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Effect of Floral Organ Sizes on Female Reproductive Success in *Erythronium japonicum* (Liliaceae)

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We examined the effects of floral organ size on female reproductive success in self-incompatible *Erythronium japonicum*. We measured tepal size and anther-stigma separation and investigated the relationship between these measurements and fruiting and seeding success. We found that tepal length was positively correlated with fruiting success and the number of seeds per fruit. This suggests that pollinator attraction is affected by tepal length in *E. japonicum* and that the number of pollinator visits affects female reproductive success. Anther-stigma separation was the most variable floral trait measured and was not correlated with either fruiting or seeding success in this species, suggesting that the proportion of outcross pollen deposited on stigmas by pollinators does not increase with anther-stigma separation. This is inconsistent with a previous report on *Etyyhtonium. grandiflorum*. *Pollinator* size might explain this interspecific difference in the effect of anther-stigma separation on female reproductive success.

Keywords: anther-stigma separation, *Erythronium*, femal reproductive success, pollinator attraction, tepal size

Pollinator-mediated selection in angiosperms is thought to modify floral characteristics, such as shape, size, color, and scent (Darwin, 1859; Stebbins, 1970). Animal-pollinated flowers typically consist of attraction-related organs, such as sepals and petals, and mating-related organs, such as stamens and pistils. It has been suggested that the sizes of these organs sometimes evolve under different selection pressures (Galen and Stanton, 1989; Conner, 1997; Ushimaru and Nakata, 2001). For example, floral attractiveness is often influenced by petal or sepal size, and larger petals or sepals increase pollinator visits per flower in several taxa of flowering plants (Bell, 1985; Galen, 1989; Galen and Stanton, 1989; Campbell et al., 1991; Eckhart, 1991; Conner and Rush, 1996). In such angiosperms, pollinator-mediated selection should favor larger attraction-related organs. On the other hand, the sizes of the stamens and pistils often affect pollen dispatch and receipt (Thomson and Stratton, 1985; Murcia, 1990; Motten and Stone, 2000; Ushimaru and Nakata, 2001). In some plant species, stigmas that are more prominent relative to the anthers receive more outcross-pollen (Thomson and Stratton, 1985; Murcia, 1990), and anther-stigma separation affects the outcrossing rate (Motten and Stone, 2000). Thus, the sizes of both attraction-related organs and mating-related organs would affect reproductive success in flowering plants.

This paper briefly reports on how floral organ size affects female reproductive success in the spring ephemeral, *E.japonicum*. This species is self-incompatible and needs insect vectors for pollination. Substantial variation in anther-stigma separation was reported in closely related *E. grandiflorum*, in which flowers with more anther-stigma separation received more outcross-pollen (Thomson and Stratton, 1985). We measured the sizes of several floral organs to examine whether tepal (petal and sepal) size and anther-stigma separation affect female reproductive success in *E. japonicum*.

MATERIALS AND METHODS

Study Species and Site

E. japonicum Dence (Liliaceae) occurs on the floor of cool temperate forests in Japan. This perennial herb rarely exhibits clonality and reproduces sexually via ant-dispersed seeds (Ohkawara et al., 1996). In April, sexual individuals of *E. japonicum* produce a single flowering shoot, which has a pinkish, bisexual flower that lasts about two weeks (Ishii and Sakai, 2000). In dichogamous *Ipomopsis aggregata*, time spent in the

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pistillate phase affects the stigma position in flowers during anthesis (Campbell, et al. 1994). Flowers of *E. japonicum* are almost adichogamous (Ishii and Sakai, 2000) and anther-stigma separation is not affected by dichogamous habit. The species is pollinated by several species of large bees, including *Xlocopa*, *Tetralonia*, *Nomada*, and *Bombus* species (Utech and Kawano, 1975) and small solitary bees, such as the Halictidae (Ishii HS personal communication). The species produces mature fruits, which are rarely damaged by predispersal seed predators, approximately one month after anthesis.

We investigated *E. japonicum* in an old-growth deciduous forest in Ogawa Forest Reserve (36°56'N, 140°35'E, 610-660 m elevation), Kitaibaragi City, Ibaraki Prefecture, Japan, in 2002. The blooming season began in late March and lasted until late April. We observed small solitary bees visiting flowers of *E. japonicum* at our site.

