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THE ROLE OF AUTONOMOUS SELF-POLLINATION IN FLORAL LONGEVITY IN VARIETIES OF *IMPATIENS HYPOPHYLLA* (BALSAMINACEAE)[1]

HISASHI SATO[2]

Biotron Institute, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka-shi, Fukuoka 812-8581, Japan

The floral longevity of unpollinated, hand self-, and hand cross-pollinated flowers was compared in two varieties of *Impatiens hypophylla*, which contrasts with their mating systems. When flowers were emasculated and hand-pollinated every day after anthesis, their longevity was reduced. In the absence of emasculation and hand pollination, the staminate phase of the flowers of both varieties was 1 d longer. After the staminate phase, flowers of the outcrossing variety dropped their androecium, exposing the stigma and initiating the pistillate phase, which lasted for ~2 d. In contrast, flowers of the mixed-mating variety self-pollinated autonomously and then terminated their flowering. Under great seasonal variation in the pollinator visitation rate, which was observed in their natural populations, the outcrossing variety should maximize expected outcross success through the phenology of floral sex phases, whereas the mixed-mating variety self-pollinated ovules that were not outcrossed within the staminate phase. Based on these results, I suggest that the autonomous self-pollination in *I. hypophylla* induced differences both in the selfing coefficient and in floral longevity between the varieties.

Key words: autonomous self-pollination; Balsaminaceae; floral longevity; *Impatiens hypophylla*; mating system; plasticity.

Floral longevity varies considerably among angiosperm species (reviewed by Primack, 1985). To explain this variation, models of evolutionary stable strategy have been developed that incorporate both the costs and outcrossing benefits of maintaining a flower (Primack, 1985; Ashman and Schoen, 1994, 1995; Schoen and Ashman, 1995). These models predict that long-lived flowers are selected when either cross-pollination rates or floral maintenance costs are low, whereas short-lived flowers are selected when cross-pollination rates and maintenance costs are high.

This prediction is consistent with the observed variation in floral longevity in eleven species of ten families, although considerable variation remains unexplained (Ashman and Schoen, 1994, 1995). Both the above models and the empirical studies by Ashman and Schoen (1994, 1995) considered outcrossing species only. Although flowers generally last longer for outcrossing species than for their selfing relatives (Wyatt, 1984; Primack, 1985; Ritland and Ritland, 1989; Dole, 1992), variation in floral longevity among species with different mating systems remains unexplained.

In self-compatible hermaphroditic species, unfertilized ovules are often fertilized by autonomous self-pollination after a flower has been receptive to outcross pollen for several days. This delayed self-pollination (Lloyd and Schoen, 1992) is prevalent among mixed-mating and selfing species and has been regarded as a mechanism of reproductive assurance under pollinator limitation (Motten, 1982; Piper et al., 1986; Lloyd and Schoen, 1992; Rathcke and Real, 1993; Kalisz et al., 1999; but see Leclerc-Potvin and Ritland, 1994; Eckert and Schaefer, 1998). Thus, the ability or timing of autonomous self-fertilization should largely influence both the selfing rate and floral longevity, because fewer ovules should remain unfertilized after an extended outcrossing period. This study examines the consequences of autonomous self-pollination on variations in floral longevity between varieties of the same species with a different inbreeding coefficient.

In numerous species, floral longevity depends partly on successful pollination (Devlin and Stephenson, 1984; Richardson and Stephenson, 1989; Proctor and Harder, 1995; Clayton and Aizen, 1996; Yasaka et al., 1998; and reviewed by Stead, 1992; van Doorn, 1997). Shortened flowering under high pollination intensity reduces the cost of floral maintenance. Thus, realized cross-pollination decreases floral longevity and selfing rates by terminating flowering before autonomous self-fertilization. If this is the case, the roles of autonomous self-fertilization suggested above would not function under high pollination intensity. To examine this, I investigated changes in floral longevity following cross-pollination, as well as variation in longevity under natural conditions in response to changes in pollination frequency.

Impatiens hypophylla Makino is a self-compatible annual species, which includes two varieties. The varieties differ in floral longevity and inbreeding coefficient (Sato and Yahara, 1999); flowers of the variety *hypophylla*, which has a lower inbreeding coefficient, last longer than those of the variety *microhypophylla*, which has a higher inbreeding coefficient. This study addresses the following specific questions: (1) Does the ability or timing of autonomous self-pollination differ between the varieties? (2) How does floral longevity change with outcross treatment and fluctuations in pollination frequency in natural populations? (3) Can the ability or timing of facultative autonomous self-pollination explain the differences in floral longevity and in inbreeding coefficient between the two varieties?

