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Gynodioecy in *Plantago maritima* L.; no compensation for loss of male function

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SUMMARY

Size, allocation of biomass, seed production of hermaphrodites and male steriles as well as germination, growth rate and survival among their progeny were compared between male fertile and male sterile cytoplasmic genotypes of *Plantago maritima.* The cytoplasmic genomes in angiosperms are predominantly maternally inherited. Thus we used the progeny originating from the male fertile and male sterile mothers in order to examine the relative success of the mothers. The progeny was grown together at three different densities in a greenhouse competition experiment. The results were analysed in a hierarchical model with siblings as replicates of mothers and mothers as replicates of sex-morphs. Differences between sex morphs were very small, but they were consistent in that the progeny from male steriles always performed less well than did the progeny from hermaphrodites. Male sterile progeny matched the hermaphrodite progeny best at the lowest density, where there was no effect of sex type on the level of individual performance. One explanation why hermaphrodites perform relatively better at higher densities could be that they are more plastic in their response to competition induced stress. This was indicated by density dependent allocation pattern of biomass to different parts of the plants, where the progeny of hermaphrodites appeared to be more plastic. The results from this experiment, and other studies, supports the idea that male sterility, if nucleo-cytoplasmically determined, can persist in a population even without any fitness advantages for females.

Key-words: cytoplasmic male sterility, female fecundity, germination, growth rate, maternal inheritance, survival.

INTRODUCTION

The location of genetic information and the mode of compatibility are two important factors for the interpretation of gynodioecious breeding systems in angiosperms. With the premise that male sterility is nuclear, it is possible to make hypotheses about the evolution of dioecy from a hermaphrodite ancestry via unstable gynodioecy (Ross 1970, 1978; Lloyd 1975; Ho & Ross 1973; Charlesworth & Charlesworth 1978). If, however, male sterility is cytoplasmically or nucleo-cytoplasmically determined (Saumitou-Laprade *et al.* 1994), gynodioecy could be regarded as a stable breeding system (Lewis 1942; Ross 1978; Gregorius & Ross 1987) (but see also Maurice *et al.* 1993, 1994 and Schultz 1994 for alternatives). In self-compatible species inbreeding depression can

facilitate the spread of gynodioecy, which in this case promotes out-breeding (Kesseli & Jain 1984; Shykoff 1988; Ågren & Willson 1991; Maki 1993). In self-incompatible species selection for out-crossing will not necessarily favour females on behalf of hermaphrodites, as all the hermaphrodites also are out-crossed. We then need to invoke alternative explanations for maintenance of male sterility such as a selfish cytoplasm (Gouyon & Couvet 1985) or pollen-pistil interference (Kikuzawa 1989).

Cytoplasmically male sterile individuals experience a reduced fitness due to reduced gene flow by loss of pollen donation. However, this is applicable only for the nuclear part of their genome; the cytoplasmic part will have the same fitness as the cytoplasmic genome of hermaphrodites due to the maternal inheritance of cytoplasm. Reduction in fitness due to loss of the male function can not restrict the introduction of a male sterile genotype unless pollen limitation reduces the female success of the male steriles more than it does for the hermaphrodites. Hence, if there is no selective pressure against a cytoplasmically determined trait, it could be maintained in a population just by means of maternal inheritance.

The chance for a male sterile lineage to persist in a population may depend on its chance to reach a sufficient number, allowing for avoidance of extinction due to demographic stochasticity. If a population is at equilibrium, the chance for male sterility to increase in numbers is closely related to the selective advantage of male sterility. This agrees with the suggestion of Lewis (1941) that females need a slight advantage to persist in a population. However, in an expanding population, the demand for a selective advantage for the male steriles is reduced, this since intra-specific competition is low or even absent (Begon *et al.* 1996), and even inferior genotypes may invade (Ferrière & Fox 1995). Thus, for a male sterile trait to invade a population at equilibrium we postulate a need for some selective advantage, but to enter an expanding population this should not be necessary.

Couvet *et al.* (1986) suggested a non-equilibrium model based on migration and differences in female fecundity between male steriles and hermaphrodites to explain high frequencies of male sterility in newly founded populations. Due to founder effects, sterile cyto-types could exhibit absence of nuclear restorer genes resulting in high degrees of male sterility, which later on would be balanced by introduction of nuclear restorer genes in the population (Belhassen *et al.* 1989). If there are no differences in fecundity between sex morphs, very high frequencies of male steriles are unlikely. However, it is still possible to express female phenotypes in a low proportion if the total population is heterogeneous or divided into subpopulations which undergo extinction and recolonization (compare with Couvet *et al.* 1986).

