

POLLEN MOVEMENT IN A NATURAL POPULATION OF *ARISAEMA SERRATUM* (ARACEAE), A PLANT WITH A PITFALL-TRAP FLOWER POLLINATION SYSTEM¹

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Arisaema serratum possesses a pitfall-trap flower pollination system. However, little is known about the efficiency and pattern of pollen movement in *A. serratum*. Thus, the aims of this study are to (1) determine the paternal parents of the seeds and (2) elucidate pollen movement in a natural population. Paternity analysis using microsatellite markers was performed. Seeds were collected from a natural population of *A. serratum* in 2001 at Horigane, Japan. Small midges became trapped in female spathe tubes during the flowering period. We found that (1) seeds in a fruit were fertilized by multiple sires; (2) seeds sired by a paternal parent were either clumped, exclusively, or randomly distributed on the spadix, depending on the parent; (3) to a great extent, a few males contributed as sires; (4) distance from a female was not a factor in the inequality of reproductive success among males; (5) male reproductive success was not correlated with its size. We conclude that pollen carryover and the trap-flower pollination system are likely to result in multiple paternity and inequality in male success.

Key words: Araceae; *Arisaema*; Japan; male success; microsatellite; paternity analysis; pollen carryover; trap flower.

The aroids (the family Araceae) can be easily recognized by their unique spathe and spadix inflorescence. The spadix is a long cylindrical inflorescence with minute, sessile, and sometimes unisexual flowers. The spathe is a large bract, usually conspicuous in color and shape, that subtends and partly surrounds the spadix. Various diversifications are found in aroid inflorescences, not only in the design and architecture of the spathe and spadix, but also in the arrangement of male and female flowers on an axis. These diversifications of inflorescence have been believed to be closely related to pollination systems (Grayum, 1990).

One of the more conspicuous diversifications is a trap system for pollinators. Kugler (1955) defined a trap as the architecture of a flower or an inflorescence that prevents pollinators from escaping immediately by capturing them briefly until pollination is completed. The trap system in aroids is a modification of the whole inflorescence (Richards, 1997).

A well-known trap system in aroids is the pitfall trap in the genus *Arisaema* (van der Pijl, 1953; Vogel and Martens, 2000). In this system, species always form a spathe tube, and

usually form a spadix appendage, that has been believed to attract pollinators (Vogel and Martens, 2000; Gusman and Gusman, 2002). In *Arisaema*, individual plants produce male or female inflorescences depending on the size of the plant (Schaffner, 1922; Maekawa, 1924; Policansky, 1981; Bierzychudek, 1982, 1984a, b; Lovett Doust and Cavers, 1982a, b; Kinoshita, 1986, 1987; Takasu, 1987), and there is a distinguishing difference between the sexes: the male has a small hole at the bottom of the spathe tube, and the female does not (Barnes, 1935; Vogel and Martens, 2000; Fig. 1).

The presence or absence of this hole is critical to the fate of pollinators. Some pollinators directly enter the spathe tube from the tube's mouth, while others land on the spadix appendage and slide to the bottom of the tube, because abrasive wax particles cover both the inner wall of the tube and the surface of the spadix appendage (van der Pijl, 1953; Vogel and Martens, 2000). Thus, pollinators cannot escape from the spathe mouth. In the male spathe tube, pollinators can escape through the exit hole (Vogel and Martens, 2000). In the female spathe tube, no escape is possible because there is no exit hole. Because the female plant bears many apetalous flowers tightly on an axis, pollinators may walk around on the flowers to find an exit while depositing pollen grains on the stigma.

In entomophilous plants, pollinator behavior influences the efficiency and distance of pollen movement, seed set, and so on (Richards, 1997). A trap flower or inflorescence is a system that forms a very specialized relationship with the pollinator to ensure pollination (Proctor et al., 1996). For many plants with trap systems, however, little is known about the efficiency and pattern of pollen movement in natural populations.

The advantages of microsatellite markers have recently been reported in reproductive ecology (Queller et al., 1993; Ashley and Dow, 1994; Jarne and Lagoda, 1996; Schlötterer and Pemberton, 1998). One of them is paternity analysis, in which the paternal parent of a seed can be determined. This method

