ORIGINAL PAPER

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How pollinator-mediated mating varies with population size in plants

Abstract In most higher plants sexual interactions are mediated by animal pollinators that affect the number and differential reproductive success of mates. The number and sex of breeding individuals in populations are central factors in evolutionary theory, but the quantitative effect of plant population size on pollinator-mediated mating is understudied. We investigated variation in pollen removal (male function) and fruit set (female function) among flowering populations of different size of two bumblebee- and one butterfly-pollinated, rewardless, pollen-limited, hermaphroditic orchid species in Sweden. As the amount of pollen removed from plants by insects (either absolute or proportional) increased, so did the number of pollinations, whereas the proportions of plants with different pollinator-designated functional sex (male, female, hermaphrodite) depended primarily on the ratio between the amount of fruit set and pollen removed within populations. A larger population size was found to have several effects: (1) the total numbers of pollinia removed and fruits set increased; (2) the proportion of pollen removed from plants decreased; (3) the proportion of flowers pollinated decreased in the butterfly- but was not affected in the bumblebee-pollinated species; (4) the ratio between fruits set and pollinia removed increased linearly in the bumblebee-pollinated species but reached a maximum at c. 80 individuals in the butterfly-pollinated species; (5) the numbers of pollinator-designated pure male and hermaphrodite individuals increased; and (6) the variance in pollinium removal, but not fruit set, increased among individuals. These findings empirically verify the basic importance of population size for the mating structure of outcrossing plants, and indicate that selection for female sexual traits is reinforced when population size is smaller while selection for male sexual traits is reinforced when population size is larger.

Introduction

The importance of population size for mating structure in relation to evolution was emphasized by Wright (1931, 1938), who modelled the amount of genetic drift in populations of varying size. Apart from its effect on stochastic genetic depletion and amount of inbreeding, population size in higher plants influences the pollen gene flow that occurs within and between populations and thus may moderate the effects of both genetic drift and selection on such plants (Bateman 1947; Handel 1983; Crawford 1984; Levin 1984; Ellstrand 1992). The pattern of pollen gene flow within and between angiosperm plant populations is often governed by animal pollinators (Levin 1979; Schmitt 1980; Waser and Price 1983). But what effect does plant population size have on the mating structure when a purely ecological factor, viz. animal pollination, accounts for all matings? Although central in evolutionary plant biology, this issue has received little empirical attention.

A decrease in population size may automatically reduce mate diversity, increase the variance among genotypes for compatible mates (Byers and Meagher 1992), and have a series of ecological consequences. In animals, small population sizes reduce opportunities for individuals to find mates and reproduce (Shaffer 1981; Lande 1988). In animal-pollinated plants, analogously, actual mate numbers become smaller and may attain a critical minimum below which pollinator service deteriorates and populations go extinct (McKey 1989; Bronstein et al. 1990). A smaller population or patch size may reduce the attractiveness of the plant to animal pollinators (Sih and Baltus 1987; Sowig 1989), and if plant density decreases concomitantly, pollination may decrease (Levin and Kerster 1974; Beattie 1976; Lloyd 1980a).

Study of the quantitative variation in pollinator-mediated reproductive success in plant populations requires

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estimates of both the male and female components. In outcrossing hermaphroditic plant individuals, reproductive success can be achieved through both pollen removal with subsequent pollen donation (male function) and pollen reception with subsequent seed production (female function). Male and female reproductive success has been studied using pollen analogues (Campbell 1989a), microtags on pollinia (Nilsson et al. 1992), or molecular methods (Meagher 1986; Broyles and Wyatt 1990). Because of the difficulties associated with assessing male function, at best patterns of fitness within single populations have been investigated by such techniques, leaving variation in patterns among populations unexplored. Male reproductive success can be estimated indirectly by assessment of the amount of pollen removed from flowers by pollinators (Lloyd 1980b; Cruzan et al. 1988; Broyles and Wyatt 1990); a significant relationship between pollinium removal and donation success or seeds sired has been found in both asclepiads and orchids (Broyles and Wyatt 1990; Nilsson et al. 1992). Consequently, pollinium removal can be used as a measure of male reproductive success and pollinium receipt as a measure of female reproductive success in such plants.

The present work used this technique on orchids to explore quantitative sexual relationships at the population level in plants. The following major questions were asked: (1) Does pollinator-mediated mating vary with population size in plants, and, if so, (2) are there differential effects on male and female reproductive success, and (3) what predictions can be made from the pattern of reproductive success about the evolutionary consequences for small populations of outcrossing plants?

Materials and methods

Populations of three species of orchids were studied on the Baltic island of Gotland, Sweden, during the summers of 1988–1990 (for details see Appendix). The plants studied were *Orchis spitzelii* Sauter ex. Koch (ten populations), *O. palustris* Jacq. (eight populations), and *Anacamptis pyramidalis* (L.) Rich. (ten populations). All three are non-clonal, non-autogamous, nectarless deceivers with low levels of natural fruit set on Gotland, where experimentally fully pollinated individuals approach 100% fruit set, indicating that resources do not limit within-season fruit production in any of the species (A.-L. Fritz, unpublished work). Only flowering individuals were included in the study. *O. spitzelii* grows in open, dry pine woods and flowers from mid-May to mid-June. Flowering plants display 1–39 (mean=10.9, SD=4.1, n=1188) light-purple flowers on a single, elongate spike. On Gotland this orchid is mainly pollinated by bumblebee queens of the species *Bombus pascuorum* (Scop.) (Fritz 1990). *O. palustris* usually grows in sedge-dominated fens, and flowers from mid-June to mid-July. Flowering plants display 1–22 (mean=6.7, SD=2.8, n=1201) purple flowers on a loose, elongate spike. On Gotland it is pollinated primarily by workers of *B. pascuorum* (A.-L. Fritz unpublished work). *A. pyramidalis* grows mainly in grazed sea-shore and dry inland ("alvar") meadows, and flowers from mid June to mid July. Flowering plants present 4–75 (mean=27.2, SD=11.2, n=330) bright-purple flowers on a pyramid-shaped spike. This species is pollinated on Gotland by a variety of butterflies, e.g. *Melitaea cinxia* (L.) and *Mesoacidalia aglaja* (L.) (Nymphalidae), *Maniola jurtina* (L.) (Satyridae), and especially *Aporia crataegi* (L.) (Pieridae) (A.-L. Fritz and L. A. Nilsson, unpublished work).

All three orchid species bear two pollinia per flower. In *Orchis*, one or both can be removed during an insect visit; the pollinia are separate and attach to the clypeus or frons of the bumblebee pollinators (Fritz 1990; A.-L. Fritz, unpublished work). In *A. pyramidalis*, however, the two pollinia are connected and are removed at the same time, attaching to the proboscis of the butterfly pollinators (Darwin 1862).

