

Phenotypic plasticity and genetic differentiation of demographic characteristics in some *Plantago* species

1. INTRODUCTION

This study was initiated as part of a multidisciplinary project on the relationship between demographic, physiological and genetic properties of *Plantago* species and the characteristics of their environment. In this project, carried out in the Netherlands, the differences in life history between and within five *Plantago* species are being studied (Van der Aart 1979).

In addition to Van Delden and Lambers *et al.*, (these proceedings) this paper reports on differences in life-history characteristics of populations of *Plantago maritima* L. and *P. major* ssp. *pleiosperma* Pilger in relation to differences in environmental factors. The main purpose of this study was to investigate if the differences between populations within these species could be ascribed to phenotypic plasticity or to genetic differentiation. The relative importance of both phenomena as well as the relationship between the nature of different population characteristics within a species and the distance between their sites in the field will be discussed. Therefore two adjacent populations of *P. maritima* and three populations of *P. major* ssp. *pleiosperma* from different areas were studied. Gene flow between populations would be most likely in the case of *P. maritima*, an outcrossing species, whereas no exchange of genes between the populations was expected in the case of the three *P. major* ssp. *pleiosperma* populations (plants with a high degree of selfing).

2. STUDIES WITH *PLANTAGO MARITIMA*

The two neighbouring populations of *P. maritima* were studied in the nature reserve Kwade Hoek, situated on the island of Goeree in the southwest of the Netherlands. The vegetation of this tidal area ranges from halotypic communities on heavy clay soils to communities growing on inland dunes with a sandy soil (cf. Beeftink 1975; Blom 1983). As a form of management part of the area is grazed by cattle from May to October. One of the two *P. maritima* populations occurs on the edge of the inner sand dunes and the other on the flooded sandy clay soils. Relatively large differences in life-history characteristics between the two neighbouring populations were observed, and besides this the two habitats had different soil types, tidal influences and grazing intensities (Table 1). Preliminary studies on allozyme

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Table 1. Characteristics of two populations of *P. maritima* in two habitats at the salt marsh Kwade Hoek (The Netherlands).

Site	Soil	Grazing	Tidal influences	Vegetation layer	Life span	Biomass	Propagation	Seedlings
Population I:								
Edge of inner dunes	sand: 4.4 mg Cl ⁻ per 100 g dry soil	rabbits (winter)	seldom	open	c. 3 yrs.	low	seed	many
Population II:								
Along a creek	sandy clay: 1195 mg Cl ⁻ per 100 g dry soil	cattle (summer)	frequent	dense	c. 10 yrs.	high	daughter rosettes	few

variation and morphological features suggested that the differences between the two populations probably do not have a genetic basis. Therefore it was postulated that the differences between these two populations are caused by the different environmental factors. To test this hypothesis - which disagrees with that of Gregor & Lang (1950) and Jefferies (1977) findings in other *P. maritima* populations - a number of field and laboratory experiments were carried out (cf. Blom 1983).

The distance between both sample sites was about 300 m. In 1970 a causeway was built in a neighbouring estuary with the result that at Kwade Hoek the highwater mark was raised. It is probable that the innerdune population of *P. maritima* in the upper parts of the area has become established since then. In order to explain the differences in demographic characteristics between the two populations, two series of experiments were conducted.

The aim of the first experiment was to compare emergence, establishment, and survival characteristics of seedlings from sown seeds with those of seedlings that occur naturally.

Table 2 shows the results of the sowing experiments using seeds from both populations. At the edge of the inner dunes significantly more of the sown seeds emerged than at the grazed and regularly flooded sites ($P < 0.05$). During the first year no differences were found in the survival rate of seedlings on both sites, but after three years significant differences in the adult survival rate were observed ($P < 0.05$). When we compare these results with the results of demographic studies on plants that occur naturally (Table 2) it appears that the survival rate of these seedlings on the edge of inner dunes is within the same range as that observed in the sowing experiment. Fewer naturally occurring seedlings survived on the grazed sites than in the sown plots. No important differences were observed between the survival rate of the naturally occurring adults and of those from sown seeds. The differences in emergence were not caused by the differences in substrate (sand or sandy clay) but by the salinity of the soil (Blom 1983).

Table 2. Emergence and survival of *P. maritima* in a sowing experiment in the field. In brackets: plants that occur naturally.

