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# Outcrossing rates and male sterility in natural populations of *Plantago coronopus* *

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**Summary.** Outcrossing rates were estimated in three populations of the gynodioecious species *Plantago coronopus* by means of electrophoresis of adult plants and their natural progenies. A multilocus estimation procedure was used. Heterogeneity among the pollen-pool allele frequencies did not exist either in space of in time. Differences between populations in mean outcrossing rates were large (range: 0.34–0.93), probably caused by differences in densities of flowering plants. In addition, there was considerable variability between individuals, which was at least partly caused by the presence of male sterility. Population density may, via its influence on outcrossing rates, be a factor influencing the maintenance of male sterile plants in the population. The level of outcrossing in hermaphrodites was not low enough to explain the maintenance of male steriles. Outcrossing rates in two populations, established via progeny analysis, were much lower than calculated with the fixation index, possibly indicating heterozygote advantage in these natural populations.

**Key words:** *Plantago coronopus* – Outcrossing rates – Male sterility – Population density

## Introduction

For an understanding of the genetic structure of a species, extensive knowledge of the mating system is necessary. The mating system is influenced by many characteristics of the plant and its environment. Some of the obvious components causing obligate outcrossing are dioecy and self-incompatibility. Many studies have been devoted to other components of the mating system in the last twenty years using allozymes as markers. Outcrossing rates may, for instance, be influenced by variability in flower color and structure. Flower color in *Ipomea purpurea* induces a specific pollinator behaviour (Ennos and Clegg 1983). Stigma excertion in *Lycopersicon pimpinellifolium* (Rick et al. 1977) and stigma-anther distance in *Ipomea purpurea* (Epperson and Clegg 1987) have been shown to influence outcrossing rates. A strong influence of flower morphology was also found in *Impatiens capensis* (Waller 1980), where two types of flowers, chasmogamous and cleistogamous, may be present: plant size and light quantities determine the percentage of chasmogamous flowers and an increase of this flower type increases outcrossing rates per plant.

Protogyny or protandry, present in many species, may have a considerable influence on the mating system (Lloyd and Webb 1986). In *Gilea achilleifolia*, a positive correlation between the degree of protandry and outcrossing rate was shown (Schoen 1982a). This was also found in a study on *Limnanthes* species (Kesseli and Jain 1985). Marshall and Abbott (1982) showed that in *Senecio vulgaris* the variability in outcrossing rate was caused by differences in levels of protogyny, associated with a polymorphism for flower morphology: the radiate flowers were protogynous, giving a higher outcrossing rate. The distance to the neighbouring male flowering individuals may also be of importance. In Ponderosa pine, a lower density of trees was correlated with a lower outcrossing rate (Farris and Mitton 1984). In Douglas fir, on the other hand, this correlation was not observed (Neale and Adams 1985), but the height of the crown affected the outcrossing rates, trees with intermediate height having the highest outcrossing rates (Shaw and Allard 1982).

The presence of male sterile plants in a predominantly selfing species may considerably increase 'overall'

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outcrossing rates, since male sterile plants are obligately outcrossed. Conversely, outcrossing rates, and the factors discussed above influencing outcrossing rates, affect the frequency of male steriles, provided that inbreeding depression occurs in the progeny of selfing hermaphrodites (Lloyd 1975). Therefore, the fitness disadvantage of male steriles, relative to hermaphrodites may be (partly) overcome by avoiding inbreeding. Gregorius et al. (1982) have shown that not only the average level of outcrossing in a population is important, but also genotypic variation in outcrossing rates.

The outcrossing rate of a population can be estimated from the fixation index (Brown 1979). This measure is, however, not only determined by the actual levels of outcrossing, but is affected by differential survival of individuals and the existence of population substructuring (Wahlund effect). In many species outcrossing rates estimated from the fixation index are in fact overestimates. Overestimation in several cases has been related to the presence of heterozygote advantage: a higher germination of more heterozygous (outcrossed) seeds was found in Jack pine (Cheliak et al. 1985) and in Ponderosa pine (Farris and Mitton 1984), and a higher survival of the more heterozygous plants during the juvenile stages was found in *Gilia achilleifolia* (Schoen 1982b) and *Lens culinaris* (Skibinski et al. 1984).

