18	4.21	-	-	(16)
	Low	0.09	0.90	-	0.27	3.53	-	-	
	Average	0.09	0.92	0.75	0.23	3.87	-	-	
Damnacanthus major	High	0.06	0.90	-	0.10	9.71	-	-	(16)
	Low	0.09	0.90	-	0.17	4.23	-	-	
	Average	0.07	0.90	0.59	0.13	6.97	-	-	
Declieuxia fruticosa	High	0.10	0.92	-	0.49	1.25	86.70	-	(114)
	Low	0.05	0.85	-	0.23	1.44	53.30	-	
	Average	0.08	0.89	0.61	0.36	1.35	-	-	
Faramea occidentalis	High	0.18	0.94	-	0.80	0.04	28.00	-	(20)
	Low	0.07	0.83	-	0.31	0.10	20.00	-	
	Average	0.13	0.88	0.61	0.56	0.07	-	-	
Faramea suerrensis	High	0.10	0.92	-	0.19	2.10	-	-	(21)
	Low	0.00	0.72	-	0.00	5.71	-	-	
	Average	0.05	0.82	0.51	0.10	3.91	-	-	
Gaertnera vaginata	High	0.06	0.92	-	0.17	17.79	-	-	(22)
	Low	0.06	0.90	-	0.08	12.40	-	-	
	Average	0.06	0.91	0.75	0.12	15.10	-	-	
Gaertnera vaginata	High	0.06	0.92	-	0.17	18.13	-	-	(22)
	Low	0.10	0.92	-	0.25	9.33	-	-	
	Average	0.08	0.92	0.75	0.21	13.73	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Gaertnera vaginata	High	0.07	0.91	-	0.09	22.60	-	-	(22)
	Low	0.13	0.91	-	0.09	9.00	-	-	
	Average	0.10	0.91	0.69	0.09	15.80	-	-	
Gaertnera vaginata	High	0.03	0.89	-	0.08	29.48	-	-	(22)
	Low	0.16	0.93	-	0.33	5.92	-	-	
	Average	0.10	0.91	0.68	0.21	17.70	-	-	
Gaertnera vaginata	High	0.05	0.92	-	0.04	17.83	-	-	(22)
	Low	0.09	0.89	-	0.17	15.21	-	-	
	Average	0.07	0.91	0.75	0.11	16.52	-	-	
Gaertnera vaginata	High	0.06	0.93	-	0.13	14.12	-	-	(22)
	Low	0.16	0.94	-	0.29	5.17	-	-	
	Average	0.11	0.94	0.86	0.21	9.65	-	-	
Gaertnera vaginata	High	0.10	0.93	-	0.16	14.45	29.00	-	(22)
	Low	0.06	0.89	-	0.04	11.64	21.00	-	
	Average	0.08	0.91	0.76	0.10	13.04	-	-	
Gaertnera vaginata	High	0.07	0.91	-	0.17	18.52	49.00	-	(22)
	Low	0.13	0.91	-	0.28	7.27	39.00	-	
	Average	0.10	0.91	0.71	0.23	12.90	-	-	
Glandora diffusa	High	0.07	0.92	-	0.70	4.55	-	-	(25)
	Low	0.06	0.88	-	0.63	3.91	-	-	
	Average	0.07	0.90	0.66	0.67	4.23	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	
Glandora diffusa	High	0.09	0.94	-	0.88	1.90	-	-	(25)
	Low	0.12	0.93	-	0.94	0.98	-	-	
	Average	0.10	0.93	0.82	0.91	1.44	-	-	
Glandora moroccana	High	0.08	0.93	-	0.74	5.48	47.00	12.00	(25, 138)
	Low	0.12	0.92	-	0.80	2.72	76.00	19.00	
	Average	0.10	0.92	0.74	0.77	4.10	-	-	
Glandora moroccana	High	0.09	0.93	-	0.69	5.51	-	-	(25)
	Low	0.13	0.92	-	0.76	2.04	-	-	
	Average	0.11	0.93	0.77	0.72	3.78	-	-	
Glandora oleifolia	High	0.04	0.92	-	0.17	6.31	-	-	(25)
	Low	0.13	0.90	-	0.90	2.58	-	-	
	Average	0.08	0.91	0.72	0.53	4.44	-	-	
Glandora oleifolia	High	0.09	0.94	-	0.73	3.45	27.00	7.00	(25, 138)
	Low	0.08	0.90	-	0.31	3.01	30.00	8.00	
	Average	0.09	0.92	0.81	0.52	3.23	-	-	
Glandora rosmarinifolia	High	0.10	0.95	-	0.58	1.88	-	-	(25)
	Low	0.08	0.92	-	0.36	1.44	-	-	
	Average	0.09	0.93	0.85	0.47	1.66	-	-	
Glandora rosmarinifolia	High	0.06	0.93	-	0.48	5.95	-	-	(25)
	Low	0.10	0.92	-	0.63	2.32	-	-	
	Average	0.08	0.92	0.75	0.55	4.14	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	R & K	SAN	L & B	ARM	Fruit set	Seed set	
Goniolimon italicum	High	0.08	0.94	-	0.07	0.95	-	-	(28)
	Low	0.21	0.96	-	0.36	0.44	-	-	
	Average	0.15	0.95	0.91	0.22	0.69	-	-	
Houstonia caerulea	High	0.04	0.88	-	0.28	2.63	-	-	(34)
	Low	0.10	0.86	-	0.28	0.83	-	-	
	Average	0.07	0.87	0.47	0.28	1.73	-	-	
Houstonia caerulea	High	0.05	0.87	-	0.28	3.33	-	-	(34)
	Low	0.22	0.92	-	0.59	0.31	-	-	
	Average	0.13	0.89	0.38	0.43	1.82	-	-	
Houstonia caerulea	High	0.06	0.89	-	0.31	3.02	-	-	(34)
	Low	0.14	0.89	-	0.39	0.77	-	-	
	Average	0.10	0.89	0.51	0.35	1.89	-	-	
Houstonia longifolia	High	0.06	0.91	-	0.26	1.14	-	-	(34)
	Low	0.23	0.93	-	0.71	0.14	-	-	
	Average	0.15	0.92	0.72	0.48	0.64	-	-	
Houstonia longifolia	High	0.05	0.91	-	0.18	1.10	-	-	(34)
	Low	0.31	0.95	-	0.52	0.09	-	-	
	Average	0.18	0.93	0.75	0.35	0.60	-	-	
Houstonia procumbens	High	0.13	0.94	-	0.56	1.84	-	-	(34)
	Low	0.16	0.92	-	0.50	1.13	-	-	
	Average	0.14	0.93	0.81	0.53	1.49	-	-	