MATERIALS AND METHODS

Biology of Impatiens hypophylla—*Impatiens hypophylla* is a chasmogamous annual species that lives in forest understories or along forest margins in eastern Japan (central Honshu, Shikoku, and central Kyushu). *Impatiens*

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[2] Phone and Fax: +081-(0)92-642-3069; e-mail: hisashi@agr.kyushu-u.ac.jp.

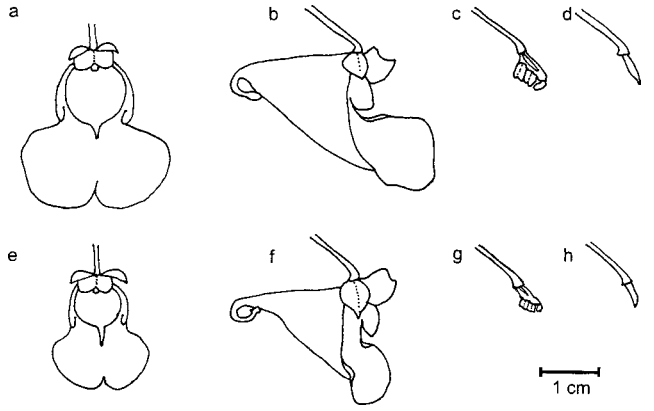

Fig. 1. Flowers of *Impatiens hypophylla* Makino var. *hypophylla* (a–d) and var. *microhypophylla* (e–h). Front view of the perianth (a and e); side view of the perianth (b and f); androecium within the perianth (c and g); pistil within the androecium (d and h).

hypophylla consists of two varieties, var. *hypophylla* and var. *microhypophylla*, which display parapatric distributions in my study areas (central Kyushu). From late August to mid-October, both varieties produce large, self-compatible hermaphroditic flowers, which hang from axillary racemes bearing several flowers. Morphologically, var. *hypophylla* and var. *microhypophylla* differ only in their floral traits; var. *hypophylla* flowers are larger and pale reddish purple, whereas those of var. *microhypophylla* are smaller and white (Akiyama, 1998). Flowers of the two varieties also differ in longevity under open-pollination, from 2.6 d for var. *hypophylla* to 2.1 d for var. *microhypophylla* (Sato and Yahara, 1999). Initially during the life of an individual flower in both varieties, the androecium covers the pistil (Fig. 1), which drops before or at the time of perianth abscission. I define the flowering stages before and after androecium drop as the staminate and pistillate phases, respectively.

Preparation of potted plants for common garden experiments—I performed all experiments with potted plants placed in the field. During early June of 1998 and 1999, I collected ~100 seedlings of each variety from natural populations in Oike (var. *hypophylla*; 33°8′ N and 131°17′ W) and Mt. Hane (var. *microhypophylla*; 33°15′ N and 131°7′ W), Oita Prefecture, Japan. The Oike and Mt. Hane populations are located ~25 km apart. To equalize environmental conditions during growth, I planted each seedling in an 18-cm pot and raised them to maturity in the forest understory near Kuju Joint-Training-Center for National Universities in the Kyushu area, ~10 km from the natural population of var. *hypophylla* and ~15 km from that of var. *microhypophylla*. Plants were watered when needed, and fertilized with 5 : 10 : 5 N : P : K solution every other week. During 1998 and 1999, I randomly selected 30 experimental plants of each variety during late August, just prior to flowering. Each year, I made an experimental array by placing potted plants in the forest understory, where they were raised.

Common garden experiments—The common garden experiments comprised two experiments. In Experiment 1, I compared self-compatibility and the timing of stigma maturation between the varieties, using the potted plants of 1998. I randomly selected six floral buds on each plant during mid-September of 1998 and randomly assigned them to one of six pollination treatments: three classes of cross- and self-pollination by hand (performed once on the morning of anthesis, once on the morning of the day after anthesis, once each morning from the day of anthesis until the day before perianth abscission). When the assigned flowers matured into fruits, I counted the number of mature seeds per fruit. For a flower that did not produce a fruit, I scored seed production as zero.