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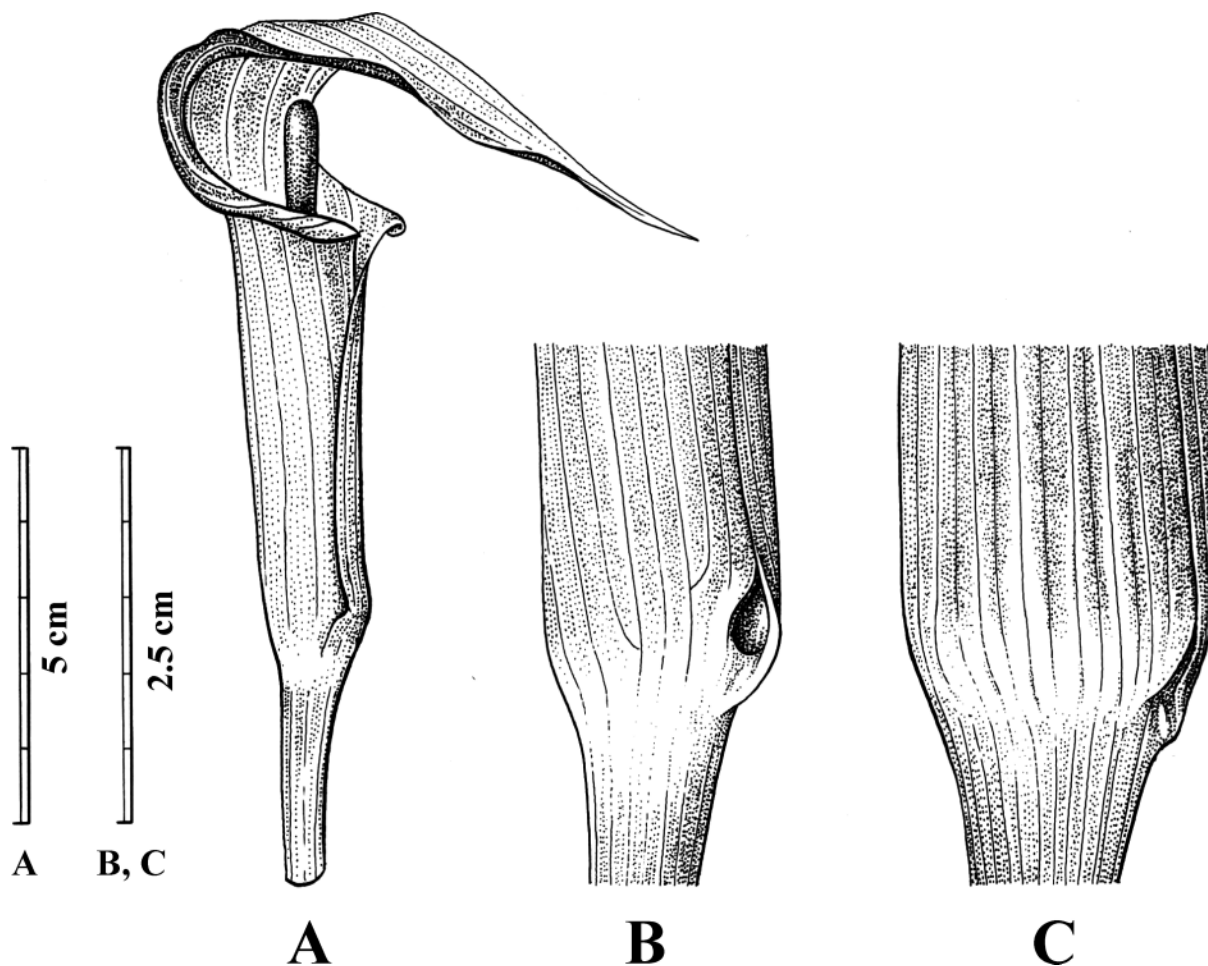


Fig. 1. Inflorescence of *Arisaema serratum*. A. Whole inflorescence. B. Lower section of male spathe with an exit hole. C. Lower section of female spathe with a closed hole.

makes it possible to trace pollen movement in a population of plants with a trap flower or inflorescence.

The present study shows the results of paternity analysis obtained by employing microsatellite markers for *Arisaema serratum* (Thunb.) Schott, the most common *Arisaema* species in Japan. The present study has two aims: (1) to determine the paternal parents of the seeds on a spadix and (2) to elucidate pollen movement in a natural population. We also comment on the relationship between plant size and reproductive success in relation to the size-advantage hypothesis (Kinoshita and Harada, 1990).

MATERIALS AND METHODS

The plant—*Arisaema serratum* (Thunb.) Schott is a perennial herb growing in the understory of forests. Sex expression in *A. serratum* depends on the size of the plant and changes from male to female and vice versa throughout the plant's lifetime (Maekawa, 1924; Kinoshita, 1986, 1987). This species also possesses a pitfall-trap flower (Fig. 1). The blooming period of *A. serratum* ranges from mid-April to late May in central Honshu (Kinoshita, 1986, 1987, 1994). The onset of flowering differs between males and females: females usually begin to flower several days later than males (Kinoshita, 1986, 1994).

Field survey—A field survey was carried out at Tajiri Shrine in Horigane, northwest of Matsumoto, Nagano Prefecture, Japan, the same site where a

permanent quadrat was established in 1981; demographic studies have been performed there since then (Kinoshita, 1986, 1987). In mid-May of both 1988 and 1990, a spathe of some females was cut off and then pollen grains of one male were hand-pollinated with a powder puff. As controls, other females were left to be pollinated by insects. We used this permanent quadrat for the present study, which we expanded to 10×11 m and subdivided into 1×1 m subquadrats. A code number was given to each reproductive individual.

To reduce cryptic gene flow (the siring of ovules by pollen parents outside the quadrat), the following process was carried out in early May, just before the spathe-tube mouth of the male plant begins to open. The inflorescences (spadix + spathe) were cut off all the individuals growing outside the quadrat within ca. 50 m from the center of the quadrat.

For the flowering individuals, the pseudostem diameter at the ground level, sex expression, and the position in the quadrat were recorded at the time of flowering in 2001. Pollinators trapped in the female spathe tube were collected in early June, when pollination had finished.

Molecular analysis—Total DNA was extracted from the leaves of all the flowering individuals in the quadrat and from all the seeds produced in 2001. DNA was extracted from the leaf samples according to the modified CTAB method (Kobayashi et al., 1998). From the seeds, total DNA was extracted only from the embryo tissue following the CTAB method described by Doyle and Doyle (1987). Before extracting DNA from the seeds, we recorded the position of each fruit on the spadix on the section paper (Fig. 2). The fruits were dried at room temperature and the seeds were obtained by removing the sarcocarp. The seed coat was broken with pliers and then soaked in distilled