Table S2 continued.

		Reciprocity index values					**Reproductive success**		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Houstonia procumbens	High	0.11	0.93	-	0.75	2.90	-	-	(34)
	Low	0.13	0.91	-	0.32	2.44	-	-	
	Average	0.12	0.92	0.71	0.54	2.67	-	-	
Houstonia procumbens	High	0.15	0.95	-	0.30	1.82	-	-	(34)
	Low	0.07	0.89	-	0.26	2.64	-	-	
	Average	0.11	0.92	0.79	0.28	2.23	-	-	
Jasminum fruticans	High	0.01	0.84	-	0.00	16.20	-	9.26	(35, 139)
	Low	0.09	0.90	-	0.22	2.70	-	9.41	
	Average	0.05	0.87	0.45	0.11	9.45	-	-	
Jasminum fruticans	High	0.04	0.89	-	0.09	8.74	-	8.56	(35, 139)
	Low	0.04	0.84	-	0.13	6.71	-	10.37	
	Average	0.04	0.86	0.43	0.11	7.73	-	-	
Jasminum fruticans	High	0.00	0.84	-	0.00	12.32	-	-	(35)
	Low	0.08	0.90	-	0.19	2.32	-	-	
	Average	0.04	0.87	0.62	0.09	7.32	-	-	
Jasminum fruticans	High	0.04	0.87	-	0.11	10.74	-	10.48	(35, 139)
	Low	0.10	0.88	-	0.32	5.11	-	10.64	
	Average	0.07	0.88	0.38	0.22	7.92	-	-	
Jasminum fruticans	High	0.00	0.84	-	0.00	12.01	-	-	(35)
	Low	0.21	0.95	-	0.36	0.60	-	-	
	Average	0.11	0.90	0.60	0.18	6.30	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	R & K	SAN	L & B	ARM	Fruit set	Seed set	
Jasminum fruticans	High	0.00	0.84	-	0.00	14.55	-	8.94	(35, 139)
	Low	0.14	0.92	-	0.25	2.15	-	17.23	
	Average	0.07	0.88	0.50	0.13	8.35	-	-	
Jasminum fruticans	High	0.02	0.86	-	0.09	11.35	-	-	(35)
	Low	0.14	0.91	-	0.45	1.79	-	-	
	Average	0.08	0.89	0.47	0.27	6.57	-	-	
Jasminum fruticans	High	0.02	0.84	-	0.08	14.06	-	15.43	(35, 139)
	Low	0.16	0.92	-	0.50	1.54	-	11.97	
	Average	0.09	0.88	0.39	0.29	7.80	-	-	
Jasminum fruticans	High	0.15	0.94	-	0.40	3.36	-	-	(35)
	Low	0.13	0.92	-	0.22	2.16	-	-	
	Average	0.14	0.93	0.80	0.31	2.76	-	-	
Jasminum fruticans	High	0.06	0.90	-	0.14	6.68	-	-	(35)
	Low	0.10	0.89	-	0.17	2.69	-	-	
	Average	0.08	0.89	0.60	0.15	4.68	-	-	
Jasminum fruticans	High	0.12	0.94	-	0.38	3.05	-	-	(35)
	Low	0.08	0.86	-	0.29	5.55	-	-	
	Average	0.10	0.90	0.66	0.34	4.30	-	-	
Kalmiopsis fragrans	High	0.04	0.92	-	0.10	6.80	64.29	-	(38)
	Low	0.02	0.81	-	0.05	12.17	64.29	-	
	Average	0.03	0.86	0.52	0.07	9.48	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	R & K	SAN	L & B	ARM	Fruit set	Seed set	
Linum aretioides	High	0.00	0.82	-	0.00	5.10	71.06	-	(40)
	Low	0.12	0.90	-	0.13	0.56	73.02	-	
	Average	0.06	0.86	0.35	0.07	2.83	-	-	
Linum aretioides	High	0.06	0.84	-	0.18	5.73	-	-	(40)
	Low	0.06	0.83	-	0.23	2.92	-	-	
	Average	0.06	0.84	-0.09	0.20	4.33	-	-	
Linum campanulatum	High	0.17	0.95	-	0.48	2.48	-	-	(35)
	Low	0.09	0.89	-	0.13	5.67	-	-	
	Average	0.13	0.92	0.80	0.30	4.07	-	-	
Linum suffruticosum	High	0.15	0.93	-	0.34	1.52	-	-	(45)
	Low	0.13	0.88	-	0.44	1.39	-	-	
	Average	0.14	0.90	0.65	0.39	1.46	-	-	
Linum tenue	High	0.18	0.94	-	0.41	0.84	-	-	(45)
	Low	0.00	0.72	-	0.00	3.72	-	-	
	Average	0.09	0.83	0.60	0.21	2.28	-	-	
Linum tenuifolium	High	0.12	0.94	-	0.51	1.49	-	-	(46)
	Low	0.13	0.93	-	0.64	1.16	-	-	
	Average	0.13	0.93	0.81	0.58	1.33	-	-	
Luculia pinceana	High	0.19	0.96	-	0.27	7.51	-	-	(140)
	Low	0.27	0.97	-	0.37	3.14	-	-	
	Average	0.23	0.96	0.94	0.32	5.33	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Luculia pinceana	High	0.25	0.97	-	0.28	4.63	-	-	(140)
	Low	0.24	0.96	-	0.20	4.14	-	-	
	Average	0.25	0.97	0.96	0.24	4.39	-	-	
Luculia pinceana	High	0.39	0.98	-	0.40	1.57	-	-	(140)
	Low	0.34	0.97	-	0.43	2.03	-	-	
	Average	0.36	0.98	0.98	0.42	1.80	-	-	
Melochia nudiflora	High	0.08	0.93	-	0.30	1.99	-	-	(49)
	Low	0.20	0.94	-	0.45	0.56	-	-	
	Average	0.14	0.94	0.85	0.38	1.27	-	-	
Melochia pyramidata	High	0.14	0.94	-	0.91	0.47	-	40.05	(49, 141)
	Low	0.10	0.90	-	1.00	0.83	-	30.97	
	Average	0.12	0.92	0.76	0.96	0.65	-	-	
Melochia savannarum	High	0.08	0.91	-	0.77	0.65	-	-	(49)
	Low	0.02	0.80	-	0.39	0.97	-	-	
	Average	0.05	0.85	0.41	0.58	0.81	-	-	
Melochia savannarum	High	0.06	0.88	-	0.80	1.79	-	15.66	(49, 141)
	Low	0.08	0.87	-	0.53	1.00	-	7.13	
	Average	0.07	0.87	0.37	0.67	1.40	-	-	
Melochia savannarum	High	0.11	0.92	-	0.91	2.80	-	-	(49)
	Low	0.13	0.89	-	0.89	1.80	-	-	
	Average	0.12	0.91	0.69	0.90	2.30	-	-	