In this study, we will focus on outcrossing rates in the gynodioecious species *Plantago coronopus*. *Plantago* species show a diversity of outcrossing rates, with selfing levels varying from 0%–100%. The diversity in outcrossing rates is mediated by the existence of a self-incompatibility system in some of the species, by diverse levels of protogyny (Bos et al. 1985) and by the widespread existence of male steriles (gynodioecy) in this genus (Van Damme 1983). The species *Plantago coronopus* is self-compatible and wind-pollinated. High outcrossing rates might be expected because of the occurrence of male sterile and partially male sterile phenotypes (J.M.M. Van Damme, in preparation) and because the species shows relatively strong protogyny, expressed as a time delay of about 5 days between female receptance and male dehiscence (greenhouse measurements; Bos et al. 1985). Electrophoretic studies have shown high levels of heterozygosity, and estimates based on the fixation index and on population structure analysis indicate very low levels of selfing of about 0%–2% (Van Dijk et al. 1988). As some non-trivial assumptions underlie these calculations, e.g. no selection, a more direct estimation is highly desirable. The outcrossing rate of a population is best determined by electrophoresis of mothers and their natural progenies. Some of the statistical problems connected with methods for estimating the maternal genotype and the pollen pool allele frequencies from the progeny genotypes (Brown et al. 1975) can be solved in this species. Male steriles can be used to measure pollen-pool allele frequencies (Valdeyron et al. 1977) and adult plants can be sampled together with ripe spikes to directly establish the maternal genotype. This accurate estimation procedure was performed in one population. To establish whether the outcrossing rates in different populations are comparable, several other populations were sampled in a less extensive way.

## Materials and methods

### *The populations studied*

Three populations from coastal habitats were sampled. From electrophoresis of the plants sampled and from earlier results (Van Dijk et al. 1988) the allozyme variability of the populations was known. The population from Kwade Hoek (KH) had the highest variability and was therefore the most extensively studied population. KH is a salt meadow, extensively grazed by cattle and occasionally flooded by sea water. The soil is relatively nutrient rich and the vegetation is dense but short, with a local high density of *P. coronopus* plants ($\pm 40$ flowering plants per $m^2$). Less extensively sampled were the populations from Oostvoornse Meer (OM) and from Schiermonnikoog (SD). OM is a former beach plain, embanked in 1965. It is frequently inundated in winter by rain water. The soil is relatively nutrient poor with a sparse and low vegetation and a low density of *P. coronopus* plants ($\pm 10$ per $m^2$). The plants were sampled at the edge of a relatively higher vegetation with shrubs where flowering plants were present. Schiermonnikoog (SD) is a grassland, irregularly mown, at the foot of a dike at the Waddensea coast of this island. The vegetation was dense with a relatively high grassland vegetation and only a low number of *P. coronopus* plants was present ($\pm 6$ per $m^2$). For more detailed descriptions of the habitats of KH and OM see Lotz and Blom (1986).