Site at Kwade Hoek	Emergence (%)	Seedling survival (%)	Adult survival After 3 years (%)
Population I:			
Edge of inner dunes	33.7	66.3 (74.8)	39.2 (32.7)
Population II:			
Along a creek	18.3	60.0 (30.3)	72.5 (81.1)

Seedling mortality was highest in the sea-water flooded sites which was also found by Jerling (1982).

The results showed that at Kwade Hoek emergence and survival of *P. maritima* is related to the site. There was no important difference between the performance of plants from the natural seedbank and those from sown seeds; environmental factors determine the emergence, establishment and survival. No significant differences between the results of a reciprocal sowing test and the experiment with mixed seeds were observed.

The aim of the second series of experiments carried out in the greenhouse was to investigate the effects of environmental factors on growth and way of reproduction, as striking differences in these characteristics were observed between both populations in the field (Table 1). In the field, grazing and inundation were the main differentiating habitat factors between both populations. In the greenhouse both factors were simulated, trampling and clipping as a simulation of grazing effects were applied, separately and in combination (Fig. 1 and 2).

Trampled plants produced more biomass and more daughter rosettes than untrampled ones (Fig. 1). Fig. 2 shows that plants inundated with sea-water (which is a simulation of tidal influences) formed about the same amount of biomass as the controls. However, more daughter rosettes were found. All plants used in these experiments came from a mixed seed sample and it was concluded that vegetative and generative growth depended on the treatments. More or less the same phenomena as found in this experiment were observed in demographic studies in the field (Table 1). Individuals without daughter rosettes occur at the edge of the inner dunes where trampling and flooding are nearly absent; these plants reproduce mainly by seed. On the grazed and flooded sites along the creek, only a few seedlings were found and reproduction occurred vegetatively. Furthermore, in the field just as in the experiments, the trampled plants produced more biomass per individual than the untrampled plants on the higher sites of the area.

It should be noted that the reaction of the Sea Plantain to a high degree of salinity depends on the stage in the life cycle. Seedlings are very susceptible to a high chloride content in the soil (Jerling 1982) whereas adult plants are very resistant to this environmental factor (Cooper 1982). This underlines that in studies on the reaction of plants to changing environmental conditions all stages in the life cycle of that plant should be considered (Blom 1977; Watkinson & Gibson, these proceedings).

Gregor & Lang (1950) and Jefferies (1977) postulated for *P. maritima* that the European populations are highly variable and differences between populations are mainly genetically determined. However, this study suggests that in spite of the contrasting selection pressures, no genetic differences between both populations exist at present. This can be explained by the fact that in this regularly flooded salt marsh, with the activities of wild and domestic grazing animals, seeds of the sea plantain will be dispersed over relatively large surfaces. Furthermore, the population in the inner dunes probably has only been established ten years ago. Changes in the environment caused changes in the behaviour of individual plants of this species. Because of the relatively long-distance dispersal and the mixing of the seeds from both populations, selection for plasticity in characteristics such as survival, growth and form of reproduction (vegetative or generative) may have occurred. However, in isolated areas, it is possible that different environmental factors such as different grazing intensities or frequencies of inundation may lead to genetic differentiation between populations of *P. maritima*, which explains the findings of e.g. Jefferies (1977).

3. STUDIES WITH *PLANTAGO MAJOR* SSP. *PLEIOSPERMA*

Three populations of *P. major* ssp. *pleiosperma* were also studied. In the Netherlands two subspecies of *P. major* occur; the common subspecies *major* and the less abundant subspecies *pleiosperma*. Marked differences in allozyme frequencies between these subspecies have been described by Van Dijk & Van Delden (1981). Kuiper (1982, 1983) distinguished a high degree of phenotypic plasticity in *P. major* ssp. *pleiosperma* and a low degree of plasticity in *P. major* ssp. *major*. In the present study the hypothesis was tested that between populations of *P. major* ssp. *pleiosperma* differences in survival, growth rate and reproductive effort are determined mainly by environmental factors. As in the Sea Plantain, the experimental studies with *P. major* ssp. *pleiosperma* were based on demographic investigations and soil analyses. The three populations originated from three different areas. No exchange of genes was expected. Table 3 shows some soil and demographic characteristics of the populations.

