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EVOLUTION OF FLOWER ALLOMETRY AND ITS SIGNIFICANCE FOR POLLINATION SUCCESS IN THE DECEPTIVE ORCHID *POGONIA JAPONICA*

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Pollinator-mediated selection in the deceptive orchid *Pogonia japonica* was studied by comparing the amount of variation in and the relative size relationships between the petals, sepals, and gynostemium. The gynostemium length had a significantly lower slope in log-log regression on the indicator of the flower size for its length than did the petals or sepals. This indicates that pollinator-mediated selection may lead to low phenotypic variation of gynostemium length. Pollinia removal by pollinators and pollinia-pollinated stigma were rarely observed in the field. Flowers whose pollinia were removed had a significantly smaller gynostemium than flowers with intact pollinia. There was no significant difference in the sizes of floral organs between pollinated and unpollinated flowers. These results indicate that the size of the gynostemium in *Pogonia* has evolved to increase male reproductive success.

Keywords: deceptive orchid, flower allometry, gynostemium, pollination success, size variation.

Introduction

Natural selection has brought about a wonderful diversity of flower shape, size, color, and scent in angiosperms. Pollen transport in many flowering plants depends on specialized animal pollinators, and this specialization is considered to be one reason for the diversity of flower morphology observed (Grant and Grant 1965; Stebbins 1970; Dodson 1980; Crepet 1983; Lloyd and Barrett 1996).

The flowers of animal-pollinated plants are composed of attraction-related organs, such as the petals, and mating-related organs, such as the stamens and the pistils. Attraction-related organs may have evolved to increase the number of pollinator visits and to determine the type of pollinator, whereas mating-related organs evolved to increase pollen dispatch and receipt. Several studies have found a strong correlation in size between attraction-related and mating-related organs, which has been attributed to maximization of pollination success (Galen and Stanton 1989; Wolfe and Barrett 1989; Murcia 1990; Kohn and Barrett 1992; Conner and Sterling 1995; Conner 1997). For example, Conner and Sterling (1995) examined correlations among floral traits in some tubular-flowered plants and found that the filament-corolla tube correlation was the strongest. They suggested that the position of the anthers relative to the opening of the corolla tube is important for pollination success (but see Conner 1997). These results indicate that pollinator-mediated selection modifies the correlations in size among floral organs.

Orchid species have a unique mating-related organ called the gynostemium (column), which is a fusion of the stamen, or anther, and the pistil (Dressler 1981). There is considerable variation in the size and form of the gynostemium among different species of orchid. Many orchids are pollinated by specialized pollinators, whose size and pollinating behavior also vary (Dressler 1981; Johnson et al. 1998). Gynostemium variation among orchids is considered to reflect adaptations of each orchid species to its pollinator species (Dressler 1981). Therefore, all flowers of the same species that depend on the same pollinator species are expected to have gynostemia whose sizes fit the specific pollinator. In other words, the "optimal" gynostemium size is that which maximizes pollination success. However, since large flowers attract pollinators more effectively than small ones (Murren and Ellison 1996), a plant that can enlarge its flowers (petal and sepal size), as much as the flower's resource conditions allow, should be selected. However, the resources available to individual flowers within a species are often variable (Diggle 1995). Following the above logic, it can be predicted that within a single species (i) the size of the gynostemium varies less than the size of the attraction-related organs, and (ii) size relationships between attraction-related and mating-related organs are weak.

In this study, we measured the size of the floral organs (petals, sepals, and gynostemium) of a deceptive orchid, *Pogonia japonica*, to test the above predictions. Relationships between pollination success and the sizes of attraction-related and mating-related organs were examined. The adaptive significance of these relationships is discussed.

