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Genetic variability in *Plantago* species in relation to their ecology

4. Ecotypic differentiation in *P. major**

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Summary. Twelve *Plantago major* plants, good representatives of their populations, appeared to be genetically different for several characters which are important for adaptation to the respective habitat conditions. These characters are: juvenile growth, leaf morphology, production of secondary rosettes, flowering time, seed production, seed size and adult leaf production. The adaptive value of some of these characters was investigated by transplantation experiments in the field and by intraspecific competition experiments. The roadside type of ssp. *major* was adapted to trampling by being erect and elastic. The lawn type of ssp. *major* was adapted to a short, frequently cut, vegetation by being prostrate and by producing leaves with short petioles throughout the growth season. In the natural situations in which ssp. *pleiosperma* occurs, growth rate and first-year seed production of this subspecies were considerably higher than that of ssp. *major*. In a number of experiments, F_1s and F_2s were included, derived from crosses between the original plants. The F_1s were generally rather well adapted to both parental habitats, whereas the F_2s appeared to be less fit. The various alternatives in spending resources relevant for fitness optimization in different habitats are discussed.

Key words: Ecotypes – *Plantago major* – Adaptive Strategies – Natural hybrids – Resource allocation

Introduction

Differences in adaptive strategy are usually investigated by comparing different species; much less research has been done at the intra-species level. A main distinction has been made between r- and K-strategies representing emphasis on reproduction (r) or maintenance of the individual (K). A refinement for plants has been represented by Grime (1977), who divided these strategies into three components: ruderal, competitive and stress-tolerant. A further subdivision, relevant for the species under examination, is possible. 'Disturbance', which means the destruction of biomass (Grime 1977), plays an important role in habitats of the various *P. major* types. The lawn type of ssp. *major* is able to maintain its competition with grasses and tall herbs only if the vegetation is frequently cut by mowing or grazing. The trampling-resistant (roadside) type of this subspecies is adapted to a different kind of disturbance: combined pressure and torsion. These disturbances happen during the growth season; tolerance means that a perennial lifetime can be realized. In the third type, represented by the subspecies *pleiosperma*, the disturbance is low during the growth season but very severe at the end, leaving only a low probability for winter survival, thus favouring annuality.

Populations of *P. major* are mostly very homogeneous in their morphological phenotype, suggesting that differences in strategy mainly occur among populations. The species is built up of genetically different populations, each of them occupying a relatively small niche. The small-scale gene flow within populations is rather low, partly due to a high selfing rate (Van Dijk et al. 1988). In a heterogeneous environment, differential selection will easily lead to sharp boundaries between different ecotypes. For these reasons the individuals sampled are good representatives of their (sub)populations. The existing ability for long-distance seed transport (by means of stickiness of the seeds) will nevertheless lead to a situation in which a reasonably well-adapted genotype will show up soon when a new habitat is created.

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This paper deals with the analysis of the characteristics of the various ecotypes of *P. major* which are met in nature, illustrating the way in which a wide ecological amplitude is realized by genetically different populations. An idea of the underlying adaptive mechanisms will be attained by measuring fitness components of different types in different environments and by competition experiments. Because hybrids between ecotypes are met at their boundary regions, F_1s and F_2s are included in some of the experiments. A certain degree of clustering on the chromosomes of genes important for ecotypic differentiation has been described earlier (Van Dijk 1984), which may be of importance for the fitness of F_2 and later generations in either parental habitat and also in eventual new habitat types. This gene clustering will influence the rate of forming of new combinations of characters.

Materials and methods

Origin and some characteristics of the parental plants

The twelve parental plants studied were collected from the localities shown in Van Dijk and Van Delden (1981), except for K. which is situated about 7 km northeast of W. Six of the 12 plants – G_1, H_{19}, S_8, A_2, H_{44} and Z_2 – were genetically analysed earlier (Van Dijk 1984) as far as leaf and inflorescence morphology and developmental characters are concerned.

In Table 1 the 12 plants are classified into five groups according to morphology and habitat. This classification will be used throughout this paper. The mean number of seeds per capsule is also given in Table 1, being the main distinctive trait for the subdivision into subspecies. Finally a short description of the original habitat is included.

