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7 Genotypic and Phenotypic Variation Between *Plantago* Populations and the Relation with the Habitat

7.1 Phenotypic Plasticity in *Plantago maritima*

7.1.1 Introduction

Plantago maritima L. is a halophytic species occurring on beach plains, mud flats, salt marshes, sea dikes, coastal grasslands and sea cliffs. A great variety in morphological characteristics can be found; based on differences in leaf characters, Heukels (1910) distinguished three types.

Changes in morphological characters can be environmentally or genetically induced, referring to phenotypic plasticity and genetic differentiation, respectively (Bradshaw 1965). The nature of adaptive variation between individuals of plant populations was already discussed by Turesson (1922), who introduced the term ecotype as the genotypic response of a species to a particular habitat.

In this section the problem is discussed whether differences in the morphological properties *P. maritima* plants are caused by phenotypic plasticity or by genetic differentiation.

7.1.2 Morphological Differences Between Populations in Relation to Distance

Gregor (1939) found differences in morphological characteristics of 52 populations of *Plantago maritima*, collected in eight regions in North America and Europe. The American populations differed from the European ones in bract, sepal and seed indices, and in anther, scape and spike lengths. Differences were also found between the American and European populations for spike density, number of seeds per capsule, leaf indices, leaf tooth grade, spots on leaves and ciliation of the floral parts. The American and Greenland sea plantains were self-fertile, and those of Europe self-sterile. Based on significant differences in morphological characteristics, Gregor (1939) distinguished these populations taxonomically into five groups of sea plantain: some of these were allied to *P. maritima*.

In a further study, Gregor and Land (1950) compared the range of morphological differences between populations from different areas in Britain (inland, island and coastal areas) with those between *P. maritima* populations within a single area, a coastal area in Britain. Between the populations of the three

areas no significant difference was observed in the scape spread/height ratios. Surprisingly, great differences were found in scape and leaf length and in the ratios of scape spread and height between the populations of a mud, a salt marsh, a drained mud and a coastal meadow, all situated within the same coastal area. The latter results showed that in a particular coastal area the distribution of the sea plantain was continuous only in a relative sense; probably not all suitable habitats were occupied by the species.

7.1.3 Experiments on the Nature of Morphological Differences Between Populations

Experimental studies under controlled conditions are necessary to determine whether morphological differences between plants of different habitats are due to phenotypic plasticity or to genetic differentiation. Turesson (1930) showed in transplant experiments with *P. maritima* plants from salt marshes and sea cliffs that differences in height were reproducible every year with only very small fluctuations. These results indicate the existence of ecotypes. Other experiments are described by Gregor (1956); in an exposed area, occupied by a population of dwarf plants, shallow depressions occurred. The leaves and spikes of plants of *P. maritima* in the depression were taller than those of the higher parts of the area. Under experimental conditions, however, the differences disappeared in five out of six samples examined, indicating that changes in characteristics were mainly due to phenotypic plasticity.

This chapter describes the nature of relatively large differences in life characteristics between two adjacent populations of *P. maritima* (Blom 1983). The study was carried out at Kwade Hoek, a salt marsh on the island of Goeree, The Netherlands. The vegetation of the tidal area ranged from halophytic communities on heavy clay soils, to communities growing on inland dunes with a sandy soil (Westhoff et al. 1961). The boundaries between the communities of some sites were quite distinct, due to the presence of tidal creeks; on the more interior sites the transition was more gradual, as described for other salt marshes (Ranwell 1972, Beeftink 1975, 1977; Mahall and Park 1976). Part of Kwade Hoek is grazed by cattle from May to October.

Population I of *Plantago maritima* occurred mainly in the Armerion maritimae community with taxa like *Glaux maritima* L., *Juncus gerardii* Loisl., *Festuca rubra* L., *Aster tripolium* L. and *Artemisia maritima* L. The community was found on sandy clay soils, containing approximately 1200 mg Cl⁻ per 100 g dry soil.

Population II of *P. maritima* occurred in low numbers at transitions between the above community and the Saginetum maritimae community with *Sagina maritima* G. Don, *Plantago coronopus* L., *Cochlearia danica* L., *Honckenya peploides* (L.) Ehrh., *Centaurium littorale* (Turner) Gilmour, *Sagina nodosa* (L.) Fenzl. and *Festuca rubra* L. This community was found on a sandy soil with 4–5 mg Cl⁻ per 100 g dry soil.