### *The sampling procedure*

In a $2 \times 2$ meter plot in the KH population 161 plants were marked and their flowering followed weekly for 6 weeks, starting with the beginning of the flowering period at the end of June. Each flowering spike was marked in the field and the sex phenotype was noted. Male sterility in *Plantago coronopus* has been described by J.M.M. Van Damme (in preparation). Complete male sterility (MS) is genetically determined and characterised by small brown anthers without any pollen. Partial male sterility (PMS) is usually expressed as a mixture of sterile and fertile flowers on a plant and is largely environmentally determined. PMS plants are most likely genetically hermaphrodites (H), showing plasticity in pollen production. The frequencies of MS, PMS and H plants in the study populations are shown in Table 1. Later, the classification was checked in the laboratory by observing ripe spikes with a stereomicroscope and by assessing the sex phenotype of the plants after transplantation into the greenhouse. The allozyme genotypes of about 100 plants were determined. Plants for progeny analysis were selected for three criteria: flowering date, sex phenotype and allozyme homozygosity. Plants with only one spike flowering from the 2nd week of the field observation period were selected as well as plants flowering 3 weeks later. The latter often had more than one spike flowering at that moment. As for sex phenotype, as many MS plants as possible were used to get a good approximation of the pollen-pool allele frequencies. Furthermore, for allozyme genotype the most homozygous plants were selected because

**Table 1.** Frequencies of sex phenotypes (%), the corresponding sample sizes, density of flowering plants (no. per $m^2$) and vegetation height (cm) in three *P. coronopus* populations

| Population | MS | PMS | H | Sample size | Density | Veg. height |
|---|---|---|---|---|---|---|
| KH | 8 | 13 | 79 | 147 | 40 | 2–5 |
| OM | 9 | 20 | 71 | 107 | 10 | 0–10 |
| SD | 46 | 26 | 28 | 207 | 5 | 10–25 |

**Table 2.** Enzyme loci used for the determination of outcrossing rates in the analyses of natural progenies, means used (+) and not used (−) for electrophoretic determination

| Locus | Population | | |
|---|---|---|---|
| | OM | SD | KH |
| 6-Pgd-1 | + | + | + |
| Pgm-2 | + | − | + |
| Idh | − | − | + |
| Acph-1 | + | + | − |
| Acph-2 | − | + | − |
| Lap-2 | − | − | + |
| Adh | − | − | + |

from the progenies of these plants outcrossing rates could be determined relatively accurately. Five H and four MS plants flowering in the 2nd week and ten H, six PMS and nine MS plants flowering in the 5th week were selected. Each plant was used in only one sample. Per selected plant, 8–16 descendants were grown and used for electrophoresis. In a 3 × 3 meter plot, 40 plants from the OM population and 20 adult plants from the SD population with a ripe spike were sampled and transplanted into the greenhouse. The sex phenotypes of the plants were not determined in the field, but this was done later on in the laboratory for the OM population. For the SD population it was not possible to assess the sex phenotypes definitely; most spikes appeared to have some MS flowers. The number of adult plants selected was six for OM (probably one MS, one PMS and four H) and four for SD (sex phenotype unknown). Per maternal plant 18–130 descendants were grown.

Germination percentages of the seeds from the KH population was only 65%. Seeds from OM and SD had higher germination percentages (about 90%), partly caused by artificially rupturing the seed coat at the root tip end of the seed.

*Electrophoresis and estimation of outcrossing rates*

Electrophoresis was carried out as described by Van Dijk and Van Delden (1981) and by Van Dijk et al. (1988). The mode of inheritance of the polymorphic enzyme loci of this species was established by Van Dijk et al. (1988). The enzyme loci used for electrophoresis of the adult plants were phosphoglucomutase (Pgm-2), 6-phosphogluconate-dehydrogenase (6-Pgd-2), isocitrate-dehydrogenase (Idh), leucineamino-peptidase (Lap-2), acid-phosphatase (Acph-1 and Acph-2) and alcohol-dehydrogenase (Adh). For Adh, root material was used instead of leaf material. Depending on the allozyme variation present in the population, progenies were assayed for a number of these enzyme systems (Table 2). Outcrossing rates for the populations were determined using the multi-locus estimation procedure of Shaw et al. (1981) and individual plant multi-locus estimates were calculated in a comparable way (Neale and Adams 1985). For one plant from SD, heterozygous at two loci, a minimal $\chi^2$-iteration method was used. Following this method the number of genotypes found in the progeny was compared with the expected numbers using a $\chi^2$ calculation, starting with outcrossing rate t = 1. The value of t was then lowered in small steps until a minimal $\chi^2$ was reached. The value of t belonging to the minimal $\chi^2$ was the best approximation.