Material and Methods

Study Species

Pogonia japonica Reichb. f. (Orchidaceae) is a perennial herb that grows in oligotrophic wetlands, such as peat bogs, in central and northern Japan, Korea, and China and that blooms from late May to early June. Each floral shoot (ramet) has a single leaf and a single flower; the upper petals and sepals

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Table 1

Mean, Coefficients of Variation (Untransformed Data), Allometric Values (Slopes When Regressed on Lip Length), Correlation Coefficients (*r*), SE of Estimate for These Regressions, and CV′ Values for 10 Characters in *Pogonia japonica*

Part	Mean (mm)	Coefficient of variation	Slope of log-log regression	*r* value	CV′
Length:					
Lip	22.81	7.4			
Upper petal	22.91	8.0	0.997	0.927	3.00
Upper sepal	25.06	8.6	1.032	0.889	3.94
Lateral sepal	23.90	9.3	1.066	0.898	4.09
Gynostemium	12.74	5.2	0.364	0.521	4.44
Width:					
Lip	7.83	12.4	0.778	0.448	11.09
Upper petal	9.12	9.8	0.665	0.502	8.48
Upper sepal	6.98	11.6	0.808	0.511	9.97
Lateral sepal	6.02	13.5	0.992	0.522	11.51
Gynostemium	3.05	9.0	0.514	0.419	8.17

Note. All slopes and correlation coefficients were significantly different from 0 ($P < 0.001$).

are pale pink, and the lip has yellowish spots. A single flower has two pollinia, which are granular and lack a viscidium. Previous study revealed that the size of the petals increases with the size of the vegetative organs (K. Matsui et al., unpublished data). The longevity of unpollinated flowers is 1–2 wk. Fruit set of outcross-pollinated, self-pollinated, and pollinator-excluded flowers has been found to be 75%, 80%, and 0%, respectively (K. Matsui et al., unpublished data). These results indicate that this species is self-compatible and herkogamous (herkogamy, spatial separation of anthers and stigma; see Webb and Lloyd 1986) and requires an animal vector for pollination. *Pogonia japonica* produces no nectar, and pollinators are rarely observed in the field, as is the case for other deceptive orchids (Neiland and Wilcock 1998). In our study, *Ceratina flavipes* (Hymenoptera, Xylocopinae) was the only pollinator observed to carry the pollinia of *P. japonica* (A. Ushimaru, personal observations).

Study Site

We studied *P. japonica* growing on a ca. 3-ha floating peat mat in the middle of Mizorogaike Pond Nature Reserve in the northern part of Kyoto City, Japan (35°04′N, 135°45′E), on June 5, 1999. This species is often found in patches composed of many clones. We chose 12 relatively large patches (more than 9 m^2) and randomly collected 10 flowers (10 ramets) from each patch.

Floral Organ Allometry

We examined the relative size relationships of the floral organs using allometric analyses. The lengths and widths of the lips, upper petals, upper sepals, lateral sepals, and gynostemia of 120 flowers were measured with digital calipers. Hereafter, we call the petals (the lip and upper petals) and sepals attraction-related organs. The lip length was arbitrarily used as the indicator of flower size because the lip is often considered a beacon that attracts the insect and a landing place for the insect. All size data were log-transformed (base 10), and linear regressions were performed; the slopes of these regressions are unaffected by the units of measurement of different structures (Smith 1980) and are routinely used in allometric analyses (Gould 1966; Niklas 1994; Eberhard et al. 1998). Log-

Table 2

Results of Comparisons of Regression Slopes

	Length				Width				
	Upper petal	Upper sepal	Lateral sepal	Gynostemium	Lip	Upper petal	Upper sepal	Lateral sepal	Gynostemium
Length:									
Upper petal	...	ns	ns	$P < 0.001$	ns	$P < 0.01$	ns	ns	$P < 0.001$
Upper sepal	...	...	ns	$P < 0.001$	ns	$P < 0.01$	ns	ns	$P < 0.001$
Lateral sepal	...	...	...	$P < 0.001$	ns	$P < 0.001$	ns	ns	$P < 0.001$
Gynostemium	...	...	...	...	ns	$P < 0.05$	$P < 0.01$	$P < 0.001$	ns
Width:									
Lip	...	...	...	...	...	ns	ns	ns	ns
Upper petal	...	...	...	...	...	...	ns	ns	ns
Upper sepal	...	...	...	...	...	...	...	ns	ns
Lateral sepal	...	...	...	...	...	...	...	...	$P < 0.01$
Gynostemium	...	...	...	...	...	...	...	...	...

Note. ns = not significant.