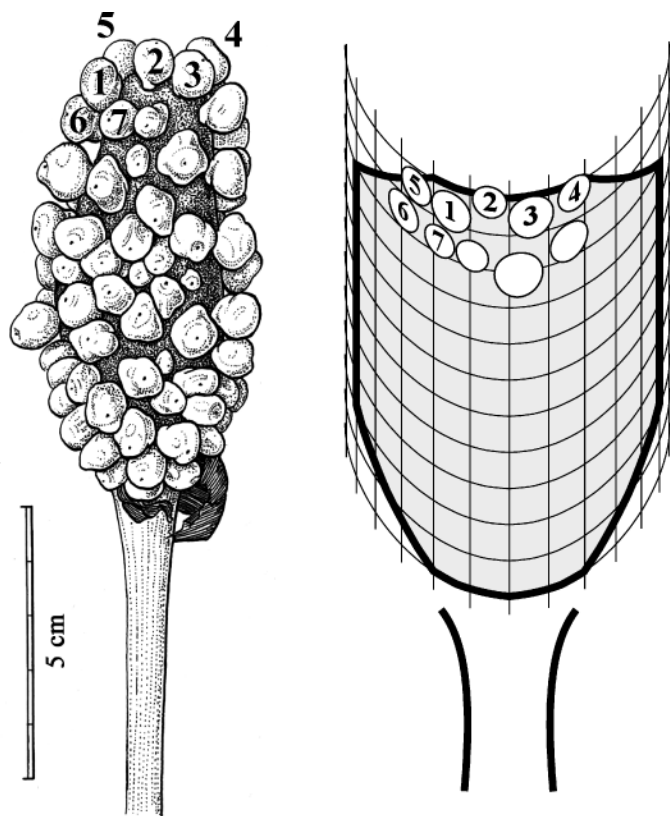


Fig. 2. A diagram of the projection of each fruit of *Arisaema serratum* onto plane coordinates. The cylindrical spadix is expanded, and the position of each fruit is recorded on the section paper.

water for about 24 h. After the seed had absorbed the water, the embryo was extracted by tweezers and deposited in a microtube.

The genotype of each DNA sample was scored using six microsatellite loci. Five of the six were developed by Nishizawa et al. (2003): As10, As24, As37, As45, and As49. We designed a new primer pair, As48 (5'-GGCAGGACAACCTTTATGGAC-3', 5'-GGGCAGTATCTCTCAATCTTC-3'; DDBJ accession number AB183867), based on the sequences of the clones containing microsatellite loci, which were screened in previous research (Nishizawa et al., 2003).

Polymerase chain reaction (PCR) was conducted in a total volume of 20 μ L containing 20 ng of template DNA, 0.2 μ M of each primer, 0.2 mM of each dNTP, 1 \times PCR buffer, 1.5 mM $MgCl_2$, and 0.625 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, California, USA). PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems) with the optimal cycling profile investigated by Nishizawa et al. (2003). The PCR products were electrophoresed using an ABI Prism 310 genetic analyzer (Applied Biosystems), and fragment analysis was carried out with GeneScan analysis software (Applied Biosystems).

Microsatellite data analysis—Paternity was assigned with the use of the software program CERVUS 2.0 (Marshall et al., 1998). This program is based on a likelihood paternity assignment algorithm (Meagher, 1986).

Apparent genotype mismatches between the known mother and its progeny (seed embryos) were occasionally observed at the As10, As37, and As48 loci. These mismatches were interpreted as the results of null alleles in the known mother. This is because the mismatches were observed only when the known mother had a homozygous genotype at these loci. In this study, to avoid excluding the true pollen parent from paternity, all the individuals homozygous in the three loci were treated as heterozygotes with null alleles. In this approach, the frequency of the heterozygote was overestimated, but on the other hand, true pollen parents are not excluded from paternity.

The use of CERVUS for paternity analysis allowed us to evaluate the statistical confidence of likelihood-based paternity. In the assignment of paternity with CERVUS, the confidence was set at a relaxed level (80%), because Marshall et al. (1998) stated that paternities assigned with 80% confidence were more accurate than those obtained by the simple exclusion method. For three females that produced remarkably numerous fruit, the error rates of typing based on the frequency of mismatches between the known mother and progeny pairs were estimated to be 0, 0.0155, and 0.0061, respectively. The mean value of the three pairs (0.008) was employed as the error rate in the simulation to statistically evaluate the confidence in the paternity of candidates.

Paternal distribution on a spadix—We performed a spatial autocorrelation analysis and calculated a standard normal deviate (SND) (Sokal and Oden, 1978) to evaluate the spatial distribution of the seeds sired by paternal parents on a spadix. A cylindrical spadix was expanded, and the position of each fruit was projected onto the plane coordinates (Fig. 2). In our analysis, a distance class was determined every 10 or 20 mm on the coordinates, depending on the size of each inflorescence. The SND values were calculated using the program PSAwinD version 1.1.1 (Takahashi, 2003). The SND values in the long-distance classes were ignored because the position of each fruit was determined based on the expanded coordinates.

Statistical analyses—Data was statistically analyzed using SPSS for Windows version 11.0.1J (SPSS, Chicago, Illinois, USA; SPSS, 2001).

RESULTS

Flowering and fruiting—In 2001, 145 individuals flowered: 107 males and 38 females. Only six females (16%) bore mature fruit in autumn. Of these six females, three (nos. 1, 2, and 3) produced markedly more fruit than the others (nos. 4, 5, and 6) (Table 1). The mean number of fruit produced per female was 45.7. The fruit production in the six fruiting females was not correlated with the pseudostem diameter at the ground level ($r = -0.516$, $N = 6$, $P = 0.294$).

Fruit production from 1987 to 1995 is shown in Table 2 (for fruit production before 1987, see Kinoshita, 1987). The percentage of females producing fruit ranged from 20.9 to 63.8%. In 1988 and 1990, when hand-pollination experiments were carried out, 44.0 and 39.5%, respectively, of the control females bore fruit. A significant difference in the proportion of fruiting females was found between the controls and the hand-pollination group (1988 $\chi^2 = 9.25$, $df = 1$, $P = 0.002$; 1990 $\chi^2 = 21.63$, $df = 1$, $P < 0.001$).