Table S2 continued.

		Reciprocity index values					**Reproductive success**		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Melochia tomentosa	High	0.08	0.92	-	0.25	3.53	80.00	-	(142)
	Low	0.05	0.78	-	0.17	4.44	83.00	-	
	Average	0.06	0.85	0.49	0.21	3.99	-	-	
Melochia tomentosa	High	0.07	0.88	-	0.62	3.90	-	9.59	(49, 141)
	Low	0.15	0.92	-	1.00	0.86	-	22.96	
	Average	0.11	0.90	0.53	0.81	2.38	-	-	
Melochia tomentosa	High	0.07	0.92	-	0.00	3.89	82.00	-	(143)
	Low	0.28	0.95	-	0.30	0.66	78.00	-	
	Average	0.17	0.93	0.80	0.15	2.28	-	-	
Melochia villosa	High	0.08	0.92	-	0.81	1.70	-	6.83	(49, 141)
	Low	0.08	0.90	-	0.38	1.22	-	16.48	
	Average	0.08	0.91	0.73	0.59	1.46	-	-	
Melochia villosa	High	0.02	0.81	-	0.36	7.14	-	-	(49)
	Low	0.15	0.91	-	0.67	1.18	-	-	
	Average	0.08	0.86	0.17	0.52	4.16	-	-	
Menyanthes trifoliata	High	0.05	0.88	-	0.06	11.86	-	-	(51)
	Low	0.11	0.90	-	0.22	4.06	-	-	
	Average	0.08	0.89	0.49	0.14	7.96	-	-	
Mussaenda decipiens	High	0.04	0.91	-	0.04	21.86	-	-	(53)
	Low	0.26	0.96	-	0.30	2.74	-	-	
	Average	0.15	0.93	0.81	0.17	12.30	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Mussaenda divaricata	High	0.25	0.97	-	0.32	2.25	-	-	(53)
	Low	0.00	0.77	-	0.00	45.67	-	-	
	Average	0.13	0.87	0.84	0.16	23.96	-	-	
Mussaenda erosa	High	0.01	0.91	-	0.00	16.11	-	-	(53)
	Low	0.00	0.84	-	0.00	15.20	-	-	
	Average	0.01	0.88	0.74	0.00	15.65	-	-	
Mussaenda hainanensis	High	0.00	0.84	-	0.00	61.86	-	-	(53)
	Low	0.00	0.64	-	0.00	63.99	-	-	
	Average	0.00	0.74	0.45	0.00	62.92	-	-	
Mussaenda hirsutula	High	0.04	0.91	-	0.00	16.09	-	-	(53)
	Low	0.04	0.88	-	0.06	14.92	-	-	
	Average	0.04	0.90	0.77	0.03	15.50	-	-	
Mussaenda kwangsiensis	High	0.09	0.91	-	0.17	22.93	-	-	(53)
	Low	0.00	0.67	-	0.00	44.93	-	-	
	Average	0.05	0.79	0.22	0.08	33.93	-	-	
Mussaenda kwangtungensis	High	0.13	0.94	-	0.12	13.25	-	-	(53)
	Low	0.00	0.56	-	0.00	121.79	-	-	
	Average	0.07	0.75	0.51	0.06	67.52	-	-	
Mussaenda lancipetala	High	0.10	0.93	-	0.15	17.01	-	-	(53)
	Low	0.19	0.94	-	0.37	4.29	-	-	
	Average	0.15	0.93	0.80	0.26	10.65	-	-	

Table S2 continued.