The first area was Kwade Hoek, described in the first part of this paper. Relatively high levels of organic matter and of total nitrogen were found in the upper soil layers at the *P. major* ssp. *pleiosperma*-site. The vegetation layer was dense which means that competition between herbs and grasses may be an important factor. The biomass production and seedling recruitment of *P. major* ssp. *pleiosperma* are low. A perennial life cycle was observed and only a few plants of this species were found per square metre.

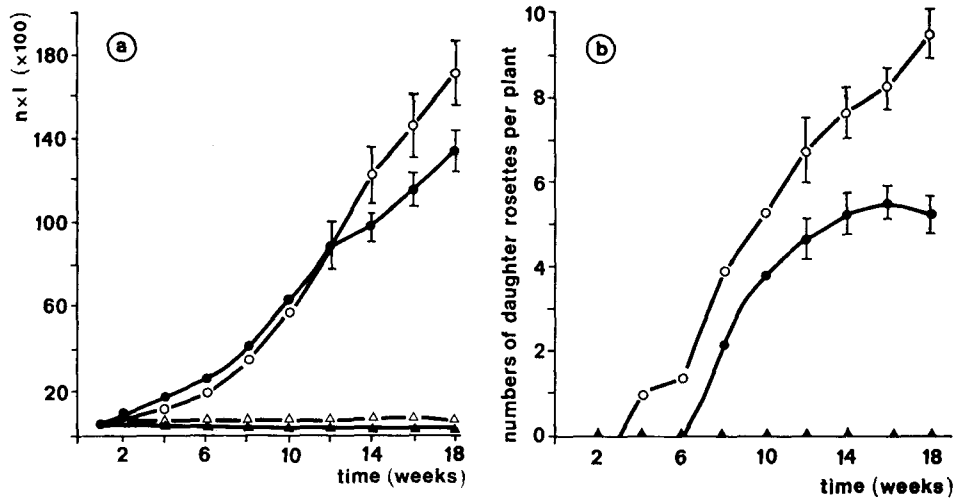


Fig. 1. a,b. The effects of clipping and trampling on biomass production (a) and on the mean numbers of daughter rosettes per plant (b) of *Plantago maritima* grown separately in pots in the greenhouse. The biomass is approximated by the multiplication of the mean numbers of leaves per plant (n) and the length of the longest leaf per plant (l).

○ trampling treatment, ● no treatment, △ clipping treatment, ▲ combined clipping and trampling, [2 S.E.

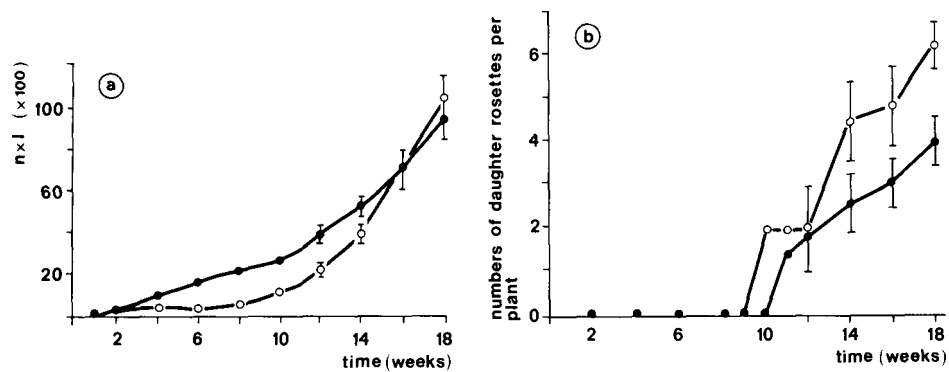


Fig. 2. a,b. The effects of inundation with sea water as well as with fresh water on the biomass production (a) and the mean number of daughter rosettes per plant (b) of *Plantago maritima* grown separately in pots in the greenhouse. The biomass is approximated by the multiplication of the mean number of leaves per plant (n) and the mean length of the longest leaf (l).

○ Inundated with sea water, ● inundated with fresh water (controls), [2 S.E.

Table 3. Soil and demographic characteristics in three populations of *Plantago major* ssp. *pleiosperma*.