Our measure of male reproductive success was pollinium removal as determined at the termination of flowering in each population. Our measure of female reproductive success was fruit production, assessed 2 weeks after flowering, when ovaries of any pollinated flowers were distinctly expanded. In order to evaluate the accuracy of this measure in relation to the actual pollinator-mediated pollinium reception, we selected one population per species and marked naturally pollinated flowers, which were later checked for fruit production. In *O. spitzelii* 15 (3.3%) out of 454, in *O. palustris* 5 (1.3%) out of 396, and in *A. pyramidalis* 0 out of 84 naturally pollinated flowers did not set fruit. Consequently, fruit set closely tracked pollination and was a fair estimate of female reproductive success.

We used four measures to explore the pollinator-mediated reproductive success and pollination efficiency in populations of different species and size:

1. The number of fruits set and number of pollinia removed.
2. The proportion of fruits set in relation to flowers produced and the proportion of pollinia removed in relation to pollinia produced.
3. The ratio between overall female and male reproductive success, as an equivalence factor (Lloyd 1980 b), E, estimated for each population as:

$$E = \frac{\sum f_i}{\frac{1}{2} \sum m_i},$$

where f_i and m_i are respectively the number of fruits set (female reproductive success) and the number of pollinia removed (male reproductive success) in individual i. The factor $1/2$ adjusts the functional female–male ratio per flower for flowers with two pollinia. The higher E is, the higher the reproductive efficiency through the pollination system.

4. The number and proportion of different pollinator-generated sexual categories or pure genders (Nilsson 1992a). A plant individual was classified as a hermaphrodite when it had ≥ 1 pollinium removed and ≥ 1 fruit, as a female when it had ≥ 1 fruit but no pollinia removed, as a male when it had ≥ 1 pollinium removed but no fruit, and as a neuter when it had neither pollinium removal nor fruit set.

In order also to obtain a quantitative measure of functional gender G for individuals within populations we used Lloyd's formula (1980b):

$$G = \frac{f_i}{f_i + (m_i \times E)}$$

The following events affected our study populations and data. Local frost occurred in May 1989 in *O. spitzelii* population B. One-third of the flowering individuals collapsed and were excluded. Frost in *O. spizelii* populations B and D in May 1990 resulted in about two flowers per individual never opening; we included these plants. A very large number of concurrently flowering, nectarless *Dactylorhiza incarnata* (L.) Soó (Orchidaceae) appeared intermingled with *O. palustris* population D in 1989, and interfered negatively with pollination in *O. palustris* (both taxa attract the same pollinating bumblebees and have very similar flower morphology with large stigmas open to each others' pollinia, in 1990, however, the number of flowering *D. incarnata* was about half the number in 1989. In *O. palustris*, almost the same proportion (c. 30%) of pollinia was removed in 1989 and 1990, but fruit set in 1989 was only about half of that in 1990, indicating that much of the pollen in 1989 was lost to *Dactylorhiza* stigmas. We excluded *O. palustris* population D in 1989 from the regression analysis of equivalence factor on population size (see below). In *A. pyramidalis* pop-
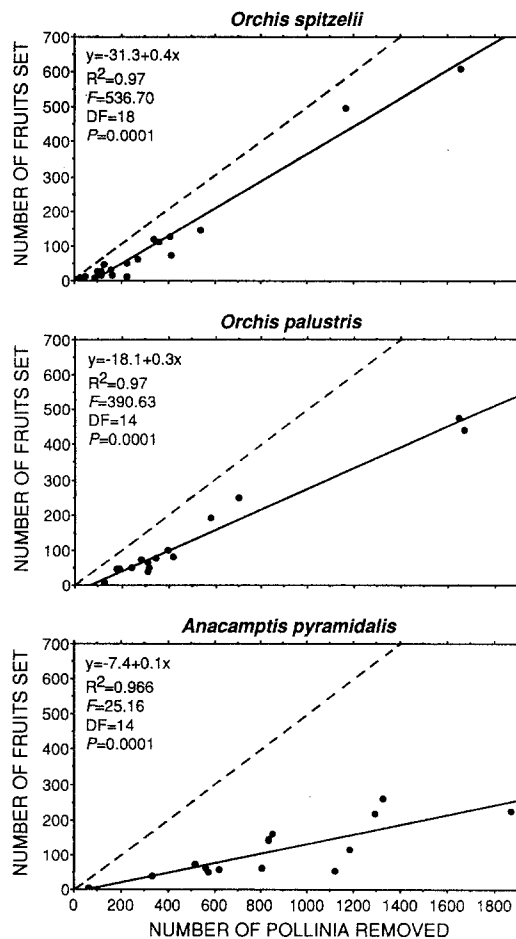
Fig. 1 Number of fruits set in relation to number of pollinia removed in populations. The *dashed lines* illustrate the hypothetical function of equal pollinium removal (taking into account that there are two pollinia per flower) and fruit set
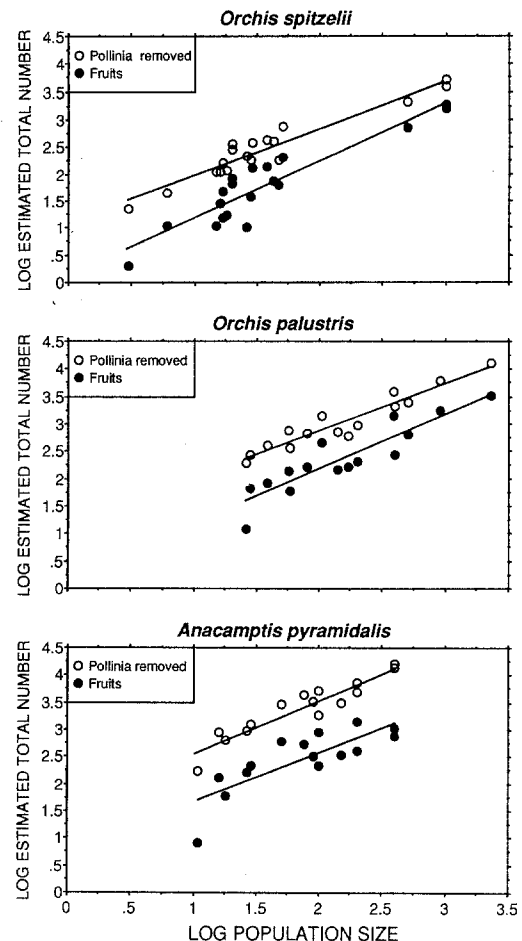

Fig. 2 Estimated total numbers (mean of sampled plants× population size) of pollinia removed (*open circles*) and fruits set (*filled circles*) in relation to population size. For equations and statistics, see Table 1A

ulation C, sheep successively reduced the flowering plants to four in 1989. We excluded this population from the analyses in specified cases.

The size of flowering populations was exactly determined in small and moderately large delimited populations by counting all the flowering individuals. The size of large, unevenly distributed populations was simply estimated, except for *O. palustris* where the exact sizes of large populations were available from Högström (1991). The closest distances between populations were 610 m in *O. spitzelii*, 500 m in *O. palustris*, and 540 m in *A. pyramidalis*.