Table 1. The parental types

Type	Mean seed no. per capsule	Origin
ssp. *major* lawn type		
G_1	9	lawn, frequently mown
ssp. *major* erect type		
G_7	9	roadside, mown once a year, not trampled
H_{19}	11	dike around sedimentary fields of a sugar factory, grazed by sheep
S_8	8	sandy shore of a newly made lake
W_9	12	dune valley near lake; extensively grazed
ssp. *pleiosperma* coastal type		
K_1	16	seashore meadow, occasionally flooded
L_6	20	sea-dike, between basalt bricks
V_{13}	14	muddy seashore, frequently flooded
ssp. *pleiosperma,* inland type; small-seeded		
A_2	19	side of new ditch; heavy clay soil
H_{44}	21	see H_{19}
Z_2	27	riverbank; edge of drinking pool for cattle
ssp. *pleiosperma,* inland type; large-seeded		
A_4	15	see A_2

The plants were mostly good representatives of the populations from which they were sampled. Only in H. a mixed ssp. *major*/ssp. *pleiosperma* population, and in A. containing two types – types like A_2 and types like A_4 did a distinct intrapopulational variability for relevant morphological characters exist. In the Z population, seed number per capsule varied between 20 and 30. G_1 and G_7 were collected from different populations in the same area.

The seeds used for the experiments

All parental plants appeared to be highly homozygous for ecologically relevant morphological characters, as was concluded from the uniformity of the progeny following selfing. The homozygosity for allozyme loci is also very high in natural populations (Van Dijk et al. 1988); the plants chosen were all completely homozygous for the allozyme loci studied. The parental plants were selfed for a few generations. The seeds obtained from the last generation were used for the various experiments, representing the parental types. F_1s and F_2s were obtained as described by Van Dijk and Van Delden (1981). A few F_1 plants appeared to be selfing products. In the greenhouse experiments they were nevertheless used, but were added to the appropriate set of parental plants after their genotypes were determined by electrophoresis (thus the numbers of the parental types are sometimes larger than nine, and in the F_1s are consistently smaller). In the transplantation experiment, false F_1 plants were not used.

The experiments

The greenhouse experiment. In this experiment pots with turf, each containing nine seeds of a particular parental type, F_1 or one of the reciprocal F_2s were placed at 25°C and 16 h light daily. Germination occurred in four (large seeds) to five (small seeds) days and was 100%. Four weeks after sowing, the plants were measured and transplantated to separate pots. After another 3 weeks they were measured again. During the remaining experimental period (until 20 weeks after sowing), inflorescences were harvested as soon as all capsules were ripe.

The transplantation experiment. In May 3-week-old juveniles were implanted in four different plots in terrains where *P. major* occurs naturally: a path, a lawn, a riverbank and a dune valley. The position of all plants (the various genetic types were mixed up in a constant way for each square meter) could be recovered by laying a transparent plastic sheet with labeled holes over the appropriate square meter. The plants were scored for survival and seed production.

The garden trampling experiment. In this experiments 100 seeds of each of the twelve parental types were sown as a mixture in summer. The area (2 × 1 m) was trampled thoroughly about twice a week during summer, autumn and the following spring. The established plants were identified by electrophoresis.

The competition experiments. Seeds of the five types, G_1, H_{19}, K_1, Z_2 and A_4, were laid to germinate in the greenhouse in containers with turf at regular distances of 2 cm in a 12 × 12 grid in such a way that each type was surrounded directly by all four other types. Germination was 100%. To avoid edge effects, only the inner square of 10 × 10 plants was evaluated for this experiment (20 plants per type per container). Four different regimes were applied: (1) no special treatment (called unlimited growth);

(2) cutting at 2 cm height twice a week; (3) trampling five times a week; (4) application of large amounts of NaCl in the soil, ranging from about 200 to 300 m*M*. After completion of seed production, seeds were collected and shoots were harvested for the determination of dry weight values.

Electrophoresis

Each of the 12 parental types was allozymatically unique. Electrophoresis, used for identification, was carried out as described by Van Dijk and Van Delden (1981), applying the modification described in Van Dijk et al. (1988).

Statistical operations

In the greenhouse experiment, all differences between the 12 parental types were tested for significance by ANOVA, followed by Tukey's test for comparison of all means. In Tables 2–5 the types are arranged according to their mean values, increasing from the left to the right; non-significant combinations are connected by common underlining. In the transplantation experiments, differences between subspecies and generations were tested by the same method.

Results

The series of experiments decribed below were performed to enlighten the various genetically determined differences in strategy between the parental types. The greenhouse experiment shows the differences in vegetative growth and reproductive output of the various types under optimal conditions. These conditions were more or less comparable with one of the field situations, the river bank which has, however, a strongly variable but always limited length of the growth season. In the transplantation experiment survival and reproduction were measured in this and in other field situations by implanting seedlings. Additionally, one experiment was carried out starting with seeds: the garden trampling experiment. Finally intra-specific competition under various conditions was studied in the greenhouse, described as competition experiments. In some of the experiments, F_1s and F_2s of crosses between different types were included.