Population I occurred in low-lying zones with a layer of sandy clay in the upper soil; individuals had an estimated age of more than 10 years. The plants mainly propagated vegetatively and only few seedlings were found; mortality of seedlings was very high. There was intensive grazing by cattle and regular flooding in this zone. The plants showed a prostrate growth form (Jerling and Andersson 1982).

Population II was located at the edge of the inner dunes. The zone was only flooded when the tide was extremely high. The vegetation was rarely grazed by cattle, and in winter the grazing pressure by rabbits was relatively high. In this upper zone, the individual plants were short-lived, about 3 years old. The reproduction of the plants was mainly by seed, which germinated rapidly, and seedlings were easily established. Most of the plants had an erect growth form.

The distance between population I and population II was about 300 m. In 1970 a dam was built in a neighbouring estuary and the high-water mark increased. It is probable that population II became established after this event.

Studies on allozyme variation and morphological characteristics suggested that the differences between the two populations did not have a genetic basis. Furthermore, there was a continuous mixing of seeds between both populations, due to spring-tide and the activities of wild and domestic animals. Therefore, we investigated if the differences in life-history characteristics could be explained as phenotypic plasticity. Since flooding with seawater and grazing were regular events at this site and in other sites with *P. maritima*, we investigated the effects of flooding and grazing on life-history characteristics.

The results of a sowing experiment (mixed seed sample) on sites of the habitats of both populations showed that emergence of *P. maritima* seedlings was significantly higher at the edge of the inner dunes (population II) than along the creek (population I); no difference was observed in seedling survival (Table 7.1). After 3 years, however, significantly more plants from sown seeds survived in the sites along the creek. Furthermore, plants from sown seeds flowered in significantly greater numbers at the edge of the inner dunes than along

Table 7.1. Emergence, survival and reproduction of *P. maritima* in sowing experiments in the field (Kwade Hoek, The Netherlands). In brackets: results obtained with naturally occurring plants. A mixed sample of seeds from both sites was used

Sowing sites at Kwade Hoek	Emergence (%)	Seedling survival (%)	Adult survival after 3 yrs ^a (%)	Reproduction after 3 yrs ^a	
				Generative (%)	Vegetative (%)
Along a creek (Population I)	18.3	60.0 (30.3)	72.5 (81.1)	12.9 (5.3)	48.9 (85.9)
Edge of inner dunes (Population II)	33.7	66.3 (74.8)	39.2 (32.7)	60.7 (72.4)	27.5 (16.1)

^a The age of the naturally occurring plants along the creek is estimated to be 10 yrs.

the creek, where more daughter rosettes were found. Differences in reproduction between adjacent populations of *P. maritima* have also been described by Jefferies (1977): the start of the flowering period of a population on a beach plain was about 2 weeks earlier than that in an upper marsh. Even such relatively small differences in flowering time reduced gene flow between populations (Jefferies 1977).

The results of the sowing experiments on seedling survival at the edge of the inner dunes were in accordance with those of demographic studies of *P. maritima* plants at Kwade Hoek. At the grazed sites fewer (naturally occurring) seedlings were found than in the sown plots. No significant differences between survival of the naturally occurring adults and adults from sown seeds were observed. Between both habitats, the differences in form of reproduction were more pronounced than those observed in the sowing experiments. The differences in emergence were not due to the substrate (sand vs. sandy clay) but possibly to the salinity of the soil (Blom 1983). Germination of *P. maritima* was inhibited at relatively high NaCl concentrations, which can be found in the upper soil layer of the sites of population I (along the creek), whilst 25% sea water caused a significant decrease in emergence. In the field, seedling emergence increased after periods of rainfall, undoubtedly due to a decrease in the salinity of the soil. Jerling (1981) showed that the survival of *P. maritima* seedlings strongly depended on the microtopography of the soil. Mortality due to inundation has been ascribed to light deficiency and to toxic effects of H₂S. Seedling establishment and survival of *P. maritima* were also strongly reduced by trampling (Blom 1983).

