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Gene flow and population structure in *Armeria maritima*

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Gene flow distances and population structure were studied in populations of *Armeria maritima* (Mill). Willd. in Denmark. Gene flow was studied by direct methods using seed and pollen dispersal distances. Pollen flow in four experimental populations, where honey-bees were pollinators, surpassed the diameter of each experimental population. Seed dispersal was restricted and was used to partition a coastal grassland population into local populations. The structure of this population was analysed with a population genetic model that formulated a sequence of hypotheses about the action of natural selection and the geographical subdivision of the total population into local populations. The variation at a morphological marker locus was used for this purpose: the presence of hairs on the flower stalk in this species is determined by a dominant allele. The estimated pollen pools in the six local populations were homogeneous, and there was no evidence of deviation from random mating in any of the local populations. The genotypic distributions were homogeneous in the local populations and accorded with Hardy–Weinberg proportions. There was no evidence of selection at the studied locus, accordingly, the studied population was panmictic and its genotypic distribution could be described with a single parameter, the allele frequency at the marker locus.

Keywords: *Armeria maritima*, gene flow, population structure, seed and pollen dispersal, selection components.

Introduction

The level of gene flow is an important factor determining the population structure of a species. Gene flow has often been considered to be low in plants, enabling populations either to diverge through drift and/or to adapt to a local environment. Well known examples of the latter are heavy-metal tolerant plant species growing on polluted mine areas, where significant genetic differentiations can be found over very short distances (Jain & Bradshaw, 1966). However, recent studies have shown that a low level of gene flow in plants is not a general rule (Ellstrand & Marshall, 1985; Golenberg, 1987; Devlin & Ellstrand, 1990).

In the species *Armeria maritima* (Mill.) Willd. gene flow distances in natural populations on zinc–lead mine wastes have been found to be short. A few metres' distance was sufficient to reduce gene flow to a very low level resulting in local populations with differentiated gene frequencies (Lefèbvre, 1976, 1985). Natural selection was probably the dominant evolutionary force for this observed divergence. Populations in less extreme environments than mine areas are expected to show a lower differentiation on the same scale.

In this paper we study gene flow in *A. maritima* by two different methods. Firstly, we make a direct estimate of seed and pollen dispersal distances. Secondly, we use an indirect method based on genetic variation at a morphological marker locus (hairiness on the flower stalks). This character is determined by one locus with two alleles or two groups of closely linked alleles (Philipp, 1974). This character does not seem to be correlated with ecological features such as drought, zinc- and lead tolerance and is considered to be neutral (Lefèbvre & Kakes, 1978; Lefèbvre, 1985). By analysing the population structure we can study the impact of gene flow on the genetic differentiation of a group of local populations at a coastal area in Denmark. The population structure was analysed by a modification of the selection component analysis of Christiansen & Frydenberg (1973).

Materials and methods

Materials

Armeria maritima is a perennial plant species which forms tussocks; it has no vegetative propagation. When

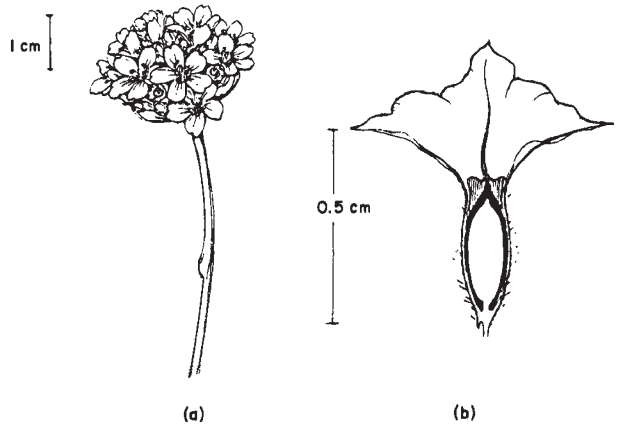

Fig. 1 Inflorescences of *Armeria maritima* (a). Diaspore of *A. maritima* with well developed seed inside the calyx (b). Drawn by Kirsten Madsen.

grown from seed in the experimental field it flowered in its first year with several inflorescences (Fig. 1). It is pollinated by a large number of different insect groups: beetles, flies, bees and butterfies (Madsen, 1987). It has a heteromorphic incompatibility system with two mating types, called A and B (Baker, 1966). No selfing was found in our material, contrary to what Richards *et al.* (1989) and Vekemans *et al.* (1990) have found. Each flower has one ovule and when pollinated it is open for one day. Seeds of *A. maritima* are dispersed enclosed in the calyx. The upper part of the calyx is membranaceous (area 0.25 cm^2) and bends back when the seed ripens, thus functioning as a parachute-like structure (Fig. 1). [The weight of the diaspore is 2 mg ($n = 61$, S. E. = 0.07).]. The seeds are thereby adapted to wind dispersal. In addition, hairs on the calyx enable the calyx with the seed to be attached to animals but this is probably a rare event.