Prior to decisions on final data analysis for the three species we performed analyses of covariance (ANCOVA) to explore the effect of flower number on two components of reproductive success (relative pollinium removal and relative fruit set) with the class variables of year and population nested within year, and the continuous variable of flower number and the interaction of flower number×year (see Campbell 1989b). Significance levels for the ANCOVAs were based on type IV sums of squares from the GLM procedure of SAS (SAS Institute Inc., Cary, N.C., USA, 1985) . For all three species there was a highly significant relationship ($P<0.0001$) between relative pollinium removal or relative fruit set and flower number. In all cases there was no significant ($P>0.05$) flower number×year interaction with relative fruit set, and also no significant interaction with relative pollinium removal in *O. spitzelii* and *A. pyramidalis*. In *O. palustris* there was a barely significant ($P<0.02$) flower number×year interaction with relative poll-

inium removal. We therefore decided that data from the same population for the three species in different years could be treated as independent.

Since populations may differ in flower production, which could perhaps influence pollination efficiency in addition to population size, we performed a multiple regression of variance in pollinia removed or variance in number of fruits set on population size and mean flower number. In all three species mean flower number had no significant effect ($P>0.12$) on the variance in pollinia removed or fruit set among populations. We therefore concluded that flower production differences between populations could not explain any differences in pollination efficiency, but that these, if any, were explained by other factors such as population size.

We then went on to use regression to determine relationships between variables. We explored each combination of data sets with simple, quadratic and cubic regression models. Prior to statistical analysis, proportions were arcsine transformed and population sizes were log transformed. Statistical packages used were StatView SE+Graphics (Abacus Concepts Inc., Berkeley, Calif., USA) for regressions and graphics, SYSTAT 5.2 (SYSTAT Inc., Evanston, Ill., USA) for Lilliefors and Tukey tests, and SAS for ANCOVA.

Table 1A, B Equations and statistics of best-fit regressions of pollinia removed (*PR*) and fruits set (*F*) on population size for *Orchis spitzelii*, *O. palustris* and *Anacamptis pyramidalis*. **A** Estimated total number (mean of sampled plants×population size) of pollinia removed and fruits set (Fig. 2). **B** Percentage pollinia removed and fruits set (Fig. 4). The variable "pollinia removed" in *O. spitzelii* is untransformed (a Lilliefors test indicated that arcsine transformation actually reduced normality in this case)

Species	Variable	Equation	r^2	F	df	P
A.						
O. spitzelii	PR	$y=1.1+0.9x$	0.92	187.52	18	0.000
	F	$y=0.1+1.1x$	0.85	99.51	18	0.000
O. palustris	PR	$y=1.2+0.9x$	0.91	134.28	14	0.000
	F	$y=0.2+1.0x$	0.82	59.47	14	0.000
A. pyramidalis	PR	$y=1.6+1.0x$	0.88	94.81	14	0.000
	F	$y=0.7+1.0x$	0.67	24.77	14	0.000
B.						
O. spitzelii	PR	$y=58.9–11.0x$	0.22	4.70	18	0.045
	F	$y=19.4–0.1x$	0.00	0.00	18	0.987
O. palustris	PR	$y=1.4–0.3x$	0.44	10.06	14	0.007
	F	$y=0.4–0.05x$	0.02	0.34	14	0.570
A. pyramidalis	PR	$y=1.4–0.3x$	0.34	6.07	13	0.030
	F	$y=0.4–0.1x$	0.32	5.56	13	0.036

Results

The numbers of flowering plants, plants and flowers examined, pollinia removed, and fruits set, along with equivalence factors and pollinator-defined gender in the populations are listed in the Appendix. Among populations of each species, there was a highly significant, positive relationship between the total number of pollinia removed and the total number of fruits set (Fig. 1). Negative departure from the hypothetical function of equal pollinium removal and fruit production was greater in the butterfly-pollinated *A. pyramidalis* than in the two bumblebee-pollinated *Orchis* species. Furthermore, the negative departure was greater in the bumblebee-worker-pollinated *O. palustris* than in the bumblebee-queen-pollinated *O. spitzelii*. The regressions predict that populations will have no fruit set, i.e. no pollinator-mediated reproductive efficiency when the number of pollinia extracted is less than 60–80. The estimated total numbers of pollinia removed and fruits set showed highly significant, linear relationships with population size (Fig. 2 and Table 1A).

The proportion of pollinia removed in populations ranged from 18 to 90%, and *O. spitzelii* had a significantly lower average than either *O. palustris* or *A. pyramidalis*, while the proportion of fruits set ranged from 3 to 58% and did not differ significantly between species (Table 2). The two variables showed significant, positive linear relationships (Fig. 3). Again, *A. pyramidalis* had the lowest pollination efficiency, the regression predicting a very low fruit set in populations with less than c. 30% pollinium removal. There were consistently significant negative relationships between the proportion of pollinia removed and population size (Fig. 4 and Table 1B). The proportion of fruits set, however, showed no dependence on population size in the two *Orchis* species, but a significant negative relationship in *A. pyramidalis* when data from population C in 1989 (see above) were excluded from the analysis.

No significant relationships appeared between the proportion of fruits set and the number of pollinia removed in any of the three species (r^2=0.003, 0.01, 0.19, and *P*=0.82, 0.72, 0.11, respectively, for *O. spitzelii*, *O.*
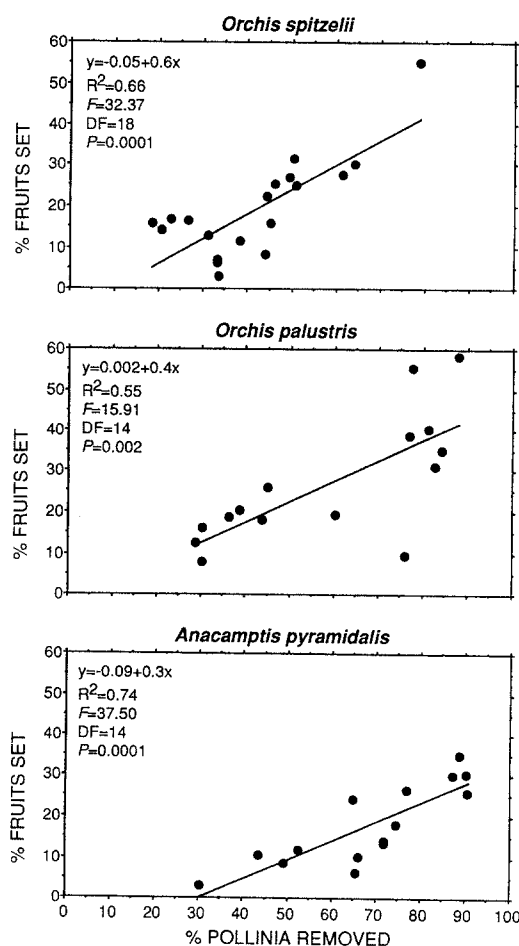
Fig. 3 Proportion of fruits set in relation to proportion of pollinia removed in populations

palustris, and *A. pyramidalis*), nor between the number of fruits set and the proportion of pollinia removed in the *Orchis* species (r^2=0.11, 0.02, and *P*=0.17, 0.60, respectively). In *A. pyramidalis*, however, the number of fruits set strongly depended on the proportion of pollinia removed (r^2=0.50, *F*=12.85, *P*=0.003).