Experiment 2 compared the ability to self-pollinate autonomously and floral longevity following daily or no outcross pollen deposition between the varieties, using the potted plants of 1999. During each of three periods (1–7, 14–18, 15–21 September) in 1999, I randomly assigned up to three floral buds from each plant to autonomous autogamy treatment and another up to three floral buds to cross-pollination treatment. ''Autonomous autogamy'' flowers were left intact in a bag, whereas flowers assigned to the cross-pollination treatment were hand-pollinated once each morning from the day of anthesis until the day before perianth abscission. Each flower was observed daily, and perianth longevity and number of mature seeds produced by a flower were recorded. For flowers assigned to the autonomous autogamy treatment, I also recorded whether the androecium fell before perianth abscission. Mean perianth longevity and seed production per flower were calculated for individuals of each treatment during each period. In this calculation, seed production by a flower that did not produce a fruit was scored as zero. For each individual involved in the autonomous autogamy treatment during each period, I also calculated the frequency of androecium drop before perianth abscission and the averages of androecium longevities.

For both Experiments 1 and 2, each floral bud assigned to a facilitated pollination treatment (i.e., all treatments except autonomous autogamy) was emasculated by gently removing the androecium with a needle on the first morning after anthesis. For each cross-pollination, the stigma was coated with pollen by gently brushing it with pollen-laden androecia from one newly opened flower from each of three plants. For self-pollination, the stigma was coated with pollen by a pollen-laden anther from a newly opened flower on the same plant, which had been kept in a nylon mesh envelope before anthesis to avoid pollen contamination. Note that the androecia were already shedding pollen at the moment of anthesis. In addition, I kept all assigned flowers in nylon mesh envelopes, except during hand-pollination.

Using data from Experiment 1, I examined whether the varieties differ in self-compatibility, as well as in the timing of stigma maturation, by testing the effects of variety, pollen source (outcross vs. self), and pollination timing on seed production. In the design of this experiment, differences between the varieties cannot be estimated independently of differences among individuals. However, comparisons among pollen sources and pollination timings are part of within-individual effects. Thus, I applied split-plot ANOVA (Winer, 1971), treating variety as a whole-plot factor and pollen source and pollination timing as within-plot factors. To facilitate analysis with split-plot ANOVA, I used individuals with complete data concerning both pollen source and pollination timing. Bartlett's test (Sokal and Rohlf, 1995) showed each set of data to be homoscedastic.

Using data from Experiment 2, I examined whether the varieties differ regarding the effect of facilitated pollination and period on floral longevity and seed production. Perianth longevity and seed production were analyzed separately by split-plot ANOVA (Winer, 1971) that considered variety as a whole-plot factor and pollination treatment and period as within-plot factors. I considered only individuals with complete data on pollination treatment and period. Before conducting the ANOVA, these data were Box-Cox transformed (Sokal and Rohlf, 1995), because Bartlett's test (Sokal and Rohlf, 1995) revealed unequal variances of perianth and seed production. For the transformation of seed production, I added 0.1 to all observations to accommodate the zeroes.

Microscopic observations of autonomous self-pollen deposition—I compared the ability and timing of autonomous self-pollination between the varieties microscopically. During 1998, I randomly selected four floral buds from each experimental plant during early September and bagged them with nylon mesh envelopes before anthesis. I collected the pistil of one flower on the day of anthesis (designated as day 0), the second (day 1) or third (day 2) day after anthesis, or on the day of perianth abscission and stored it in 70% ethanol until microscopic observation. I then noted the presence or absence of pollen on the stigma.

Field observations—Throughout the flowering phase during 1999, I observed the frequency of pollinator visits for during 11 d in the Oike population and 10 d in the Mt. Hane population. I avoided rainy days for observations. On each observation day, one or two observers marked 12–55 open flowers randomly and observed them for 1 h during the afternoon. We recorded pollinator species and the number of pollinator visits to each flower. For each

TABLE 1. The relation of seed number per capsule (mean ± 1 SE) to the timing of pollination for cross-pollinated and self-pollinated flowers (Experiment 1). All flowers were emasculated on the day of anthesis.