		Reciprocity index values					**Reproductive success**		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Mussaenda macrophylla	High	0.07	0.92	-	0.24	13.32	-	-	(53)
	Low	0.16	0.93	-	0.20	5.10	-	-	
	Average	0.11	0.93	0.81	0.22	9.21	-	-	
Mussaenda macrophylla	High	0.17	0.95	-	0.24	7.83	-	-	(53)
	Low	0.01	0.84	-	0.00	22.99	-	-	
	Average	0.09	0.89	0.68	0.12	15.41	-	-	
Mussaenda mollissima	High	0.09	0.92	-	0.07	29.18	-	-	(53)
	Low	0.07	0.87	-	0.14	29.14	-	-	
	Average	0.08	0.89	0.58	0.10	29.16	-	-	
Mussaenda multinervis	High	0.00	0.83	-	0.00	61.44	-	-	(53)
	Low	0.08	0.93	-	0.12	6.31	-	-	
	Average	0.04	0.88	0.65	0.06	33.87	-	-	
Mussaenda parviflora	High	0.00	0.80	-	0.00	20.32	-	-	(54)
	Low	0.14	0.92	-	0.47	1.77	-	-	
	Average	0.07	0.86	0.20	0.23	11.04	-	-	
Mussaenda pingbianensis	High	0.21	0.97	-	0.16	4.56	-	-	(53)
	Low	0.00	0.54	-	0.00	132.09	-	-	
	Average	0.10	0.75	0.56	0.08	68.32	-	-	
Mussaenda pubescens	High	0.16	0.95	-	0.40	2.56	-	-	(53)
	Low	0.00	0.72	-	0.00	13.08	-	-	
	Average	0.08	0.84	0.53	0.20	7.82	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Mussaenda pubescens	High	0.06	0.95	-	0.04	3.23	-	-	(53)
	Low	0.00	0.71	-	0.00	22.78	-	-	
	Average	0.03	0.83	0.72	0.02	13.00	-	-	
Mussaenda recurvata	High	0.03	0.87	-	0.00	34.60	-	-	(144)
	Low	0.04	0.82	-	0.00	15.93	-	-	
	Average	0.04	0.84	0.47	0.00	25.26	-	-	
Nivenia argentea	High	0.04	0.93	-	0.25	17.21	-	-	(61)
	Low	0.01	0.87	-	0.07	28.90	-	-	
	Average	0.02	0.90	0.68	0.16	23.06	-	-	
Nivenia corymbosa	High	0.07	0.94	-	0.46	7.93	-	-	(61)
	Low	0.08	0.92	-	0.23	4.58	-	-	
	Average	0.08	0.93	0.83	0.34	6.26	-	-	
Nivenia inaequalis	High	0.02	0.93	-	0.42	40.78	-	-	(61)
	Low	0.03	0.93	-	0.48	34.09	-	-	
	Average	0.02	0.93	0.81	0.45	37.44	-	-	
Nymphoides montana	High	0.03	0.92	-	0.05	2.94	79.00	70.00	(63)
	Low	0.24	0.96	-	0.40	0.37	84.00	83.00	
	Average	0.13	0.94	0.86	0.23	1.66	-	-	
Nymphoides montana	High	0.01	0.89	-	0.05	4.87	-	-	(63)
	Low	0.23	0.96	-	0.40	0.45	-	-	
	Average	0.12	0.92	0.77	0.23	2.66	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	R & K	SAN	L & B	ARM	Fruit set	Seed set	
Nymphoides montana	High	0.00	0.84	-	0.00	7.64	70.00	75.00	(63)
	Low	0.23	0.95	-	0.35	0.42	79.00	74.00	
	Average	0.12	0.90	0.61	0.18	4.03	-	-	
Ophiorrhiza japonica	High	0.17	0.96	-	0.46	1.90	-	-	(66)
	Low	0.17	0.93	-	0.33	2.04	-	-	
	Average	0.17	0.94	0.89	0.39	1.97	-	-	
Oplonia nannophylla	High	0.10	0.94	-	0.50	3.20	-	-	(69)
	Low	0.13	0.93	-	0.39	1.42	-	-	
	Average	0.12	0.93	0.82	0.44	2.31	-	-	
Oplonia nannophylla	High	0.07	0.93	-	0.61	3.69	-	-	(69)
	Low	0.10	0.91	-	0.42	2.68	-	-	
	Average	0.08	0.92	0.75	0.51	3.19	-	-	
Palicourea coriacea	High	0.12	0.93	-	0.39	3.93	-	-	(109)
	Low	0.11	0.91	-	0.49	4.38	-	-	
	Average	0.11	0.92	0.76	0.44	4.15	-	-	
Palicourea coriacea	High	0.11	0.93	-	0.06	3.54	-	-	(109)
	Low	0.15	0.92	-	0.40	4.24	-	-	
	Average	0.13	0.93	0.76	0.23	3.89	-	-	
Palicourea coriacea	High	0.11	0.94	-	0.58	3.30	-	-	(109)
	Low	0.14	0.94	-	0.33	2.18	-	-	
	Average	0.13	0.94	0.84	0.46	2.74	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Palicourea coriacea	High	0.11	0.94	-	0.33	3.23	-	-	(109)
	Low	0.16	0.94	-	0.32	1.84	-	-	
	Average	0.14	0.94	0.86	0.33	2.54	-	-	
Palicourea coriacea	High	0.11	0.94	-	0.52	3.27	-	-	(109)
	Low	0.14	0.94	-	0.33	2.47	-	-	
	Average	0.13	0.94	0.83	0.42	2.87	-	-	
Palicourea coriacea	High	0.11	0.94	-	0.27	3.16	-	-	(109)
	Low	0.13	0.93	-	0.29	3.28	-	-	
	Average	0.12	0.93	0.83	0.28	3.22	-	-	
Palicourea coriacea	High	0.17	0.95	-	0.58	2.34	-	-	(109)
	Low	0.13	0.93	-	0.00	2.07	-	-	
	Average	0.15	0.94	0.85	0.29	2.21	-	-	
Palicourea coriacea	High	0.12	0.94	-	0.36	3.66	-	-	(109)
	Low	0.11	0.92	-	0.47	4.18	-	-	
	Average	0.12	0.93	0.77	0.41	3.92	-	-	
Palicourea coriacea	High	0.15	0.95	-	0.11	2.33	-	-	(109)
	Low	0.14	0.93	-	0.33	2.94	-	-	
	Average	0.14	0.94	0.81	0.22	2.64	-	-	
Palicourea coriacea	High	0.10	0.94	-	0.25	3.04	-	-	(109)
	Low	0.14	0.94	-	0.36	2.44	-	-	