The greenhouse experiment

Four weeks after sowing, all plants were still in the vegetative (juvenile) stage. A measurement of growth was performed by taking leaf length and number of leaves. Leaf length affects the leafs ability to compete for light. In this respect, the ratio between length of the longest leaf and seed weight can be considered as a relevant measure of growth rate. This ratio (Table 2) appeared to be very well correlated with fresh weight relative growth rate (P. Dijkstra, unpublished results). The ratios were considerably higher for ssp. *pleiosperma* than for ssp. *major:* in spite of their usually lower seed weights, members of the former subspecies showed the longest leaves after 4 weeks. A_4, combining a high seed weight with a high

Table 2. The greenhouse experiment: juvenile growth characters measured 4 weeks after sowing

Type	*n*	Longest leaf no.[a]			Length of longest leaf (mm)	Seed[b] weight	Growth[c] rate
		3	4	5			
G_1	9	–	2	7	39	0.285	137
G_7	9	–	9	–	56	0.275	204
H_{19}	10	6	4	–	44	0.242	182
S_8	9	1	8	–	46	0.281	164
W_9	9	–	9	–	40	0.250	160
K_1	9	2	7	–	58	0.177	328
L_6	12	5	7	–	74	0.204	363
V_{13}	12	–	12	–	63	0.236	267
A_2	10	9	1	–	53	0.188	282
H_{44}	9	–	9	–	67	0.152	441
Z_2	10	3½	6½	–	59	0.146	404
A_4	9	–	9	–	102	0.305	334
F_1s[d]							
G_1Z_2	5	–	5	–	70	0.300	233
Z_2G_1	3	–	2	1	42	0.199	211
$H_{19}H_{44}$	4	–	4	–	93	0.278	335
$H_{44}H_{19}$	4	–	4	–	86	0.264	326
A_2S_8	8	3	5	–	58	0.241	241
$V_{13}G_1$	6	–	5	1	49	0.272	180
L_6V_{13}	6	–	6	–	75	0.110	682
Z_2W_9	8	3	5	–	36	0.160	225
S_8W_9	9	3½	5½	–	60	0.472	127
F_2s[d]							
G_1Z_2	9	2	6½	½	48		
Z_2G_1	9	1	7	1	39		
$H_{19}H_{44}$	9	–	9	–	88		
$H_{44}H_{19}$	9	–	8	1	91		
A_2S_8	9	4½	4½	–	85		
S_8A_2	9	4	5	–	87		
$V_{13}G_1$	9	–	7½	1½	49		

Comparison of all means:

No. of longest leaf ($F_{11,103} = 13.8$; $P < 0.001$)

A_2 H_{19} L_6 Z_2 K_1 S_8 G_7 W_9 V_{13} H_{44} A_4 G_1

Length of longest leaf ($F_{11,103} = 28.1$; $P < 0.001$)

G_1 W_9 H_{19} S_8 A_2 G_7 K_1 Z_2 V_{13} H_{44} L_6 A_4

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[a] The oldest leaf (neglecting the cotyledons) is no. 1, etc. If two leaves were equally long, ½ has been given to both leaf numbers

[b] Mean weight (mg) of the seeds in the whole batch of the appropriate cross

[c] The ratio of length of the longest leaf and seed weight (mm/mg)

[d] Progenies are indicated by the subsequent names of female and male parent

Table 3. The greenhouse experiment: leaf dimensions (mm) measured at 7 weeks after sowing (mean values)

Type	*n*	Petiole length	Lamina length	Lamina width	Total length
G_1	9	28	73	52	101
G_7	9	62	88	64	150
H_{19}	10	55	88	62	142
S_8	9	52	83	61	135
W_9	9	46	74	55	120
K_1	9	80	106	68	186
L_6	12	83	126	76	209
V_{13}	12	55	95	64	149
A_2	10	74	118	65	192
H_{44}	9	70	108	76	178
Z_2	10	54	92	54	146
A_4	9	76	124	69	199
F_1s (combined reciprocals)					
G_1Z_2	8	38	84	56	122
$H_{19}H_{44}$	8	86	118	75	204
A_2S_8	8	71	110	69	180
$V_{13}G_1$	6	37	72	52	109
L_6V_{13}	6	68	110	70	178
Z_2W_9	8	45	75	49	119
S_8W_9	9	49	90	67	139
F_2s (combined reciprocals)					
G_1Z_2	18	35	75	50	109
$H_{19}H_{44}$	17	75	110	71	185
A_2S_8	18	74	108	69	182
$V_{13}G_1$	9	40	79	56	118