In the fertile females, small insects became trapped in the spathe tube in early June, including Sciaridae, Mycetophilidae, Phoridae, Cecidomyiidae, Chironomidae, Ceratopogonidae, Sphaeroceridae, and Hemiptera (Table 1). Most of them were dipteran insects. The number of trapped insects was not correlated with the fruit/flower ratio in the six fruiting females (Wilcoxon rank test $Z = -0.105$, $P = 0.917$). In female no. 4, for example, the fruit/flower ratio was only 4%, the second lowest among the six females, but this plant trapped 13 midges, more than any other of the six females.

Thirty-two females (84%) were nonfruiting. The inflorescence had withered by early summer in some nonfruiting females. In others, the inflorescence persisted until autumn, holding sterile fruit. Insects, mostly small dipterans, were trapped in the spathe tube of 22 nonfruiting females.

Paternity assignment—Table 3 summarizes the diversity of the six microsatellite loci from 145 flowering individuals growing in the quadrat. The number of alleles per locus ranged

TABLE 1. Fruit production, no. of sires estimated from paternity analysis, and no. of midges trapped at Horigane in 2001.

Variable	Female						Total
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	
Pseudostem diameter (mm)	11.9	15.0	17.0	12.1	19.2	18.5	—
Total ovaries (A)	123	139	119	119	160	155	815
Mature ovaries (B)	116	120	22	5	2	9	274
Fruit/flower ratio (B/A)	0.94	0.86	0.18	0.04	0.01	0.06	—
No. of seeds produced in 2001	294	328	41	5	4	10	682
No. of seeds tested (C)	283	319	39	5	4	10	660
No. of seeds assigned (D)	157	208	37	5	3	0 ^b	410
Success rate (D/C)	0.55	0.65	0.95	1.00	0.75	—	—
No. of minimum sires estimated (E)	18 (27 ^a)	26 (40 ^a)	15 (17 ^a)	1	3 (4 ^a)	—	—
No. of midges trapped (F)	7	7	3	13	4	8	42
Sciaridae	6	4	1	2	0	2	15
Mycetophilidae	0	2	2	1	0	0	5
Phoridae	1	0	0	8	1	4	14
Cecidomyiidae	0	0	0	2	0	0	2
Chironomidae	0	0	0	0	0	2	2
Ceratopogonidae	0	0	0	0	1	0	1
Sphaeroceridae	0	0	0	0	1	0	1
Hemiptera	0	0	0	0	1	0	1
Unknown	0	1	0	0	0	0	1
Sire/midge ratio (E/F)	2.6	3.7	5.0	0.08	0.75	—	—

^a The number of sires assigned without considering statistical confidence.

^b PCR amplification of seed DNA failed.

from 9 to 47 with a mean of 26.0, and the expected heterozygosity varied from 0.14 to 0.95 with a mean of 0.75. The combined exclusion probability across the six loci was 0.996, which was calculated using a formula in Marshall et al. (1998, appendix 2). A significant heterozygote deficit relative to the Hardy-Weinberg equilibrium was detected at each of three loci: As10, As37, and As48. This deficit may have been caused by the presence of a null allele, because homozygote-homozygote mismatches between known mother-offspring pairs were observed only at those three loci.

Candidates for the sires of the seeds of females nos. 1, 2, 3, 4, and 5 were determined with 80% confidence, but this was not the case for female no. 6, owing to a failure in the PCR amplification of seed DNA (Table 1). Multiple males sired individual females: 18 males sired seeds in female no. 1, 26 in no. 2, 15 in no. 3, 1 in no. 4, and 3 in no. 5. Except for female no. 4, these are minimal estimates because the paternal parents of all the seeds could not be assigned with 80% confidence: in female no. 1, 55% of the sires were assigned, 65% in no. 2, 95% in no. 3, 100% in no. 4, and 75% in no. 5. When a sire was assigned without considering the statistical confidence in the candidate with the highest log-likelihood ratio (LOD score), females nos. 1, 2, 3, and 5 had 27, 40, 17, and 4 sires, respectively.

Male plants did not perform uniformly as sires. A few males sired numerous seeds, and many others a few seeds (Fig. 3): more than 60% of seeds to which paternity was assigned were sired by 4 of the 18 males (22%) with female no. 1, 4 of the 26 males (15%) with female no. 2, and 3 of the 15 males (20%) with female no. 3. This inequality of contributions among the males can also be observed in the case where the paternal candidate with the highest LOD score was assigned as a true paternal parent of a seed. In this paper, the males that sired many seeds are designated as major males and the others as minor males. Major males each account for more than 10% of all paternity-assigned seeds; the rest are the minor males.

In the three females that produced markedly numerous fruit,

the number of midges trapped inside the spathe tube was lower than the number of sires (Table 1). The sire/midge ratios were 2.6 for female no. 1, 3.7 for no. 2, 5.0 for no. 3, 0.08 for no. 4, and 0.75 for no. 5.

The positions of seeds sired by each paternal parent on the spadix—Figure 4 illustrates the positions of mature seeds on the spadix of female no. 1 projected onto plane coordinates, and their estimated sires. This analysis was performed only for females nos. 1, 2, and 3; females nos. 4 and 5 produced too few fruit or seeds, and the sires could not be determined for female no. 6. The results for females nos. 2 and 3 are not shown because their seeds were distributed randomly, as described later. The positions of most seeds sired by any individual paternal parent were intermingled throughout the spadix. This held true at the levels of both fruit and seeds for all three females. In many cases, seeds in a fruit were fertilized by more than one sire.