The studied population was situated at Strøby Egede on Zealand [Fig. 2, see also Woodell *et al.* (1977)]. The morphological variation in *A. maritima* there (as almost everywhere in Denmark) is high (Lefèbvre, 1969, 1974; Philipp, 1974; Woodell *et al.*, 1977; Lefèbvre, 1985). One of the characters that varies is the hairiness of the flower stalk, which is determined by a locus with two alleles *H* and *h*. The allele *H*

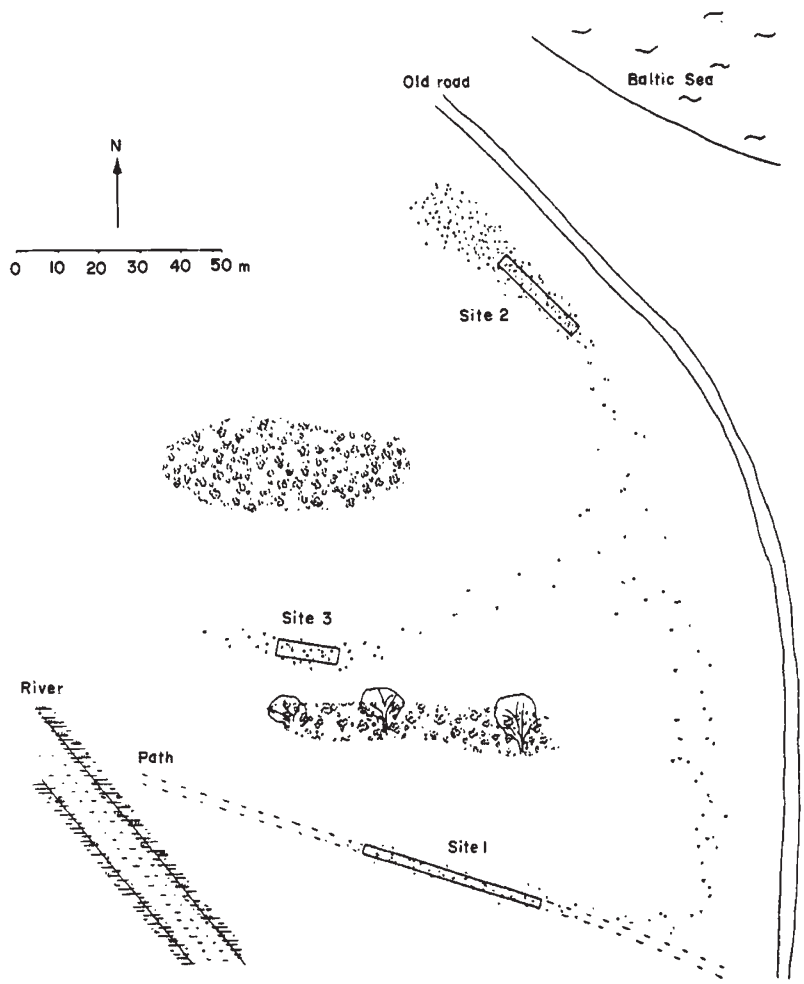

Fig. 2 The investigated area by Strøby Egede. Dots indicate the occurrence of *Ameria maritima* plants.

dominates over *h*: the genotypes *HH* and *Hh* have hairy scapes whereas the genotype *hh* has glabrous scapes.

Methods

The dispersal of seeds was studied in 1982 at Ulfshale, Møn (Madsen, 1987). In this coastal area the vegetation is dominated by *Carex arenaria*. Seed dispersal distances were measured for one *A. maritima* plant (height 38 cm). The scapes of all other *A. maritima* plants within 11 m from this plant were removed to ensure the origin of the dispersed seeds. The seeds were caught in carpets of artificial grass, which had a short open piling (2 cm) that effectively caught the seeds. The carpets were placed out from the plant so that they captured all the seeds that fell within a distance of 4 m from the centre of the plant and within an angle of ±30° from the direction of the predominant wind. The distance to the centre of the *A. maritima* plant was measured for each seed trapped on the carpet. Wind velocity at the height of inflorescences was measured with an anemometer held for 2 min at the start of the experiments.

Pollen dispersal distances were studied at the experimental field of the Botanical Garden, University of Copenhagen, in Tåstrup, where we planted a large number of plants of mating type B at interplant distances of 30 cm within a circle with a diameter of 10 m. At the centre of the circle a small group of plants of mating type A was planted. Pollen flow distances could be measured by means of the seedset in the mating type B plants.

Three sites in the study area at Strøby Egede were chosen in 1984 representing areas with high densities of *A. maritima* (Figs 2 and 3). Between site 1 and site 3 there were some shrubs and a few trees but no *A. maritima* individuals. Between site 3 and site 2 the density of *A. maritima* was low. At each site contiguous squares (1 m^2) were laid out along lines. In each square the flowering plants were counted and for each plant the number of scapes and the hairiness were noted. The size of the plants was expressed as the number of scapes (Lefèbvre & Chandler-Mortimer, 1984). The sites were subdivided into *local populations* (1–6) according to the following criteria. Firstly, local populations should be separated by 4 m, which is about the maximal observed seed dispersal distance, see below. Secondly, the local population should not be too small; that is, it should consist of at least 50 plants. Thirdly, most of the plants should be included in the local populations.