Populations of *P. maritima* growing on sea shore meadows along the Baltic are constantly subjected to disturbances by submersion. This produces populations with a zonated pattern, where the lower regions of the meadows continuously undergo local extinction and recolonization (Jerling 1982).

As a first step to analyse the mechanisms behind the presence of male sterility in populations of *P. maritima* we wanted to test if male sterile individuals had a higher female fecundity than did hermaphrodites and, further, if there were any differences in germination rates between seeds from the two sex-morphs. To investigate if the relative performance of progeny from male sterile and hermaphrodite individuals differed between phases of low and high population density, as in early and late successional stages, we used an experimental design where the progeny of male sterile and hermaphrodite individuals of *P. maritima* competed with each other in three different densities. If maintenance of male sterilite genotypes in populations of *P. maritima* is due

to higher fitness in comparison with hermaphrodite genotypes, we expect to find differences in characters related to female fecundity or growth and survival in favour of the progeny of male sterile individuals. One possible explanation for differences in performance depending on density could be the ability to alter the allocation of biomass to different parts of the plant. We have therefore also tested if the progeny of male steriles are more plastic in their growth pattern than the progeny of hermaphrodites.

METHODS

Plantago maritima

P. maritima is a wind-pollinated, self incompatible, protogynous and gynodioecious perennial herb. Genets are easily distinguished, and vegetative propagation is quite rare. Many of its congeners are gynodioecious (van Damme 1985), and the breeding system of its near relative *P. lanceolata* (van Damme & van Delden 1982; van Damme 1983, 1984a, 1984b, 1986; van Dijk 1985; and others) and *P. coronopus* (Koelwijn & van Damme 1995a, 1995b, 1996) is well documented. As well as in *P. lanceolata* and *P. coronopus*, male sterility show a nuclear-cytoplasmic inheritance pattern in *P. maritima* (van Damme 1992). In the studied population the frequency of male sterility varied between 2% and 10%, based on counts of flowering rosettes per square metre.

In the following experiments we used plants that were completely male sterile, carrying only flowers with rudimentary anthers without pollen, and hermaphrodite plants with only perfect flowers. As the mithocondrial genome, causing male sterility, is regarded to be maternally inherited in angiosperms (Corriveau & Coleman 1988; Reboud & Zeyl 1994; Mogensen 1996), we made the assumption that the cytoplasmic genotype of a mother and her offspring will be the same. Thus we regarded the offspring as individual replicates of a mothers cytoplasmic genotype. The presence of nuclear restorer genes makes it likely that the progeny from a male sterile mother contain both sex phenotypes, nevertheless all her offspring will still have the same cytoplasmic genotype. Therefore, the aim with the following experiments was to analyse the differences between the cytoplasmic genotypes disregarding the sexual phenotypes of studied individuals. Hence it was the fitness of the mothers measured as success of their progeny, irrespective of its sex type, that was of interest. When collecting male sterile phenotypes we knew that they had male sterile cytoplasmic genotypes, but when collecting hermaphrodites there was always a risk that we collected restored male sterile cytotypes. This means that our experiment was conservative in finding differences between sex-morphs.

Fecundity and germination

In the summer of 1991, 106 hermaphrodite and male sterile individuals of *P. maritima* were collected pairwise, at random, from the sea shore meadows at Tullgarn, c. 45 km south of Stockholm (58°57′N, 17°36′E). Plants were measured for size, number of spikes, size of spikes, number of seeds and seed weight. In January 1993, seeds from the different plants were sown in petri dishes and percentage germination was determined.

Competition experiment

The experiment was conducted as a competition arena with plants from mothers of the two different sex types placed in a Latin square pattern. Seedlings of equal size from 73 of the initial plants (from here named mothers) were planted in small cubic plastic pots of two different sizes, big (45 mm*45 mm, height 75 mm) and small (35 mm*35 mm,

Table 1. Initial density of *Plantago maritima* in the experiment pots, low (12 346 plants/m^2), medium (40 000 plants/m^2), and high (81 633 plants/m^2), number of plants/pot are indicated within brackets, and total number of mothers and plants in the different densities. All densities were replicated with 12 pots/density