Only the results for the major males in the spatial autocorrelation analysis are given (Table 4), although all the joins among the males that sired a female were examined. Some joins in female no. 1 had significantly positive or negative SND scores in the short-distance classes. In the case of “like pairs” of seeds with the same male, for example no. 1106 × no. 1106 and no. 79 × no. 79, the SND scores in the shortest distance class were significantly positive, with a clumped distribution. Other significant positive SND scores were observed at the joins of the no. 1106 × no. 1517 and the no. 5095 × no. 79. The positive joins of the seeds fertilized by two males indicated that these males tend to exist close to each other. The no. 1106 × no. 79 join and the no. 1517 × no. 79 join had significantly negative SND scores, with mutually exclusive distributions. However, none of the joins in females nos. 2 and 3 were significant, indicating that the seeds of each sire were distributed randomly.

TABLE 2. Fruit production by *Arisaema serratum* after natural or hand-pollination at Horigane.

Trait	Natural pollination							Hand-pollination experiment			
	Year										
	1987	1989	1991	1992	1993	1994	1995	1988		1990	
								Control	Hand-pollination	Control	Hand-pollination
Number of female plants	67	72	99	85	69	62	57	50	16	43	30
Number of female plants producing fruits	14	22	44	38	44	24	36	22	14	17	28
Percentage of female plants producing fruits	20.9	30.6	44.4	44.7	63.8	38.7	63.2	44.0	87.5	39.5	93.3
Total number of fruits produced in the plot	720	811	2131	1309	2620	1274	1307	1124	1877	310	1821
Mean number of fruit per female (\pm SD)	51.4 \pm 40.8	36.9 \pm 42.3	48.4 \pm 37.6	34.4 \pm 37.5	59.5 \pm 39.7	53.1 \pm 37.4	36.3 \pm 28.7	51.1 \pm 49.6	134.1 \pm 37.9	18.2 \pm 23.2	65.0 \pm 35.9

TABLE 3. Microsatellite allele diversity at six loci in *Arisaema serratum*.

Locus	Number of alleles	Heterozygosity	
		Expected	Observed
As10	15	0.86	0.19
As24	47	0.95	0.85
As37	25	0.89	0.48
As45	9	0.14	0.15
As48	15	0.74	0.51
As49	45	0.94	0.76
Average	26.0	0.75	0.49

Spatial distribution of paternal parents in the quadrat—Figure 5 presents the frequency distributions of all males and of the sires around a female within each distance class. The distribution patterns of potential and real pollination did not differ significantly (Fisher’s exact test: female no. 1, $\chi^2 = 5.41$, $df = 9$, $P = 0.79$; female no. 2, $\chi^2 = 1.73$, $df = 10$, $P = 0.99$; female no. 3, $\chi^2 = 4.31$, $df = 10$, $P = 0.95$).

Male reproductive success in relation to size—As for plant size, no significant difference was found between males that sired and those that did not (t test, $t = 1.965$, $P = 0.052$). Figure 6 shows that there was no significant correlation between the size of a male and the number of seeds sired ($r = 0.119$, $N = 52$, $P = 0.403$).

DISCUSSION

Specialized or unusual pollination has long been assumed to occur in *Arisaema* species because they have trap inflorescences (Vogel and Martens, 2000). We were able to identify specific paternal parents of seeds to a considerable extent, and we showed that (1) seeds in a fruit had multiple sires; (2) the seeds sired by each paternal parent had either a clumped, exclusive, or random distribution on the spadix; (3) a few males accounted for a disproportionately large share of the siring; (4) the distance from a female is not a factor in the inequality of reproductive success among males; and (5) the reproductive success of a male was not correlated with its size.

Insects trapped and pollinator limitation—Although not all seeds on a spadix were assigned sires in this study, the number of sires estimated was larger than the number of trapped midges in females nos. 1, 2, and 3 (Table 1). In contrast, the number of trapped midges exceeded the estimated number of sires for females nos. 4 and 5, which had very low seed sets. These results indicate that, given that there is no escape from the female spathe tube, a single midge transferred pollen grains of multiple males in some cases, while in other cases a single midge transferred pollen grains of one male. The former case resulted in more successful pollen transfer than the latter.

One probable factor in this difference in the sire/midge ratio can be attributed to the presence of various pollinators. Handel (1983) stated that when flowers of one plant species are visited by various insects, pollen movement may be affected by the kind of visitors. Thus, not all the insects trapped in a female spathe tube may achieve successful pollination, and some insects may be trapped by accident. Considering the kinds of insects and the number of fruit produced, Sciaridae and Mycetophilidae are likely to be effective pollinators, but Phoridae are not (Table 1). Sasakawa (1994) reported that most insects