Mature inflorescences from each plant were harvested and the seeds sown in the autumn of 1984 at the experimental field. Up to 10 seedlings from each sample were pricked out and later grown to flowering. Each offspring was inspected for hairiness on flower stalks. The genotype of their offspring could be identified (hairy *Hh*, glabrous *hh*) for all glabrous plants (*hh*).

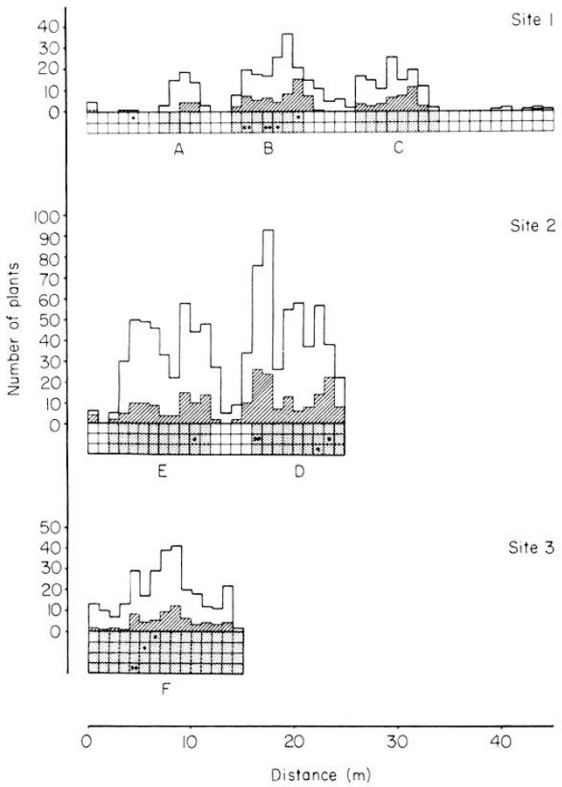

Fig. 3. Number of plants along the sites. The height of the histogram expresses the total number of plants in the underlying squares. The number of hairy plants is indicated (▨) as well as the occurrence of hairy homozygotes (★). The subdivision into local populations is indicated by the grey squares (▩).

In order to investigate the genetic structure of the populations, we determined the genotypes of the hairy individuals (*H*—). This was done through the segregation of their offspring raised at the experimental field. We included all plants with at least five offspring surviving to the age where their hairy/glabrous phenotype could be determined. With five or more offspring per plant the genotype of a hairy parental individual was determined with a probability of at least 0.94. This is the probability of observing at least one recessive offspring. It was estimated from the allele frequencies under the assumption of Mendelian segregation and that there was no selection acting on the polymorphism

$$P = 1 - \left(\frac{\mu_{21} + 1}{2}\right)^5 = 1 - \left(\frac{1.14}{2}\right)^5 = 0.94,$$

see Table 3. A number of glabrous plants with known genotype were excluded by this procedure, but the restriction of at least five offspring per plant ensures that inclusion is independent of the genotype (with the exception that less than 6 per cent of the heterozygotes are expected to have been mis-classified at homozygous *HH*).

Results

Seed and pollen dispersal

Seed dispersal was studied in two experiments of different lengths. Figure 4 shows the dispersal in the experiment where the sampling was cumulated over 45 days. As is commonly observed for seed and pollen dispersal, the dispersal curves are highly skewed with a long tail to the right. The mean dispersal distance was estimated from the log transformed data. It was 0.88 m, and the observed maximal distance that a seed was transported did not exceed 3.5 m.

A similar pattern was observed in the experiments that lasted 4 h. The dispersal distance depends on the wind velocity. During 4 h of wind with a velocity of 4 m s^{-1} the mean dispersal distance was 0.69 m, which increased to 1.09 m in an experiment with 5–6 m s^{-1}. In neither experiment did the maximum dispersal distance reach 3 m.

These results were used to partition the three sites into local populations, separated by at least 4 m with no or very low population density of the plants (Fig. 3). Using this procedure site 1 was subdivided into three local populations, site 2 into two and site 3 was kept as one local population.

It was not possible to estimate pollen dispersal with the four experiments at the experimental field where honey bees (*Apis mellifera*) were the main pollinators. Within each test area seeds were found in all plants and in high quantities, indicating that a radius of 5 m was a lower limit for the average distance of pollen dispersal.

Demographic population structure

The distributions of flower stalks per plant showed a similar distribution in all three sites and have been pooled (data not shown). The distributions for the two phenotypes are alike and indicate that new plants are regularly recruited from seeds, see Fig. 5. Lefèbvre & Chandler-Mortimer (1984) found that larger plants (>15 years) constituted 10 per cent of the total number of plants but contributed 28 per cent of the inflorescences in the population while 2–5 year-old plants contributed only 5 per cent of the inflorescences. In our study area the maximal number of flower stalks per plant was 54, see Fig. 6. Although such plants have a large seed production, the high number of smaller plants results in a substantial contribution from these to the seed production, resulting in a high genetic turnover. The smaller plants (one to four flower stalks) contributed about 30 per cent of all inflorescences, see Fig. 6.

