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The evolution of flower allometry in selfing species

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ABSTRACT

Allometric relationships of floral organs were compared in related outcrossing (herkogamous) and selfing (autonomously self-pollinated) species in the genera *Mazus* and *Hosta* to determine how floral traits have evolved under selection favouring autonomous self-pollination. Autogamous *Scutellaria dependens* was also examined. We measured several floral traits in the five species. Selfing species had a steeper slope of the log–log regression and correlation coefficient for the filament–stigma height relationship than related outcrossing species. In three selfing species, the filament–stigma correlation was stronger than the petal–filament and petal–stigma correlations. Outcrossing species had weaker filament–stigma correlations than the other correlations. These findings suggest that, in selfing species, the placement of the stigma close to the anthers has evolved under selection favouring autogamy and that filament–stigma correlations might have evolved together with mating-system evolution in flowering plants.

Keywords: comparative study, filament–stigma correlation, flower allometry, outcrossing, selfing.

INTRODUCTION

Evolutionary biologists have long been intrigued by floral diversity in angiosperms, and pollinator-mediated selection has been thought to promote interspecific variation in floral shape, size, colour and scent in nature (Darwin, 1859; Stebbins, 1970). Many researchers have shown that the positions of the stigma and anthers in flowers are often important for animal-mediated pollination success in outcrossing species (Galen and Stanton, 1989; Wolfe and Barrett, 1989; Murcia, 1990; Kohn and Barrett, 1992; Conner *et al.*, 1995; Motten and Stone, 2000; Nishihiro *et al.*, 2000; Kudo *et al.*, 2001). For example, Conner and Sterling (1995) found that, among several floral correlations (including stigma–corolla tube correlations), those between the filaments and the corolla tube were the strongest in three plant species (*Raphanus raphanistrum*, *Brassica napus* and *Phlox divaricata*). They hypothesized that the strong filament–corolla tube correlations have evolved under selection for proper anther placement relative to the opening of the tube to enhance animal-mediated pollin-

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ation success, whereas the position of the stigma deeply inserted in the tube would not affect the efficiency of pollen receipt to the same extent in these species (see also Conner, 1997; Kudo *et al.*, 2001).

In contrast, predominantly autonomously selfing species can pollinate their own stigmas without the assistance of pollinators (Darwin, 1877; Hogan, 1983; Wyatt, 1984; Dafni and Bernhardt, 1990). Therefore, it can be hypothesized that floral traits in selfing species evolved under selection favouring autonomous self-pollination, rather than pollinator-mediated selection. According to this hypothesis, the floral correlations in selfing species may be different from those in related outcrossing species. For example, the stigma should always be placed close to the anthers for autonomous self-pollination. Thus, the correlation between the anther and stigma positions in selfing species should be stronger than in outcrossing species. Meanwhile, correlations between the petal (corolla tube) and pistil or filament should be stronger in outcrossing species than in self-pollinators. It can also be hypothesized that anther–stigma correlations should be stronger than correlations between petal (or corolla tube) lengths and the lengths of either stamens or pistils in the selfers, and that the opposite should be true in outcrossers. Thus, the correlations among floral traits may have evolved differently between selfing and outcrossing species under natural selection, prompting us to measure these correlations as a test of the above predictions.

Most traits of organisms (including morphological, physiological and life-history traits) are correlated with each other more often than expected by chance (Berg, 1960; Roff, 1996). Patterns of phenotypic correlations can be caused by intrinsic developmental constraints (developmental correlation hypothesis; see Cheverud, 1984) and may also be modified by natural selection (selective correlation hypothesis; see Berg, 1960; Kudo *et al.*, 2001). Closely related taxonomic groups that share developmental patterns but are subjected to different selection regimes are ideal for testing these hypotheses (Kudo *et al.*, 2001).