	Average	0.12	0.94	0.84	0.31	2.74	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	R & K	SAN	L & B	ARM	Fruit set	Seed set	
Palicourea coriacea	High	0.12	0.94	-	0.27	3.21	-	-	(109)
	Low	0.14	0.93	-	0.24	3.31	-	-	
	Average	0.13	0.94	0.83	0.25	3.26	-	-	
Palicourea coriacea	High	0.15	0.94	-	0.31	3.26	-	-	(109)
	Low	0.14	0.94	-	0.17	1.87	-	-	
	Average	0.15	0.94	0.83	0.24	2.57	-	-	
Palicourea croceoides	High	0.03	0.91	-	0.05	7.00	54.90	-	(112)
	Low	0.15	0.93	-	0.38	3.71	69.60	-	
	Average	0.09	0.92	0.72	0.22	5.35	-	-	
Palicourea croceoides	High	0.10	0.93	-	0.24	6.67	95.00	-	(13)
	Low	0.09	0.88	-	0.36	9.13	81.25	-	
	Average	0.09	0.90	0.67	0.30	7.90	-	-	
Palicourea padifolia	High	0.21	0.96	-	0.57	2.14	49.30	36.90	(76)
	Low	0.00	0.76	-	0.00	17.75	40.50	29.70	
	Average	0.10	0.86	0.65	0.29	9.94	-	-	
Palicourea rigida	High	0.03	0.96	-	0.32	0.04	56.60	-	(78)
	Low	0.02	0.95	-	0.19	0.03	52.55	-	
	Average	0.02	0.96	0.89	0.25	0.03	-	-	
Palicourea rigida	High	0.01	0.95	-	0.15	0.06	31.80	-	(78)
	Low	0.03	0.94	-	0.26	0.05	41.70	-	
	Average	0.02	0.94	0.85	0.21	0.06	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Palicourea tetragona	High	0.21	0.96	-	0.00	22.78	-	-	(80)
	Low	0.16	0.95	-	0.20	31.97	-	-	
	Average	0.19	0.95	0.92	0.10	27.37	-	-	
Pentanisia angustifolia	High	0.09	0.92	-	0.17	16.10	28.50	-	(82)
	Low	0.11	0.91	-	0.25	11.86	33.10	-	
	Average	0.10	0.92	0.73	0.21	13.98	-	-	
Pentanisia prunelloides	High	0.11	0.93	-	0.29	10.86	61.30	-	(82)
	Low	0.14	0.93	-	0.12	5.89	61.30	-	
	Average	0.12	0.93	0.80	0.20	8.37	-	-	
persicaria wugongshanensis	High	0.02	0.95	-	0.00	0.19	-	-	(85)
	Low	0.50	0.98	-	0.85	0.01	-	-	
	Average	0.26	0.97	0.97	0.43	0.10	-	-	
Plumbago auriculata	High	0.06	0.95	-	0.07	1.81	26.72	-	(87)
	Low	0.24	0.96	-	0.42	0.78	23.11	-	
	Average	0.15	0.95	0.92	0.24	1.29	-	-	
Polygonum hastatosagittatum	High	0.25	0.97	-	0.54	0.06	-	78.20	(145)
	Low	0.11	0.92	-	0.17	0.12	-	74.10	
	Average	0.18	0.94	0.93	0.36	0.09	-	-	
Polygonum jucundum	High	0.22	0.97	-	0.45	0.07	-	-	(146)
	Low	0.00	0.83	-	0.00	0.40	-	-	
	Average	0.11	0.90	0.82	0.23	0.24	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Primula allionii	High	0.08	0.91	-	0.44	3.70	-	-	(89)
	Low	0.18	0.88	-	0.63	0.82	-	-	
	Average	0.13	0.89	0.58	0.53	2.26	-	-	
Primula chungensis	High	0.20	0.96	-	0.71	1.22	-	-	(92)
	Low	0.07	0.84	-	0.36	9.62	-	-	
	Average	0.14	0.90	0.63	0.53	5.42	-	-	
Primula marginata	High	0.07	0.90	-	0.37	4.83	-	-	(96)
	Low	0.18	0.89	-	0.60	0.87	-	-	
	Average	0.13	0.90	0.53	0.49	2.85	-	-	
Primula merrilliana	High	0.22	0.97	-	0.60	0.38	-	60.50	(97)
	Low	0.19	0.95	-	0.30	0.28	-	70.70	
	Average	0.21	0.96	0.93	0.45	0.33	-	-	
Primula mistassinica	High	0.20	0.94	-	0.56	0.60	-	-	(98)
	Low	0.29	0.95	-	0.79	0.25	-	-	
	Average	0.25	0.94	0.86	0.68	0.43	-	-	
Primula oreodoxa	High	0.16	0.95	-	0.76	1.32	90.10	-	(147)
	Low	0.23	0.95	-	0.75	0.60	88.60	-	
	Average	0.19	0.95	0.89	0.75	0.96	-	-	
Primula veris	High	0.05	0.89	-	0.20	12.97	-	57.14	(104)
	Low	0.11	0.89	-	0.27	5.90	-	66.37	
	Average	0.08	0.89	0.57	0.24	9.44	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Primula veris	High	0.07	0.91	-	0.20	8.55	-	-	(104)
	Low	0.11	0.92	-	0.31	3.31	-	-	
	Average	0.09	0.92	0.75	0.26	5.93	-	-	
Primula veris	High	0.08	0.92	-	0.30	9.25	-	-	(104)
	Low	0.09	0.90	-	0.22	4.26	-	-	
	Average	0.08	0.91	0.72	0.26	6.76	-	-	
Primula veris	High	0.05	0.90	-	0.10	11.20	-	-	(104)
	Low	0.04	0.88	-	0.15	6.20	-	-	
	Average	0.04	0.89	0.67	0.12	8.70	-	-	
Primula veris	High	0.09	0.91	-	0.28	9.75	-	-	(104)
	Low	0.13	0.91	-	0.31	4.20	-	-	
	Average	0.11	0.91	0.71	0.30	6.98	-	-	
Primula veris	High	0.17	0.95	-	0.60	3.29	-	69.35	(104)
	Low	0.16	0.93	-	0.32	2.24	-	67.86	
	Average	0.17	0.94	0.88	0.46	2.76	-	-	
Primula veris	High	0.18	0.96	-	0.41	1.85	-	-	(104)
	Low	0.23	0.95	-	0.48	1.23	-	-	
	Average	0.21	0.95	0.92	0.44	1.54	-	-	
Primula veris	High	0.16	0.95	-	0.26	3.05	-	-	(104)
	Low	0.11	0.92	-	0.24	2.97	-	-	
	Average	0.14	0.93	0.87	0.25	3.01	-	-	