The adjusted ratio between the number of fruits set and pollinia removed (the female–male equivalence fac-

Table 2 Summary statistics of population data on fruit production, pollinium removal and sexual equivalence factor (single populations data are reported in the Appendix). Mean values in between-species comparisons significantly different at the 0.05 level (Tukey's HSD test) are indicated by different letters in superscript

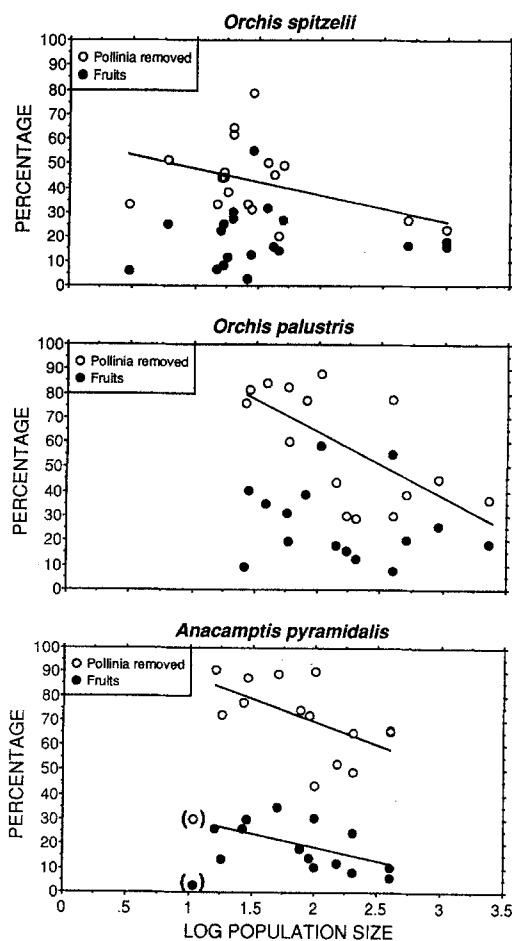| Species	n	Fruits (%)		Pollinia removed (%)		Equivalence factor E	
		$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
O. spitzelii	19	20 [a]	12	42 [a]	16	0.47 [a]	0.21
O. palustris	15	27 [a]	16	59 [b]	23	0.46 [a]	0.15
A. pyramidalis	15	18 [a]	10	68 [b]	18	0.25 [b]	0.10

Fig. 4. Proportion of pollinia removed and fruits set in relation to population size. *A. pyramidalis* population C in 1989, indicated by ( ), was excluded from the regression analyses (see Material and Methods). For equations and statistics, see Table 1B
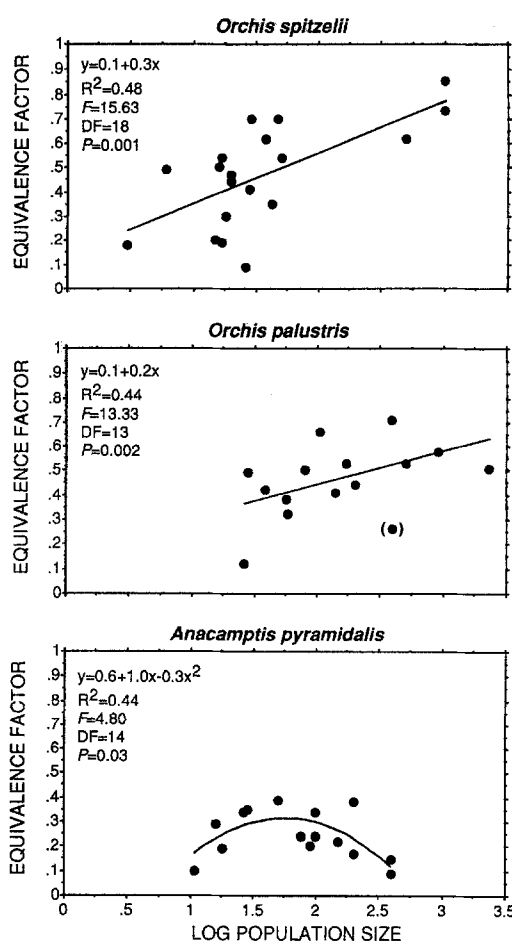
Fig. 5 Female–male sexual equivalence factor E in relation to population size. *O. palustris* population D in 1989 (*in parenthesis*) was excluded from the analysis (see Materials and methods)

Table 3 Summary statistics of population data on pollinator-generated pure genders among plants (single-population data are reported in the Appendix). Mean values in between-species comparisons were not significantly different at the 0.05 level (Tukey's HSD test)

Species	n	Neuter (%)		Male (%)		Female (%)		Hermaphrodite (%)	
		$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
O. spitzelii	19	8	9	33	22	0.5	1.3	59	22
O. palustris	15	10	11	25	13	0.6	1.2	65	20
A. pyramidalis	15	0	.	23	25	0	.	77	25

456

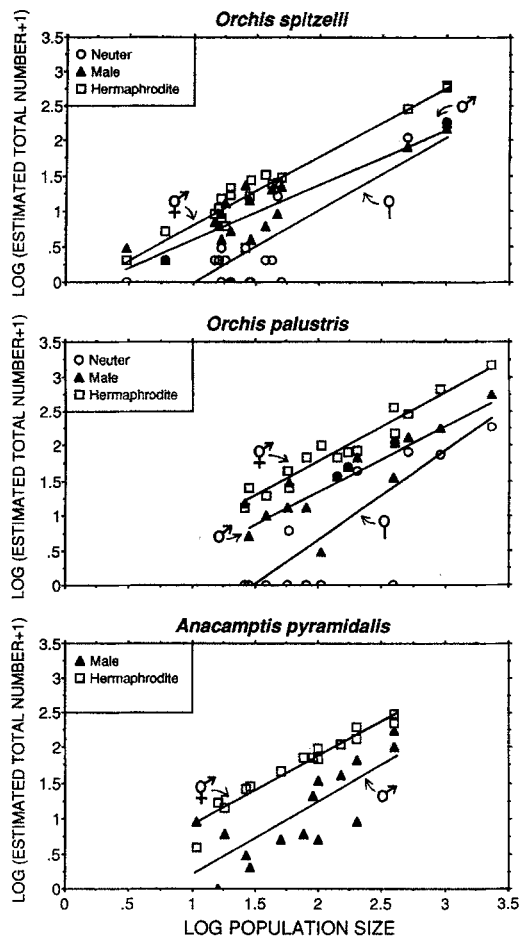**Fig. 6** Estimated total number (recorded proportion X population size) of pollinator-defined neuter (*open circles*), male (*filled triangles*), and hermaphrodite (*open squares*) plants in relation to population size. For equations and statistics, see Table 4B
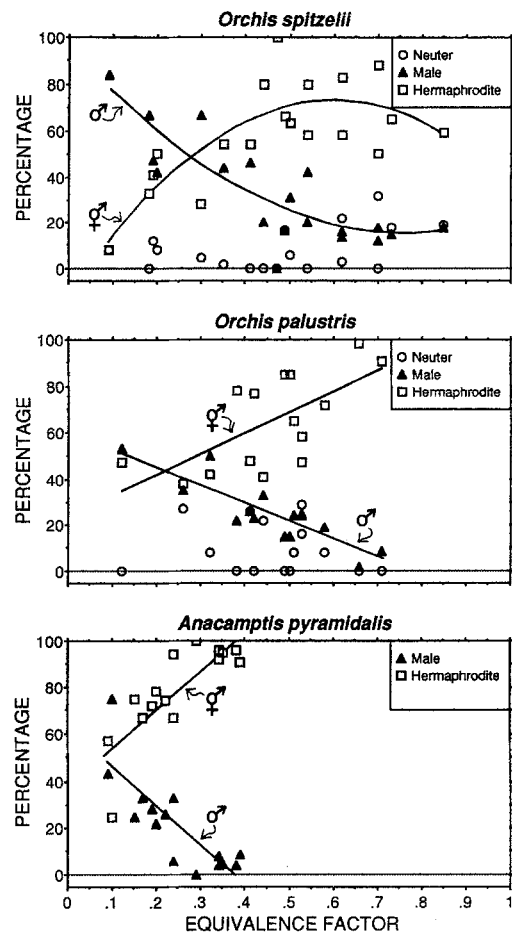