Treatment	Pollination timing			Overall
	On the day of anthesis	1 d after anthesis	Every day after anthesis	
Var. *hypophylla* (N = 22 individuals)				
Cross-pollinated	1.0 ± 0.3	1.2 ± 0.3	1.4 ± 0.3	1.2 ± 0.3
Self-pollinated	1.5 ± 0.3	1.3 ± 0.3	1.7 ± 0.3	1.5 ± 0.3
Var. *microhypophylla* (N = 16 individuals)				
Cross-pollinated	1.9 ± 0.4	2.5 ± 0.3	1.8 ± 0.4	2.1 ± 0.4
Self-pollinated	1.9 ± 0.4	2.6 ± 0.4	2.4 ± 0.3	2.3 ± 0.4

flower, I calculated the frequency of visits by *Bombus diversus, Apis mellifera,* and *Amegilla florea* (Anthophoridae). Other observed insects, such as sphingid moths, were ignored, because they barely touched the stigma or androecium. For each pollinator species of each population, I compared visit frequency among observation days with a Kruskal-Wallis test (Sokal and Rohlf, 1995). Here, I applied nonparametric analysis instead of parametric ANOVA, because Bartlett's test (Sokal and Rohlf, 1995) revealed unequal variances of visit frequencies and Box-Cox transformation (Sokal and Rohlf, 1995) did not reduce it sufficiently.

To observe the seasonal change in floral longevity and seed production in naturally pollinated flowers during a reproductive period, I randomly marked 30 plants of each variety during late August 1999 in the Oike and Mt. Hane populations from which I had collected seedlings of var. *hypophylla* and var. *microhypophylla*. I divided the reproductive phase into the following five periods: 1–7, 8–14, 15–21 September, 26 September–1 October, and 2–5 October. The first three periods correspond to those considered in Experiment 2. For each individual, I randomly selected up to three floral buds for each period and observed them every day, recording perianth longevity and whether the androecium dropped before perianth abscission. When these flowers produced fruits, I counted the mature seeds in each fruit. Seed production of a flower that did not produce fruit was scored as zero.

For each individual in each period, I calculated average perianth longevity, androecium longevity, seed production per flower, and the frequency of androecium drop before perianth abscission. Perianth longevity, androecium longevity, and seed production of each variety were analyzed separately by the Kruskal-Wallis test (Sokal and Rohlf, 1995), in which I included period as the main factor. Again, I applied nonparametric analysis instead of parametric ANOVA, because Bartlett's test (Sokal and Rohlf, 1995) revealed unequal variances of data and Box-Cox transformation (Sokal and Rohlf, 1995) did not reduce it sufficiently.

RESULTS

Experiment 1: self-compatibility and timing of stigma maturation—Both varieties of *I. hypophylla* were equally self-compatible, and their pistils were receptive on the day of anthesis. In particular, seed production by both varieties did not vary significantly with pollen source (i.e., self- or outcross-pollen) or pollination timing (Tables 1 and 2). Note that these results may misrepresent the initiation of stigma receptivity, because pollen may remain viable on the surface of unreceptive stigmas until they become receptive. On the other hand, the androecia of both varieties shed pollen on the day of anthesis, and this pollen was fertile, as evidenced by the fruit set from hand pollinations using androecia from newly opened flowers. Overall, var. *hypophylla* produced fewer seeds than var. *microhypophylla* (Tables 1 and 2), as we found during our previous study (Sato and Yahara, 1999).

Experiment 2: plasticity of floral longevity and autonomous self-fertilization ability—Perianth longevity of the varieties of *I. hypophylla* responded differently to pollination treatment (Fig. 2; variety × pollination interaction in Table 3). For both varieties, perianth longevity was shortest in flowers in the outcrossed treatment. In the absence of cross-pollination, the staminate phase of flowers of both varieties lasted ~1 d longer than the shortest floral longevity. After the staminate phase, flowers of var. *hypophylla* dropped their androecium, exposing the stigma and initiating the pistillate phase, which lasted ~2 d. In contrast, flowers of var. *microhypophylla* terminated anthesis soon after the staminate phase. In addition, significant interaction between pollination treatment and sampling period was found in the perianth longevity (Table 3).

Only var. *microhypophylla* set seed effectively by autonomous self-fertilization (Fig. 3) resulting in a strong interaction between variety and pollination treatment (Table 4). Flowers in the autonomous autogamy treatment of var. *hypophylla* produced almost no seeds (Fig. 3). In contrast, autonomous flowers of var. *microhypophylla* produced nearly as many seeds as outcrossed flowers (Fig. 3). Average seed production (±1 SE) of flowers in the outcrossed and autonomous autogamy treatments were 1.19 (±0.11) and 0.24 (±0.06) seeds in var. *hypophylla* and 1.98 (±0.15) and 2.13 (±0.13) seeds in var. *microhypophylla*, respectively. In addition, only var. microhypophylla changed the number of seeds per capsule with pe-

TABLE 2. Split-plot ANOVA of seed number for plants in Experiment 1. Mean seed production is listed in Table 1. The variety effect was tested over among-plant residual; all other effects were tested over the within-plant error.