The effects of increased salinity on growth of *P. maritima* was studied by Cooper (1982) and Blom (1983). Cooper concluded from an investigation of eight species occurring on salt marshes, that the growth of species from the upper marsh was strongly limited by salinity and by waterlogging (Table 7.2), in contrast with species of the lower marsh. The position of *P. maritima* in the salt marsh studied by Cooper (1982) was just between the upper and lower marsh. Shoot and root weights of *P. maritima* were reduced when grown under saline conditions for 2 months. This species was more tolerant to waterlogging and to salinity than *Festuca rubra*, *Juncus gerardii* and *Armeria maritima*, species from the upper salt marsh. In contrast, the growth of *Salicornia europaea* was stimulated by a high level of NaCl in the substrate (Figs. 7.1 and 7.2). Blom (1983) observed that plants grown for over 2 months under saline conditions started to produce more biomass. After about 4 months no differences were observed between the biomass of plants grown under saline and non-saline circumstances. At Kwade Hoek *P. maritima* occurred in saline and in less saline habitats. Floristic surveys of salt marshes in Britain also showed that the species occurs in both lower and higher zones of salt marshes (e.g. Gimingham 1964; Boorman 1971; Beeftink 1975; Adam et al. 1977). Under saline conditions *P. maritima* plants showed significantly more daughter rosettes than under non-saline conditions (Fig. 7.3). In both treatments the plants were grown from the same seed pool which indicates that the formation of daughter rosettes depended on the environmental circumstances.

Table 7.2a. The effects of waterlogging and salinity on the mean whole plant dry wt. yield (mg) of species from a salt marsh near Portaferry, Britain. (Results from Cooper 1982)

	Drained non-saline	Waterlogged non-saline	Drained saline	Waterlogged saline
<i>Festuca rubra</i> ^a	345.5	138.9	150.8	80.5
<i>Juncus gerardii</i>	150.5	77.3	59.4	34.8
<i>Armeria maritima</i>	278.5	158.0	189.9	81.1
<i>Plantago maritima</i>	723.6	712.8	521.6	499.7
<i>Aster tripolium</i>	904.2	908.3	740.3	489.0
<i>Triglochin maritima</i>	154.9	130.5	158.6	92.4
<i>Puccinellia maritima</i>	369.0	424.1	243.8	282.6
<i>Salicornia europaea</i>	31.6	19.4	40.7	35.4

^a Sequence of species represents a seaward progression.

Table 7.2b. Summary of analysis of variance of the data in a

	Waterlogging	Salinity	Interaction
<i>Festuca rubra</i>	**	**	NS
<i>Juncus gerardii</i>	*	**	NS
<i>Armeria maritima</i>	**	*	NS
<i>Plantago maritima</i>	NS	**	NS
<i>Aster tripolium</i>	NS	**	NS
<i>Triglochin maritima</i>	**	NS	NS
<i>Puccinellia maritima</i>	NS	**	NS
<i>Salicornia europaea</i>	*	**	NS

*, ** Significance of F values at the $P = 0.05$ and 0.01 levels respectively; NS, not significant.

Next to salinity, grazing was an important environmental factor in differentiation between the habitats of the two populations. The separate effects of trampling and clipping on biomass production and propagation of *P. maritima* have also been studied (Blom 1983). Trampling caused a significantly higher biomass production than non-trampling or clipping. Furthermore, significantly more daughter rosettes were formed by trampling (Fig. 7.4).

In treatments of simulated inundation and of grazing, the treatment determined to a large extent the morphological characters of the individual plants. The differences found in the experiments were in accordance with the differences in morphological characteristics and demographic features of the populations in the field between the non grazed and rarely inundated habitat at the edge of the inner dunes and the grazed and inundated habitat along the creek. These results suggest a plastic reaction in growth and reproduction characteristics of *P. maritima*.

Most of the differences which had been recognized between *P. maritima* populations related to morphological characteristics such as growth forms and reproductive features. There were some examples of direct evidence of genetic differentiation. Jefferies (1977) and Jefferies and Perkins (1977) stated that in