In this study, we examined two pairs of closely related selfing and outcrossing species and one additional selfing species: *Mazus japonicus, Hosta longissima* and *Scutellaria dependens* (selfers), and *M. miquelii* and *H. sieboldii* (outcrossers). We measured the sizes of several floral organs in these five species to address the following questions: (1) Is the anther–stigma height correlation stronger in *M. japonicus* and *H. longissima* than in *M. miquelii* and *H. sieboldii*, respectively? (2) Are the petal–stigma and petal–anther correlations stronger in *M. miquelii* and *H. sieboldii* than in *M. japonicus* and *H. longissima*, respectively? (3) Is the anther–stigma correlation stronger than the stigma–petal and anther–petal correlations in *M. japonicus, H. longissima* and *S. dependens* and the reverse in *M. miquelii* and *H. sieboldii*? Our results suggest that correlations among floral traits have evolved in concert with the evolution of selfing.

MATERIALS AND METHODS

Study species

We studied two *Mazus* species (Scrophulariaceae) with different pollination systems. *Mazus miquelii* Makino is a self-compatible and herkogamous perennial with a sensitive stigma that cannot set seeds in the absence of pollinators (Kimata, 1978). Several kinds of bees and beetles (*Cetonia* sp.) have been observed to pollinate this species (Kimata, 1978) and, typically, medium-sized bees crawl into its tubes to feed both on pollen and nectar, which is supplied at the bottom of each tube. *Mazus japonicus* (Thunb.) O. Kuntze is a self-

compatible, autonomously selfing annual (Kimata, 1978). No pollinator visitation was observed under natural conditions (A. Ushimaru, personal observation).

Hosta sieboldii (Paxton) J. Ingram (Liliaceae) is a self-compatible and herkogamous perennial and is mainly pollinated by bumblebees (Takahashi *et al.*, 1994). Bumblebees land on the anthers and crawl into the flower while moving forward on the filaments and the style to drink nectar supplied at the narrow part of the tepals (Takahashi *et al.*, 1994). Pollen-collecting behaviour is often observed on flowers that are laden with pollen (Takahashi *et al.*, 1994). The flowers of *H. longissima* Honda are also self-compatible, and their stigmas are mostly placed close to the anthers (Takahashi *et al.*, 1993). This perennial species produces seeds mainly through facilitated selfing (selfing caused by pollinators; see Lloyd, 1992) and autonomous selfing (Takahashi *et al.*, 1993). Outcrossing mediated by pollinators appears to occur in slightly herkogamous flowers in *H. longissima* (Takahashi *et al.*, 1993).

Scutellaria dependens Maxim. (Labiatae) is rarely visited by insects and reproduces via autonomous self-pollination (Tanaka, 1998).

Measurements

We collected one flower from each of 109 *M. miquelii* ramets and 120 *M. japonicus* individuals in May and June 1999 from the Botanical Garden of the Faculty of Science, Kyoto University, Japan (35°01′N, 135°47′E), where these species occur as natural weeds. We measured five floral characteristics using digital calipers: lip and corolla tube lengths, and stigma, long filament and short filament heights (Fig. 1).

We examined 30 and 35 flowers (1–3 flowers per plant) of *H. sieboldii* and *H. longissima*, respectively, in September 2000. Flowers of *H. sieboldii* were collected at Mt. Hiei (35°04′N, 135°51′E) and those of *H. longissima* were collected in Kyo-Tanabe City (34°48′N, 135°45′E). We did not find any insect pollinators on *H. longissima* in the field. Four floral characters (petal and sepal lengths, stigma and filament heights) were measured in these species (Fig. 1). *Hosta* has six filaments per flower; we measured two filaments and used their average height for analysis. We also averaged petal and sepal lengths as tepal length for analysis.

Floral measurements (lip length, and stigma, long filament and short filament heights) were taken on 40 flowers (1–3 flowers per plant) of *S. dependens* that were collected in Kyo-Tanabe City in September 2000.