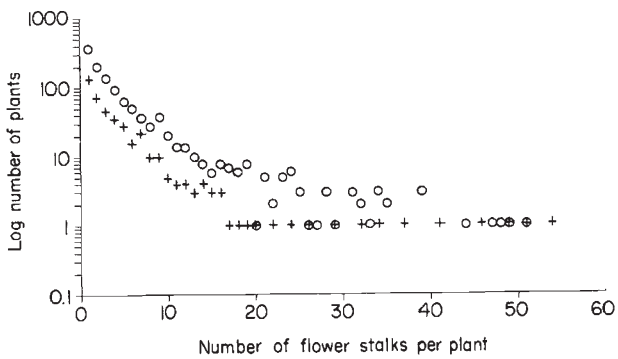

Fig. 5 Demographic population structure of the Strøby Egede population. (○) *hh*, (+) *HH* + *hh*.

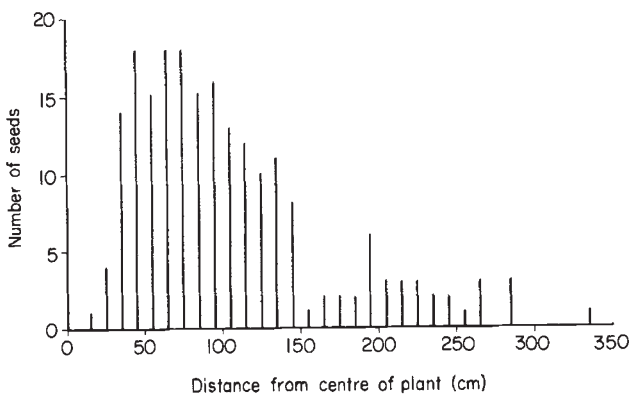

Fig. 4 Dispersal of diaspores in *Armeria maritima*. The number of seeds found within an angle of 60° around one individual over a period of 45 days.

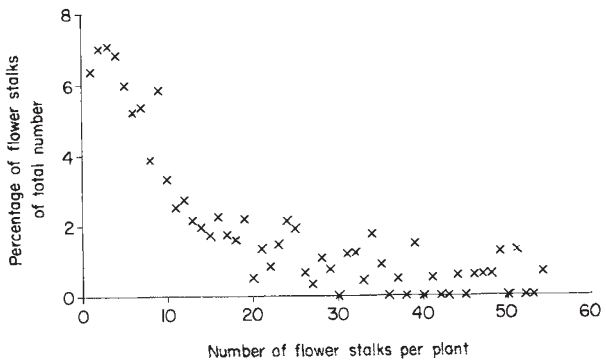

Fig. 6 The distribution of all inflorescences in the population.

Genetic population structure

The population structure is analysed with a modified version of the selection component analysis described by Christiansen & Frydenberg (1973) for an autosomal locus with two alleles. The effect of natural selection on the variation at the locus is studied by partitioning the selection into the components of gametic, sexual zygotic and fecundity selection. The selection component analysis is based on population samples that include mother–offspring combinations, where a single offspring is included for each mother. It formulates a sequence of hypotheses that puts increasingly restrictive assumptions on a model to describe the data.

The selection component analysis has been modified by Säll (1982) for plant species that are hermaphroditic and self-incompatible (like *Armeria maritima*). Säll included several offspring per mother plant and tested for geographical position effects by comparing the gene frequencies in the pollen that had fertilized individuals with the same genotype. We use another approach by partitioning the total population into local populations, which can then be compared.

The six (n) local populations consisted of plants whose genotypes have been determined through the segregation of their offspring. As there are two alleles, three genotypes are found, *HH*, *Hh* and *hh*; the number of these are $F_1(l)$, $F_2(l)$ and $F_3(l)$ in the lth local population, respectively (Table 1). The sum is $F_0(l)$. The genotypes of the offspring are known for *hh* plants. The numbers of *Hh* and *hh* offspring from these plants in the lth local population are $C_{32}(l)$ and $C_{33}(l)$, respectively. The first subscript refers to the genotype of the mother, the second to the genotype of the offspring. In heterozygous plants the *HH* and *Hh* offspring cannot be distinguished, therefore $C_{2x}(l)$ is the number of offspring with the dominant phenotype in the lth local population. There is no information about the genotype of the offspring of *HH* plants, they all have the dominant phenotype *H*— and their number is $C_{10}(l)$. The sum of offspring for plants with genotype i is $C_{i0}(l)$. As we have included a single offspring per plant this is equal to $F_i(l)$. The mother–offspring combinations for the three genotypes are given in Table 1 for a single local population. The data is presented for all local populations in Table 2.