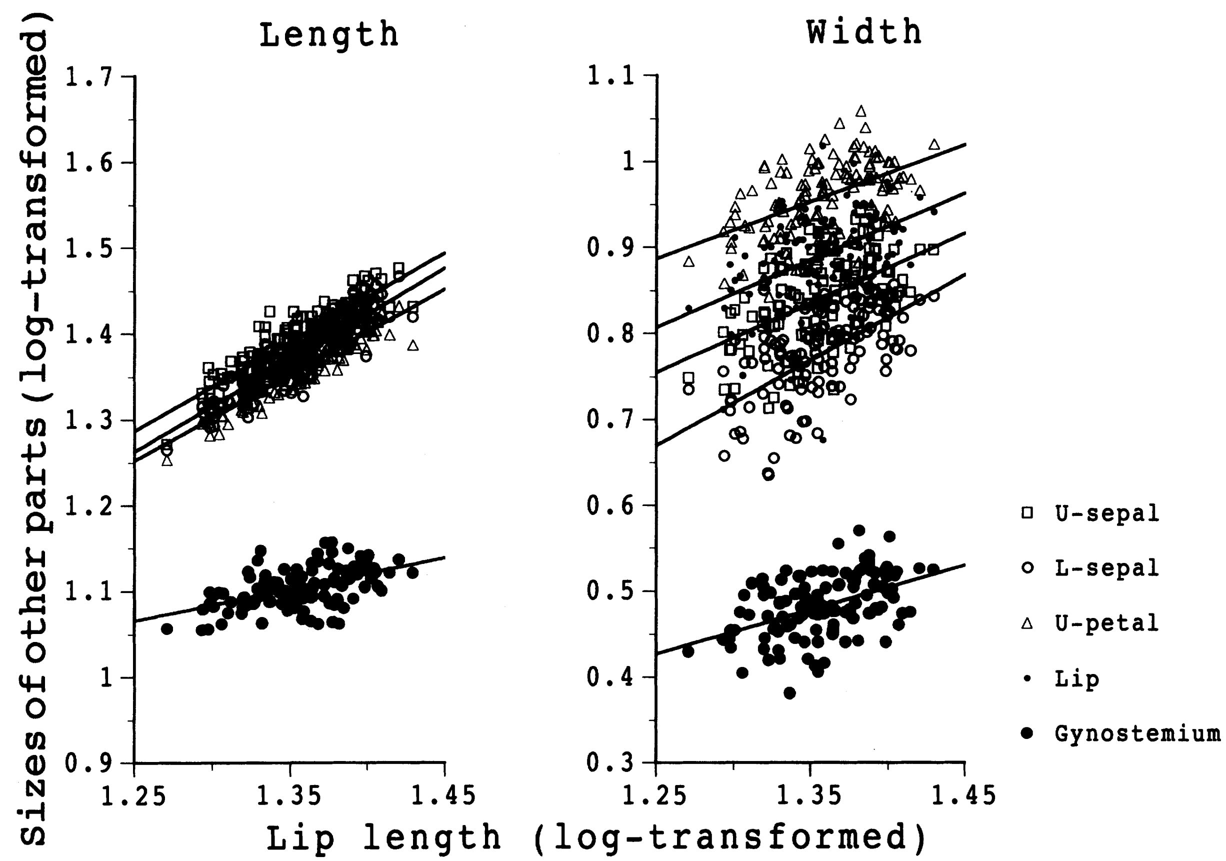

Fig. 1 The allometric relationships between floral organs and the flower size (lip length). The slope and r value for each regression refer to table 1.

transformation also reduces the statistical "noise" caused by outlying data (Niklas 1994). The slopes of the flower size regressions were compared statistically by undertaking the preliminary step of analysis of covariance (Sokal and Rohlf 1995).

It is important to note that the regressions do not imply that one variable is dependent on another. Rather, the regressions provide a means to quantify and compare relationships between different variables and yield a common reference variable (Eberhard et al. 1998). In this study, we used these regression analyses to quantify the relative size relationship of each floral organ to the flower size and to compare differences among the allometric relationships of floral organs with the flower size.