Flower allometry

Allometric analyses were used to examine floral correlations in these species. We arbitrarily used the lip length of *Mazus* and *Scutellaria* as an indicator of petal size. Tepal length was used as the petal size for *Hosta* species. The mean sizes of floral organs of all five species are shown in Table 1. Corolla tube–filament and corolla tube–stigma correlations were examined in *Mazus* species using allometric analyses. All size data were log-transformed (base 10) and linear regressions were then performed on these transformed data. The slopes of the regressions are unaffected by the units of measurement of different structures (Smith, 1980) and are often used in allometric analyses (Gould, 1966; Niklas, 1994; Eberhard *et al.*, 1998). Log-transformation also reduces statistical problems caused by some outlying data points (Niklas, 1994). In the case of allometry, regressions often do not mean that one

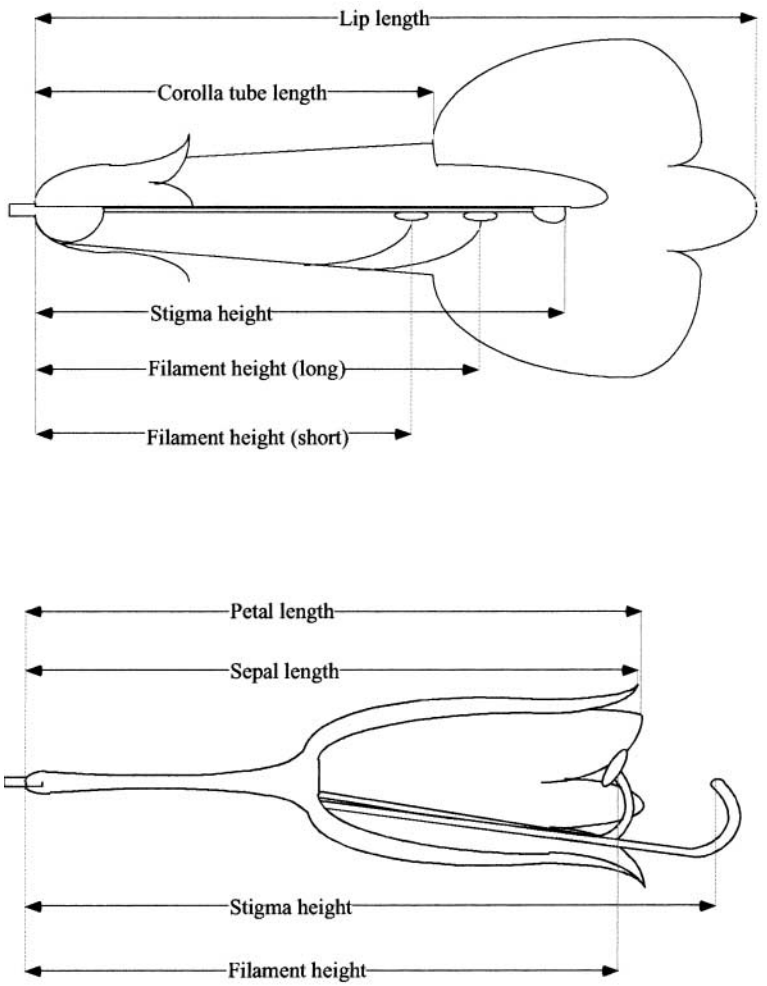

Fig. 1. Floral character measurements taken for *Mazus* and *Hosta* species. The flowers of *Scutellaria dependens* are similar to those of *Mazus*.

variable is dependent on another (Eberhard *et al.*, 1998). The regression analyses in this study were used to quantify the relative length of each floral organ and to compare intra- and interspecific differences in flower allometries.

The slope of the regression line reflects a design feature of an organism, presumably the result of selection favouring one slope over others (Eberhard *et al.*, 1998). For example, we have found evidence that pollinator-mediated selection favours low phenotypic variation in gynostemium length, independent of petal length, in the orchid *Pogonia japonica* (Ushimaru and Nakata, 2001). In this orchid, the slope of the lip–gynostemium length regression is lower than that of the lip–sepal length regression. Thus, a poor relationship between a particular floral organ and other floral organs can be expressed by a low regression slope. The correlation coefficient (r) can vary independent of the slope and may be related to genetic variation among individuals, variation in environmental factors during

Table 1. Measurements (mm) for several floral organs in *Mazus*, *Hosta* and *Scutellaria* species (mean ± standard error)