Table S2 continued.

		Reciprocity index values					**Reproductive success**		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Primula veris	High	0.21	0.96	-	0.49	1.62	-	-	(104)
	Low	0.16	0.94	-	0.19	1.78	-	-	
	Average	0.18	0.95	0.92	0.34	1.70	-	-	
Primula veris	High	0.11	0.93	-	0.17	6.17	-	-	(35)
	Low	0.14	0.89	-	0.26	4.80	-	-	
	Average	0.13	0.91	0.72	0.22	5.49	-	-	
Psychotria asiatica	High	0.18	0.93	-	0.75	0.29	14.10	-	(105)
	Low	0.11	0.85	-	0.29	0.57	0.00	-	
	Average	0.14	0.89	0.63	0.52	0.43	-	-	
Psychotria asiatica	High	0.24	0.95	-	0.73	0.22	15.40	-	(105)
	Low	0.11	0.88	-	0.28	0.49	0.00	-	
	Average	0.18	0.91	0.74	0.50	0.36	-	-	
Psychotria asiatica	High	0.21	0.94	-	0.77	0.22	-	-	(105)
	Low	0.03	0.78	-	0.06	1.12	-	-	
	Average	0.12	0.86	0.51	0.42	0.67	-	-	
Psychotria boninensis	High	0.16	0.93	-	0.60	0.48	-	-	(107)
	Low	0.15	0.89	-	0.26	0.34	-	-	
	Average	0.15	0.91	0.71	0.43	0.41	-	-	
Psychotria capitata	High	0.11	0.95	-	0.46	2.16	53.10	-	(112)
	Low	0.12	0.93	-	0.37	2.22	59.00	-	
	Average	0.11	0.94	0.86	0.41	2.19	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Psychotria capitata	High	0.10	0.94	-	0.35	2.08	80.70	-	(109)
	Low	0.22	0.94	-	0.41	0.57	80.00	-	
	Average	0.16	0.94	0.85	0.38	1.32	-	-	
Psychotria carthagenensis	High	0.08	0.92	-	0.37	1.84	30.37	-	(110)
	Low	0.12	0.93	-	0.58	0.67	24.77	-	
	Average	0.10	0.92	0.75	0.48	1.25	-	-	
Psychotria carthagenensis	High	0.00	0.79	-	0.04	7.55	3.72	-	(110)
	Low	0.10	0.93	-	0.55	0.64	9.77	-	
	Average	0.05	0.86	0.28	0.29	4.10	-	-	
Psychotria carthagenensis	High	0.11	0.93	-	0.61	1.36	4.72	-	(110)
	Low	0.15	0.93	-	0.61	0.57	9.90	-	
	Average	0.13	0.93	0.78	0.61	0.96	-	-	
Psychotria cephalophora	High	0.16	0.95	-	0.55	0.36	-	-	(111)
	Low	0.10	0.89	-	0.25	0.63	-	-	
	Average	0.13	0.92	0.80	0.40	0.50	-	-	
Psychotria colorata	High	0.11	0.95	-	0.55	5.18	73.00	-	(112)
	Low	0.17	0.95	-	0.76	2.66	77.80	-	
	Average	0.14	0.95	0.87	0.65	3.92	-	-	
Psychotria deflexa	High	0.07	0.90	-	0.38	1.59	12.50	-	(109)
	Low	0.16	0.90	-	0.62	0.39	3.60	-	
	Average	0.12	0.90	0.58	0.50	0.99	-	-	

Table S2 continued.