Size Variation of Each Floral Organ

The relative amount of variation in different floral organs can be expressed by the coefficient of variation (the standard deviation divided by the mean) (see Wolfe and Krstolic 1999). When one variable has a significant allometric relationship with another, the coefficient of variation can be influenced by two different factors: the slope of this allometric relationship and the degree of dispersion of points around the regression line (see details in Eberhard et al. 1998). We calculated the coefficient of variation that y would have if x were kept constant (CV′) as $\mathrm{CV}(y) \times (1 - r^2)^{1/2}$ in order to exclude the effect of the allometric relationship (the steepness of the slope) and to examine the effect of dispersion (see Eberhard et al. 1998). When the coefficient of variation is high but CV′ is low, we can conclude that high variation is attributable to the high slope of the regression. With these two statistics (the coefficient of variation and CV′), we compared differences in the size variation of different floral organs. The difference in the coefficient of variation was statistically compared using the conventional t-test (Sokal and Braumann 1980).

Relationship between Pollination Success and the Sizes of Floral Organs

Another 120 flowers from 12 patches were collected later in the 1999 flowering season. We selected flowers that were more than 1 wk old. First, we checked for removal of pollinia (by pollinator species) from the anthers and for the presence of pollinia on the stigmatic surface of each flower. The lip, upper sepal, and gynostemium lengths were measured in 45 of these 120 flowers to examine the effect of floral size on pollination success. We randomly subsampled 27 flowers out of the 102 unpollinated flowers with pollinia in order to balance the low sample size of pollinia-removed or pollinated flowers (18 flowers).

We repeated this study in June 2000. We collected 110 flowers from 11 patches and checked pollinia removal and polli-

Table 3

Relationship between Floral Sizes and Pollination Successes of *Pogonia* in 1999

	Pollinia removed ($n = 14$)	Pollinia not removed ($n = 31$)	t-test	Pollinated ($n = 6$)	Unpollinated ($n = 39$)	t-test
Gynostemium	12.06 ± 0.24	13.03 ± 0.11	$P < 0.001$	13.09 ± 0.11	12.68 ± 0.14	ns
Upper sepal	24.51 ± 0.47	25.21 ± 0.40	ns	26.25 ± 0.82	24.80 ± 0.33	ns
Lip	22.40 ± 0.29	22.26 ± 0.26	ns	23.09 ± 0.66	22.19 ± 0.20	ns

Note. Lengths of floral parts were compared between pollinia-removed and intact flowers and between pollinated and unpollinated flowers. The mean (mm), SE, and the results of t-test are shown; ns = not significant.

nated stigmas. We compared the average lengths and widths of the lip, upper sepal, and gynostemium between pollinia-removed and intact flowers and between pollinated and unpollinated flowers. We used all flowers in 2000 in order to check the effect of balancing sampling effort in 1999.

Results

Floral Organ Allometry

The length and width of all floral organs were correlated significantly with flower size (table 1). The slopes of the regressions on the flower size differed significantly between attraction-related organs and the gynostemium, and between the lengths and widths of all organs (table 2; fig. 1). The length of attraction-related organs had significantly higher slopes than the length of the gynostemium (table 2). For all floral organs, the slopes of the regressions were greater for their length than their width (table 1; fig. 1). We found a similar trend in the r value of these regressions (table 1).

Size Variation of Each Floral Organ

The coefficient of variation for gynostemium length was significantly lower than that determined for the lengths of attraction-related organs (df = 238, $3.7 \leq t \leq 5.9$; table 1). However, attraction-related organs had lower CV' values than the gynostemium. For each floral organ, the coefficients of variation for the width were significantly greater than those for the corresponding length (df = 238, $2.2 \leq t \leq 5.6$; table 1). The CV' values were also greater for the width than for the length of each floral organ.

Relationship between Pollination Success and the Sizes of Floral Organs

In 1999, pollinia removal and pollinated stigmas were observed in 14 (11.7%) and six (5%) flowers, respectively; only two flowers both lacked pollinia and had been pollinated. We found 12 (10.9%) pollinia-removed and five (4.5%) pollinated flowers (all pollinated flowers had their pollinia removed by pollinators) in 2000.