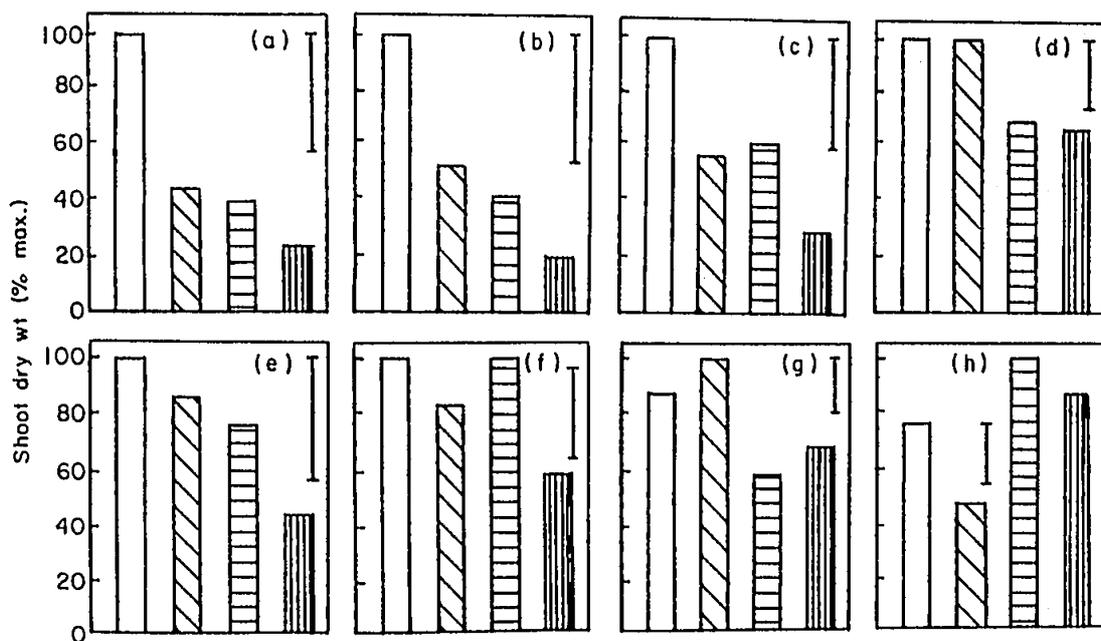


Fig. 7.1. The effects of waterlogging and salinity on the shoot dry weight yield of a *Festuca rubra*, b *Juncus gerardii*, c *Armeria maritima*, d *Plantago maritima*, e *Aster tripolium*, f *Triglochin maritima*, g *Puccinellia maritima* and h *Salicornia europaea*: □, drained non-saline; ▨, waterlogged non-saline; ▤, drained saline; ▥, waterlogged saline. Vertical bars indicate confidence intervals above which any two treatment means are significantly different. Results obtained after 2 months. (Cooper 1982)

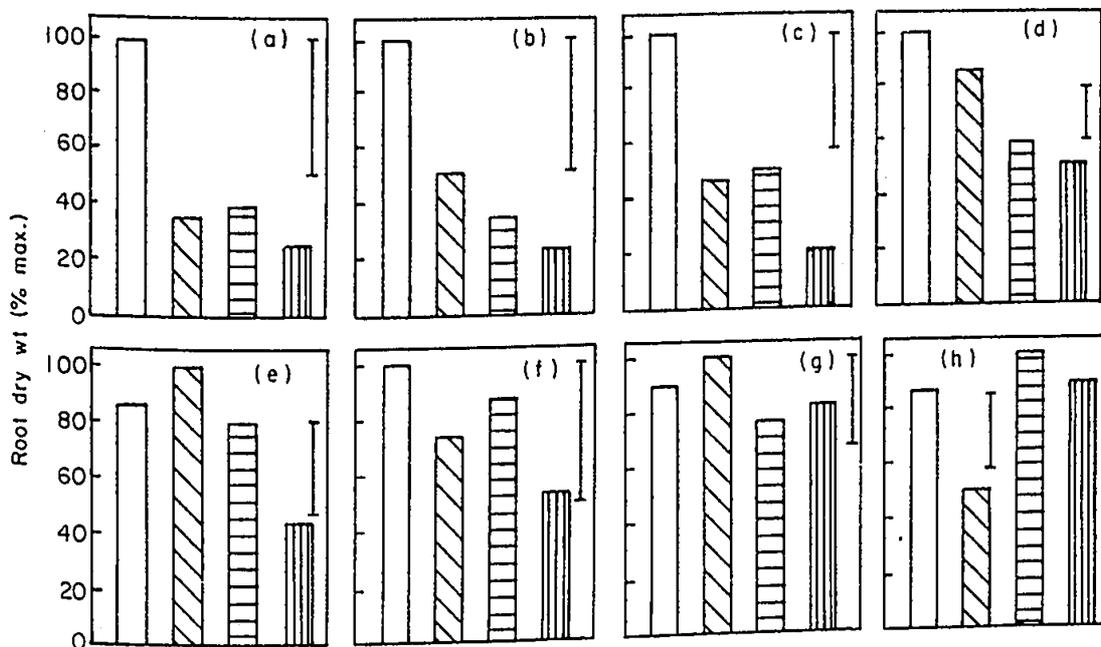


Fig. 7.2. The effects of waterlogging and salinity on the root dry weight yield of a *Festuca rubra*, b *Juncus gerardii*, c *Armeria maritima*, d *Plantago maritima*, e *Aster tripolium*, f *Triglochin maritima*, g *Puccinellia maritima* and h *Salicornia europaea*: □, drained non-saline; ▨, waterlogged non-saline; ▤, drained saline; ▥, waterlogged saline. Vertical bars indicate confidence intervals above which any two treatment means are significantly different. Results obtained after 2 months. (Cooper 1982)