		Reciprocity index values					**Reproductive success**		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Psychotria deflexa	High	0.11	0.93	-	0.50	1.10	25.00	-	(114)
	Low	0.11	0.90	-	0.40	0.81	30.00	-	
	Average	0.11	0.91	0.74	0.45	0.96	-	-	
Psychotria deflexa	High	0.01	0.86	-	0.03	6.76	12.20	-	(148)
	Low	0.00	0.77	-	0.00	4.78	3.60	-	
	Average	0.00	0.81	0.38	0.01	5.77	-	-	
Psychotria gracilenta	High	0.08	0.91	-	0.42	1.57	-	-	(112)
	Low	0.04	0.79	-	0.30	3.33	-	-	
	Average	0.06	0.85	0.30	0.36	2.45	-	-	
Psychotria hoffmannseggiana	High	0.07	0.92	-	0.33	1.06	84.80	-	(112)
	Low	0.07	0.90	-	0.30	0.83	86.00	-	
	Average	0.07	0.91	0.72	0.32	0.95	-	-	
Psychotria hoffmannseggiana	High	0.12	0.93	-	0.51	0.65	80.40	-	(109)
	Low	0.14	0.90	-	0.53	0.51	85.20	-	
	Average	0.13	0.91	0.71	0.52	0.58	-	-	
Psychotria homalosperma	High	0.11	0.92	-	0.24	23.30	-	-	(116)
	Low	0.03	0.80	-	0.03	42.54	-	-	
	Average	0.07	0.86	0.43	0.14	32.92	-	-	
Psychotria homalosperma	High	0.09	0.88	-	0.20	43.45	-	-	(116)
	Low	0.03	0.80	-	0.07	47.33	-	-	
	Average	0.06	0.84	0.17	0.14	45.39	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Psychotria jasminoides	High	0.12	0.94	-	0.26	3.25	-	-	(149)
	Low	0.16	0.93	-	0.34	2.48	-	-	
	Average	0.14	0.94	0.79	0.30	2.86	-	-	
Psychotria leiocarpa	High	0.05	0.87	-	0.33	2.37	80.00	-	(148)
	Low	0.07	0.81	-	0.22	0.95	80.10	-	
	Average	0.06	0.84	0.23	0.27	1.66	-	-	
Psychotria nervosa	High	0.04	0.94	-	0.03	44.43	4.67	-	(118)
	Low	0.27	0.97	-	0.34	5.71	4.40	-	
	Average	0.16	0.96	0.92	0.19	25.07	-	-	
Psychotria nervosa	High	0.10	0.97	-	0.17	16.59	4.89	-	(118)
	Low	0.30	0.97	-	0.30	4.63	4.82	-	
	Average	0.20	0.97	0.96	0.23	10.61	-	-	
Psychotria peoppigiana	High	0.02	0.88	-	0.00	14.24	-	-	(80)
	Low	0.15	0.90	-	0.17	5.20	-	-	
	Average	0.08	0.89	0.58	0.08	9.72	-	-	
Psychotria poeppigiana	High	0.13	0.94	-	0.36	9.77	-	-	(21)
	Low	0.18	0.93	-	0.43	4.86	-	-	
	Average	0.16	0.93	0.82	0.39	7.31	-	-	
Psychotria serpens	High	0.14	0.94	-	0.40	0.50	-	-	(120)
	Low	0.20	0.92	-	0.38	0.19	-	-	
	Average	0.17	0.93	0.81	0.39	0.34	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	
Psychotria serpens	High	0.20	0.96	-	0.46	0.20	-	-	(120)
	Low	0.14	0.90	-	0.21	0.30	-	-	
	Average	0.17	0.93	0.88	0.34	0.25	-	-	
Psychotria serpens	High	0.14	0.94	-	0.28	0.46	53.40	-	(120)
	Low	0.17	0.91	-	0.46	0.29	40.20	-	
	Average	0.16	0.92	0.80	0.37	0.38	-	-	
Psychotria suerrensis	High	0.14	0.93	-	0.29	9.22	-	-	(21)
	Low	0.19	0.93	-	0.33	2.68	-	-	
	Average	0.17	0.93	0.78	0.31	5.95	-	-	
Psychotria suterella	High	0.16	0.95	-	0.39	4.48	46.10	-	(122)
	Low	0.17	0.94	-	0.43	3.08	62.00	-	
	Average	0.17	0.95	0.88	0.41	3.78	-	-	
Psychotria trichophora	High	0.11	0.94	-	0.40	3.39	31.40	-	(109)
	Low	0.12	0.91	-	0.31	2.59	19.70	-	
	Average	0.11	0.92	0.79	0.35	2.99	-	-	
Psychotria trichophora	High	0.18	0.96	-	0.52	1.41	33.20	-	(148)
	Low	0.23	0.94	-	0.44	1.05	19.30	-	
	Average	0.20	0.95	0.90	0.48	1.23	-	-	
Pulmonaria angustifolia	High	0.07	0.94	-	0.10	2.85	-	-	(124)
	Low	0.23	0.95	-	0.50	0.54	-	-	
	Average	0.15	0.94	0.84	0.30	1.69	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	R & K	SAN	L & B	ARM	Fruit set	Seed set	
Pulmonaria angustifolia	High	0.13	0.95	-	0.40	1.56	-	-	(124)
	Low	0.08	0.88	-	0.20	1.39	-	-	
	Average	0.11	0.91	0.79	0.30	1.48	-	-	
Pulmonaria collina	High	0.09	0.94	-	0.10	1.93	-	-	(124)
	Low	0.16	0.93	-	0.27	0.67	-	-	
	Average	0.13	0.94	0.86	0.19	1.30	-	-	
Pulmonaria collina	High	0.05	0.91	-	0.00	7.09	-	-	(124)
	Low	0.18	0.94	-	0.30	0.94	-	-	
	Average	0.12	0.92	0.69	0.15	4.01	-	-	
Pulmonaria longifolia	High	0.16	0.96	-	0.10	1.01	-	-	(124)
	Low	0.17	0.93	-	0.20	0.81	-	-	
	Average	0.16	0.95	0.90	0.15	0.91	-	-	
Pulmonaria longifolia	High	0.12	0.94	-	0.18	2.76	-	-	(35)
	Low	0.20	0.93	-	0.45	1.10	-	-	
	Average	0.16	0.94	0.84	0.31	1.93	-	-	
Pulmonaria mollis	High	0.08	0.93	-	0.10	3.48	-	-	(124)
	Low	0.14	0.92	-	0.10	1.24	-	-	
	Average	0.11	0.93	0.77	0.10	2.36	-	-	
Pulmonaria obscura	High	0.09	0.95	-	0.00	1.56	-	-	(124)
	Low	0.19	0.93	-	0.20	0.69	-	-	
	Average	0.14	0.94	0.87	0.10	1.13	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Pulmonaria obscura	High	0.04	0.92	-	0.10	3.40	-	-	(124)
	Low	0.18	0.93	-	0.20	0.70	-	-	
	Average	0.11	0.93	0.80	0.15	2.05	-	-	
Pulmonaria obscura	High	0.15	0.95	-	0.10	1.64	-	-	(124)
	Low	0.16	0.92	-	0.10	0.85	-	-	
	Average	0.15	0.94	0.86	0.10	1.25	-	-	
Pulmonaria officinalis	High	0.14	0.96	-	0.39	1.72	-	61.50	(150)
	Low	0.03	0.90	-	0.00	1.82	-	59.30	
	Average	0.08	0.93	0.87	0.19	1.77	-	-	
Pulmonaria officinalis	High	0.08	0.94	-	0.11	3.11	-	61.50	(150)
	Low	0.07	0.92	-	0.05	1.42	-	59.30	
	Average	0.07	0.93	0.83	0.08	2.26	-	-	
Pulmonaria officinalis	High	0.07	0.92	-	0.14	5.44	-	-	(124)
	Low	0.02	0.90	-	0.00	2.58	-	-	
	Average	0.05	0.91	0.70	0.07	4.01	-	-	
Pulmonaria officinalis	High	0.07	0.92	-	0.10	4.17	-	-	(124)
	Low	0.15	0.92	-	0.30	1.00	-	-	
	Average	0.11	0.92	0.73	0.20	2.58	-	-	
Pulmonaria officinalis	High	0.12	0.96	-	0.21	1.76	-	-	(124)
	Low	0.04	0.90	-	0.00	1.77	-	-	
	Average	0.08	0.93	0.87	0.11	1.76	-	-	