	M. japonicus	*M. miquelii*
Lip	9.73–0.12	21.06–0.17
Corolla tube	4.91–0.06	8.51–0.07
Stigma	6.68–0.08	13.24–0.11
Long filament	5.62–0.06	10.23–0.10
Short filament	4.30–0.05	8.34–0.09
	H. longissima	*H. sieboldii*
Tepal	49.91–0.34	53.96–0.65
Stigma	42.75–0.29	54.44–0.73
Filament	42.15–0.31	44.04–0.41
	S. dependens	
Lip	8.04–0.08	
Stigma	5.20–0.05	
Long filament	5.64–0.05	
Short filament	4.74–0.05	

particular stages of growth, or other similar factors (Eberhard *et al.*, 1998). Low slopes and *r*-values indicate a weak relationship between the organs (Eberhard *et al.*, 1998).

Statistical comparisons of slopes between and within species were carried out in the same way as the preliminary step of an analysis of covariance (see Sokal and Rohlf, 1995). Two correlation coefficients (the *r*-values) of the floral organs were also compared between and within species using *z*-transformations (Sokal and Rohlf, 1995; Kudo *et al.*, 2001). Their levels of significance were then adjusted with a sequential Bonferroni test (Rice, 1989).

We also compared allometries of stigma height to filament heights between the two *Mazus* species to examine the hypothesis that the filament–stigma correlation is stronger in selfing species than in outcrossing species. Because pollen grains from lower anthers are seldom transferred to the stigma in *M. japonicus* (Kimata, 1978), it can be hypothesized that the short filament–stigma correlation should be lower than that of the long filament–stigma correlation. To test this hypothesis, we examined the allometric relationship between the stigma and short filament heights. We also did this for *S. dependens*, whose stigmas are mainly autonomously pollinated with pollen from lower anthers (Tanaka, 1998).

RESULTS

Interspecific differences in *Mazus*

In both *M. miquelii* and *M. japonicus*, the sizes of all floral organs were significantly correlated with petal size and corolla tube length (Table 2). The slopes and *r*-values of the regressions on flower size were not significantly different between species (Table 2). Furthermore, the slopes and *r*-values for the corolla tube–stigma and corolla tube–filament regressions did not differ between species (Table 2).

Table 2. Slope of log–log regression and correlation coefficients (r) of regressions in three autonomously self-pollinated and two outcross-pollinated plant species

Regressions	Slope of log–log regression	r		Slope of log–log regression	r	Interspecific comparison: Slope[a]	Interspecific comparison: r[b]
	M. japonicus			*M. miquelii*			
Lip–corolla tube	0.768	0.802	**	0.783	0.791	0.31	1.22
Lip–stigma	0.797	0.787		0.685	0.680	1.27	1.74
Lip–long filament	0.729	0.797	***	0.855	0.756	2.04	0.77
Lip–short filament	0.791	0.814	***	0.956	0.733	2.88	1.52
Corolla tube–stigma	0.822	0.777		0.647	0.634	2.93	2.16
Corolla tube–long filament	0.756	0.790	***	0.940	0.822	4.38	−0.68
Corolla tube–short filament	0.845	0.833	***	0.982	0.745	1.96	1.76
Long filament–stigma	0.932	0.842		0.574	0.644	17.0***	3.45**
Short filament–stigma	0.780	0.748		0.510	0.659	9.52*	1.32
	H. longissima			*H. sieboldii*			
Tepal–stigma	0.531	0.550		0.474	0.636	0.08	−0.51
Tepal–filament	0.452	0.429		0.565	0.742	0.32	−1.90#
Filament–stigma	0.678	0.739		0.467	0.477	0.95	1.64
	S. dependens						
Lip–stigma	0.650	0.687					
Lip–long filament	0.618	0.733					
Lip–short filament	0.650	0.609					
Long filament–stigma	0.957	0.852					
Short filament–stigma	0.807	0.895					

Note: The results of intra- and interspecific comparisons of slopes and r-values between congeneric species are shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, # $P < 0.10$. All slopes and correlation coefficients were significantly different from zero. [a]F-values are shown; [b]t_s-values are shown.