Source	df	Mean square	F
Among-plant comparisons			
Variety	1	6.57	8.54**
Among-plant residuals	36	0.77	
Within plants			
Pollen source	1	4.21	2.34 NS
Pollination timing	2	1.87	1.04 NS
Variety × pollen source	1	0.17	0.09 NS
Variety × pollination timing	2	2.97	1.65 NS
Pollen source × pollination timing	2	0.56	0.31 NS
Variety × pollen source × pollination timing	2	0.74	0.41 NS
Within-plant error	180	1.80	
Total	227		

** $P < 0.01$; NS: not significant.

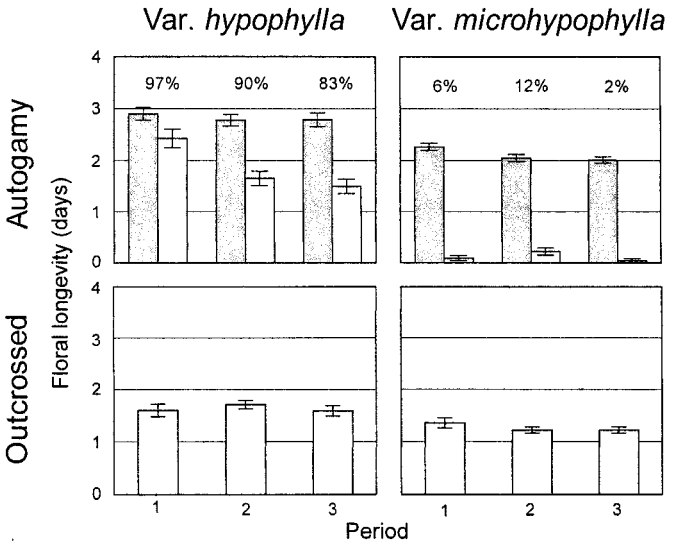

Fig. 2. Floral longevity of autonomous autogamy and outcrossed treatments during three experimental periods (Experiment 2). Shaded and open bars indicate staminate and pistillate phases, respectively. Outcrossed flowers expressed only the pistillate phase, because they had been emasculated on the day of anthesis. Error bars indicate 1 SE. Percentages above the bars indicate observed frequency of androecium drop before perianth abscission. Sample sizes of var. *hypophylla* and var. *microhypophylla* were 26 and 23 individuals, respectively.

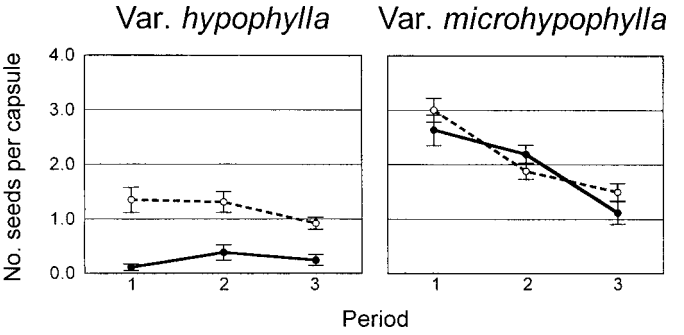

Fig. 3. Seed production of autonomous autogamy and outcrossed treatments during three experimental periods (means ± 1 SE; Experiment 2). Closed circles and solid lines indicate flowers receiving autonomous autogamy treatment, whereas open circles and dashed lines indicate flowers receiving outcross treatment. Sample sizes of var. *hypophylla* and var. *microhypophylla* were 18 and 20 individuals, respectively.

riod (Fig. 3) resulting in a highly significant interaction between variety and period (Table 4).

Autonomous self-pollination—Autonomous self-pollination seldom occurred in flowers of var. *hypophylla*, no matter when the pistil was collected (Table 5). In contrast, for var. *microhypophylla*, the proportion of flowers with self-pollen increased from 0.18 the day after anthesis to 0.86 on the third day.

Seasonal changes in pollinator visitation frequency—The frequency of bee visits fluctuated among observation periods in natural populations of both varieties (Fig. 4). *Bombus diversus* was the most abundant pollinator species throughout the observation periods. In addition, *Apis mellifera* visited flowers of var. *hypophylla*, whereas *Amegilla florea* visited flowers of var. *microhypophylla*. The effect of observation periods on visitation rate was highly significant for each pollinator species of each population (Kruskal-Wallis test, $P < 0.001$ for all tests). When the pollinator species of each population were combined, pollinator visitation frequencies were lowest during the middle flowering periods for both populations.