Table S2 continued.

		Reciprocity index values					Reproductive success		
Species	**Level**	**COC**	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	**Reference**
Pulmonaria officinalis	High	0.10	0.94	-	0.10	3.10	-	-	(124)
	Low	0.08	0.89	-	0.10	2.23	-	-	
	Average	0.09	0.91	0.79	0.10	2.66	-	-	
Pulmonaria officinalis	High	0.08	0.92	-	0.10	4.29	-	-	(124)
	Low	0.07	0.90	-	0.10	1.47	-	-	
	Average	0.07	0.91	0.73	0.10	2.88	-	-	
Pulmonaria officinalis	High	0.14	0.95	-	0.47	1.82	-	-	(126)
	Low	0.16	0.93	-	0.57	1.03	-	-	
	Average	0.15	0.94	0.85	0.52	1.42	-	-	
Pulmonaria saccharata	High	0.08	0.92	-	0.20	4.49	-	-	(124)
	Low	0.17	0.93	-	0.10	0.98	-	-	
	Average	0.13	0.93	0.75	0.15	2.73	-	-	
Pulmonaria saccharata	High	0.11	0.94	-	0.10	3.01	-	-	(124)
	Low	0.14	0.91	-	0.20	1.93	-	-	
	Average	0.13	0.93	0.82	0.15	2.47	-	-	
Salvia brandegeei	High	0.18	0.96	-	0.62	1.42	-	52.00	(130, 151)
	Low	0.13	0.93	-	0.46	1.66	-	49.00	
	Average	0.16	0.94	0.88	0.54	1.54	-	-	
Schizomussaenda henryi	High	0.06	0.93	-	0.20	23.53	-	-	(53)
	Low	0.22	0.96	-	0.28	6.88	-	-	
	Average	0.14	0.94	0.86	0.24	15.20	-	-	

Table S2 continued.

Species	Level	Reciprocity index values					Reproductive success		Reference
		COC	**R & K**	**SAN**	**L & B**	**ARM**	**Fruit set**	**Seed set**	
Turnera scabra	High	0.10	0.93	-	0.21	4.36	-	-	(134)
	Low	0.02	0.81	-	0.00	8.13	-	-	
	Average	0.06	0.87	0.64	0.11	6.24	-	-	
Turnera subulata	High	0.21	0.96	-	0.52	1.48	-	33.72	(134, 152)
	Low	0.20	0.92	-	0.34	1.48	-	33.47	
	Average	0.21	0.94	0.88	0.43	1.48	-	-	
Turnera subulata	High	0.11	0.92	-	0.16	5.65	97.00	43.40	(153)
	Low	0.17	0.91	-	0.19	2.35	87.00	71.95	
	Average	0.14	0.92	0.74	0.18	4.00	-	-	
Turnera ulmifolia	High	0.11	0.93	-	0.40	3.79	-	-	(134)
	Low	0.07	0.88	-	0.10	3.64	-	-	
	Average	0.09	0.90	0.74	0.25	3.72	-	-	
Vismia guianensis	High	0.64	0.98	-	1.00	0.00	23.00	-	(136)
	Low	0.65	0.96	-	1.00	0.00	17.00	-	
	Average	0.65	0.97	0.97	1.00	0.00	-	-	

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