## Results

*Allele frequencies in adult plants and in the pollen-pool*

The allele frequencies of both the adult plants and the pollen-pool in KH are given in Table 3. To test whether allele frequencies were significantly different, $\chi^2$ values were calculated from a 2 × 2 contingency table for each locus. These values were compared to critical chi-square values for each locus separately and for all loci together. It appeared that the allele frequencies in the pollen-pool, as calculated from outcrossed progenies of homozygous MS plants, and the adult plants in KH were not significantly different ($P > 0.05$) for each locus separately and for all loci together (for all loci: adults vs pollen in 2nd week: $\chi^2_3 = 5.81$, adults vs pollen in 5th week: $\chi^2_4 = 8.20$). The adult plant allele frequencies in the KH population were thus representative for pollen-pool allele frequencies. We therefore will consider the adult plant allele frequencies in OM and SD as being representative for pollen-pool allele frequencies as well.

It was further examined whether there was a heterogeneity in time or in space in pollen-pool allele frequencies in KH. For the examination of temporal variability the allele frequencies of both periods, if available, in the 2nd and 5th week were compared (Table 3), but no significant difference was found ($\chi^2_3 = 1.94$). For the purpose of testing spatial heterogeneity in space, two groups of MS progenies were distinguished on the basis of the original position of their parents in the plot (distance of group centres: 1.1 m.). Allele frequencies of one part of the plot were not significantly different from the frequencies of the other part of the plot ($\chi^2_4 = 7.99$).

*Outcrossing rates*

For the KH population the outcrossed progenies of MS plants were used for calculating pollen-pool allele frequencies, whereas in OM and SD the frequencies of the adult plants were used. In Table 4 the values of the outcrossing rates determined in the different populations are given, together with the fixation indices and the outcrossing rates ($t_F$) as calculated from the number of expected

**Table 3.** Allele frequencies of the pollen-pool in the KH population and in adult plants for all populations studied (no. of plants given in brackets; (−) = not determined)

| Locus | Allele | Populations | | | | |
|---|---|---|---|---|---|---|
| | | OM (40) adults | SD (24) adults | KH adults | KH pollen 2nd wk | KH pollen 5th wk |
| 6-Pgd-2 | S | 0.45 | 0.45 | 0.30 (105) | 0.24 (55) | 0.28 (114) |
| | F | 0.55 | 0.55 | 0.70 | 0.76 | 0.72 |
| Pgm-2 | N | 0.85 | 1.0 | 0.94 (103) | 0.92 (53) | 0.94 (84) |
| | S | 0.15 | | 0.06 | 0.08 | 0.06 |
| Idh | N | 0.99 | 1.0 | 0.92 (104) | 0.93 (55) | 0.92 (107) |
| | S | 0.01 | | 0.08 | 0.07 | 0.08 |
| Acph-1 | N | 0.94 | 0.93 | 0.96 (93) | – | – |
| | S | 0.06 | 0.07 | 0.04 | | |
| Acph-2 | N | 1.0 | 0.85 | 1.0 (93) | – | – |
| | S | | 0.15 | | | |
| Lap-2 | N | 1.0 | 1.0 | 0.96 (105) | 0.89 (55) | 0.89 (90) |
| | S | | | 0.04 | 0.11 | 0.11 |
| Adh | N | 1.0 | 1.0 | 0.68 (25) | – | 0.73 (105) |
| | S | | | 0.28 | | 0.19 |
| | F | | | 0.04 | | 0.08 |