Fig. 7 Proportion of pollinator-defined neuter (*open circles*), male (*filled triangles*), and hermaphrodite (*open squares*) plants in relation to female–male sexual equivalence factor E in populations. For equations and statistics, see Table 4C

tor, E) ranged between 0.09 and 0.85, and *A. pyramidalis* had a significantly lower average than the two *Orchis* species (Table 2). E and population size showed significant relationships in each species (Fig. 5). In small populations E was low, indicating that relatively many of the pollinia removed were lost. The larger the populations of *O. spitzelii* or *O. palustris*, the greater the E attained. In contrast, E dropped in large populations of *A. pyramidalis* (and a quadratic term significantly improved the regression over a simple linear model), suggesting that pollinator-mediated E reached a maximum at a population size of c. 80 plant individuals.

The proportions of pollinator-generated pure gender among populations did not differ significantly between species (Table 3). Hermaphrodites or males made up between 0 and 100%, while females were few and recorded only in *Orchis* (see Appendix). Neuters made up 0–32% in *Orchis* while none were found in *A. pyramidalis*. The estimated total numbers of individuals with particular genders showed strong linear relationships with population size (Fig. 6 and Table 4C). In contrast, the proportions of genders showed significant relationships with population size only in two cases (Table 4A). In *O. spitz-*

elii, there was a positive linear relationship for neuters; in the largest populations c. 20% were neuters. In *A. pyramidalis*, the proportion of hermaphrodites decreased with population size when data from population C in 1989 were excluded from the analysis (reciprocally, there was an almost significant positive relationship for males). In *O. palustris*, a nearly significant relationship appeared when the data for neuters were fitted to a quadratic regression model.

Regression of proportions of pure genders on female–male reproductive equivalence factor E showed that the higher the E, the higher the proportion of hermaphrodites, but this relationship levelled off with increasing E in *O. spitzelii* (Fig. 7 and Table 4B). Reciprocally, the lower the E, the higher the proportion of males. In populations with high Es, the proportion of males dropped to c. 16% in *O. spitzelii*, 17% in *O. palustris*, and 6% in *A. pyramidalis*. There was no significant relationship between the proportion of neuter individuals and E in *Orchis* (in *A. pyramidalis* there were no neuters).

Regression of the population variance in individual male, female, and total reproductive success (male plus

Table 4A–C Equations and statistics of best-fit regressions of pollinator-generated pure genders (N neuter, M male, H hermaphrodite) on population size and female–male sexual equivalence factor in populations. **A** Gender proportions in relation to population size. **B** Estimated total number of different genders (recorded proportionpopulation size) in relation to population size (Fig. 6). **C** Gender proportions in relation to female–male equivalence factor E (Fig. 7). The variables H in *O. spitzelii* and M in *O. palustris* are untransformed (a Lilliefors test indicated that arcsine transformation reduced normality in those cases)

Species	Gender	Equation	r^2	F	df	P
A.						
O. spitzelii	N	$y=-0.03+0.07x$	0.26	5.82	18	0.028
	M	$y=0.6-0.1x$	0.13	2.52	18	0.131
	H	$y=0.6-0.02x$	0.00	0.03	18	0.869
O. palustris	N	$y=-0.9+0.8x-0.2x^2$	0.38	3.61	14	0.060
	M	$y=0.4-0.05x$	0.05	0.63	14	0.441
	H	$y=0.9-0.1x$	0.02	0.19	14	0.668
A. pyramidalis	M	$y=-0.1+0.2x$	0.28	4.69	13	0.051
	H	$y=1.7-0.3x$	0.30	5.10	13	0.043
B.						
O. spitzelii	N	$y=-1.0+1.0x$	0.77	58.23	18	0.000
	M	$y=-0.2+0.8x$	0.76	54.13	18	0.000
	H	$y=-0.2+1.0x$	0.91	167.49	18	0.000
O. palustris	N	$y=-1.8+1.3x$	0.58	19.23	14	0.001
	M	$y=-0.5+0.9x$	0.75	39.12	14	0.000
	H	$y=-0.2+1.0x$	0.94	216.67	14	0.000
A. pyramidalis	M	$y=-0.9+1.0x$	0.64	23.18	14	0.000
	H	$y=-0.1+1.0x$	0.94	219.56	14	0.000
C.						
O. spitzelii	N	$y=-0.001+0.2x$	0.17	3.56	18	0.001
	M	$y=1.0-2.3x+1.4x^2$	0.72	21.00	18	0.000
	H	$y=-2.8+226.8x-168.8x^2$	0.60	11.77	18	0.010
O. palustris	N	$y=0.1-0.1x$	0.02	0.34	14	0.570
	M	$y=58.5-69.5x$	0.75	39.19	14	0.000
	H	$y=0.2+1.2x$	0.46	11.10	14	0.005
A. pyramidalis	M	$y=0.6-1.7x$	0.63	22.50	14	0.000
	H	$y=0.3+2.7x$	0.66	24.90	14	0.000

Table 5 Equations and statistics of best-fit regressions for variance in relative pollinia removed (*Rel PR*), relative fruits set (*Rel F*), the sum of these two variables ("total fitness", Rel PR+Rel F), and quantitative functional gender G in relation to population size. All variables were log transformed before analysis