Area	Soil (0-15 cm)			Demography			
	Organic matter (%)	Total N g.m ⁻²	Total P g.m ⁻²	Vegetation layer	Vegetative growth g/g day	Seedling recruitment	Life cycle
Kwade Hoek (salt marsh)	26.4	710	75	dense	0.023	low	perennial
Oostvoornse Meer (former beach plain)	1.3	115	42	open	0.021	low	short-lived
Angeren (river bank)	2.4	200	190	open	0.069	high	annual or short-lived perennial

Table 4. Reciprocal transplant experiment with three populations of *P. major* ssp. *pleiosperma*. (Results of greenhouse experiment in brackets). Analysis of variance: Dependent variables are log-transformed, except of RGR (shoots). Components of variance (sum of squares) are given as proportion of total variance (100%).

Dependent variable:	Independent variables		
	Site (nutrient level)	Population	Interaction
Vegetative development:			
dry weight of shoots	84.6 (88.9) *** (***)	1.3 (7.8) *** (***)	0.8 (2.3) *** (***)
RGR (shoots)	27.9 (90.9) *** (***)	2.3 (-) **	- (-)
Generative development:			
dry weight of seeds	a (97.1) (***)	a (0.2) (*)	a (1.1) (***)
Reproductive effort:			
seed dry weight/shoot dry weight	a (67.9) (***)	a (12.4) (***)	a (12.5) (***)

* P < 0.05, ** P < 0.01, *** P < 0.001, - no significance

a No analysis of variance performed: variance of the cells not normally distributed, because variance within 4 cells equals zero.

At the second area, Oostvoornse Meer, a former beach plain, and at the third, Angeren, a river bank exposed to alternating erosion and sedimentation, the organic matter content of the soil and the level of total N were relatively low, but at the latter site a high total amount of P was found. At Angeren the vegetative growth was higher than at the other sites. Growth and reproduction were significantly correlated with the amount of total phosphate in the soil as will be shown in a paper by Lotz & Blom (in prep.). Seedling recruitment was high at Angeren and an annual or short-lived perennial life cycle was observed at both sites. The number of *P. major pleiosperma* individuals per square metre was much higher at Angeren and Oostvoornse Meer than at Kwade Hoek. In order to investigate the nature of differences in growth and reproduction between the three populations of *P. major ssp. pleiosperma* a transplant and a greenhouse experiment were carried out.

The two experiments were conducted with seeds collected in 1982 from several plants in the three populations. The first experiment was a reciprocal transplant test and the second a greenhouse experiment in which different levels of mineral nutrients were supplied (optimal, intermediate, low). At the end of May 1983 in each of the three habitats, 70 seedlings of each population were planted alternately in a grid. The survival rate of the transplants depended on the study site ($P < 0.001$). The survival rate was highest at Angeren and lowest at Kwade Hoek. At Kwade Hoek and Oostvoornse Meer the survival rate of transplants from the Angeren population was lower than those of the other populations ($P < 0.001$). The relatively high survival rate at Angeren may be ascribed to low competition and to the relatively high level of nutrients in the soil of this site, and the low survival rate at Kwade Hoek probably to the effects of a high degree of competition due to the dense vegetation.

Statistical analysis of the results of the transplant experiment (Table 4) shows that the variance in vegetative development was mainly affected by the independent variable "site" (thus environmentally induced).

Table 4 also summarizes the results of the greenhouse experiment. The variance in vegetative and generative development was mainly due to nutritional factors. Dry weight of shoots also contained an important component of variance due to the factors "population" and "interaction of site and population". The variance in reproductive effort (expressed as the ratio of seed dry weight and shoot dry weight) was explained by nutrition as well as by population and by the two-way interaction. In order to illustrate the main factor effects and their interaction, the data of the reproductive effort are shown in Fig. 3. Reproductive effort was highest when nutrients were in optimal supply. The effect of mineral nutrition depended on the population. For example, at the lowest level of nutrition the reproductive effort of the Angeren population (corresponding with a nutrient-rich habitat) is significantly lower than that of the Oostvoornse Meer population (corresponding with a nutrient-poor habitat). Furthermore, the distribution of flowering frequencies (first dates of flowering) depended significantly on "population" (Lotz & Blom, in prep.).