Comparison of all means:

Petiole length ($F_{11,103}=56.0$; $P<0.001$)

G_1 W_9 S_8 Z_2 H_{19} V_{13} G_7 H_{44} A_2 A_4 K_1 L_6

Lamina length ($F_{11,103}=44.0$; $P<0.001$)

G_1 W_9 S_8 H_{19} G_7 Z_2 V_{13} K_1 H_{44} A_2 A_4 L_6

Lamina width ($F_{11,103}=18.2$; $P<0.001$)

G_1 Z_2 W_9 S_8 H_{19} V_{13} G_7 A_2 K_1 A_4 H_{44} L_6

Total length ($F_{11,103}=54.8$; $P<0.001$)

G_1 W_9 S_8 H_{19} Z_2 V_{13} G_7 H_{44} K_1 A_2 A_4 L_6

growth rate, was the largest type of the whole set. After 4 weeks, the third or fourth leaf was usually the longest one, as indicated in Table 2. In the lawn type G_1, however, the fifth leaf was generally the longest one. Obviously, G_1 produced more – and shorter – leaves than the other types. Particularly because the petioles were relatively short in this type, one may conclude that G_1 is able to build up a closed rosette. The F_1 plants were usually intermediate in their juvenile growth characters; the maternal effect of seed weight was strongly shown in those cases where both parents produced seeds with distinctly different individual weights. The F_2 data are only partially presented in Table 2 because no data on parental seed weights were available.

Seven weeks after sowing, almost all types were already flowering. Their vegetative development had been more or less completed: all types had already produced their longest leaves and were investing a considerable part of their effort in reproduction. Only G_1, and to a lesser extent V_{13}, continued making leaves to maintain their rosettes. The final maximum leaf lengths of the various types were not directly correlated with the juvenile growth rate. The moment of slowing down vegetative growth in favour of reproductive investment was also important for the ultimate leaf size and varied among types. W_9, V_{13} and Z_2 were, in their groups (ssp. *major*, coastal *pleiosperma* and inland *pleiosperma*, respectively), smaller than the other representatives (Table 3). Generally, ssp. *pleiosperma* tended to be larger than ssp. *major*, especially when lamina length is considered. Table 3 also shows the very short petioles of G_1. In the F_1s and F_2s, leaf morphology and leaf dimensions were sometimes intermediate between their parents, sometimes close to one of them.

The slowing down of the growth of the central rosette was associated with the formation of inflorescences at lower nodes, or occasionally with the formation of secondary rosettes. In Table 4 the number of flowering spikes, developing spikes and secondary rosettes is presented. After 7 weeks, the inland *pleiosperma* types, and especially A_4, were already abundantly flowering. The coastal *pleiosperma* types showed a considerably larger number of secondary rosettes than the other *pleiosperma* types. Looking at the number of developing inflorescences, however, the coastal *pleiospermas* surpassed the other groups because the secondary rosettes started to produce inflorescences too. In the F_1s, the characters of the reproductively most active parent appeared to dominate in most cases. In the F_2s, and especially in $G_1 \times Z_2$ and $H_{19} \times H_{44}$, a return towards the midparent value of reproductive investment could be noticed.

As is to be expected, the seed production started first in the early flowering inland *pleiosperma* types (Table 5). In the first week of seed ripening, which is the 12th week after sowing, Z_2 produced the largest number of seeds and A_4 the greatest seed biomass. A few weeks later, the ssp. *major* and the coastal *pleiosperma* types also started to ripen seeds on a larger scale. At the end of the growing period, 2,000–2,500 mg of seed biomass had been produced on the average by most of the types. The early starting Z_2, which was still rather small vegetatively because of its small initial seed weight and early switch-over to generative growth, was only able to produce about 1,000 mg of seed biomass. G_1, which was constantly

Table 4. The greenhouse experiment: inflorescences and secondary rosettes produced 7 weeks after sowing