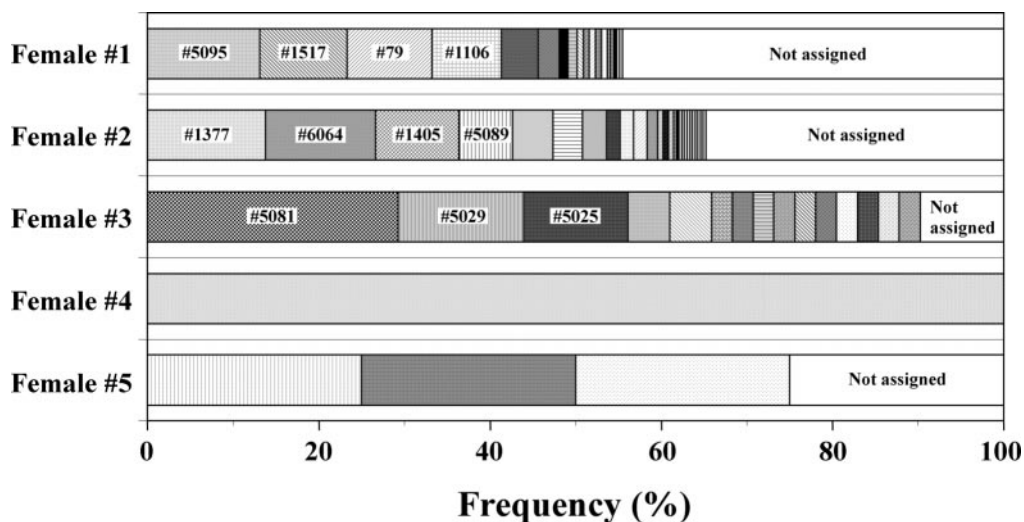


Fig. 3. The proportion of seeds on a spadix sired by each male of *Arisaema serratum*. A code number is present only for the major males that sired females nos. 1, 2, and 3.

trapped in the female spathe tube of *A. serratum* growing in Toyama and Chiba prefectures were Mycetophilidae and Sciaridae. Bierzychudek (1982) also reported in *A. triphyllum* that pollen was carried by many species of fungus gnats, including Mycetophilidae and Sciaridae. Vogel and Martens (2000) also stated that these two families dominated in *Arisaema*. Other insects may be trapped accidentally.

In this study, about 70% of the nonfruiting females were actually visited by insects. Three probable causes of unsuccessful seed production in this study can be identified: (1) the insect was not a genuine pollinator but was trapped accidentally; (2) the insect was a genuine pollinator but was carrying no pollen grains; (3) the insect was a genuine pollinator with sufficient pollen grains, and the sterility was attributable to

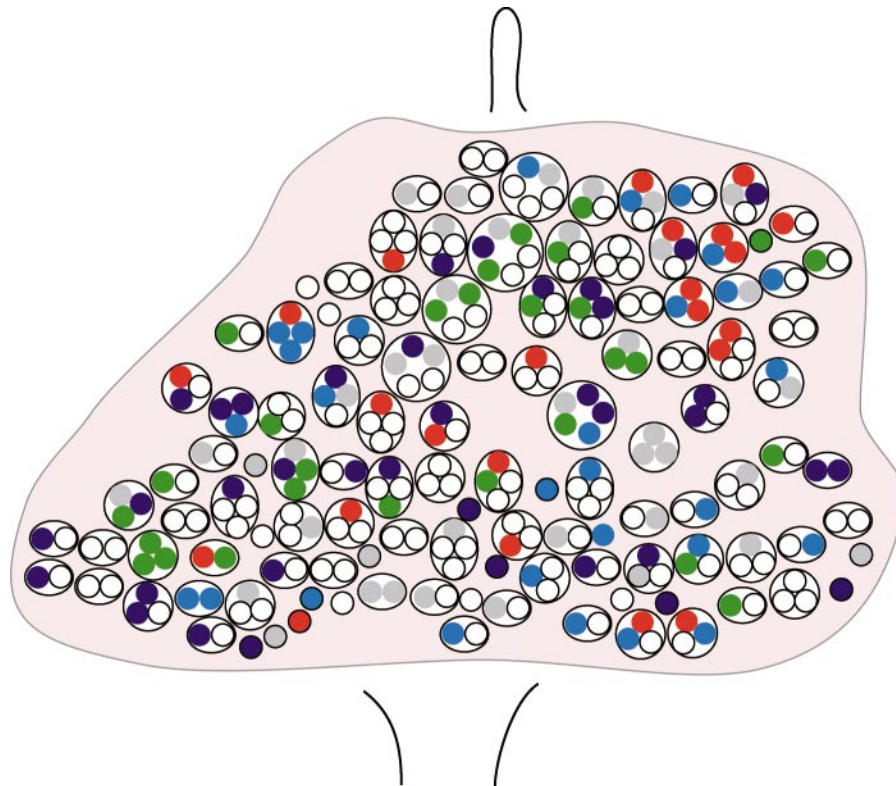


Fig. 4. Spatial distribution of sires on a spadix of female no. 1 of *Arisaema serratum*. The small circles correspond to seeds, and the large circles surrounding the small ones correspond to fruit. The four major males are represented by the following colors: purple (no. 5095), blue (no. 1517), green (no. 79), red (no. 1106). The seeds sired by minor males are gray. The small open circles represent the seeds for which a paternal parent could not be assigned with 80% confidence.

TABLE 4. Spatial distribution of seeds sired on the spadix of *Arisaema serratum* revealed by spatial autocorrelation analysis. The joins for paternal parents that sired relatively many seeds are indicated. Each distance class was 20 mm in females nos. 1 and 2, and 10 mm in female no. 3.

Female	Joins for paternal parents	Distance class		
		1	2	3
No. 1	1106 × 1106	2.89**	0.02	−2.05
	1106 × 1517	3.21**	−0.84	−1.56
	1106 × 5095	−0.35	−0.95	−0.33
	1106 × 79	−2.03*	2.11*	0.58
	1517 × 1517	0.61	−0.79	−2.13
	1517 × 5095	−1.12	−0.11	−0.90
	1517 × 79	−2.26*	−0.04	0.65
	5095 × 5095	−1.69	−0.06	0.50
	5095 × 79	2.09*	0.69	0.00
	79 × 79	2.70**	−1.04	0.51
No. 2	1377 × 1377	−1.35	−0.73	−0.22
	1377 × 1405	0.25	−0.65	0.27
	1377 × 5089	0.41	−0.09	−1.31
	1377 × 6064	0.85	0.23	0.71
	1405 × 1405	−0.12	0.55	0.07
	1405 × 5089	0.03	0.76	−0.69
	1405 × 6064	0.32	0.56	0.83
	5089 × 5089	0.23	−1.95	0.51
	5089 × 6064	0.51	0.27	−0.10
	6064 × 6064	−1.52	0.99	0.96
No. 3	5025 × 5025	−0.04	−1.29	−1.23
	5025 × 5029	−1.32	−0.27	−0.10
	5025 × 5081	−0.56	−1.23	−0.74
	5029 × 5029	1.27	−1.84	1.08
	5029 × 5081	1.93	−0.22	−1.03
	5081 × 5081	0.97	1.79	−1.16

Note: Significance levels: * $P < 0.05$, ** $P < 0.01$.

factors involving the female plants, such as predation, disease, and physical damage.