Table 1 Mother–offspring combinations in the lth local population

Mothers		Number of offspring			
Genotype	Number	*H*—	*Hh*	*hh*	Sum
HH	$F_1(l)$	$C_{10}(l)$			$C_{10}(l)$
Hh	$F_2(l)$	$C_{2x}(l)$		$C_{23}(l)$	$C_{20}(l)$
hh	$F_3(l)$		$C_{32}(l)$	$C_{33}(l)$	$C_{30}(l)$

We have chosen to include a single offspring per mother plant as in the original selection component analysis; the higher number of offspring is then exclusively used to determine the genotype of the mother plants. The main difference consists of the geographically structured sample and of the fact that the variation is determined by a dominant locus.

The hypotheses were tested with likelihood ratio tests; twice the difference of the logarithm of the likelihoods of two hypotheses is approximately χ^2-distributed with degrees of freedom equal to the difference in the numbers of parameters used in the hypotheses. The estimation procedures are given in the Appendix. The parameters needed to describe the sample are introduced sequentially.

Hypothesis 1. Random mating in each local population. The hypothesis of random mating compares the frequencies of the pollen that has fertilized the different genotypes of the adult plants. Because there is no information about the offspring of *HH* plants, the hypothesis is limited to a comparison of the pollen that has fertilized *Hh* and *hh* plants.

Table 2 Mother–offspring combinations for all local populations and the total population. The expected distribution is given for Hypothesis 5, see text

Population	$C_{10}(l)$	$C_{2x}(l)$	$C_{23}(l)$	$C_{32}(l)$	$C_{33}(l)$	$F_1(l)$	$F_2(l)$	$F_3(l)$
1	0	3	1	3	26	0	4	29
2	5	11	9	7	41	5	20	48
3	0	7	6	7	33	0	13	40
4	3	31	22	22	140	3	53	162
5	3	21	17	12	100	3	38	112
6	4	25	24	24	127	4	49	151
Total	15	98	79	75	467	15	177	542
Expected	15	100.89	76.11	75.85	466.15	14.38	176.69	542.93

The offspring of plants with genotype 3 (hh) segregate into Hh and hh with the frequencies of the alleles H and h in the pollen pool. These frequencies are named $\mu_{31}(l)$ and $\mu_{32}(l)$ for the lth local population $[\mu_{31}(l)+\mu_{32}(l)=1]$. The first subscript refers to the genotype of the mother plant, the second to the allele in the pollen pool.

Heterozygous plants are fertilized by pollen with the allele frequencies $\mu_{21}(l)$ and $\mu_{22}(l)$. Philipp (1974) has shown that heterozygotes segregate the alleles H and h in a Mendelian way. Therefore, one half of the offspring of heterozygotes are expected to be heterozygotes themselves

$$\{\tfrac{1}{2}\mu_{21}(l)+\tfrac{1}{2}\mu_{22}(l)=\tfrac{1}{2}[\mu_{21}(l)+\mu_{22}(l)]=\tfrac{1}{2}\}.$$

The remaining half part of the offspring is partitioned into HH and hh genotypes with the frequencies of H and h in the pollen, see Table 3.

We expect with random mating that there is no difference in allele frequencies in the pollen that have fertilized plants of genotype 2 and 3, that is

$$\mu_{2i}(l)=\mu_{3i}(l)=\mu_i(l) \quad \text{for } i=1, 2 \text{ and } l=1, \ldots, n.$$

This hypothesis is tested with a single degree of freedom in each local population. The estimated gene frequencies are given in Table 4. None of the test statistics are significant and neither is the sum, so there is no evidence that plants of genotype 2 and 3 are fertilized by pollen with different allele frequencies. There is random mating in each local population.

Table 3 Expected offspring distributions with Mendelian segregation in the lth local population

Mother	Expected frequency of offspring		
	$H-$	Hh	hh
HH			
Hh	$[\mu_{21}(l)+1]/2$		$\mu_{22}(l)/2$
hh		$\mu_{31}(l)$	$\mu_{32}(l)$

Table 4 Estimated allele frequencies in the pollen pool and tests for random mating in all local populations. The last line is the estimated common pollen pool for the total population

Population	$\mu_1(l)$	$\mu_2(l)$	χ^2	d.f.	P
1	0.109	0.891	0.67	1	0.41
2	0.144	0.856	0.04	1	0.84
3	0.171	0.829	0.12	1	0.73
4	0.137	0.863	0.06	1	0.81
5	0.107	0.893	0.00	1	1.00
6	0.153	0.847	0.91	1	0.34
Sum			1.80	6	0.94
Total	0.137	0.863	1.89	5	0.86

Hypothesis 2. Pollen pool is common to all local populations. In this hypothesis we restrict our assumptions further. If the pollen pool is common to all local populations we expect that

$$\mu_i(l)=\mu_i \quad \text{for } i=1, 2 \text{ and } l=1, \ldots, n.$$

Instead of the n $\mu_i(l)$, the pollen can now be described with a single parameter, μ_i, common to all local populations leaving $n-1$ degrees of freedom for the test, see Table 4. There is no evidence that the allele frequencies in the pollen pool are heterogeneous among the six local populations.