Seasonal changes in floral longevity and seed production—In their natural populations, floral longevity varied significantly among observation periods for both varieties (Fig. 5): the average duration (± SE) of the staminate and pistillate phases was 1.85 (±0.08) and 0.51 (±0.14) d for var. *hypophylla* and 1.31 (±0.10) and 0.02 (±0.01) d for var. *microhypophylla*, respectively. The androecium commonly fell before perianth abscission in var. *hypophylla*, especially during the third and fifth observation periods, whereas this seldom occurred in var. *microhypophylla*. Kruskal-Wallis tests showed that the longevity of both the perianth and the androecium varied significantly with observation period for both varieties. Results of the test for perianth and androecium longevity were $P < 0.001$ ($H = 46.7$) and $P < 0.05$ ($H = 12.5$) for var. *hypophylla* and $P < 0.001$ ($H = 20.3$) and $P < 0.001$ ($H = 20.0$) for var. *microhypophylla*, respectively.

For both varieties in their natural populations, seed production decreased from period 1 to period 3 and then increased

TABLE 3. Split-plot ANOVA of perianth longevity for plants in Experiment 2 (see Fig. 2 for summary of observations). The variety effect was tested over among-plant residual; all other effects were tested over the within-plant error.

Source	df	Mean square	*F*
Among-plant comparisons			
Variety	1	3.74	176.67***
Among-plant residuals	47	0.02	
Within plants			
Pollination	1	64.36	965.95***
Period	2	0.41	6.11**
Variety × pollination	1	6.62	99.36***
Variety × period	2	0.00	0.04 NS
Pollination × period	2	0.23	3.40*
Variety × pollination × period	2	0.21	3.16 NS
Within-plant error	235	0.07	
Total	293		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS: not significant.

TABLE 4. Split-plot ANOVA of seed production for plants in Experiment 2. Mean seed production values are shown in Fig. 3. The variety effect was tested over among-plant residual; all other effects were tested over the within-plant error.

Source	df	Mean square	*F*
Among-plant comparisons			
Variety	1	15.08	104.43***
Among-plant residuals	36	0.14	
Within plants			
Pollination	1	9.22	20.74***
Period	2	9.84	22.13***
Variety × pollination	1	19.76	44.41***
Variety × period	2	6.55	14.72***
Pollination × period	2	0.91	2.05 NS
Variety × pollination × period	2	0.92	2.06 NS
Within-plant error	180	0.44	
Total	227		

*** $P < 0.001$; NS: not significant.

TABLE 5. Percentage of bagged flowers with autonomous self pollination during the field observation. The numbers of flowers sampled are shown in parentheses.

Taxon	Flowers with autonomous self-pollination (%)			
	No. days after anthesis			Perianth abscission
	0	1	2	
Var. *hypophylla*	0 (28)	0 (28)	4 (28)	0 (27)
Var. *microhypophylla*	0 (26)	18 (28)	86 (29)	81 (27)

during period 4 (Kruskal-Wallis test, $P < 0.001$ for var. *hypophylla* and $P < 0.05$ for var. *microhypophylla*): the average seed production (± SE) was 2.58 (±0.44) for var. *hypophylla* and 3.05 (±0.24) for var. *microhypophylla* (Fig. 6). Thus, var. *hypophylla* produced fewer seed with more variation between periods.

DISCUSSION

Phenology of floral sex phases—The varieties differed considerably the response of floral longevity to hand pollination (Fig. 2). In both varieties, flowers that were emasculated and hand pollinated daily from the day of anthesis lasted shorter periods than flowers from which pollinators were excluded. Without facilitated pollination, the staminate phase of flowers of both varieties lasted ~1 d longer than the shortest longevity. After the staminate phase, var. *hypophylla* entered the pistillate phase, which lasted for 1–2 d, whereas var. *microhypophylla* self-pollinated and terminated flowering. Both varieties were self-compatible and had male and female organs that could function from the day of anthesis (Tables 1 and 2). Therefore the difference in self-pollination ability likely resulted from autonomous self-pollination, which occurred only in var. *microhypophylla* 1–2 d after anthesis (Table 5).