One feature of this population may be a low percentage of females producing fruits as compared with other populations at Kanazawa (T. Nishizawa, unpublished data); the percentage of fruiting females in this population ranged from 20.9 to 63.8% between 1981 and 1995 (Table 2; Kinoshita, 1987). The fact that 2001 had the lowest fruit production (16%) may have been a result of the experimental manipulation of plants to assess paternity, specifically, the removal of the inflorescences growing outside the quadrat (Tables 1 and 2). A significant difference in the proportion of fruiting females was found between the controls and the hand-pollinated plants (Table 2). This indicates that pollinator limitation, not resource limitation, is involved in the high percentage of nonfruiting females in this population; effective pollinators are not enough to fertilize all females (ovules) in a population. Bierzychudek (1982, 1984a) reported pollinator limitation in *A. triphyllum*.

Female success is limited by the availability of resources for reproduction (e.g., Arnold, 1994; Wilson et al., 1994). Kinoshita (1986, 1987) showed that corm weight and ovule number were closely correlated with plant size and that female success (submatrix B) increased with size, indicating that female success in *A. serratum* is primarily limited by size (i.e., by resources). However, fruit production was not correlated with plant size in the present study. This may be due to an insufficient number of plants studied. Thus, to properly evaluate the reproductive success of male and female plants, pollinator behavior as well as of pollinator quality and quantity

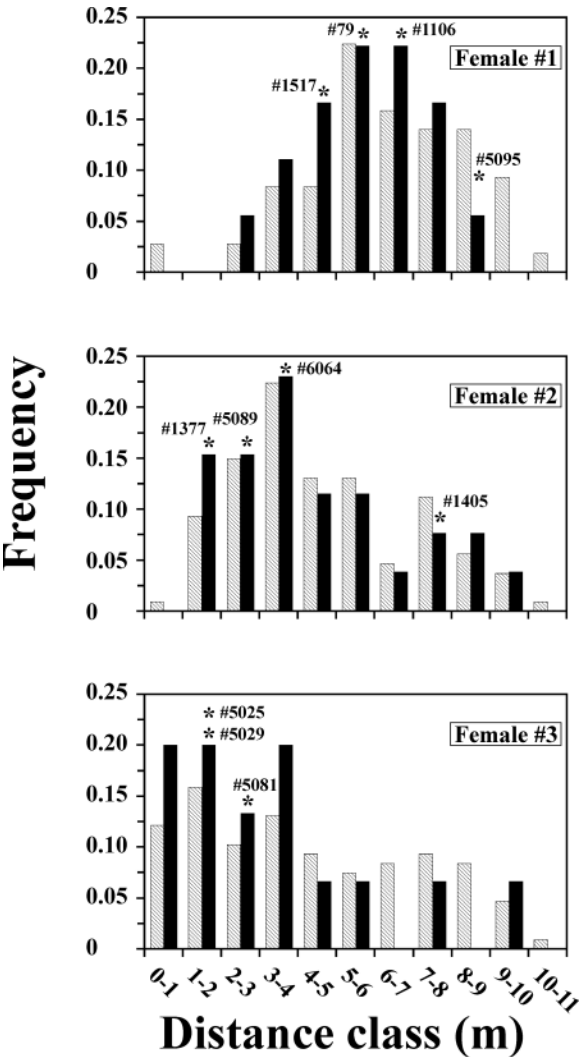


Fig. 5. Frequency of the males and the sires around a female of *Arisaema serratum* in each distance class. The black columns show the frequencies of the sires assigned, the gray columns the frequencies of potential males. The positions of the major males in each female are indicated by asterisks.

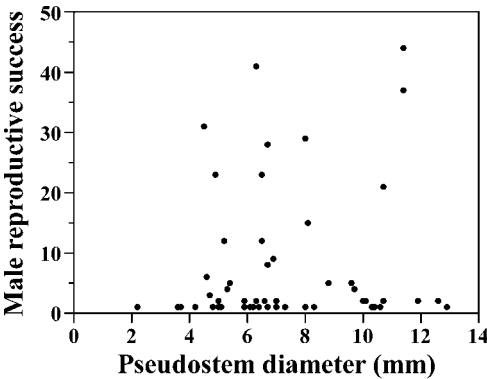


Fig. 6. Relationship between male size (pseudostem diameter at ground level) and reproductive success (the number of seeds each male sired) in *Arisaema serratum*.

must be elucidated. We discuss pollinator behavior and pollen movement in the following sections.

Pollen carryover and multiple paternity—The presence of pollen from more than one male in the same plant can be readily assumed by the observation of numerous midges trapped inside the spathe tube at the end of the flowering season (Fig. 3). Although this evidence does not necessarily lead to an inference of multiple paternity, paternity assignment showed that seeds in a fruit were sired by more than one male in many cases (Fig. 4). Two mechanisms of multiple paternity have been considered: pollen from different donors may be deposited separately during sequential visits by different pollinators (sequential pollen deposition); and different types of pollen may be deposited simultaneously by a single vector carrying pollen from several plants (simultaneous pollen deposition or pollen carryover) (Marshall and Ellstrand, 1985).