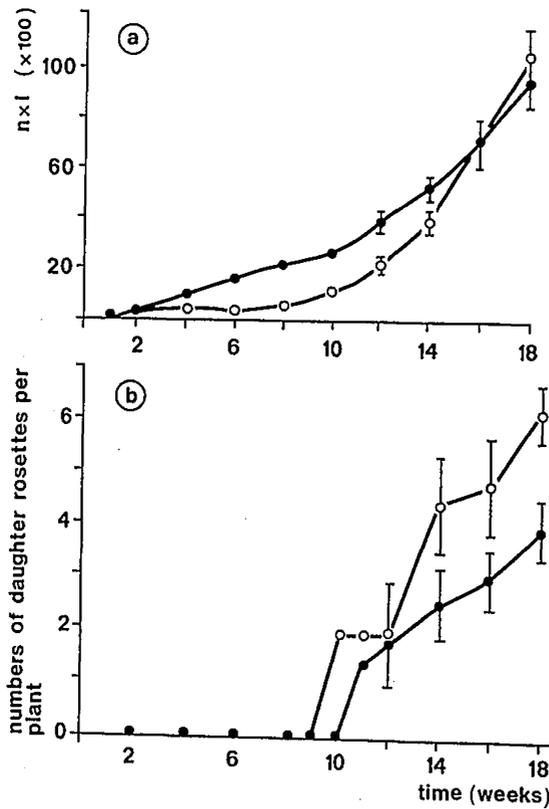


Fig. 7.3a, b. The effects of inundation with sea water as well as with fresh water on the biomass production (a) and the mean number of daughter rosettes per plant (b) of *Plantago maritima* grown separately in pots in the greenhouse. The biomass is approximated by the multiplication of the mean number of leaves per plant (n) and the mean length of the longest leaf (l). \circ — \circ , inundated with sea water; \bullet — \bullet , inundated with fresh water (controls). I 2S.E. $n = 20$. (Blom 1983)

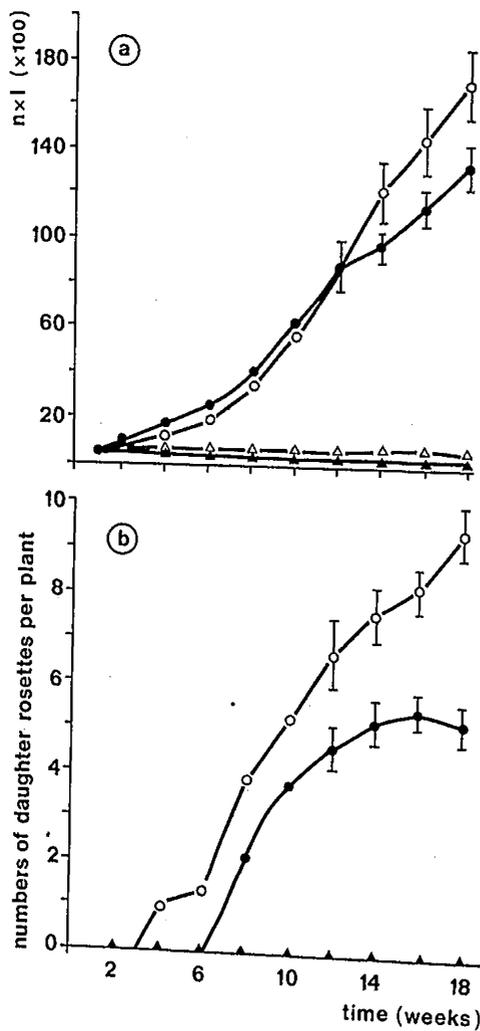


Fig. 7.4a, b. The effects of clipping and trampling on biomass production (a) and on the mean number of daughter rosettes per plant (b) of *Plantago maritima* grown separately in pots in the greenhouse. The biomass is approximated by the multiplication of the mean number of leaves per plant (n) and the length of the longest leaf per plant (l). Δ — Δ , clipping treatment; \circ — \circ , trampling treatment; \blacktriangle — \blacktriangle , combined clipping and trampling; \bullet — \bullet , controls, I 2S.E. $n = 20$. (Blom 1983)