**Table 4.** Outcrossing rates ($t_m$) as calculated with a multi-locus estimation procedure for three populations, with the standard deviations between brackets; $n$ = total no. of descendants analysed. Also presented are the fixation index (F) and outcrossing rates ($t_F$) as calculated from the number of heterozygotes observed and expected

| | Population | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | OM | $n$ | SD | $n$ | KH 2nd wk | $n$ | KH 5th wk | $n$ |
| $t_m$ | 0.64 (0.06) | 202 | 0.34 (0.04) | 250 | 0.75 (0.09) | 124 | 0.93 (0.06) | 42 |
| F | −0.009 | | −0.184 | | +0.080 | | +0.080 | |
| $t_F$ | >1 | | >1 | | 0.85 | | 0.85 | |

**Table 5.** Outcrossing rates ($t_m$) for population OM and KH (5th week) for the different sex phenotype groups, standard deviations between brackets; $n$ = no. of descendants analysed. The mean of the outcrossing rates over sexes ($t_m$) of the maternal plants used in this study is given as well as an estimation of the actual outcrossing rates ($t_a$) and a weighted average ($t_g$). For further explanation see text

| Sex phenotype | Population | | | |
|---|---|---|---|---|
| | OM | $n$ | KH | $n$ |
| MS | >1.0 (0.19) | 26 | 0.97 (0.09) | 77 |
| PMS | 0.89 (0.16) | 31 | 0.99 (0.14) | 55 |
| H | 0.45 (0.06) | 145 | 0.90 (0.09) | 110 |
| $t_m$ | 0.64 (0.06) | 202 | 0.93 (0.06) | 242 |
| $t_a$ | 0.62 | | 0.92 | |
| $t_g$ | 0.55 | | 0.91 | |

**Table 6.** Outcrossing rates ($t_m$) together with standard deviations (sd) and sex phenotype (OM only) for individual plants in the populations OM and SD; $n$ is the no. of descendants analysed for that individual

| Population | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| OM | | | | | SD | | | |
| Plant no. | t | sd | n | Sex type | Plant no. | t | sd | n |
| 1 | >1.0 | 0.19 | 26 | MS | 1 | 0.54* | | 43 |
| 2 | 0.89 | 0.16 | 31 | PMS | 2 | 0.61 | 0.09 | 39 |
| 3 | 0.46 | 0.20 | 18 | H | 3 | 0.23 | 0.10 | 38 |
| 4 | 0.19 | 0.09 | 43 | H | 4 | 0.25 | 0.05 | 130 |
| 5 | 0.71 | 0.13 | 46 | H | | | | |
| 6 | 0.42 | 0.12 | 38 | H | | | | |

* $\chi^2$ method used, no standard deviation given

and observed heterozygotes. It appeared that for the KH population the outcrossing rates were lower for the spikes sampled in the 2nd week than for those sampled in the 5th week (Student's $t$-test: $P<0.001$). The differences between populations were large (all $P<0.001$). Outcrossing rates as determined by progeny analyses were, for populations OM and SD, much lower than the figures calculated from the fixation index (Table 4).

Differences in outcrossing rates between plants from different sex phenotype groups were also present as shown in Table 5: in both the KH and the OM population the MS plants and the PMS plants had higher outcrossing rates than the H plants (Student's $t$-test: $P<0.001$). It must be noted that the mean outcrossing rates ($t_m$) of the populations presented here are influenced by the ratios of H, PMS and MS plants included in this study. The actual outcrossing rate ($t_a$; Table 5) was estimated with the ratios of H, PMS and MS plants observed in the field and using the outcrossing rates for each sex phenotype as given in Table 5.

For populations OM and SD it was also possible to calculate outcrossing rates for individual plants separately, because higher numbers of progeny per adult plant were analysed (Table 6). This showed large differences between individual plants, not only between between plants having different sex types but also between individual hermaphrodites.