Species	Variable	Equation	r^2	F	df	P
O. spitzelii	Rel PR	$y=0.1+0.08x$	0.29	7.08	18	0.016
	Rel F	$y=0.5-0.04x$	0.02	0.32	18	0.580
	Rel (PR+F)	$y=0.6+0.04x$	0.02	0.40	18	0.533
	G	$y=0.1-0.02x$	0.13	2.49	18	0.133
O. palustris	Rel PR	$y=0.01+0.07x$	0.18	2.91	14	0.112
	Rel F	$y=0.02+0.04x$	0.04	0.50	14	0.490
	Rel (PR+F)	$y=0.2+0.12x$	0.13	1.99	14	0.182
	G	$y=0.03-0.001x$	0.001	0.01	14	0.928
A. pyramidalis	Rel PR	$y=0.2+0.41x$	0.27	4.76	14	0.048
	Rel F	$y=0.4-0.02x$	0.01	0.10	14	0.763
	Rel (PR+F)	$y=0.4+0.04x$	0.02	0.20	14	0.666
	G	$y=0.02+0.001x$	0.002	0.02	14	0.882

female) as well as functional gender on population size indicated no effect on the variance for any of the last three variables (Table 5). In contrast, the larger the population, the greater the variance in pollinium removal among individuals in *O. spitzelii* and *A. pyramidalis* (in *O. palustris* the relationship approached significance).

Discussion

Since plants were non-clonal, non-autogamous, and strongly pollen/pollinator limited, the null hypothesis was that there was no relationship between reproductive success and population size. This study shows that species of plant and/or pollinator, extent of pollen removal, and population size are factors that influence variation in sexual success at the population as well as the individual level in hermaphroditic plants. Indeed, any change in

population size may change overall pollen flow and thus the female–male mating structure within (and between) populations (Wright 1938).

The results indicated that a varying proportion of removed pollinia never encountered stigmas within the populations. However, once the pollen reached a stigma, the probability of male reproductive success was high: 97–100% of such flowers set fruit. The three study species are thus strongly pollen/pollinator limited, a condition that seems to prevail in orchids (Gill 1989; Zimmerman and Aide 1989; Nilsson 1992b). Pollen export (male function) in plants may be pollinator-visit, pollen-removal or pollen-pool limited (Stanton and Preston 1988), while pollen import (female function) may be pollinator-constancy, pollen-transportation, or pollen-receipt limited. The orchids studied were more limited by pollen import than by pollen export (pollinator-visit or pollen-removal), e.g. regressions predicted that 60–80 pollinia must be removed before any pollination with subsequent fruit set occurred in populations.

Pollinator-mediated female–male reproductive efficiency increased with greater pollinium removal (absolute or proportional) and different plant species operated at different levels of reproductive efficiency. Clearly, the more pollen in circulation, the more pollinations (and different plant species may differ in, for instance, pollinator or functional morphology). Estimated total numbers of pollinia removed and fruits set showed that pollinator service decreased with smaller plant population size. Thus, a decrease in population size may have serious negative sexual consequences for animal-pollinated plant populations.

The variation in functional female–male reproductive equivalence (E) showed that pollinator-mediated reproductive efficiency exhibits certain relationships with population size in the plant species studied. Whereas the value for the two *Orchis* species was still increasing at the largest population size studied, that for *A. pyramidalis* reached a maximum at moderate population sizes, c. 80 individuals. Evidently, a high E is not dependent on high proportions of pollen removal per se: *O. spitzelii* had the lowest pollinium removal but the highest E, while *A. pyramidalis* had the highest pollinium removal but the lowest E, and *O. palustris* was intermediate. This suggests that pollen waste, e.g. by specific pollinator or floral morphology, strongly affects the female–male reproductive efficiency and is one important evolutionary factor in plant populations (cf. Lloyd 1984).

The species-specific departures from hypothetical equal pollinium removal and fruit set in populations (Fig. 1) may reflect different "pollen-wasteful" movement patterns of the pollinators. Butterflies fly longer distances than bumblebees between successive visits to flowers and plants (Schmitt 1980; Waser 1982). The main pollinator of *A. pyramidalis* on Gotland, the large pierid butterfly *Aporia crataegi*, probably moves long distances; on the adjacent island of Öland, one individual *Ap. crataegi* marked in one *A. pyramidalis* population was observed 2 h later in another population 6 km away

(Lind 1992). Accordingly, it is predicted that pollinia will be moved out of populations more frequently in the largely *Ap. crataegi*-pollinated *A. pyramidalis* than in the two bumblebee-pollinated *Orchis* species, and thus will frequently not contribute to fruit production in the population of origin, or at all. *A. pyramidalis* had the greatest departure from equal pollinium removal and fruit set.

Levin (1988) suggested that, given the same plant density, the number of potential mates will be smaller in a population pollinated by an animal that forages over a narrow range than in a population pollinated by a vector species moving longer distances. If this is the case, the number of potential mates is expected to be smaller in the two bumblebee-pollinated *Orchis* species than in the butterfly-pollinated *A. pyramidalis*. This is supported by our data because *A. pyramidalis* populations had no neuters. However, the number of potential mates among plants will also be influenced by metapopulation structure (cf. Ellstrand et al. 1989), density of pollinator populations (Jennersten 1988), and level of floral constancy of individual pollinators in response to floral reward (Heinrich 1975, 1979).

For plant species offering no reward, as in the orchids considered here, there is no initiation of floral constancy and thus pollinator movement may be determined by the density of profitable surrounding foodplants rather than by the density of conspecifics (Laverty and Plowright 1988; Levin 1988). The lower the density of food-plants, the greater the mean flight distance (Levin and Kerster 1969), and the more often a pollinator encounters a rewardless orchid, the more quickly it will learn to avoid the plant and the site (Heinrich 1975). The nectar-rich, frequently bumblebee-visited *Arctostaphylos uva-ursi* (L.) Spreng. (Ericaceae) often occurred as clonal carpets around *O. spitzelii* (Fritz 1990). In contrast, bumblebee-exploited flowers were quite sparse in the *O. palustris* fens (while abundant in the surroundings). Bumblebees would be predicted to fly longer distances and waste relatively more pollinia in *O. palustris* than in *O. spitzelii*, a pattern supported by our data. In the *A. pyramidalis* populations foodplants for pollinators were usually abundant, but butterflies may still have had an inherent tendency to fly relatively long distances.

While the proportion of pollinia removed decreased with larger population size (a simple effect of pollinator limitation), the proportion of fruits set was independent in *Orchis* and showed a negative relationship in *A. pyramidalis* (Fig. 4). Probably this is another reflection of the different effects of sedentary bumblebee and far-flying and thus less efficient butterfly pollinators.

Several lines of evidence indicated that while the numbers of individuals with particular pollinator-designated genders primarily depend on population size, their proportions depend mainly on the pollinator-mediated level of relative female–male reproductive efficiency within populations. The number of individuals with any particular pollinator-designated gender increased with population size, whereas the higher the female–male equivalence factor E, the larger the proportion of her-

maphrodites and the smaller the proportion of males; however, this relationship levelled off at high Es in *O. spitzelii* because in the largest populations there were more neuters due to reinforced pollinator-visit limitation. The butterfly-pollinated *A. pyramidalis* was evidently either so frequently visited, or had such an effective pollinium removal mechanism (cf. Darwin 1862), that no neuters or females occurred; still, there was a negative relationship between the proportion of hermaphrodites and population size (Table 4A), an effect of pollen-import limitation.