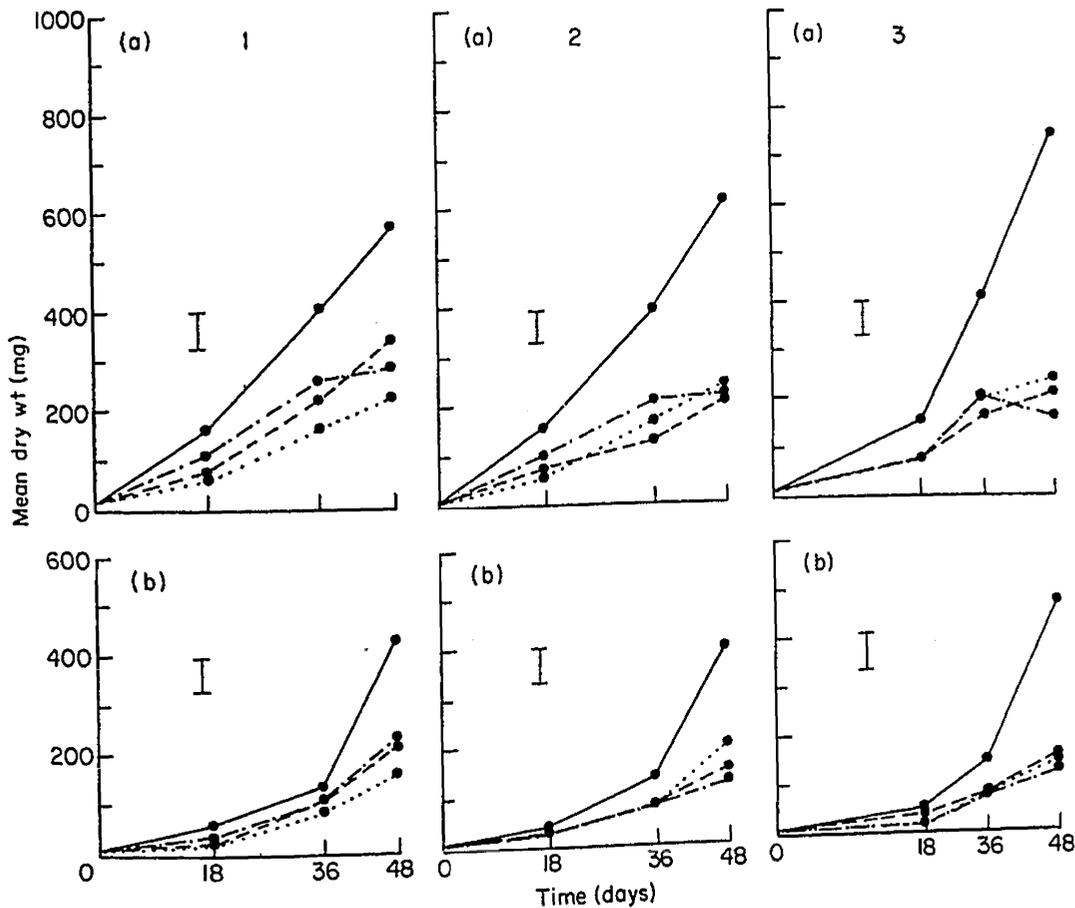


Fig. 7.5. The mean dry weight of plants from populations of *Plantago maritima* in sand cultures which received different concentrations of inorganic nitrogen. 1 population from strand line of a lower marsh; 2 population from upper marsh (high elevation site); 3 population from bank of a drainage channel in upper marsh; a shoot dry weight; b root dry weight. Treatments as follows: ●—●, 10^{-3} M nitrate-nitrogen; ●---●, 10^{-4} M nitrate-nitrogen; ●-●, 10^{-4} ammonium-nitrogen; ●···●, 10^{-3} M nitrate-nitrogen. The vertical bars represent the least significant difference between means. (Jefferies 1977)

the upper levels of some coastal marshes the growth of certain species was limited by the availability of nitrogen. In a greenhouse experiment significant differences were found in growth response to nitrogen between populations of *P. maritima* (Jefferies 1977). Shoots of plants from the lower marsh populations showed significantly faster growth than those of plants from the upper marsh (Fig. 7.5). Significant differences in growth response were also found between populations within the upper marsh. These differences had developed between populations which were separated by a distance of less than 10 m (Fig. 7.6). Analyses of variance showed significant population X treatment interactions, demonstrating genetic differentiation (Table 7.3). This phenomenon was also observed for other salt marsh species as *Aster tripolium*, *Salicornia europaea* and *Triglochin maritima*. Jerling (1985) reported on genetic differentiation in fitness-related characters in *Plantago maritima* along a gradient situated on a Baltic sea shore meadow. Grazing intensities and flooding frequencies varied along the gradient and differences in densities of *P. maritima* and in covering