Initial density	Sex	No. mothers	No. plants
Low (25)	Male sterile	29	152
	Hermaphrodite	29	148
Medium (25)	Male sterile	31	151
	Hermaphrodite	26	149
High (36)	Male sterile	30	218
	Hermaphrodite	36	214
Total		73	1032

height 60 mm).The seedlings used germinated at the same time and carried only primary leaves when planted. We planted in three different initial densities, low (12 346 plants/m^2, big pots), medium(40 000 plants/m^2, small pots) and high(81 633 plants/m^2, small pots—at the highest density we did only use a subsection of the pots covering 21 × 21 mm), with 12 replicates of each density. The substrate was a mixture of 25% sand and 75% garden soil and the pots were watered daily from underneath. In May the pots were transferred to the garden and lowered down into the soil. During summer months the pots were watered only occasionally. In September, when flowering had stopped, the experiment was halted and plants were harvested. As far as possible we tried to ensure that each mother was represented by one seedling in at least six pots in each density, so that it was possible to analyse the experiment both with seedlings as replicates of mothers within treatment, and pots as replicates of treatments. The main aim was to analyse the outcome of competition between cytoplasmic sex genotypes replicated by mothers. The initial numbers of mothers and seedlings in the beginning of the experiment are listed in Table 1.

The experiment was monitored twice. Thirty to 31 days after the start, all the plants were measured for length of all leaves. At the end of the experiment roots extending outside the pots were cut off (because of difficulties of extracting full-length roots from the soil) and all plants were separated into root, stem, leaf and spikes. The different parts of the plants were dried to constant weight and weighed individually to the nearest 0·001 mg. There is a good correlation between leaf number, leaf length and plant weight in *P. maritima*, shown in earlier studies (Noë & Blom 1982, Blom 1983).

The three densities were chosen to resemble the density of emerging seedlings under natural conditions. Seeds are often dispersed in clumps when spikes are felled to the ground by trampling of animals or by heavy precipitation. The seeds also have a sticky coating which attaches them to the vegetation and prevents secondary movements. The highest density is reached occasionally during periods of seedling establishment in small disturbances created in the zone where *P. maritima* is growing at a very high density. The natural disturbances are mainly due to flooding, cow droppings and rooting from wild boar. The lower densities are normally found during seedling establishment within already established stands. The density of adult rosettes varies from 50 to 100 m^2 nearest to the sea, to nearly 1000 m^2 in the older stands (Jerling 1982).

The experiment was designed without controls for cytoplasmic sex genotype within density. This means that we can not estimate the magnitude of the effect of intrasexual competition at each density; we can only look at the differences between the male fertile and male sterile cytoplasmic genotypes at each density. However, we will argue that even if differences between sex types are not due to exploitation competition, the different morphs will be represented in future generations mainly as a result of the traits we have examined in this experiment. Therefore, in this experiment we regard the differences between the hermaphrodite and the male sterile offspring as competitive ability.

As all the mother plants were naturally pollinated in the field and thereafter randomly sampled, and as there is no difference in phenology between sex morphs, we regard the experiment as controlled for fitness differences depending on paternal effects.

Statistics

Differences in size and reproduction between the initially collected mothers were analysed with Wilcoxon's signed ranks test, and the mortality of their progeny were analysed using the Mann–Whitney *U*-test. Log seed number per plant was analysed as a function of sex type with plant size as covariate. We also analysed the effect of seed weight on germination for the different sex types with linear regressions. Difference in regression slopes were tested with one-way ANCOVA with sex type as fixed affect and seed weight as covariate.

Sizes and weights from the first and second recording of the experiment were analysed with two-way mixed model ANOVA with maternal plant sex type as a fixed effect and individual mothers nested within sex. In this way we analysed differences between maternal sex-morph with mothers as the independent observations, not their progeny. Weights from the second reading were log transformed prior to statistical analysis to improve normal distribution. Mortality was calculated as the percentage of dead seedlings per mother.

Allocation of biomass to the different parts of the plants were compared within maternal sex morph between densities, and analysed using the Kruskal–Wallis test. To reduce the likelihood of type-I error we performed a sequential Bonferroni test within maternal sex-morph to assess the tablewide significance levels (Rice 1989). We also compared the flowering ability of the progeny of the different mothers, both between sex types and between densities. Differences in flowering capacity was tested using the χ^2 test. Weights of reproductive tissue (spikes with capsules containing seeds) were compared as proportions of total plant weights in a two-way ANOVA as a function of sex type and density. Spike weights were square root transformed to improve normality.