Type	*n*	No. of inflorescences																				No. of sec. rosettes					
		Flowering										Developing															
		0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5
G_1	9	–	6	3	–	–	–	–	–	–	–	–	–	3	6	–	–	–	–	–	–	5	2	2	–	–	–
G_7	9	4	5	–	–	–	–	–	–	–	–	–	–	8	1	–	–	–	–	–	–	4	4	1	–	–	–
H_{19}	10	9	1	–	–	–	–	–	–	–	–	9	1	–	–	–	–	–	–	–	–	8	2	–	–	–	–
S_8	9	8	1	–	–	–	–	–	–	–	–	–	4	5	–	–	–	–	–	–	–	9	–	–	–	–	–
W_9	9	1	3	5	–	–	–	–	–	–	–	–	–	7	2	–	–	–	–	–	–	5	3	1	–	–	–
K_1	9	5	3	1	–	–	–	–	–	–	–	–	–	–	3	5	1	–	–	–	–	–	1	2	5	1	–
L_6	12	2	–	5	4	1	–	–	–	–	–	–	–	1	4	2	4	–	1	–	–	–	4	1	5	2	–
V_{13}	12	–	9	3	–	–	–	–	–	–	–	–	–	–	1	1	5	1	2	2	–	–	–	6	4	2	–
A_2	10	–	–	3	4	2	1	–	–	–	–	–	–	8	2	–	–	–	–	–	–	7	1	2	–	–	–
H_{44}	9	–	–	2	1	6	–	–	–	–	–	–	–	1	7	1	–	–	–	–	–	–	4	3	2	–	–
Z_2	10	–	1	2	3	4	–	–	–	–	–	–	–	5	5	–	–	–	–	–	–	8	2	–	–	–	–
A_4	9	–	–	–	–	–	–	4	1	2	2	–	–	–	4	1	2	–	1	–	1	2	3	4	–	–	–
F_1s (combined reciprocals)																											
G_1Z_2	8	–	–	–	4	2	2	–	–	–	–	–	–	3	5	–	–	–	–	–	–	6	2	–	–	–	–
$H_{19}H_{44}$	8	–	–	–	6	2	–	–	–	–	–	–	–	6	2	–	–	–	–	–	–	–	4	3	1	–	–
A_2S_8	8	1	–	2	4	1	–	–	–	–	–	–	–	8	–	–	–	–	–	–	–	3	4	1	–	–	–
$V_{13}G_1$	6	1	2	2	1	–	–	–	–	–	–	–	–	1	5	–	–	–	–	–	–	2	1	3	–	–	–
L_6V_{13}	6	–	–	6	–	–	–	–	–	–	–	–	–	–	2	2	–	1	–	1	–	–	–	2	4	–	–
Z_2W_9	8	1	3	2	1	1	–	–	–	–	–	–	1	6	1	–	–	–	–	–	–	5	3	–	–	–	–
S_8W_9	9	–	2	5	2	–	–	–	–	–	–	–	–	7	2	–	–	–	–	–	–	4	3	2	–	–	–
F_2s (combined reciprocals)																											
G_1Z_2	18	4	1	2	9	1	1	–	–	–	–	–	4	9	5	–	–	–	–	–	–	16	2	–	–	–	–
$H_{19}H_{44}$	18	2	1	4	3	7	1	–	–	–	–	–	–	10	6	2	–	–	–	–	–	2	5	9	1	–	1
A_2S_8	18	–	1	3	10	4	–	–	–	–	–	–	1	10	6	1	–	–	–	–	–	7	8	3	–	–	–
$V_{13}G_1$	9	1	3	5	–	–	–	–	–	–	–	–	–	6	2	1	–	–	–	–	–	6	1	1	1	–	–

Comparison of all means:

No. of flowering inflorescences ($F_{11,103} = 54.5$; $P < 0.001$)

H_{19} S_8 G_7 K_1 V_{13} G_1 W_9 L_6 Z_2 A_2 H_{44} A_4

No. of developing inflorescences ($F_{11,103} = 24.1$; $P < 0.001$)

H_{19} S_8 G_7 A_2 W_9 Z_2 G_1 H_{44} K_1 L_6 A_4 V_{13}

No. of secondary rosettes ($F_{11,103} = 17.2$; $P < 0.001$)

S_8 H_{19} Z_2 A_2 W_9 G_1 G_7 A_4 H_{44} L_6 K_1 V_{13}

making new leaves, also stopped at about 1,000 mg of seed biomass. To a lesser extent, V_{13} also expended more resources in favour of leaves at the cost of seed production. For both latter types, a delay in seed ripening was noticed, caused by shading of the prostrate spikes by new leaves.