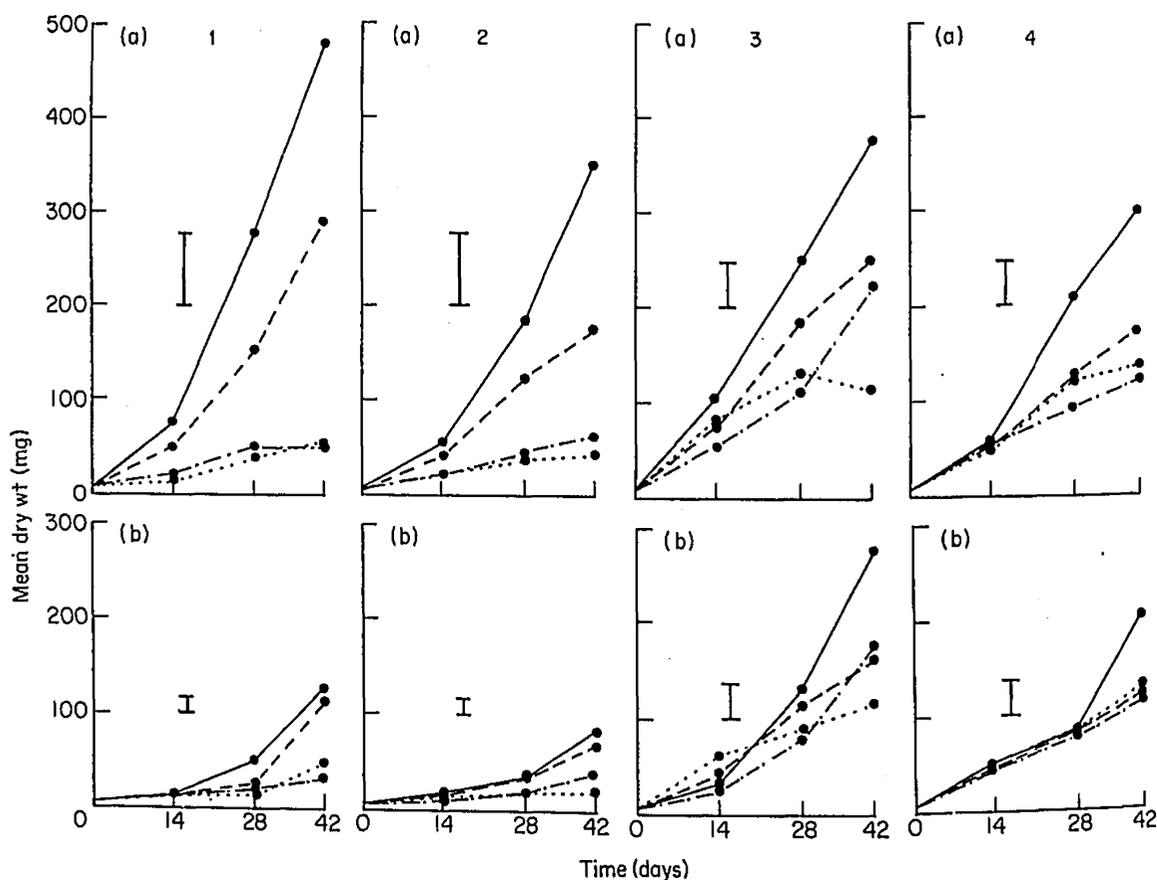


Fig. 7.6. The mean dry weight of plants from populations of *Plantago maritima* in sand cultures which received different concentrations of inorganic nitrogen. 1 population from bank of a drainage channel in upper marsh; 2 population from upper marsh (high elevation site); 3 plants with erect growth habit; 4 plants with prostrate growth habit; a shoot dry weight; b root dry weight. Treatments as follows: ●—●, 10^{-3} M nitrate-nitrogen; ●---●, 10^{-4} M nitrate-nitrogen; ●-●, 10^{-4} M ammonium-nitrogen; ●····●, 10^{-5} M nitrate-nitrogen. The vertical bars represent the least significant difference between means. (Jefferies 1977)