RESULTS

Fecundity and germination

Among the initially collected individuals, there were no differences in total plant size between male steriles and hermaphrodites (Table 2). Male steriles produced more seeds per capsule than did hermaphrodites; however, hermaphrodites produced heavier seeds (Table 2). Correction for differences in plant weight between sex morphs, as deduced from number and length of leaves (Noë & Blom 1982), did not reveal any significant difference in produced number of seeds between the two. There was no significant difference in germination rates between the sex-morphs (Table 2), but there was a

Table 2. Data from plants of the two sex-morphs of *Plantago maritima*. Plant size was measured as length of leaves. Reproductive tissue was measured as spike length divided with total plant length. Seeds per capsule are the total no. of seeds divided by the length of the spike, which is a good approximation of number of capsules per spike. All data are mean values ± standard deviations. Inferential statistics were done with Wilcoxon's signed rank test. Log number of seeds per plant did not differ between sex types but there was a positive effect of plant size (sex type $F_{1,98}=2{\cdot}386$, $P>0{\cdot}10$; plant size $F_{1,98}=10{\cdot}618$ $P<0{\cdot}01$ ANCOVA)

Variable		Plant size	Reprod.	No. seeds	Seed weight	No. seeds	Germination
Sex	*N*	(cm)	tissue (%)	per plant	(mg)	per capsule	rate (%)
Male steriles	53	11·08 ± 8·43	4·4 ± 4·6	34·3 ± 31·5	0·47 ± 0·15	1·1 ± 0·6	49·2 ± 25·6
Hermaphrodites	53	13·67 ± 12·94	3·9 ± 4·3	30·7 ± 29·7	0·56 ± 0·14	0·9 ± 0·4	55·7 ± 27·1
Z-value	*Z*	− 0·323	0·401	0·826	− 2·722	2·495	− 1·725
Probability	*P*	>0·75	>0·50	>0·25	<0·01	<0·05	<0·05

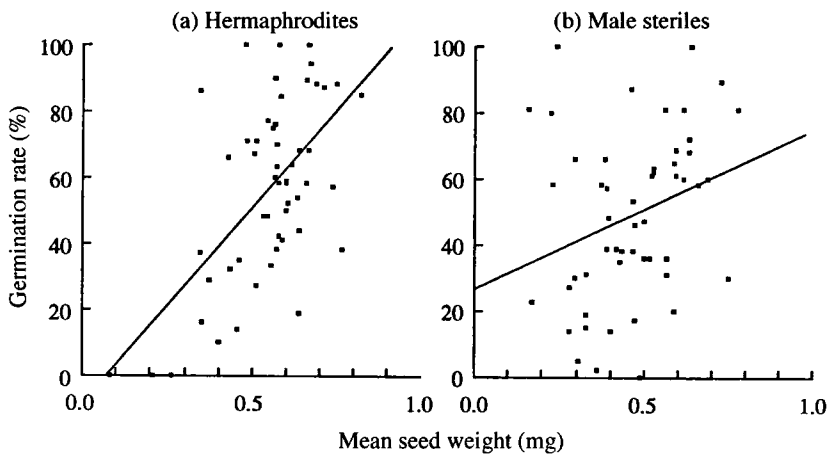

Fig. 1. The percentage of germinated seeds in relation to the mean seed weight of different mothers of *Plantago maritima*. (**a**) Hermaphrodites; $F(1,52)=28{\cdot}423$, $P<0{\cdot}0001$, $r^2=0{\cdot}353$. (**b**) Male steriles; $F(1,50)=4{\cdot}459$, $P<0{\cdot}05$, $r^2=0{\cdot}082$.

positive effect of seed weight on the probability of germination for both sexes (Fig. 1). There was also a significant interaction between sex-morphs and seed weight affecting germination rates (Table 3). This interaction was due to the lower partial regression coefficient for male steriles compared to the hermaphrodites, and it seems that male sterile seeds were capable of germinating at lower seed weights than were the seeds from the hermaphrodites (Fig. 1).