On the other hand, *M. japonicus* had a significantly steeper slope and larger correlation coefficient for the long filament–stigma regression than *M. miquelii* (Table 2). The slope of the short filament–stigma regression was also significantly higher in *M. japonicus* than in *M. miquelii* (Table 2).

Intraspecific differences in *Mazus*

In *M. japonicus*, both the slope and *r*-value of the short filament–stigma regression were lower than those of the long filament–stigma regression, although these differences became non-significant when we adjusted their levels alter applying a sequential Bonferroni test (Table 2). The slopes and correlation coefficients of the two regressions also did not differ in *M. miquelii* (Table 2).

The slope and *r*-value for the long filament–stigma regressions were higher than those of the other floral regressions in *M. japonicus*, whereas the opposite patterns were found in *M. miquelii* (Table 2). The difference in the slopes was significant between the long filament–stigma and lip–long filament regressions and between the long filament–stigma and corolla tube–long filament regressions in *M. japonicus* (Table 2). In *M. miquelii*, the slope of the long filament–stigma regression was significantly lower than the slopes of the lip–filament and corolla tube–filament regressions (Table 2). Also, the short filament–stigma regression had a significantly lower slope than the lip–corolla tube, lip–filament and corolla tube–filament regressions (Table 2). The differences in the *r*-values, however, were not significant in either species (Table 2).

Interspecific differences in *Hosta*

Stigma and filament heights were significantly correlated with tepal size in both species of *Hosta* (Table 2). The slope and correlation coefficients of the filament–stigma regression were higher in *H. longissima* than in *H. sieboldii* (Table 2). *Hosta sieboldii* had a higher slope and *r*-value of the tepal–filament regression than *H. longissima* (Table 2). The difference in the *r*-values of the tepal–filament regression was marginally significant (Table 2). However, other interspecific differences in the slope and *r*-values of all regressions were not statistically significant.

Intraspecific differences in *Hosta*

The slope and *r*-value of the filament–stigma regression were higher than those of the tepal–stigma and tepal–filament regressions in *H. longissima*, while the reverse was true in *H. sieboldii* (Table 2). In *H. longissima*, the *r*-values were significantly different between the tepal–filament and filament–stigma regressions (Table 2; $t_s = -1.96$, $P < 0.05$). However, other intraspecific differences were not significant in the two species (Table 2).

Intraspecific differences in *S. dependens*

The slopes of the filament–stigma regressions were higher than those of the lip–stigma and lip–filament regressions in *S. dependens* (Table 2). However, the differences in the slopes were not significant. The *r*-value of the short filament–stigma regression was the highest among all regressions (Table 2). The differences in *r*-values were significant between the

lip–stigma and short filament–stigma regressions and between the lip–short filament and short filament–stigma regressions (Table 2; $t_s = 2.60$, $P < 0.05$ and $t_s = 3.18$, $P < 0.01$, respectively); all other differences were not significant. The slope and *r*-value did not differ between the long filament–stigma and short filament–stigma regressions.

DISCUSSION

Filament–stigma correlation in selfing and outcrossing species

The slope and *r*-value of the filament–stigma regression were both higher in autonomously selfing species than in outcrossing species in the genera *Mazus* and *Hosta*, although the differences were not always significant. This is consistent with our selective correlation hypothesis, which predicted that the filament–stigma correlation would be greater in selfers than in outcrossers. We also found a tendency for the long filament–stigma correlation to be higher than the short filament–stigma correlation in *M. japonicus*. This also suggests that the high correlations between long filaments and stigma heights have evolved under selection favouring autonomous self-pollination in this species.