Frequency-dependent selection in a given sexual population is predicted to favour differential evolution of reproductive traits (e.g. sex-related ones) until the female-male reproductive efficiency ratio reaches a point where there is overall equal investment in offspring production through male and female function (Fisher 1958; Maynard Smith 1971; Charnov 1982). However, natural populations of a species may, for a number of reasons (e.g. historical, stochastic), deviate from the species' optimal population size and sexual proportions for such overall equal investment.

The three orchid species studied here were more pollen-import (female function) than pollen-export (male function) limited, and increasingly so with smaller population size. Moreover, variance in pollen export, but not pollen import, increased with population size, suggesting that the intensity of sexual selection for male traits was greater with larger population size (e.g. Stephenson and Bertin 1983). Consequently, this yields the following major prediction: beneficially female sexual traits will be increasingly favoured by density-dependent selection when population size decreases and causes a greater pollen waste, i. e. a factor of uncertainty increasingly associated with pollen import that may promote allocation of resources to female function. Although small populations of outcrossing plants may have reduced phenotypic variability as a result of genetic erosion (Ouborg et al. 1991), intensified inbreeding due to decreasing population size tends to increase the number of genes which are expressed and become subject to phenotypic selection (Lloyd 1965). Indeed, in a small population of the composite *Senecio integrifolius* heritabilities of a number of female reproductive traits were greater than in a large population (Widén and Andersson 1993). Both predictions and data thus suggest that in outcrossing plants selection may be an important evolutionary force even in small populations. Whether plants in small, sexually suboptimal populations shift sex allocation patterns, respond to selection and become exaggerated in their female sexual traits (e.g. stigma size and receptivity schedule) relative to plants in larger populations is clearly testable and deserves further study.

Using a simulation model where, however, fruit production was not pollen but resource limited and mating was random, Nakamura et al. (1989) predicted that in large plant populations male and female reproductive success in hermaphroditic individuals will be nearly equal, while in small populations most individuals will reproduce more through male than through female function. This still awaits an empirical test. Here we have provided evidence that larger populations of pollen/pollinator limited species have greater female–male reproductive equivalence than smaller populations and that there is some pollinator-mediated optimum in pollination efficiency in relation to population size variation. We conclude that animal pollinators make small populations of pollen- or pollinator-limited plants functionally male-biased in terms of pollen removal but selectively female-biased due to reduced pollen-import success.

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Appendix The number of flowering plants, plants and flowers examined, fruit set, pollinium removal, equivalence factor (E), and pollinator-generated pure gender in populations of three nectarless orchids in Gotland, Sweden. The numbers of flowering plants of *Orchis palustris* marked with an asterisk are from Högström (1991). Universal Transverse Mercator (UTM) coordinates for population localities are given

Population (UTM coordinates)	Year	Flowering plants, no.	(n)	Flowers in sample, no.	Fruits %	(no.)	$\bar{X}\pm SD$	Pollinia removed %	(no.)	$\bar{X}\pm SD$
Orchis spitzelii										
A (63,21,0;6,62,0)	1988	1000	(296)	3186	15.6	(496)	0.14±0.17	18.3	(1164)	0.19±0.18
	1989	1000	(319)	3641	16.7	(608)	0.16±0.17	22.8	(1657)	0.22±0.20
	1990	500	(236)	680	16.5	(112)	0.17±0.41	26.7	(363)	0.23±0.29
B (63,76,1;3,61,5)	1989	50	(36)	549	26.8	(147)	0.27±0.29	49.4	(542)	0.50±0.25
	1990	26	(26)	333	3.0	(10)	0.04±0.14	33.5	(223)	0.35±0.22
C (63,80,8;3,62,4)	1989	47	(34)	316	14.2	(45)	0.17±0.23	20.4	(129)	0.26±0.26
	1990	15	(12)	133	6.8	(9)	0.06±0.10	33.1	(88)	0.31±0.22
D (63,19,1;3,65,1)	1989	43	(43)	456	15.8	(72)	0.15±0.20	45.2	(412)	0.45±0.21
	1990	17	(17)	182	8.2	(15)	0.09±0.12	44.2	(161)	0.42±0.26
E (63,17,9;3,68,3)	1989	38	(36)	406	31.5	(128)	0.29±0.23	50.5	(410)	0.49±0.22
	1990	20	(15)	179	27.4	(49)	0.26±0.20	61.7	(221)	0.61±0.27
F (63,11,5;3,49,2)	1989	29	(26)	216	55.1	(119)	0.53±0.29	78.9	(341)	0.80±0.19
	1990	16	(16)	126	22.2	(28)	0.21±0.19	44.4	(112)	0.44±0.29
G (63,21,5;3,33,2)	1989	28	(24)	250	12.8	(32)	0.12±0.16	31.0	(155)	0.30±0.14
	1990	3	(3)	33	6.1	(2)	0.05±0.09	33.3	(22)	0.33±0.11
H (63,93,2;3,45,3)	1989	6	(6)	44	25.0	(11)	0.29±0.27	51.1	(45)	0.55±0.30
	1990	18	(18)	149	11.4	(17)	0.10±0.19	38.3	(114)	0.39±0.22
I (63,13,5;3,56,9)	1989	17	(10)	107	25.2	(27)	0.24±0.22	46.3	(99)	0.44±0.24
J (63,81,3;3,62,3)	1989	20	(15)	208	30.3	(63)	0.20±0.28	64.4	(268)	0.59±0.29
Total no.		2893	(1188)	11194		(1990)			(6526)	
Orchis palustris										
A (63,11,6;3,72,0)	1988	511*	(350)	2158	20.4	(440)	0.20±0.22	38.7	(1672)	0.38±0.27
	1989	923*	(252)	1827	25.9	(473)	0.26±0.23	45.0	(1645)	0.47±0.28
	1990	202*	(76)	6011	2.6	(76)	0.13±0.17	28.8	(346)	0.29±0.27
B (63,38,9;3,30,0)	1989	2325*	(71)	5441	8.6	(101)	0.20±0.18	36.4	(396)	0.39±0.25
	1990	139*	(46)	272	18.0	(49)	0.18±0.23	43.9	(239)	0.44±0.35
C (63,47,7;3,32,5)	1988	104*	(44)	331	58.3	(193)	0.57±0.26	87.8	(581)	0.90±0.14
	1989	392*	(70)	452	55.3	(250)	0.54±0.27	77.6	(702)	0.77±0.21
	1990	58	(50)	262	19.5	(51)	0.20±0.26	60.3	(316)	0.59±0.28
D (63,38,5;3,29,6)	1989	400	(60)	513	7.8	(40)	0.08±0.12	30.2	(310)	0.28±0.24
	1990	170	(49)	292	16.1	(47)	0.16±0.22	30.3	(177)	0.28±0.24
E (63,60,3;6,32,6)	1989	39	(30)	183	35.0	(64)	0.35±0.25	84.2	(308)	0.85±0.14
	1990	26	(17)	84	9.5	(8)	0.13±0.15	76.2	(128)	0.77±0.19
F (63,49,1;3,33,1)	1989	57	(32)	254	31.1	(79)	0.31±0.24	82.7	(420)	0.84±0.17
G (63,39,5;3;30,1)	1990	80	(34)	184	38.6	(71)	0.38±0.28	76.9	(283)	0.78±0.22
H (63,12,1;3,65,6)	1990	28	(20)	117	40.2	(47)	0.40±0.23	81.2	(190)	0.82±0.19
Total no.		5454	(1201)	8074		(1989)			(7713)	
Anacamptis pyramidalis										
A (63,73,7;3,66,6)	1989	200	(23)	652	24.4	(159)	0.23±0.16	65.0	(848)	0.62±0.22
B (63,69,9;3,67,8)	1989	150	(27)	533	11.8	(63)	0.10±0.11	52.5	(560)	0.49±0.18
C (63,73,6;3,66,2)	1989	11	(4)	103	2.9	(3)	0.02±0.04	30.1	(62)	0.34±0.24
D (63,18,1;3,78,2)	1989	100	(18)	385	10.4	(40)	0.09±0.10	43.5	(335)	0.44±0.20
	1990	90	(32)	823	14.1	(116)	0.11±0.11	71.9	(1184)	0.70±0.14
E (63,62,7;6,30,5)	1989	100	(25)	714	30.5	(218)	0.30±0.22	90.3	(1289)	0.89±0.11
	1990	50	(22)	746	35.0	(261)	0.33±0.21	88.9	(1326)	0.88±0.11
F (63,02,8;3,45,3)	1989	77	(33)	1255	17.9	(225)	0.19±0.16	74.5	(1870)	0.72±0.19
	1990	400	(28)	855	6.1	(52)	0.06±0.07	65.5	(1120)	0.64±0.24
G (63,63,0;6,30,3)	1989	16	(9)	284	26.0	(74)	0.24±0.13	90.5	(514)	0.90±0.04
	1990	29	(19)	475	30.3	(144)	0.31±0.20	87.4	(830)	0.87±0.13
H (63,11,6;3,71,6)	1989	18	(18)	432	13.6	(59)	0.13±0.14	72.0	(622)	0.71±0.23
	1990	27	(24)	541	26.4	(143)	0.26±0.20	76.9	(832)	0.75±0.22
I (63,64,7;3,47,9)	1990	200	(24)	582	8.4	(49)	0.09±0.10	49.2	(573)	0.50±0.24
J (63,85,0;3,68,4)	1990	400	(24)	606	10.2	(62)	0.09±0.08	66.3	(804)	0.65±0.12
Total no.		1868	(330)	8986		(1668)			(12769)	