Competition experiment

Growth. The results of the experiment showed very few, and quite small, differences between the progeny of the two maternal sex-morphs. However, there was a trend in the data that the progeny of hermaphrodites performed slightly better than did those of male steriles (Table 4). After 30 days the progeny of the hermaphrodite sex-morph had grown significantly larger in all three densities, but there was also a significant effect of mother within sex-morph in all three densities. At the end of the experiment, the relative mean

Table 3. Effects of seed weight on germinability for the two sex-morphs of *Plantago maritima*. Differences in germinability are analysed with ANCOVA with sex as fixed effect and seed weight as covariate

Source	d.f	MS	*F*	P
Sex	1	0·218	3·914	0·051
Seed weight	1	1·500	26·946	<0·001
Sex*seed weight	1	0·251	4·514	0·036
Error	102	0·056		

weight differences between progeny of sex-morphs were quite large, but the variance was also very large so there were no significant differences in mean total weights at any of the densities. At the low density there was a significant variance component between mothers within sex (Table 4), but none between sex-morphs. At the medium density there was a trend that the progeny of the hermaphrodites were heavier than the progeny of the male steriles, but the difference was not significant (Table 4). However, at medium density the hermaphrodite progeny had a significantly higher root weight (Table 4). At the highest density hermaphrodites had a significantly higher stem weight (Table 4). However, differences in root weights were only significant at the level of mother at this density (Table 4). This may be interpreted as a shift in allocation from roots to stem, between the middle density, where light competition was lower, and the highest density where it was important to elongate to compete for light. For mean total weights at the highest density there are no significant variance components at any level (Table 4).

Mortality. There were no differences in mortality rates between progeny of different maternal sex-morphs in any of the three densities (low, $U=214$, $P>0{\cdot}90$; medium, $U=134$, $P>0{\cdot}25$; high, $U=246$, $P>0{\cdot}25$, Mann-Whitney U-tests). There was only a significant decrease in survival of plants, independent of sex type, with increased density (no inferential statistics; Fig. 2).

Allocation of biomass

There were significant differences in total plant weights between the low density and the two higher ones (Table 5), but there was no significant difference in weights between medium and high density (Table 5). If we look instead at the different parts of the plants, the allocation of biomass between root, stem and leaf differs both between progeny of maternal sex-morphs and among densities (Table 6). The proportion of stem weight increased significantly from low to medium density, for both sex-morphs (Table 6). For the hermaphrodites progeny, the proportion of stem weight also increased in the density step from medium to high, which was not the case for the progeny of male steriles (Table 6). For male steriles progeny the proportion of root weight tended to decrease from low to medium density, whereas the proportion of root weight in the hermaphrodites progeny tended to decrease from medium to high density (Table 6). However, none of these differences were significant at the table wide significance level. The proportion of leaf weight was approximately the same at all densities.

Flowering

Density had a clear effect on both the frequency of flowering plants (Table 7) ($\chi^2=13{\cdot}510$, d.f.$=2$, $N=167$, $P<0{\cdot}001$), and on the proportion of biomass allocated to

Table 4. Size and weight of the progeny of male sterile (MS) and hermaphrodite (H) plants of *Plantago maritima*, from the two readings of the experiment. Data were analysed with two-way ANOVA with mothers nested under sex type for each density, respectively. Data from the second reading were log transformed to improve normal distribution

Character	Density	Sex	Mean ± SD		Source	d.f.	MS	*F*	P
Leaf length in mm after 30 days	12 000/m²	MS	644 ± 144		sex	1	293692	6·231	0·016
		H	695 ± 157		mothers {sex}	56	47177	2·764	<0·001
					error	240	17070		
	40 000/m²	MS	505 ± 135		sex	1	528120	16·04	<0·001
		H	576 ± 131		mothers {sex}	55	39932	2·344	<0·001
					error	222	14047		
	80 000/m²	MS	397 ± 124		sex	1	264071	9·223	0·003
		H	465 ± 109		mothers {sex}	64	28632	2·671	<0·001
					error	314	10751		
Weight in mg at end of experiment	12 000/m²	MS	62 ± 42	root	sex	1	0·196	0·210	0·649
		H	78 ± 57		mothers {sex}	55	0·934	1·754	0·003
					error	206	0·533		
		MS	25 ± 28	stem	sex	1	0·072	0·084	0·774
		H	32 ± 23		mothers {sex}	55	0·860	2·040	<0·001
					error	206	0·422		
		MS	87 ± 59	leaf	sex	1	0·040	0·039	0·844
		H	108 ± 80		mothers {sex}	55	1·025	1·784	0·002
					error	206	0·574		
		MS	174 ± 114	total	sex	1	0·217	0·250	0·619
		H	219 ± 154		mothers {sex}	55	0·869	1·838	<0·001
					error	206	0·473		
	40 000/m²	MS	38 ± 36	root	sex	1	3·719	4·595	0·037
		H	50 ± 48		mothers {sex}	46	0·809	1·006	0·473
					error	146	0·804		
		MS	18 ± 15	stem	sex	1	2·157	3·258	0·078
		H	23 ± 20		mothers {sex}	46	0·622	1·170	0·240
					error	146	0·566		
		MS	54 ± 52	leaf	sex	1	2·057	2·970	0·092
		H	69 ± 64		mothers {sex}	46	0·693	0·868	0·706
					error	146	0·798		
		MS	110 ± 112	total	sex	1	2·539	3·880	0·055
		H	141 ± 130		mothers {sex}	46	0·654	0·954	0·561
					error	146	0·686		
	80 000/m²	MS	34 ± 36	root	sex	1	1·593	1·270	0·264
		H	41 ± 46		mothers {sex}	59	1·255	1·560	0·020
					error	123	0·804		
		MS	16 ± 14	stem	sex	1	3·376	4·743	0·033
		H	22 ± 24		mothers {sex}	59	0·712	1·648	0·011
					error	123	0·432		
		MS	50 ± 65	leaf	sex	1	2·969	2·848	0·097
		H	62 ± 87		mothers {sex}	59	1·042	1·218	0·181
					error	123	0·856		
		MS	100 ± 112	total	sex	1	2·379	2·496	0·119
		H	125 ± 153		mothers {sex}	59	0·953	1·417	0·054
					error	123	0·672		