Although the stigma of var. *hypophylla* is receptive from the day of anthesis, it is covered by the androecium during the staminate phase (Fig. 1). Thus, outcross-pollination seems most likely when the androecium drops, uncovering the stigmatic surface. However, naturally pollinated flowers may receive outcross-pollen during the staminate phase if frequent pollinator visits remove most of the pollen from the anthers, exposing the stigmatic surface. Indeed, during periods 1, 2, and 4 in the natural population of var. *hypophylla*, most flowers produced many seeds (Fig. 6), even though their androecium seldom dropped before perianth abscission (Fig. 5). Thus, cross-pollination must have occurred during the staminate phase, causing floral senescence before the flowers drop their androecium. Consistent with this suggestion, the frequencies of androecium drop fluctuated with the frequency of pollinator visits in the natural population of var. *hypophylla*, except during period 5; frequencies of androecium drop increased gradually from periods 1 to 3 and then decreased during period 4 (Fig. 5), whereas the pollinator visitation rate exhibited the opposite pattern (Fig. 4). Therefore, var. *hypophylla* probably abscised the androecium before the perianth dropped primarily when pollinators visited so infrequently that the stigmas had not received sufficient cross-pollen.

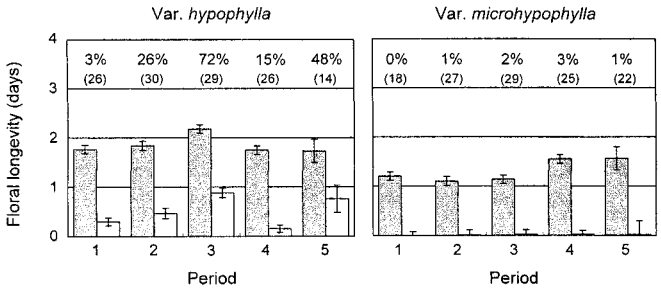

Fig. 5. Changes in floral longevity of naturally pollinated flowers. Shaded and open bars indicate the duration of the staminate phase and pistillate phase, respectively. Error bars and numbers in parentheses indicate SE and sample size (plant number), respectively. Percentages above the bars indicate observed frequency of androecium drop before perianth abscission.

Under unpredictable pollination intensity in natural populations, var. *hypophylla* should enhance outcross success by controlling androecium drop. For example, without dropping of the androecium before perianth abscission, a flower requires many visits by a pollinator to expose the stigmatic surface. Before that happens, pollinator visits increase only male success. For *Impatiens capensis*, a single pollinator visit results in much lower pollen removal than female success occurring at the same time (Bell, 1985). Thus, when pollinators visit so infrequently that pollen deposition on the stigmatic surface does not occur within some period, the limited residual male success may not justify keeping the flower open, because the estimated nectar cost of a flower in 1 d is very high in var. *hypophylla* (mean ± SD = 4.83 ± 1.67 mg/d of sugar; Sato and Yahara, 1999). In this case, the flower can enhance total outcross success by dropping the androecium and exposing the stigmatic surface, at the expense of some residual male success.

In var. *microhypophylla*, the androecium rarely dropped before perianth abscission (Fig. 2). Thus, outcross-pollen deposition on the stigmatic surface can occur only after pollinators remove enough self-pollen from the androecium to expose the stigmatic surface. In the natural population of var. *microhy-*

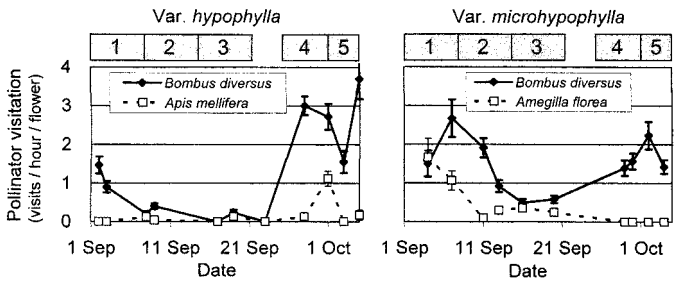

Fig. 4. Changes in pollinator visitation rate in the natural populations of var. *hypophylla* and var. *microhypophylla*. Error bars indicate SE. Numbers in boxes correspond to periods in the Figs. 5 and 6.