The final number of seeds produced at the end of the growing period varied from about 15,000 per plant for the ssp. *pleiosperma* types A_2, A_4, H_{44} and L_6 to less than 5,000 per plant for G_1. The F_1s were usually producing more seeds than either of the parents, the F_2s being closer to the midparent value. The weight of individual seeds differed up to a factor of 3.6 between the largest (S_8) and the smallest (Z_2) seeds initially produced (Table 6). Ssp. *major* types produced the largest seeds and maintained their seed size reasonably well during the ripening period. In ssp. *pleiosperma,* seed size was lower and decreased considerably in some cases, especially in A_4 (Table 6) in which the first seeds were over 250 µg and the last seeds at the end of the period were less than

Table 5. The greenhouse experiment: cumulative seed production after 12, 13, 14, 16 and 20 weeks. Numbers (*n*) and biomass (mg); mean values per plant

Type	Week 12		Week 13		Week 14		Week 16		Week 20	
	n	mg	*n*	mg	*n*	mg	*n*	mg	*n*	mg
G_1	0	0	0	0	41	11	886	205	4,551	1,012
G_7	0	0	219	60	1,009	269	4,495	1,180	8,463	1,987
H_{19}	0	0	0	0	127	33	2,362	606	12,811	2,635
S_8	0	0	45	16	408	146	3,195	1,114	6,372	2,123
W_9	0	0	364	92	1,360	323	5,764	1,265	8,644	1,796
K_1	0	0	77	16	911	152	8,106	1,280	13,211	1,957
L_6	0	0	810	136	2,361	397	9,146	1,441	16,518	2,510
V_{13}	0	0	0	0	168	32	3,676	663	10,621	1,675
A_2	118	24	1,232	235	3,617	633	10,167	1,657	16,702	2,493
H_{44}	323	54	2,220	390	4,836	769	12,152	1,763	14,687	2,083
Z_2	1,118	115	3,109	312	4,876	496	7,926	814	12,093	1,188
A_4	716	206	2,602	706	4,726	1,167	12,805	2,357	15,108	2,604
F_1s (combined reciprocals)										
G_1Z_2	567	96	1,779	298	2,310	383	4,959	781	10,012	1,533
$H_{19}H_{44}$	376	111	1,292	500	3,693	1,156	8,067	2,179	11,946	2,935
A_2S_8	155	51	1,000	355	2,012	661	5,703	1,621	9,830	2,534
$V_{13}G_1$	0	0	111	37	111	37	1,995	581	6,073	1,487
L_6V_{13}	0	0	0	0	98	29	2,313	566	9,972	2,226
Z_2W_9	65	11	655	104	1,266	202	4,123	659	7,167	1,077
S_8W_9	105	44	398	142	1,591	586	5,358	1,756	8,203	2,631
F_2s (combined reciprocals)										
G_1Z_2	86	14	511	89	936	168	3,611	635	6,055	1,021
$H_{19}H_{44}$	151	39	1,166	322	2,585	699	7,462	1,728	10,208	2,273
A_2S_8	143	45	1,067	319	2,917	791	7,846	1,865	11,590	2,631
$V_{13}G_1$	0	0	0	0	0	0	1,786	429	5,968	1,358

Comparison of all means:

n after 12 weeks ($F_{11,103}=8.5$; $P<0.001$)

G_1 G_7 H_{19} S_8 W_9 K_1 L_6 V_{13} A_2 H_{44} A_4 Z_2

n after 13 weeks ($F_{11,103}=18.3$; $P<0.001$)

G_1 H_{19} V_{13} S_8 K_1 G_7 W_9 L_6 A_2 H_{44} A_4 Z_2

n after 14 weeks ($F_{11,103}=19.7$; $P<0.001$)

G_1 H_{19} V_{13} S_8 K_1 G_7 W_9 L_6 A_2 A_4 H_{44} Z_2

100 µg. In the F_1s and F_2s, seed sizes were usually higher than the midparent values and sometimes higher than either of the parents.

After finishing their seed production, most plants exhibited a 'winter rosette' appearance (a circle of small leaves at the base of each rosette, while the large leaves died). Only G_1 and V_{13} still had fresh green leaves. Most A_4 plants died, which can be interpreted as a lack of investment in winter survival, while expending everything in favour of reproduction.

The transplantation experiment

One month after the implantation in May, nearly all plants were still found in good condition. At the end of August only a minor portion of the plants on the path were still alive, whereas in the other three plots, the plants which died did so mainly because of aspecific disturbance caused by cattle, rabbits, moles etc., usually leaving most plants of each type alive. The surviving types on the path appeared to be mainly ssp. *major* types and progenies of

Table 6. The greenhouse experiment: mean seed weights (μg) over the whole production period, over the first 2 weeks of production and the last 2 weeks of production