In the trap-flower pollination system in *Arisaema*, the female inflorescence is literally a dead end, while the male inflorescence has an exit hole for pollinators (Fig. 1). Thus, it is most probable that a pollinator may pass through numerous male spathe tubes before finally becoming imprisoned in a female spathe tube. Based on the sire/midge ratio (5.0) in female no. 3, in which the paternal parents of 95% of the seeds were identified (Table 1), a pollinator may have passed through several males. The higher sire/midge ratios may be partly due to the male-biased sex ratio (107/145). In addition, male plants begin to flower earlier than females (Kinoshita, 1986, 1987, 1994). From the midge's point of view, for several days from the onset of flowering, the population is composed almost entirely of male plants. Hence, multiple paternity in *A. serratum* is mainly due to pollen carryover, which is consistent with the multiple paternity in wild radish (Marshall and Ellstrand, 1985).

Spatial autocorrelation analysis apparently suggests pollen carryover as well. In female no. 1, significant positive SND scores for male no. 1106 \times no. 1517 and male no. 5095 \times no. 79 suggest that the pollen grains of nos. 1106 and 1517 were carried by the same pollinator, and the pollen grains of nos. 5095 and 79 were also carried simultaneously (Fig. 4, Table 4). The negative SND scores in no. 1517 \times no. 79 and no. 1106 \times no. 79 strongly suggest that the pollen grains of each pair were carried by different midges. These findings lead to the assumption that in female no. 1, only two midges could carry the pollen grains of the four major males, accounting for about 70% of the seeds to which paternity was assigned.

The random distribution in females nos. 2 and 3 may be due in part to the wandering of midges within the female spathe tube, because the trapped midges probably walk around to look for an exit. If there is a time lag between the unfolding of the spathe tube and the pollen receptivity of a stigma, pollen grains from multiple males would be fully mixed on the surface of a stigma during the wanderings of the midges.

Pollinator behavior, pollen movement, and inequality in male success—It has been generally considered that the foraging behavior of insect pollinators, particularly bees, follows the nearest-neighbor pollination rule (Levin and Kerster, 1974; Waser, 1982). More pollen from plants of *Silene latifolia* were distributed to close neighbors than to more distant ones (Wright and Meager, 2004). In the present study, however, the pollination behavior of midges did not follow the nearest-neighbor pollination rule; the major males were not necessarily

growing near the females. The flight patterns of midges are nearly random, because the distribution pattern of the sires around a female did not significantly deviate from that of the males as a whole (Fig. 5). Thus, the paternal parents that sire a female are primarily determined according to the density around the female and not on the distance from the female; distance is not a factor in the inequality of reproductive success among males.

Male reproductive success has been thought to be highly variable (e.g., Meagher, 1986; Smouse et al., 1999; Elle and Meager, 2000; Wright and Meager, 2004). This holds true in the present study; major males and minor males were identified based on the number of seeds that a male sired (Fig. 3). It is a conspicuous feature in *A. serratum* that male success is independent of interplant distance (Levin and Kerster, 1974; Waser, 1982; Wright and Meager, 2004). One explanation for the strong siring predominance of a relatively few males is the combination of the trap-flower pollination system and pollen carryover. A midge may have passed through several males and brought pollen grains from all of them. It is generally known that pollen grains adhering to the body of a pollinator tend to fall off as the pollinator moves (Thomson and Plo-wright, 1980; Thomson et al., 1982; Campbell, 1985; Thomson, 1986; de Jong et al., 1992; Richards et al., 1999). While midges walk around seeking an exit in a male spathe tube, pollen grains of previous males may detach from the midge's body and be replaced by those of this male. Thus, a major male may be the last male that visited before a midge is trapped in a female inflorescence, and thus a minor male would be one of the males that visited previously. At present, we have no idea whether or not other attributes, such as those of the spathe, appendix size, or pollen production (male flower number), may work as attractive features as reported for many entomophilous pollinations (cf. Wilson et al., 1994).

Size-independent male reproductive success in relation to the size-advantage hypothesis—Sex lability in *Arisaema* has fascinated biologists for years. The labile sex in this species has been interpreted within the framework of the size-advantage hypothesis (Policansky, 1981; Bierzychudek, 1984a; Kakehashi and Kinoshita, 1990; Kinoshita and Harada, 1990); size-dependent sex lability will be favored when there is a discrepancy between males and females in reproductive success with respect to size (Ghiselin, 1969; Warner, 1975, 1988; Warner et al., 1975; Munoz and Warner, 2003).

Although we found a large variance in male siring successes, in which a few males sired the majority of seeds, we found no correlation between male plant size and success (Fig. 6). Kinoshita (1986, 1987) showed size-dependent female success, and Kinoshita and Harada (1990) showed that the sex-changing schedule of *A. serratum* at Horigane agreed closely with the expectations based on the size-advantage hypothesis, where size-independent male success was assumed. Our results strongly support Kinoshita and Harada's assumption.

Conclusion—This study sheds light on pollen movement and multiple paternity in a trap-flower pollination system; pollen carryover and the trap-flower pollination system are likely to result in multiple paternity and inequality in male success. However, not all sires were determined with high confidence, owing to the paucity of resolution power of the microsatellite markers. Thus, additional microsatellite markers need to be developed to elucidate completely pollen movement within a

population. In addition, we cannot explain why the distribution was clumped or exclusive in some cases and random in others. It will be necessary to conduct field investigations into midge behavior, studies of the time lag between the unfolding of a spathe tube and the pollen receptivity of a stigma, and so on. Such studies would reveal the pollination syndrome in trap-flower pollination or provide a basis for testing the size-advantage hypothesis in *A. serratum*.

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