Flowers whose pollinia were removed had significantly shorter gynostemia in both 1999 and 2000 (tables 3, 4). However, the lengths and widths of the lips and upper sepals were not significantly different between flowers with intact pollinia and those in which the pollinia had been removed (tables 3, 4). There were no significant differences in the sizes of floral organs between pollinated and unpollinated flowers (tables 3, 4).

Discussion

The coefficient of variation for gynostemium length was lower than that of the attraction-related organs. This is consistent with our prediction. The low slope and r value and high CV' value for the gynostemium relative to those of attraction-related organs indicate that there is little phenotypic variation in the gynostemium length as a result of a low slope of the regression. Furthermore, we found that the flowers from which the pollinia had been removed had a shorter gynostemium, implying that selection would prevent the gynostemium from being longer because it would reduce male fitness. There was no apparent difference in the size of the gynostemium between pollinated and unpollinated flowers. This finding indicates that

Table 4

Relationship between Floral Sizes and Pollination Successes of *Pogonia* in 2000

	Pollinia removed ($n = 12$)	Pollinia not removed ($n = 98$)	t-test	Pollinated ($n = 5$)	Unpollinated ($n = 105$)	t-test
Length:						
Gynostemium	11.75 ± 0.24	12.22 ± 0.07	$P < 0.05$	11.63 ± 0.33	12.19 ± 0.34	ns
Upper sepal	24.37 ± 0.46	25.62 ± 0.27	ns	23.90 ± 0.21	25.66 ± 0.26	ns
Lip	22.81 ± 0.27	22.66 ± 0.20	ns	22.88 ± 0.12	22.66 ± 0.19	ns
Width:						
Gynostemium	3.05 ± 0.47	2.95 ± 0.02	ns	3.09 ± 0.07	2.95 ± 0.02	ns
Upper sepal	6.96 ± 0.18	6.94 ± 0.09	ns	6.80 ± 0.17	6.95 ± 0.08	ns
Lip	8.15 ± 0.18	8.23 ± 0.07	ns	8.25 ± 0.27	8.22 ± 0.07	ns

Note. Lengths and widths of floral organs were compared between pollinia-removed and intact flowers and between pollinated and unpollinated flowers. The mean (mm), SE, and the results of t-test are shown; ns = not significant.

the low phenotypic variation in gynostemium size may have evolved to increase male rather than female function, but further sampling is required to confirm this hypothesis.

In some orchid species, it has been reported that flower visitation and fruit set increase with increasing display size (the flower size or inflorescence size) (Schemske 1980; Firmage and Cole 1988; Murren and Ellison 1996). In this study, the sizes of attraction-related organs did not seem to affect pollination success in *Pogonia japonica*. However, we cannot conclude that the petal and sepal sizes did not affect the attraction function because the relationship between the size of attraction-related organs and the number of pollinator visits was not directly examined. This matter also requires further examination.

The strength of the correlations was greater and the amount of variation lower for the length of each floral organ than for their widths. Furthermore, we could not find any evidence that the widths of the floral organs affected pollination success. It appears that selection has acted more strongly on the length than on the width of the floral organs.

Pogonia japonica has no nectar, and its pollination success is pollinator limited; pollinia removal and pollinated stigma were rarely observed. These patterns have been recorded for many other deceptive orchids (Janzen et al. 1980; Firmage and Cole 1988; Zimmerman and Aide 1989; Johnson and Nilsson 1998; Neiland and Wilcock 1998). In deceptive orchids such as *Pogonia*, floral traits such as size, form, and color should have evolved to make any rare chance of pollination successful.

In summary, we found low phenotypic variation for a mating-related organ relative to attraction-related organs. This may be attributable to dependence on a single pollinator species. Wolfe and Krstolic (1999) found that the intraspecific variation in flower size was smaller in species with bilaterally symmetrical flowers than in those with radially symmetrical flowers. They attributed this lower variance in flower size in zygomorphic species to adaptation to specialized pollinators. Thus, it is expected that relatively large size variation will be found in the mating-related organs (such as gynostemium) of species that are pollinated by several different pollinators.

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