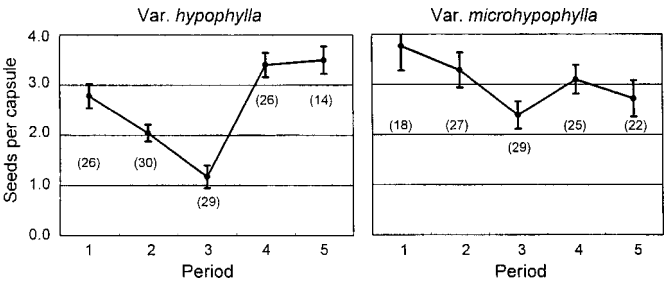

Fig. 6. Changes in seed production in the natural population of var. *hypophylla* and var. *microhypophylla*. Error bars and numbers in parentheses indicate SE and sample size (plant number), respectively.

pophylla, relatively frequent and stable pollinator visitation throughout the flowering season (Fig. 4) may result in cross-pollination before self-pollination. Indeed, plants produced many seeds and tended to terminate flowering before the timing of autonomous self-pollination (Figs. 5, 6). Moreover, average floral longevity within the natural population was equivalent to that of the pollination treatment in Experiment 2 (Figs. 2, 5), in which flowers were emasculated on the day of anthesis and outcrossed every day from the time of anthesis. This comparison indicates that stigmas naturally receive cross pollen on the day of anthesis, thus shortening floral longevity. Therefore, autonomous self-pollination of var. *microhypophylla* probably occurs only when pollinators visit infrequently, resulting in conditional selfing of ovules that had not been cross-fertilized.

Role of autonomous self-pollination in the differentiation in floral longevity and the inbreeding coefficient—As discussed previously, the phenology of floral sex phases var. *hypophylla* should enhance outcross success, whereas that of var. *microhypophylla* should allow autonomous autogamy in the absence of cross-pollination. This difference in flowering phenology corresponds to the difference in inbreeding coefficient between the varieties, which we described previously (Sato and Yahara, 1999). Because male and female organs of both varieties can function from the day of anthesis (Tables 1, 2), the difference in phenology of floral sex phases is probably the proximate cause of the difference in inbreeding.

This difference in flowering phenology could also account for the longer floral longevity of var. *hypophylla* in the natural population. However, two additional factors could also expand flower life in this variety: (1) a relatively lower frequency of pollinator visits (Fig. 4) and (2) a longer minimum floral longevity (Fig. 2). The latter factor, at least, seems to contribute the differences. Because floral longevity was longer for var. *hypophylla*, even for the periods 4 and 5 (Fig. 5), frequencies of pollinator visits were high in both populations (Fig. 4).

The within-flower phenology of var. *hypophylla* and var. *microhypophylla* should be mutually exclusive evolutionary options. Autonomous self-pollination cannot occur after androecium drop, and few unfertilized ovules should remain after autonomous self-pollination. Thus, the evolution of autonomous self-pollination in *I. hypophylla* should sacrifice the alternative flowering phenology, which maximizes expected outcross success by dropping the androecium and elongating floral longevity. In turn, the evolution of autonomous self-pollination in *I. hypophylla* should mediate an association between increased selfing and the decreased floral longevity, as was observed in the varieties.

Floral structure of var. *hypophylla*, where by the stigma is covered with the androecium (Fig. 1), caused the phenomenon of the extension of outcross periods being accompanied by androecium drop. For other self-compatible species, an extension of outcross periods could be achieved simply by delaying autonomous self-pollination, with a concurrent increase in floral longevity. If this were the case, autonomous self-pollination could mediate both the evolution of floral longevity and the mating system. The only empirical study of the effect of autofertilization on flower longevity was made by Karle and Boyle (1999). They showed that flower longevity of self-compatible cytochimeras of Easter cactus was determined by the stage of floral development in which autogamy commences. This result clearly supports my suggestion, although more rigorous testing of this issue in future studies is needed using numerous pairs of related taxa with contrasting mating systems.

It should also be pointed out that this is a restricted hypothesis, because the situation considered here involved only one of several modes of self-pollination (Lloyd and Schoen, 1992). Facilitated, rather than autonomous self-pollination, could occur in *Impatiens hypophylla*, because it is adichogamous and a self-compatible species. Facilitated self-pollination should be accompanied by outcross-pollen deposition during the staminate phase, because the stigma and androecium of *I. hypophylla* lie in close proximity. Whether such self-pollination affects floral longevity remains to be examined.

The purpose of this study was to examine the hypothesis that the ability or timing of autonomous self-pollination largely determines both the selfing rate and floral longevity. Based on the results, I suggest that the autonomous self-pollination in *I. hypophylla* induced, partially at least, differences both in the selfing coefficient and in floral longevity between the varieties. The results suggest that autonomous self-pollination influences an important role in the evolution of floral longevity. Future studies into the evolution of floral longevity of self-compatible hermaphrodite species should consider this role of autonomous self-pollination.

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