Substantial variation in anther–stigma separation within a population was observed in outcrossing (herkogamous) *Erythronium grandiflorum* and *Ipomoea trichocarpa* (Thomson and Stratton, 1985; Murcia, 1990), implying weak anther–(filament–) stigma correlations in these species, similar to what we found for the outcrossers *M. miquelii* and *H. sieboldii*. Moreover, flowers with greater anther–stigma separation received more outcross pollen in *E. grandiflorum* and *I. trichocarpa* (Thomson and Stratton, 1985; Murcia, 1990). In addition, Motten and Stone (2000) found a variation in anther–stigma separation that correlated positively with outcrossing rates for single flowers in mixed-mating *Datura stramonium*. The relative position of the stigma to the anthers, therefore, appears to be able to affect outcrossing rates in flowering plants. In several species, anther and stigma heights have heritable bases and are considered to be sensitive to natural selection (Ennos, 1981; Carr and Fenster, 1994; Anderson, 1996; Campbell, 1996; Motten and Stone, 2000). Hence, anther–stigma correlations may have evolved together with mating systems in angiosperms.

Petal–filament and petal–stigma correlations in selfing and outcrossing species

We found that most slopes of the relationships between flower size and either stigma or filament height did not differ between selfing and outcrossing *Mazus* species. The corolla tube–long filament correlation was stronger in the selfer *M. miquelii* than in *M. japonicus*, but *M. japonicus* had stronger corolla tube–short filament and corolla tube–stigma correlations than *M. miquelii.* We cannot explain the high slopes and correlations seen in *M. japonicus*, although developmental constraints caused by ancestry may be responsible.

In contrast, herkogamous *H. sieboldii* had a stronger tepal–filament correlation than autogamous *H. longissima*, although the difference was only marginally significant. This marginal result is in the same direction predicted by our hypothesis. Interspecific differences in tepal–stigma correlations were not significant, suggesting that pollinator-mediated selection may have modified the relative lengths of the anthers to the tepal but not that of the stigma. A similar floral correlation pattern was reported by Conner and Sterling (1995). In some tubular-flowered species, the heights of anthers and stigmas positioned at the opening of the corolla tube often have strong correlations with the tube lengths, but those

that are deeply inserted in the tube do not (Conner and Sterling, 1995; Kudo *et al.*, 2001). In *H. sieboldii*, the anthers are located at the opening of the corolla tube, while the stigmas project out of it. Based on our hypothesis, the difference in the position of anthers or stigmas relative to the corolla opening should affect the correlation patterns. The developmental correlation hypothesis, which predicts a strong correlation between petal size and stamen size as a developmental constraint (reviewed by Delph *et al.*, 1996), cannot explain the weaker tepal–filament correlation in *H. longissima*.

Evolution of floral traits in selfing species

Comparative studies have shown that the evolutionary shift from outcrossing to selfing is often accompanied by several changes in floral traits, such as flower size, floral morphology and the pollen:ovule ratio (Cruden, 1977; Morgan and Barrett, 1989). Consistent with previous investigations (Wyatt, 1984; Morgan and Barrett, 1989; Jacquemart and Thompson, 1996), selfing species in our study had smaller flowers and less stigma–anther separation than outcrossing species in the genera *Mazus* and *Hosta*. We also found that the floral correlations were different between outcrossing and selfing species.

In the three autonomously selfing species (*M. japonicus, H. longissima* and *S. dependens*), the correlations between filament and stigma height were stronger than those between petal size and filament or stigma heights. Opposite trends were found in the two outcrossing species (*M. miquelii* and *H. sieboldii*). It is often difficult to determine which correlation pattern is ancestral (Conner, 1997). However, there is limited evidence that selfing species have arisen from outcrossing ancestors in independent phylogenetic lineages (e.g. Stebbins, 1950; Grant, 1981; Schoen *et al.*, 1997; for a review, see Takebayashi and Morrell, 2001). If this were the case, then patterns of floral correlation seen in outcrossing species would be ancestral, whereas those in selfing species would be derived under natural selection.

The evolution of phenotypic correlations among floral traits in selfing species has rarely been investigated. This may be because the small flowers of selfing species are sometimes more difficult to study than the relatively large flowers of outcrossing species. Our results suggest that consistent differences in phenotypic correlations among floral traits could exist between outcrossing and selfing species. To further evaluate our findings, more comparisons between closely related outcrossing and selfing species are required.

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