Hypothesis 3. Local populations are homogeneous. The total population was partitioned into six local populations that (at least for the sites) are geographically separated. We now ask whether the genotype distributions of the adult plants are the same in all local populations. That is

$$\phi_i(l)=\phi_i \quad \text{for } i=1, 2, 3 \text{ and } l=1, \ldots, n,$$

where $\phi_i(l)$ and ϕ_i are the parameters describing the frequency of genotype i in the lth and in the total population, respectively. This hypothesis is tested with the usual test for homogeneity, which has $(n-1)\times 2$ degrees of freedom. The estimates of the genotypic distribution in the total population are given by

$$\phi_1=0.020, \quad \phi_2=0.241, \quad \phi_3=0.738.$$

The test statistic is not significant (Table 5), so we accept the hypothesis of a homogeneous total female population.

Hypothesis 4. No male reproductive selection. As in the original selection component analysis of Christiansen & Frydenberg (1973), male sexual selection and male gametic selection are confounded and their effects on the polymorphism are measured through the combined component male reproductive selection.

Table 5 Summary of the test statistics for all five hypotheses

Hypotheses	χ^2	d.f.	P
1	1.80	6	0.94
2	1.89	5	0.86
3	13.45	10	0.20
4	0.05	1	0.82
5	0.01	1	0.93

If plants of the three genotypes produce pollen in equal quantity and heterozygotes segregate *H* and *h* in the ratio 1:1, we expect that the frequencies of the two alleles in the pollen are given by

$$\mu_1 = \alpha_1 + \alpha_2/2$$
$$\mu_2 = \alpha_3 + \alpha_2/2,$$

where α_i is the frequency of adult plants in the population. It is a combined estimate of the genotypes of plants that serve as both mothers and pollen donors. The number of parameters is now two, since $\alpha_1 + \alpha_2 + \alpha_3 = 1$. This is a reduction by one: in hypothesis 3 we used three parameters, two for the adult female plants, ϕ_1, ϕ_2, ϕ_3 and one for the pollen allele frequency. Thus, the hypothesis is tested with one degree of freedom. The estimated adult genotypic distribution is

$$\alpha_1 = 0.020, \quad \alpha_2 = 0.240, \quad \alpha_3 = 0.740.$$

This genotypic distribution is very close to the one we found in hypothesis 3, and the test is not significant (Table 5). We therefore accept the hypothesis that the pollen has been produced by the plants that have been included as mothers in the analysis.

Hypothesis 5. No zygotic selection. If there is zygotic (viability) selection that affects the variation at the studied locus and the population is at the equilibrium, we should detect this as a deviation of the genotypic distribution in adults from Hardy–Weinberg proportions with the allele frequencies π_1 and π_2 estimated from the whole population sample, that is

$$\alpha_1 = \pi_1^2, \quad \alpha_2 = 2\pi_1\pi_2, \quad \alpha_3 = \pi_2^2,$$

where the allele frequencies are estimated to be $\pi_1 = 0.140$ and $\pi_2 = 0.860$, so that the expected adult genotypic distribution is

$$\alpha_1 = 0.020, \quad \alpha_2 = 0.241, \quad \alpha_3 = 0.740.$$

This distribution is virtually identical to the one found in hypothesis 4, so there is no evidence that zygotic selection affects this polymorphism, (Table 5). The genotypic distributions are very close to the expected Hardy–Weinberg proportions (Table 2).

Discussion

In the selection component analysis the test of a hypothesis only makes sense if the hypotheses it depends on have been accepted. That is, if hypothesis 2 (the pollen pool is common to all local populations) has been rejected one cannot test hypothesis 4 which postulates that there is no male reproductive selection. Hypothesis 3 does not depend on any previous hypothesis and can be tested in any case. A rejection of hypothesis 2 could be due to selection or (which is more likely) to a limited pollen dispersal among the local populations that might differ in allele frequencies. In this case, the selection component analysis could continue by formulating new hypotheses. These are outlined in the Appendix.

As all adult plants produced seeds there was no need to include the hypothesis about female sexual selection, which was part of the original selection component analysis. This hypothesis is a homogeneity test of two groups of adult plants, one producing offspring and the other without offspring. It would be difficult to include it in the same way as in the analysis of Christiansen & Frydenberg (1973) if some of the plants are without seeds because we have used the offspring to determine the genotypes of their mothers. This hypothesis would have to be restricted to a comparison of the phenotypic distribution of plants that produced seeds with those that did not produce seeds. We did not count the seeds produced by the plants. Therefore, it was not possible to test for fecundity selection.