## Discussion

### *Measuring outcrossing rates*

Accurate estimations of outcrossing rates are difficult to establish in a species like *P. coronopus* because of its low electrophoretic variability. The basic assumptions of the method used were fulfilled, but with respect to the levels of environmental and individual variability some simplifying assumptions were made. The requirement of mixed mating, opposite to the case of a single-pollen parent, as with insect pollination (Schoen and Clegg 1986), is probably satisfied as *P. coronopus* is a wind-pollinated species. Heterogeneity of gene frequencies in time, which might give an underestimate of outcrossing rates (Ennos and Clegg 1982), was not observed. In our study, in contrast to what has been found by Bijlsma et al. (1986), male sterile plants (comparable to their detasseled plants) did show 100% outcrossing. Therefore, differences in flowering period of different allozyme genotypes, as observed in maize, seems highly unlikely for natural populations of *P. coronopus*. The possibility of local differences in allele frequencies, although not apparent in this study can, however, not totally be disregarded when larger areas should have been sampled. Van Dijk et al. (1988) revealed considerable population structuring, implying deviations from random mating, in populations from larger areas than used in this study. All mechanisms, apart from selfing, that promote inbreeding will provide an underestimation of outcrossing rates, especially when single-locus estimation methods are used. Fortunately, the multi-locus method used in this study is much less influenced (Shaw and Allard 1982). The fact that Acph-1, Acph-2 and Adh are probably situated in the same linkage group (Van Dijk et al. 1988) tends to give underestimates of outcrossing rates (Shaw et al. 1981). Variability in outcrossing rates between individuals are found in many studies (e.g. Humphereys and Gale 1974), which may bias estimates for the population as a whole. Differential seed abortion may induce differences between individuals in apparent outcrossing rates (Levin 1984; Ellstrand and Foster 1983). Further environmental and genetical influences on outcrossing rates of individual plants can not be excluded: as for the latter, Dommeé (1981) has shown outcrossing rates in *Thymus vulgaris* to be dependent on the genotype of the mother and indications of gamete and zygote selection have both been found in particular crosses and selfings in *P. coronopus* (K. Wolff, unpublished results: J.M.M. Van Damme, in preparation).

Outcrossing rates calculated from the fixation index are all almost 1.0 or have unrealistic values higher than 1.0. For the OM and SD populations progeny-tests gave much lower values, but this was not the case for KH. Several processes may be responsible for this result. Local differentiation gives an underestimate of t, and may work out differently in the fixation index method than in the multi-locus analysis method. This would, however, work out in the same direction in all three populations. Heterozygote advantage or reduced fitness of one of the homozygotes, e.g. for higher germination or seedling survival, can be postulated to explain the observation; this has been found for several species (Cheliak et al. 1985; Farris and Mitton 1984). The low fraction of germinating seeds of the KH population as found in the laboratory (65%) may have contained a disproportionate number of heterozygotes and this in turn would cause the relatively high outcrossing rates in this population.

### *Outcrossing rates and plant density*

The differences in outcrossing rates between populations are striking. This may be caused by many factors, but as the populations strongly differ in density, this is probably a major factor. The OM and SD populations have a low density of *P. coronopus* plants and lower outcrossing rates than KH. A positive correlation of plant density and outcrossing rates was present [Spearman rank correlation: $r_s=+1.0$ ($P=0$) and Pearson correlation coefficient $r=+0.92$ ($P=0.09$)]. Influences of density on out-

crossing rate are found in other species, e.g. in Ponderosa pine (Farris and Mitton 1984) and in *Helianthus annuus* (Ellstrand et al. 1978): a lower density of plants was associated with a lower outcrossing rate. For a related species, *P. major*, several studies have shown a strong relation between outcrossing rates and plant density (Van Dijk et al. 1988; K. Wolff, in preparation). In natural populations of *P. major*, outcrossing rates are all close to zero, but under conditions of high plant densities and plants that have grown exuberantly (e.g. under favourable conditions), outcrossing rates of up to 0.5–0.6 (Van Dijk et al. 1988) and 0.6–0.7 (M. Bos, personal communication) were found.