Flower Measurements

In 2002, we measured petal and sepal lengths and stigma and anther heights in 144 flowers to examine how flower size and anther-stigma separation affect female reproductive success. Stigma height means style length while anther height is filament length. Flowers were collected one day after opening. We flattened petal and sepals and then measured their lengths. We measured anther height of long and short stamens. The flowers were tagged on 13 April and fruit and seed maturation was examined on 11 May.

Calculations

We averaged petal and sepal lengths as tepal length. We calculated stigma-anther separation as follows: anther-stigma separation = stigma height - long stamen anther height.

We calculated the mean size and coefficient of variation (CV = standard deviation / mean) for each character and also examined the Pearson product-moment correlations between tepal length and the sizes of other floral organs.

Satatistical Analyses

The difference in CV was compared statistically using the conventional *t*-test (Sokal and Braumann, 1980). We compared the average tepal length and anther-stigma separation between flowers that fruited and those that did not fruit using the *t*-test. Using a logistic regression analysis, we also examined the relationship between tepal length and anther-stigma separation and fruiting success with fruiting (1) or non-fruiting (0) as the dependent variable. We also examined the Pearson product-moment correlations between tepal length and anther-stigma separation and the number of (developed) seeds per flower. We included the data for immature fruits; the number of seeds per flower for non-fruited flowers was 0.

RESULTS

Stigma and anther heights were significantly correlated with tepal length (Table 1), while anther-stigma separation did not correlate with tepal length and was the most variable of the floral characters measured (Table 1). The differences in the CV between anther-stigma separation and the other floral measurements were statistically significant (d.f. = 286, $10.1 < t < 10.7$, $P < 0.001$).

Ninety five flowers (66%) set fruit in the field. Logistic regression analysis revealed that there was a significant relationship between tepal length and fruiting success (d.f. = 1, $\chi^2 = 5.61$, $P < 0.05$) and that anther-stigma separation did not affect fruiting success (d.f. = 1, $\chi^2 = 1.33$, $P > 0.05$). The mean tepal length was significantly larger in fruiting flowers than in non-fruiting flowers (Table 2). There was no significant difference in anther-stigma separation between fruiting and non-fruiting flowers (Table 2).

For the 95 fruiting flowers, the number of seeds per

Table 1. Mean (±SD) sizes, the coefficients of variation (CV), and correlation coefficients of floral organs (N = 144). The correlation coefficients were calculated between petal size and other floral organs

	Size (mm)	CV (%)	Correlation coefficient
Tepal length	46.36 ± 4.81	10.4	
Stigma height	28.36 ± 2.62	9.2	0.577***
Anther height (long stamen)	24.73 ± 2.24	9.1	0.527***
Anther height (short stamen)	18.70 ± 2.23	12.3	0.476***
Anther-stigma separation	3.64 ± 2.28	62.8	0.139

Significance level was adjusted with a sequential Bonferroni test: ***, $P < 0.001$.

Table 2. The relationships between floral sizes and fruiting success. Mean size ± SE was shown. The sampling number is shown in parenthesis.

Organ	Size of floral organs (mm)		T-test
	Fruiting (95)	Unfruiting (49)	
Tepal	47.06 ± 4.56	45.02 ± 5.03	*
SA separation	3.80 ± 2.32	3.33 ± 2.20	ns

*, $P < 0.05$; ns, $P > 0.05$.

flower was significantly correlated with tepal length (Fig. 1: $r = 0.20$, $P < 0.05$), but not with the number of ovules per flower ($r = 0.18$, $P > 0.05$) or anther-stigma separation ($r = 0.034$, $P > 0.05$).

DISCUSSION

Individuals with large flowers within a population tend to receive more pollinator visits (Bell, 1985; Galen, 1989; Galen and Stanton, 1989; Campbell et al., 1991; Eckhart, 1991; Conner and Rush, 1996). Some reports have documented that large floral displays are correlated with male success (Bell, 1985; Broyles and Wyatt, 1990, 1995) or that flower size is important for female success (Campbell, 1989; Stanton et al., 1991; Johnson et al., 1995; Conner et al., 1996a, 1996b). In this study, we found that the flowers with larger tepals had a higher fruit set and more seed per fruit in *E. japonicum*. Therefore, female reproductive success increased with the tepal length in *E. japonicum*, supporting the previous finding. Pollinator attraction should increase with tepal size and the number of pollinator visits might affect fruiting success in the Ogawa Forest Reserve. However, we did not observe pollinator visitation in this Reserve, which would help confirming this directly. In the deciduous-coniferous forest on Mt. Aoba, tepal size did not affect fruiting success of *E. japonicum* (Ishii HS personal communication). Thus, the effects of tepal size on female success differ between sites in *E. japonicum*. The difference might be caused by differences in pollinator activity between sites. Larger individuals tend to produce larger flowers in some flowering plants (Matsui et al., 2001). Thus, there is another possibility that larger flowers have more resource so that they can produce more seeds in *E. japonica*, though the idea cannot explain the difference in fruiting pattern between the Ogawa Forest Reserve and Mt. Aoba. The rate of allocation to reproductive structures, which does not correlate with plant size, is known to affect the seed production of *E. japonicum* (Kawano et al., 1982). The relationship between the reproduction allocation and flower size should be investigated in future research.