The selection component analysis of the *Armeria maritima* population concluded that the total population is panmictic and homogeneous over the local populations and that there is no evidence of any selection on the variation at the locus for hairiness. The genetic variation can be described with the allele frequencies of *H* and *h* found in hypothesis 5. They are 0.140 and 0.860, respectively. Woodell *et al.* (1977) have studied the same population at Strøby Egede. They found that the frequency of the recessive genotype *hh* was 0.37 in 1974. Assuming Hardy–Weinberg proportions in the population, this amounts to a frequency of 0.608 of the *h* allele. The number of plants collected in 1974 is so high that the gene frequencies are significantly different in 1974 and 1984. One might now ask how such a large difference could have been produced, if the reported gene frequency is correct. A possible explanation could be selection against the dominant homozygote *HH*. The fitness of the the *HH* genotypes would have to be about half the fitness of the other two genotypes in order to cause a change in gene frequency of allele *h* from 0.608 to 0.840 in 10 generations. Such large differences seem very unlikely. The allele *H* would have been eliminated very quickly, leaving the population monomorphic for allele *h*. In order to test for selection against *HH* genotypes, we crossed *Hh* plants with others of the same genotype. The genotype distribution of the offspring was 110 *H*— and 55 *hh*, which differs significantly at the 2 per cent level from the expected 3:1 ratio. The frequency of the recessive homozygotes is too high and could be

explained with selection against *HH* homozygotes. The very good fit of the genotypic distribution with Hardy–Weinberg proportions, which resulted in a very low test statistic in hypothesis 5, does not support such an explanation. Thus, the evidence for selection against *HH* genotypes is very weak. Would such a large fitness difference have been detected by the selection component analysis? If the genotype *HH* survived with only half the probability of the two other genotypes, the number of plants in the study should have been about twice as large in order to detect this selection with a probability of 50 per cent. The reason for this weak resolution of the selection component analysis is the biased gene frequencies of the two alleles. Had the frequencies been closer to 0.5, fewer plants would have been necessary to detect such large fitness differences. We are not able to offer an explanation for the large difference in gene frequencies between 1974 and 1984.

A major conclusion of the genetic analysis of the population structure is that the six local populations are homogeneous. Thus, the gene flow among the local populations is sufficiently large to prevent any differentiation among them. Seed dispersal is limited to within a few metres and makes only a minor contribution to gene flow among the populations that we have grouped as 'local'.

It should be emphasized that our conclusions about gene flow and the absence of selection refers to the *H* locus only. Despite the high gene flow suggested by the data for variation at the *h* locus, it is possible that genes at other loci may show substantial differentiation if the selection pressure is sufficiently high.

The genetic analysis suggests that pollen flow has a large homogenizing impact on the genetic population structure. How does the evidence from pollination ecology studies fit with the genetic analysis?

1 The experiment with the circular plantation at the experimental field showed that in the presence of honey bees the pollen flow distance is at least 5 m. This exceeds the minimum distance we have used for the separation of local populations.

2 Woodell (1978) has investigated the pollinator movement pattern in a population of *A. maritima* in England. He found that bumblebees did not move randomly, but specifically upwind. The bumblebees could visit up to 295 inflorescences on one foraging flight. Directionality means that the distance of pollen transfer is much larger than the pollinator flight distances between inflorescences suggest (Schaal, 1980).

3 The dimorphic sporophytic incompatibility system itself might help to increase the pollen flow distance (Glover & Barrett, 1986). In principle every second plant visited by the insect is non-compatible, and this might mean that incompatible pollen grains, which do not adhere well to incompatible stigmas, are carried further away than if all the plants were compatible.

4 Pollen dispersal is limited if the pollinators visit more than one flower and/or more than one inflorescence per plant. In *A. maritima* three characteristics counteract this effect. Firstly, 30 per cent of the plants have only 1–4 inflorescences. Secondly, the number of open flower per inflorescence is below eight for 80 per cent of the inflorescences. Thirdly, in a single visit pollinators only exploit a few of the available flowers per inflorescence: bumblebees 2.1, syrphids 1.6, and flies 0.9 (Madsen, 1987).

Based on the pollination ecology, we conclude that several factors point to a comparatively long pollen dispersal distance; that is, the direction of the pollinator movement, the self-incompatible mating system, a limited number of flowers visited per plant, and the presence of bumblebees and butterflies among the pollinators.

In addition, the success of a plant as pollen donor can be dependent on the distance to the ovule donor (Willson & Burley, 1983; Levin, 1984; Sobrevila, 1988). *Armeria maritima* is an outbreeding species; therefore, inbreeding depression would be expected when related individuals are crossed. As neighbouring plants can be closely related, inbreeding depression could sort out pollen tubes from closely placed plants and zygotes produced by them, which could help to increase the pollen flow distance.

The gene flow distance in *A. maritima* is thus composed of a short seed dispersal and a long pollen flow distance resulting in a homogeneous population structure at the studied locality. This supports the view of Ellstrand & Marshall (1985, 1989) that pollen dispersal distances seem to be longer than expected from pollen carryover studies when the distances are measured from the actual pollen receiving plant. Gene flow in plants may thus be higher than is commonly believed.

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Appendix

The logarithm of the likelihood for the population sample from the lth local population is given by

$$\log[L(l)] = C_{2x}(l)\log\{[1+\mu_{21}(l)]/2\} + C_{23}(l)\log[\mu_{22}(l)/2] + C_{32}(l)\log[\mu_{31}(l)] + C_{33}(l)\log[\mu_{32}(l)] + \sum_{i=1}^{3} F_i(l)\log[\phi_i(l)]$$

The total logarithmic likelihood function is found by summing over all n local populations

$$\log(L) = \sum_{l=1}^{n} L(l),$$

where n is the number of local populations. The maximum likelihood estimators are either given explicitly or are found with the *EM* algorithm of Dempster *et al.* (1977).