of the adjacent vegetation were found. Results of demographic as well as experimental studies strongly suggest a genetically determined variation in seed size, germinability, growth patterns, and allocation of dry matter among plants of *P. maritima* occurring in the separated parts of the gradient. Selection due to grazing and competition resulted in genetic differentiation. Life-history traits of some genotypes appeared to be of adaptive value. Restricted gene flow and variation in male sterility also affected genetic differentiation in the gradient studied by Jerling (1988).

7.1.4 Conclusion

P. maritima is considered to be a highly variable species, especially in morphological characters as growth form (erect versus prostrate) and form of reproduction (generative versus vegetative). In spite of the evidence of a certain genetic differentiation in some physiological processes and the existence of

Table 7.3. Analysis of variance of the growth responses of *Plantago maritima* populations at Stiffkey Marsh (Britain) to different levels of nitrogen. For treatments see Figs. 7.5 and 7.6. (Results from Jefferies 1977)

Sources	<i>Plantago maritima</i>			<i>Plantago maritima</i> (upper marsh, high elevation site and drainage channel populations only)			<i>Plantago maritima</i> (erect and prostrate populations)		
	d.f.	Shoots significance level	Roots significance level	d.f.	Shoots significance level	Roots significance level	d.f.	Shoots significance level	Roots significance level
Treatments (T)	3	***	***	3	***	***	3	***	***
Harvests (H)	2	***	***	2	***	***	2	***	***
Population (P)	2	*	NS	1	*	***	1	***	***
TxH	6	***	***	6	***	***	6	NS	NS
PxT	6	**	NS	3	NS	*	3	NS	NS
PxH	4	NS	NS	2	NS	**	2	***	**
PxTxH	12	*	NS	6	NS	NS	6	NS	NS
Error	180			120			120		
Total	215			143			143		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

d.f. degrees of freedom.

ecotypes in separate areas, other results of experimental work on adjacent populations indicate that differences in morphological characters were due to phenotypic plasticity. Furthermore, the results described in the section showed that the reaction of *P. maritima* plants to flooding with sea water and trampling depended on the stage in the life cycle: relatively extreme variations in environmental factors may occur over distances which were too small for adaptation by genetic differentiation. If selection acted on populations without reproductive isolation, selection favoured plasticity of *P. maritima*.

7.2 Ecotypic Differentiation in *Plantago major*

7.2.1 Introduction

A number of varieties of *Plantago major* have been described by taxonomists (e.g. Pilger 1937; see also Sagar and Harper 1964). Several papers deal with the heritable component of the characters in which these varieties differ (Groot and Boschhuizen 1970; Mølgaard 1976; Warwick and Briggs 1980; Van Dijk 1984). In order to evaluate these genetic differences between varieties as ecotypic differences, a relationship between characters and environment should be presented showing that these characters are adaptive for the genotypes under the reigning environmental conditions. Warwick and Briggs (1980) described differences in fitness of the lawn type and the roadside of type ssp. *major*, showing that each type is adapted only to its own specific environment.

A genetic analysis of some of the characters which are important for ecotypic differentiation has been made by Van Dijk (1984; Sect. 6.1). Genes, contributing to several of these characters, were found in the vicinity of the *Pgm-1* locus, suggesting a gene complex around this locus.

Knowledge about the level of gene flow has been presented elsewhere (Sect. 6.1 and 6.5): in addition to short-distance gene flow, distinct long-distance seed transport occurs due to sticky seeds.

P. major is a predominantly selfing species and occasionally hybrids between different ecotypes will be formed. Because such hybrids may be important in the formation of genotypes adapted to habitats different from both parental habitats, the role of hybrids may be important in an evolutionary sense.

In a series of experiments the detailed adaptation of several ecotypes to their respective habitats was analyzed.

7.2.2 Origin and Characteristics of the Parental Plants

The 12 original parental plants were collected from various localities in The Netherlands (Van Dijk and Van Delden 1981). Six of the 12 plants: G₁, H₁₉, S₈, A₂, H₄₄ and Z₂, were genetically analyzed (Van Dijk 1984) as far as leaf and inflorescence morphology and developmental characters were concerned. According to morphology and habitat the 12 plants were classified into five