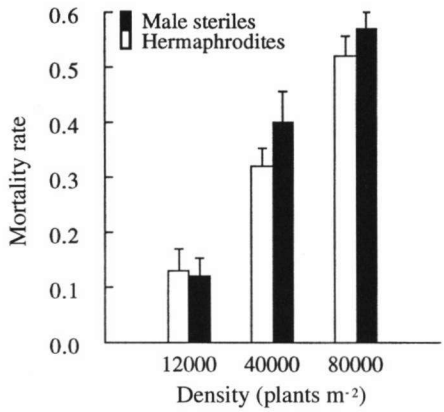

Fig. 2. Mortality rates for the progeny of hermaphrodite (open bars) and male sterile (solid bars) individuals of *Plantago maritima* in three different densities, low (12 000 plants/m^2), medium (40 000 plants/m^2), and high (80 000 plants/m^2). Error bars represent one-sided SE.

Table 5. Differences in total plant weight of *Plantago maritima* among densities (low, medium and high). Total plant weights from the second reading of the experiment are analysed by two-way ANOVA with pots nested within density. Pairwise comparisons were made with Tukey–Kramer HSD test. Weights were log transformed prior to analyse to improve normal distribution

Source	d.f.	MS	*F*	P
Density	2	22·468	19·881	<0·001
Pot{density}	33	1·130	1·774	<0·01
Error(plant)	604	0·637		
Pairwise comparisons	low=medium	low=high	medium=high	
Probability	<0·001	<0·001	0·115	

reproductive tissue in those plants that flowered (Table 8). Maternal sex-morph had no effect at all, neither on the number of flowering plants (Table 7)(χ^2=0·080, d.f.=1, N=167, P>0·75) nor on the allocation of biomass (Table 8). When total plant weight decreased due to increased density, the relative weight of reproductive tissue in those plants that flowered increased (Fig. 3).

DISCUSSION

The main aim of this study was to look for obvious functional differences between individuals carrying male sterile cytoplasm and individuals that did not. We compared reproductive parameters such as number of seeds, seed weights, reproductive effort in terms of biomass and germination rates between the progeny of male sterile and hermaphroditic individuals of *P. maritima* and we tested if the progeny of male sterile individuals were better competitors for space, light and nutrients than the progeny of hermaphrodite individuals from the same population. The results were clear, indicating no support for any superiority of females compared to hermaphrodites. On the contrary, the trend is that the progeny of hermaphrodites perform slightly better than

Table 6. Proportions of biomass allocated to root, stem and leaf in the progeny of male sterile and hermaphrodite plants of *Plantago maritima,* in three different densities. Differences were analysed using the Kruskal–Wallis test. *Denotes the table wide significance level of $P<0.05$ assessed by sequential Bonferoni test within sex type ($k=9$)