Another indication of the importance of the density of flowering plants comes from the difference in outcrossing rate determined in spikes sampled in the 2nd and 5th week. Plants sampled in the 2nd week, having only one flowering spike, had lower outcrossing rates than plants from the 5th week in which many of the plants had more than one flowering spike (Table 4). The latter plants probably had a better opportunity to fertilize their own ovules. In the beginning of the flowering period only 20%–50% of the plants were flowering, while after a few weeks almost all plants flowered. Thus, apparently the higher density of flowering plants in the 5th week is more important than multiple flowering spikes per plant, as was the case in the 5th week.

The relation of plant density and outcrossing rate for *P. coronopus*, observed both between populations and between sampling dates, makes density as the main cause of differences in outcrossing rates highly probable. The difference in outcrossing rates between the populations SD and OM may (partly) be explained by the higher vegetation in the SD population. The high vegetation and the low density of *P. coronopus* plants in this population may restrict gene flow and therefore reduce the outcrossing rate in the SD population.

### *Outcrossing rates and male sterility*

In this study the MS and PMS plants had higher outcrossing rates than H plants, as may be expected in general from MS and PMS plants. Statistically, the presence of MS plants in a population will increase the observed mean outcrossing rate to a considerable extent in those populations where H plants have low outcrossing rates. This is the case in populations OM and SD, but not in KH. According to theoretical models (Lloyd 1975; Gregorius et al. 1982; Sun and Ganders 1986) male steriles, contributing only female gametes to the next generation, can be maintained in a population in spite of their fitness disadvantage by avoiding inbreeding depression. This mechanism, however, requires considerable levels of selfing and inbreeding depression. Specifically, an outcrossing rate of H plants lower than 50% is a necessary (though not sufficient) condition for the maintenance of male sterility by this mechanism alone. It should be noted that PMS plants are considered to be genetically hermaphrodites (J.M.M. Van Damme, in preparation). Since PMS plants showed a higher outcrossing rate than H plants (Table 5), plasticity in pollen production of H genotypes is expected to hinder the maintenance of male sterility. The difference in outcrossing rates between MS and H genotypes can be calculated for KH and OM through weighted averaging of values for PMS and H phenotypes (Tables 1 and 5). For neither population is the condition met ($t_g$ in Table 5; the $t_m$-value of 0.34 in Table 4 cannot be evaluated since the sex types of the plants used for this estimate are unknown). Therefore, the avoidance of the inbreeding mechanism cannot be solely responsible for the maintenance of gynodioecy and another mechanism, pleiotropic action of the MS genes (Van Damme 1984), is likely to be involved as well. Some role for avoidance of inbreeding is nevertheless indicated because populations with higher outcrossing rates in H plants seem to have lower frequencies of male steriles. Gouyon and Vernet (1982) proposed that, besides the heterosis advantage of MS plants, there is counteracting selection in favour of H plants. They proposed that selfing hermaphrodites have the property of preserving good combinations of genes (co-adapted gene complexes) that would facilitate local adaptation. Both the absence of genetic differentiation for morphological characters between populations (K. Wolff, in preparation) and low selfing rates (Tables 4 and 5) make it unlikely that this counteracting selection will be important in this species. An ecological factor that may influence the male sterile frequency in a population is plant density because of its impact on outcrossing rate. Such an impact is indicated in the present study by the KH population, which combines the highest H frequency and the highest outcrossing rate with the highest density.

The fact that outcrossing rates are strongly influenced by environmental variability in space and in time requires relatively large sample sizes to detect genetic variation in outcrossing rates within populations. For the study of gynodioecy this is especially relevant because of the importance of differences of outcrossing rates between sex genotypes (Gregorius et al. 1982). The influence of population density on outcrossing rates has clearly been shown in this experiment. Further refinements in unraveling the determination of outcrossing rates in *P. coronopus* should primarily be concentrated on the influences of genetic versus environmental factors and should pay attention to gamete, zygote and seedling selection.

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