Appendix (Continued)

	E	Pollinator-generated pure gender,% (no.)			
		Neuter	Male	Female	Hermaphrodite
Orchis spitzelii					
A (63,21,0;6,62,0)	0.85	19 (55)	18 (54)	4 (11)	59 (176)
	0.73	18 (58)	15 (47)	2 (5)	65 (209)
	0.62	22 (53)	16 (38)	4 (9)	58 (136)
B (63,76,1;3,61,5)	0.54	0	42 (15)	0	58 (21)
	0.09	8 (2)	84 (22)	0	8 (2)
C (63,80,8;3,62,4)	0.70	32 (11)	18 (6)	0	50 (17)
	0.20	8 (1)	42 (5)	0	50 (6)
D (63,19,1;3,65,1)	0.35	2 (1)	44 (19)	0	54 (23)
	0.19	12 (2)	47 (8)	0	41 (7)
E (63,17,9;3,68,3)	0.62	3 (1)	14 (5)	0	83 (30)
	0.44	0	20 (3)	0	80 (12)
F (63,11,5;3,49,2)	0.70	0	12 (3)	0	88 (23)
	0.50	6 (1)	31 (5)	0	63 (10)
G (63,21,5;3,33,2)	0.41	0	46 (11)	0	54 (13)
	0.18	0	67 (2)	0	33 (1)
H (63,93,2;3,45,3)	0.49	17 (1)	17 (1)	0	66 (4)
	0.30	5 (1)	67 (12)	0	28 (5)
I (63,13,5;3,56,9)	0.54	0	20 (2)	0	80 (8)
J (63,81,3;3,62,3)	0.47	0	0	0	100 (15)
Total no.		(187)	(258)	(25)	(718)
Orchis palustris					
A (63,11,6;3,72,0)	0.53	16 (56)	26 (90)	1 (3)	57 (201)
	0.58	8 (21)	19 (47)	1 (2)	72 (182)
	0.44	22 (17)	33 (25)	4 (3)	41 (31)
B (63,38,9;3,30,0)	0.51	8 (6)	24 (17)	3 (2)	65 (46)
	0.41	26 (12)	26 (12)	0	48 (22)
C (63,47,7;3,32,5)	0.66	0	2 (1)	0	98 (43)
	0.71	0	9 (6)	0	91 (64)
	0.32	8 (4)	50 (25)	0	42 (21)
D (63,38,5;3,29,6)	0.26	27 (16)	35 (21)	0	38 (23)
	0.53	29 (14)	24 (12)	0	47 (23)
E (63,60,3;6,32,6)	0.42	0	23 (7)	0	47 (8)
	0.12	0	53 (9)	0	47 (8)
F (63,49,1;3,33,1)	0.38	0	22 (7)	0	78 (25)
G (63,39,5;3,30,1)	0.50	0	15 (5)	0	85 (29)
H (63,12,1;3,65,6)	0.49	0	15 (3)	0	85 (17)
Total no.		(146)	(287)	(10)	(758)
Anacamptis pyramidalis					
A (63,73,7;3,66,6)	0.38	0	4 (1)	0	96 (22)
B (63,69,9;3,67,8)	0.22	0	26 (7)	0	74 (20)
C (63,73,6;3,66,2)	0.10	0	75 (3)	0	25 (1)
D (63,18,1;3,78,2)	0.24	0	33 (6)	0	67 (12)
	0.20	0	22 (7)	0	78 (25)
E (63,62,7;6,30,5)	0.34	0	4 (1)	0	96 (24)
	0.39	0	9 (2)	0	91 (20)
F (63,02,8;3,45,3)	0.24	0	6 (2)	0	94 (31)
	0.09	0	43 (12)	0	57 (16)
G (63,63,0;6,30,3)	0.29	0	0	0	100 (9)
	0.35	0	5 (1)	0	95 (18)
H (63,11,6;3,71,6)	0.19	0	28 (5)	0	72 (13)
	0.34	0	8 (2)	0	92 (22)
I (63,64,7;3,47,9)	0.17	0	33 (8)	0	67 (16)
J (63,85,0;3,68,4)	0.15	0	25 (6)	0	75 (18)
Total no.		0	(63)	0	(267)