	Root		Stem		Leaf	
Density	Male steriles	Herma-phrodites	Male steriles	Herma-phrodites	Male steriles	Herma-phrodites
Hypothesis	Amount of total biomass %x ± SD					
Low	35·8 ± 6·9	35·6 ± 7·2	15·1 ± 4·5	16·0 ± 5·6	49·0 ± 7·8	48·5 ± 8·4
Low=medium	*P=0·014*	*P=0·102*	*P<0·001**	*P=0·005**	*P=0·652*	*P=0·619*
Medium	33·1 ± 8·0	34·3 ± 6·9	18·5 ± 5·7	18·0 ± 6·6	48·4 ± 8·4	47·7 ± 8·9
Medium=high	*P=0·913*	*P=0·025*	*P=0·578*	*P=0·003**	*P=0·075*	*P=0·784*
High	33·5 ± 9·9	31·9 ± 7·1	19·6 ± 7·5	20·9 ± 7·8	46·9 ± 10·3	47·2 ± 9·1
Low=high	*P=0·006**	*P<0·001**	*P<0·001**	*P<0·001**	*P=0·046*	*P=0·453*

Table 7. Number of male sterile (S) and hermaphrodite (H) mothers flowering (F) and non-flowering (N) progeny, in the experiment. Figures within brackets are total number of flowering and non-flowering plants

Sex	Density	F	*N*
S	Low	17 (32)	11 (100)
H	Low	16 (33)	13 (98)
S	Medium	13 (18)	12 (72)
H	Medium	11 (20)	12 (84)
S	High	7 (9)	20 (77)
H	High	9 (9)	26 (89)

Table 8. Two-way analysis of variance on proportion of biomass allocated to reproductive tissue in the progeny of male sterile and hermaphrodite plants of *Plantago maritima* in three different densities. The weight proportions were square root transformed to improve normality

Source	d.f.	MS	*F*	P
Density	2	0·070	5·208	0·008
Sex	1	0·010	0·087	0·769
Density*sex	2	0·000	0·029	0·971
Error	67	0·013		

the progeny of male steriles. The only advantage found for the male steriles was that they produced slightly more seeds per capsule; however, there was no difference in total number of seeds produced per plant.

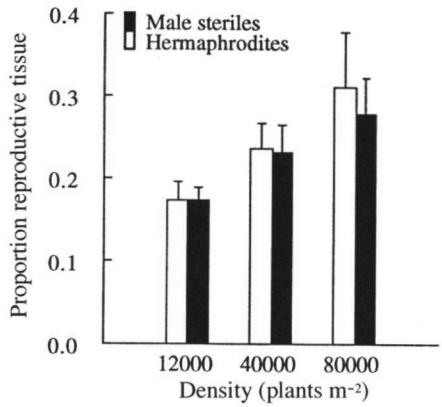

Fig. 3. Proportion of total plant weight in *Plantago maritima* allocated to reproductive tissue for the progeny of hermaphrodite (open bars) and male sterile (solid bars) plants in three different densities, low (12 000 plants/m^2), medium (40 000 plants/m^2), and high (80 000 plants/m^2). Error bars represent one-sided SE.

At the first recording of the competition experiment hermaphrodite progeny were larger irrespective of density, i.e. had grown faster than the progeny of male steriles. At the second recording, mean weights of progeny of the hermaphrodites were larger than those of male steriles, but the variance had increased compared to the first recording resulting in non-significant differences. At low density mortality was low, and growth rates of seedlings and juveniles did not seem to influence the possibility of establishing rosettes which in turn grew large. At this density, the identity of the mother plant was more important than the maternal sex-morph in determining growth rates of the progeny. At the medium density the results were reversed, and the sex-morph of the mother was more important than the identity of the mother. At the highest density, sex type was still important but maternal identity influenced the outcome more than at the medium density. It seems quite reasonable that the outcome of competition at a high density is more dependent on initial growth rates than at low density (see, for example, Harper 1977).

One lost dimension in this experiment is the absence of winter, which is the main mortality factor present in natural stands of *P. maritima* (Jerling 1982). This could be one reason why we did not find any differences in mortality rates between sex types in the experiment. Plants which were doomed to death had not yet ceased to live, thereby masking the final outcome of competition.

The allocation of biomass between root, stem and leaf reflects somewhat the behaviour of the plants when subjected to different densities. In many plants, during growth under density stress the allocation of assimilates between different structures becomes proportionally altered (Harper 1977). When density increased in this study, the experimental plants needed to elongate their stems so that leaves could protrude from a higher position. Root biomass decreased, but there was no change in leaf size. This result may be an effect of light competition with no constraints on nutrient and water levels.