Type	Whole period	First two weeks	Last two weeks
G_1	222	238	218
G_7	235	267	185
H_{19}	206	253	185
S_8	333	358	320
W_9	208	238	174
K_1	148	167	148
L_6	152	168	132
V_{13}	158	189	133
A_2	149	191	113
H_{44}	142	176	125
Z_2	98	100	74
A_4	172	271	107
F_1s (combined reciprocals)			
G_1Z_2	153	168	136
$H_{19}H_{44}$	246	387	176
A_2S_8	258	355	201
$V_{13}G_1$	245	318	233
L_6V_{13}	223	268	210
Z_2W_9	150	159	143
S_8W_9	321	357	323
F_2s (combined reciprocals)			
G_1Z_2	169	174	121
$H_{19}H_{44}$	227	276	188
A_2S_8	227	299	227
$V_{13}G_1$	228	240	234

Table 7. Survival in the transplantation experiment. Numbers implanted in May and numbers of survivors in the end of August; mean survival percentages for the two subspecies and F_1s and F_2s. In the lawn, all plants survived

Type	Path		Riverbank		Dune valley	
	n	survived	*n*	survived	*n*	survived
G_1	24	12	24	14	32	27
G_7	24	4	24	18	32	12
H_{19}	24	9	24	14	32	10
S_8	24	8	24	16	32	17
W_9	24	11	24	13	32	16
K_1	16	0	24	13	32	11
L_6	12	0	24	9	32	16
V_{13}	24	1	24	6	32	22
A_2	24	2	24	14	32	7
H_{44}	24	2	24	10	32	20
Z_2	16	0	24	14	32	11
A_4	18	1	24	14	32	17
F_1s (combined reciprocals)						
G_1Z_2	24	11	24	16	32	25
$H_{19}H_{44}$	24	10	24	15	32	21
A_2S_8	16	1	24	13	32	17
F_2s (combined reciprocals)						
G_1Z_2	96	17	96	55	128	91
$H_{19}H_{44}$	96	18	96	58	128	90
A_2S_8	96	17	96	62	128	79
ssp. *major*		37%		63%		51%
ssp. *pleiosperma*		4%		48%		46%
F_1s		31%		61%		66%
F_2s		18%		61%		68%

Comparison of all group means:

Survival at the path ($F_{3,14}=9.9$; $P<0.001$)

ssp. *pleiosperma* F_2s F_1s ssp. *major*

Survival at the riverbank ($F_{3,14}=2.8$) and dune valley ($F_{3,14}=1.7$): no significant differences between groups

the crosses (Table 7). In the lawn, all plants survived. In this first year, only a few plants produced one or a few very small spikes on the path, so that no conclusion about seed production could be drawn. Unfortunately, all plants died in the late summer after an exceptionally long period of drought that year. An idea of seed production in a trampled situation is given in the garden trampling experiment.

Survival was considerable in both the lawn and the dune valley: 50%–60%, but no inflorescences were formed during the first season. The riverbank plants, in contrast, flowered abundantly in the first summer. The production of spikes was more or less comparable with the seed production in the greenhouse experiment. According to Table 8 the relative mean production of inflorescences at the end of the season was about 1:2.3:3:2.1 for ssp. *major*, ssp. *pleiosperma*, F_1s and F_2s, respectively. Assuming a constant number of capsules per spike, and a similar weight of individual seeds for both greenhouse and riverbank, the ratio of seed numbers produced in the latter situation would be about 1:3.3:2.4:2.5.

In the next year all riverbank plants had disappeared, due to the influence of the river. A fraction of the lawn and dune valley plants were still alive and were producing seeds in some cases. In Table 8 the numbers of inflorescences produced, together with a qualitative survey of the seed production in the lawn situation, is presented. In the dune valley, no clear differences between the types could be established, whereas in the lawn, G_1 was distinctly the most successful one. The general tendency of a good survival and seed production of the F_1s and F_2s could also be noticed here.

The garden trampling experiment

Several dozens of *P. major* seedlings were detected in the experimental plot, together with some grass species

Table 8. Seed production in the transplantation experiment. Number of inflorescences at the end of the first season at the riverbank; number of surviving and flowering plants in the next year in the dune valley (quantitatively) and the lawn (qualitatively)

Type	Riverbank: mean no. of inflorescences per plant	Dune valley		Lawn[a]	
		surv.	flow.	surv.	flow
G_1	0.6	25	3	+	++
G_7	1.2	9	0	–	
H_{19}	1.3	5	0	–	
S_8	2.0	10	0	–	
W_9	1.2	16	1	+	+
K_1	2.6	11	0	–	
L_6	2.0	6	0	–	
V_{13}	3.5	14	4	–	
A_2	3.6	5	1	–	
H_{44}	3.7	15	4	–	
Z_2	2.2	11	0	–	
A_4	3.6	2	1	–	
F_1s (combined reciprocals)					
G_1Z_2	4.1	21	6	+	+
$H_{19}H_{44}$	4.0	15	0	+	+
A_2S_8	3.6	13	0	+	+
F_2s (combined reciprocals)					
G_1Z_2	1.9	77	24	+	+
$H_{19}H_{44}$	2.7	44	2	+	–
A_2S_8	3.6	66	6	+	–
ssp *major*	1.3	41%	3%		
ssp. *pleiosperma*	3.0	29%	4%		
F_1s	3.9	51.%	6%		
F_2s	2.7	49%	8%		