Hypothesis 1

The parameter estimates for the allele frequencies in the pollen that has fertilized heterozygous plants in the lth local population are

$$\mu_{21}(l) = \begin{cases} \dfrac{C_{2x}(l) - C_{23}(l)}{C_{2x}(l) + C_{23}(l)} & \text{for} \quad C_{2x}(l) > C_{23}(l) \\ 0 & \text{for} \quad C_{2x}(l) \leq C_{23}(l) \end{cases}$$

$$\mu_{22}(l) = 1 - \mu_{21}(l).$$

For plants of genotype 3 the allele frequencies in the pollen pool are found by direct counts

$$\mu_{31}(l)=\frac{C_{32}(l)}{C_{32}(l)+C_{33}(l)}$$

$$\mu_{32}(l)=\frac{C_{33}(l)}{C_{32}(l)+C_{33}(l)}.$$

These are the same estimators as in Christiansen & Frydenberg (1973).

The estimators of the allele frequencies common to both genotypes are the positive solution to a quadratic equation

$$\mu_1(l)=\frac{-B-\sqrt{B^2-4AC}}{2A}$$

$$\mu_2(l)=1-\mu_1(l)$$

where

$$A=-[C_{20}(l)+C_{30}(l)]$$

$$B=C_{2x}(l)-C_{23}(l)-C_{33}(l)$$

$$C=C_{32}(l).$$

Hypothesis 2

The estimators for μ_1 and μ_2 are found with the same procedures as in Hypothesis 1 by summing the mother–offspring combinations over all populations.

Hypothesis 3

The estimators for the common genotypic distribution of the plants in the total population are

$$\phi_i=\frac{\Sigma_{l=1}^{n}F_i(l)}{F_0} \quad \text{for } i=1, 2, 3,$$

where

$$F_0=\sum_{l=1}^{n}F_0(l)$$

is the total number of adult plants included in the study.

Hypothesis 4

The estimators for the adult plants that serve as both mothers and fathers in the population are found by iterating gene counting equations.

First a guess is made of the genotypic frequencies. These are inserted in the right-hand side of the equations to find a new and better estimate. This procedure is repeated until a sufficient degree of precision has been reached. The equations are

$$\alpha_1'=\frac{F_1+[C_1+2C_{2x}/(1+\alpha_1+\alpha_2/2)+C_{32}/(\alpha_1+\alpha_2/2)]\alpha_1}{F_0+C_{10}+C_{20}+C_{30}}$$

$$\alpha_3'=\frac{F_3+[C_1+C_{2x}/(1+\alpha_1+\alpha_2/2)+C_{23}+C_{33})/(\alpha_3+\alpha_2/2)]\alpha_3}{F_0+C_{10}+C_{20}+C_{30}}$$

$$\alpha_2'=1-\alpha_1'-\alpha_3'.$$

Normally, the procedure converges quickly.

Hypothesis 5

The estimators of allele frequencies in the total population are the positive solution to a quadratic equation

$$\pi_1=\frac{-B-\sqrt{B^2-4AC}}{2A}$$

$$\pi_2=1-\pi_1$$

where

$$A=-(2F_0+C_{20}+C_{30})$$

$$B=-(F_2+2F_3-C_{2x}+C_{23}+C_{33})$$

$$C=2F_1+F_2+C_{32}.$$

Rejection of Hypothesis 2

If Hypothesis 2 (pollen pool is common to all local populations) has been rejected, the selection component analysis can be continued in another way. Firstly, we can test whether the pollen pool is produced within the local populations,

$$\mu_1(l)=\alpha_1(l)+\alpha_2(l)/2$$

$$\mu_2(l)=\alpha_3(l)+\alpha_2(l)/2,$$

where $\alpha_i(l)$ now refers to the adults of genotype i in the lth local population. This hypothesis is the same as Hypothesis 4 applied to each local population. The next step would be to compare the adult genotypic proportions in all local populations

$$\alpha_i(l)=\alpha_i \quad \text{for } i=1, 2, 3 \text{ and } l=1, \ldots, n.$$

This hypothesis would probably be rejected because it has already been accepted that the allele frequencies in

the pollen differ among the local populations and that the pollen has been produced within the local populations. If the hypothesis is rejected, the analysis could continue by testing whether there is any zygotic selection within the local populations. This hypothesis is the same as the original Hypothesis 5 applied to each local population.

In a population with a very limited gene flow another approach could be to estimate the pollen frequency for every single plant (Säll, 1982), but instead of comparing the frequencies in the pollen pool for every genotype class over the total population, one could investigate these frequencies on a finer geographical scale. With a dominant allele in a two-allele system this procedure is restricted to the heterozygote and the recessive homozygote.

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