The progeny of hermaphrodites seemed to be more plastic than that male steriles. They kept leaf size on the same relative proportion in all densities, and the proportion of stem biomass increased both between low to medium and from medium to high density. The progeny of male sterile plants could only reallocate between low and medium densities. There were no differences in allocation to sexual reproduction between maternal sex-morphs, neither in the material collected in nature, nor in the plants grown in the experiment.

The results from this study suggests that maintenance of male sterility in *P. maritima* can not directly be deduced from functional advantages for the female individuals. Therefore we need alternatives to explain how an equal or inferior male sterile genotype can invade and persist in an hermaphrodite population. Van Damme (1984a) found two different plasmon types in populations of *P. lanceolata,* one producing male sterile individuals that were superior to hermaphrodites (ms^1), and one with male sterile individuals that were equal to hermaphrodites (ms^2), in terms of fecundity and seed weight. He also argued (Van Damme 1984b) that as well as for females of *Geranium silvaticum* (Vaarama & Jaaskelainen 1967), the persistence of females in the ms^2 plasmon type was due to secondary sex characters (germination, survival, etc.) acting in different life cycle stages. In *P. maritima*, male sterile individuals were matching the hermaphrodites best at the lowest density. This indicates that in addition to the variation in competitive ability between maternal sex-morphs in different life-cycle stages, the outcome of inter sex-morph competition may also be affected by variation in population structures, e.g. density.

In populations with fluctuating densities, both in time and space, it is possible for a genetic variant which is slightly less competitive in high densities, to persist in fragments of lower density (Ferriére & Fox 1995). In *P. maritima* it is also possible that the minute difference in seed set per capsule, in favour of the male steriles, may be important during phases of establishment. In *Thymus vulgaris* the frequencies of male sterility in new populations depended on the genetic differentiation within the founder populations and on founder effects determining the frequencies of the male sterile cytoplasms and their specific nuclear restorer genes (Belhassen *et al.* 1989). This could also be the case during seedling establishment in disturbed areas within populations of *P. maritima.*

In the studied populations of *P. maritima*, extinction and recolonization occurred mainly in the lower end of a successional gradient (Jerling 1988). Females which carry lighter seeds may distribute these over longer distances. If colonizers migrate from older parts of the same population, colonizing females will always be subjected to a high frequency of nuclear restorer genes. However, if individuals with sterile cytotypes from the upper regions of the gradient succeed to disperse slightly longer and establish in the lower regions, they will probably receive fewer of the restorer genes present in the upper parts. The reason for this is partly a reduced frequency of hermaphrodites carrying the specific restorer genes surrounding them, and partly due to low pollen transport from upper to lower regions of the gradient. Plants in upper regions flower later in the season and in addition there is a prevailing wind direction landwards (Jerling 1988).

Molina-Freaner & Jain (1992) argued that their studies of the colonizer *Trifolium hirtum* did not fit the non-equilibrium *Thymus vulgaris* model of Belhassen *et al.* (1989), because of the lack of differences in fecundity between females and hermaphrodites. However, if there are no differences in fitness, the sex ratios will depend on founder effects and drift. There may then, still be a good chance for male sterility to persist, especially if the population, or a part of it, is expanding. This, taken together with the suggestion of Couvet *et al.* (1986), that male sterility should be looked for in marginal or disturbed areas of a population, implies that we should look for high frequency of male sterility in populations of *P. maritima* at the lower and most disturbed parts of the gradients at our sea shore meadows.

The results of this experiment indicate that cytoplasmically male fertile individuals of *P. maritima* are better competitors than cytoplasmically male sterile individuals (even though a decreased overall fitness for females depending on loss of pollen spread is not

accounted for). This contrasts with studies of self-compatible species such as *Silene acaulis* (Shykoff 1988), where females are more fecund than hermaphrodites. In *Silene vulgaris* (Jolls & Chenier 1989) and *Chinographis japonica* (Maki 1993) females are more fecund than inbreeding hermaphrodites. However, at least in *S. vulgaris* there is no difference between females and out-crossed hermaphrodites (Jolls & Chenier 1989). There are also examples of self-compatible plants, such as *Iris douglasiana* (Uno 1982) and *Trifolium hirtum* (Molina-Freaner & Jain 1992), where no detectable differences between male steriles and hermaphrodites were found. Therefore, if one compares the results from self-compatible and self-incompatible species, out-crossing seems to be more an effect of male sterility than the primary reason for its persistence (Gouyon & Couvet 1985). This reasoning could hold true for both self-compatible and self-incompatible species as long as they are nucleo-cytoplasmically male sterile.

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