In the second part of this paper the nature of differences in three populations of *P. major ssp. pleiosperma* from different, isolated areas were studied. Also in this species, the vegetative and generative development of individuals within the three populations has been determined mainly

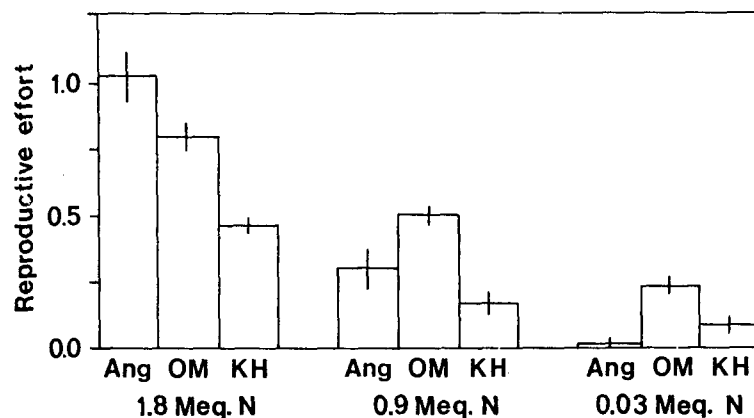


Fig. 3. Reproductive effort (dry weight of seeds/dry weight of shoots) of three populations of *Plantago major* ssp. *pleiosperma* in a greenhouse experiment (means \pm s.d. are given; $n = 6$). Three levels of Hoagland minerals were supplied. Ang: Angeren, OM: Oostvoornse Meer, KH: Kwade Hoek.

by environmental characteristics. This phenotypic plasticity may contribute to the ability of this subspecies to invade new habitats and to cope with changing environmental factors, a feature which is of great adaptive significance in the original habitat of these populations. However, some stages in the life cycle such as the survival rate of the transplants, the date of first flowering and the reproductive effort are partly genetically determined. It may be postulated that in this subspecies, selection occurs especially in these phases of the life cycle.

4. DISCUSSION

It can be stated that different life-history characteristics between both populations of *P. maritima* and between the populations of *P. major* ssp. *pleiosperma* were found. With both species a common hypothesis was that these different demographic characteristics are mainly caused by environmental factors. As was shown in this paper relatively extreme variations in the terrestrial environment, such as differences in grazing and flooding intensity, but also factors such as micro-climate, humidity, differences in light intensity and changes in chemical or physical conditions of the soil, may occur at distances which are too small for adaptation to occur by genetic differentiation (see also Bradshaw 1965). If this acts on populations without reproductive isolation selection favours plasticity as was

proved for *P. maritima*. Gregor (1956) found, too, that in an exposed area, occupied by a dwarf population of *P. maritima*, shallow depressions in the area caused taller plants than was the case with plants situated in the higher parts. In experiments under controlled conditions, however, these differences disappeared in five of the six samples examined.

If changes in space occur over reasonable distances and no exchange of genes occurs, plant species usually adapt themselves by the formation of genetically different populations. For example, the great distances between the studied sites of *P. major* ssp. *pleiosperma*, the species with the high degree of phenotypic plasticity, may explain the occurrence of some genetic polymorphism for certain characteristics.

When we finally compare the results obtained with *P. major* ssp. *pleiosperma* - a species of ruderal sites with strongly fluctuating environmental factors - with the results obtained with *P. lanceolata*, a species of grasslands with more stable environmental conditions (Lambers *et al.*, these proceedings) it appears that a certain similarity was found with respect to the phenomena genetic differentiation and fitness. For *P. lanceolata* selection during the seedling stage could not be clearly proved, which corresponds to the findings of Antonovics & Primack (1982). However, there are indications (J.M. van Groenendael, pers. comm.) that under certain circumstances selection during the first stages in the life cycle can not be totally excluded. In transplant experiments with *P. lanceolata* differences in fitness during the generative stage were found by Van der Toorn *et al.* (1984). In addition, Van Damme (these proceedings), demonstrated that in *P. lanceolata* selection for male sterility is also restricted to the adult phase. It can be concluded that for *P. lanceolata* and for *P. major* ssp. *pleiosperma*, genetic differentiation in these species occurs at least during the adult stages in the life cycle.

The question remains as to how far these results can also be applied to other plant species which are present in the *Plantago* habitats. This aspect will be investigated in the coming years.

5. ACKNOWLEDGEMENTS

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