In *E. japonicum*, we found a significantly large variation in anther-stigma separation. This finding is consistent with the previous report on *E. grandiflorum* (Thomson and Stratton, 1985). However, anther-stigma separation, which was not significantly correlated with tepal length, did not correlate positively with female reproductive success, and flowers with more prominent stigma rel-

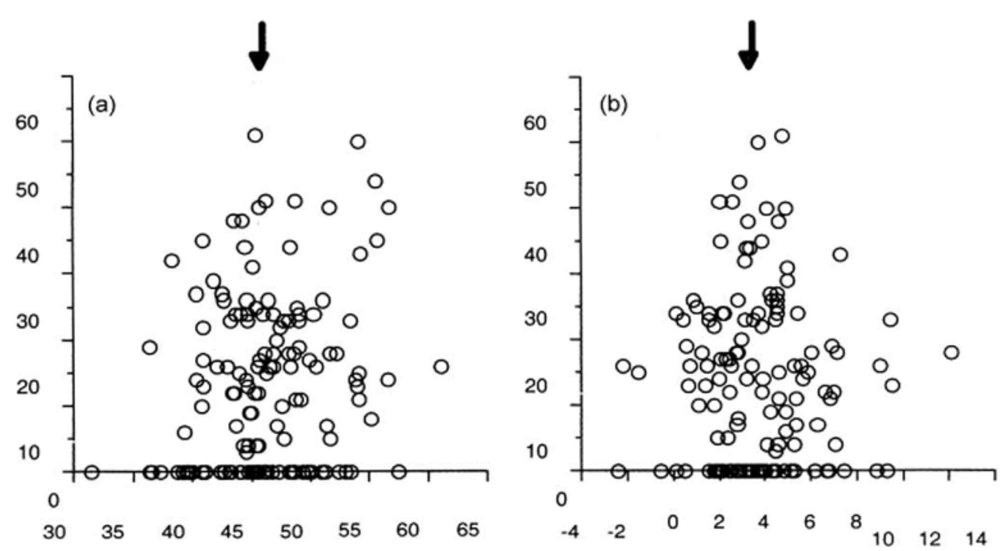

Figure 1. The relationship between tepal length (a) and anther-stigma separation (b) and the number of seeds per flower in *E. japonicum*. Arrows indicate the means of tepal length and anther-stigma separation.

ative to anthers had low fertility (Fig. 1). This seems to contrast with the results for *E. grandiflorum*, in which the proportion of outcross pollen significantly increased with anther-stigma separation that was based on bumble bee-pollinated flowers (Thomson and Stratton, 1985), while at our site small solitary bees pollinated *E. japonicum* predominantly. Thomson and Stratton (1985) proposed that the relationship between anther-stigma separation and outcrossing might be less clear in small bee-pollinated *Erythronium* flowers. Our results are consistent with this statement: Solitary bees usually landed directly on the anthers and collected pollen grains of *E. japonicum* (Ushimaru A personal observation), which is consistent with the observation of Thomson and Stratton (1985). Long anther-stigma distance might decrease the opportunity for physical contact between small pollinators and stigmas in *E. japonicum*. This idea should be examined by comparing our results with a bumble bee-pollinated *E. japonicum* population in Japan.

In this study, the size of attraction-related organs affected fruit set in *E. japonicum*, while the size of mating-related organs did not. Ushimaru and Nakata (2001) reported that the size of mating-related organs affected pollination success in *Pogonia japonica*, while tepal size did not. In addition, in *Monotropastrum globosum*, pollination success is affected by stigma area, but not by petal size (Ushimaru and Imamura, 2002). Consequently, selection on the sizes of attraction-related and mating-related organs would differ among species. The morphological and behavioral characteristics of animal vectors might explain these interspecific differences in flowering plants.

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