Comparison of all group means:

No. of inflorescences per plant at the riverbank ($F_{3,14}=12.3$; $P<0.001$)

ssp. *major* F_2s ssp. *pleiosperma* F_1s

Flowering in the dune valley ($F_{3,14}=0.9$): no significant differences between groups

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[a] – = no plants; + = a part of the plants; ++ = all plants present

Table 9. Genotypes identified in the garden trampling experiment. Original number of seeds: 100 per type

Type	Identified	Seed-producing
G_1	4	0
G_7	0	–
H_{19}	6	4
S_8	4	0
W_9	5	0
K_1	0	–
L_6	0	–
V_{13}	9	0
A_2	0	–
H_{44}	0	–
Z_2	0	–
A_4	0	–

(mainly *Poa annua*) and some other herbs. Those *P. major* plants which reached sufficient biomass to be used for electrophoresis were thus identified. Nearly all of them belonged to the subspecies *major*. All ssp. *major* types except G_7 were present, together with a few V_{13} plants. In the next spring and summer, the most vital-looking plants were H_{19} and G_1; H_{19} was the only type which produced seeds (Table 9). Its spikes were standing erect and after trampling they got back into their upright position immediately, without severe damage. Also the leaves were relatively resistant to trampling. The spikes of G_1 remained small and were damaged by the trampling such that no seed production was possible. The other identified types, S_8, W_9 and V_{13}, remained small plants and did not generate inflorescences.

The competition experiments

In accordance with the expectations derived from the greenhouse experiment, A_4 was the dominating type under the unlimited growth conditions. Only H_{19} and K_1 could slightly compete with A_4; the other two types were severely shaded and did not reach the generative phase, except for the single surviving Z_2 plant. Perhaps not only shading but also root competition was involved, but this factor was not studied. The seeds produced originated mainly from A_4, although the types H_{19} and K_1 could also produce considerable numbers of seeds (Table 10).

The container in which the vegetation was frequently cut was strongly dominated by G_1. Only this type could retain enough leaf biomass to realize a reasonable seed production. Most individuals of the other types survived but could save only a few very small spikes containing not more than a few seeds: most of their leaves and spikes were removed by the cutting.

In the trampled container, only G_1 and H_{19} survived reasonably well, as could be expected from the earlier experiments dealing with trampling. Almost all ssp. *pleiosperma* plants died in the juvenile phase. As in the garden trampling experiment, only H_{19} produced seeds, due to the elasticity and toughness of the inflorescences, whereas the G_1 spikes were fatally damaged.

The addition of NaCl to the soil had no strong influence on the competition ratios of the various types. As in the situation of unlimited growth, A_4 was dominating. The coastal type K_1, however, appeared to be more successful here compared with the situation in the first container. All types showed a high degree of succulence and survived to some extent. These findings were in agreement with the observations of a set of various types implanted in a coastal meadow daily flooded by the sea: all plants survived during the summer season and became very succulent. The better tolerance for salinity of K_1 compared with the other four types is consistent with the findings of Kuiper (1984), who described K_1 as being better salt-tolerant than G_1 and Z_2.

Table 10. The results of the competition experiment: survival, shoot dry weight per plant, seed production per plant and product of survival fraction and seed production

Type	Survived (of 20 plants)	Mean shoot dry weight (mg per plant)	Mean no. of seeds produced per plant	Product of survival and seed production
1. Unlimited growth				
G_1	13	101	0	0
H_{19}	11	304	546	300
K_1	13	369	448	291
Z_2	1	330	363	18
A_4	14	289	1,457	1,020
2. Cut at 2 cm height				
G_1	20	414	283	283
H_{19}	18	18	0	0
K_1	19	53	2	2
Z_2	10	16	0	0
A_4	20	27	1	1
3. Trampled				
G_1	9	100	0	0
H_{19}	14	106	581	407
K_1	1	139	0	0
Z_2	0	–	–	0
A_4	1	22	0	0
4. High salt concentration				
G_1	8	126	200	80
H_{19}	6	81	67	20
K_1	14	529	1,029	720
Z_2	5	80	200	50
A_4	13	702	1,981	1,288

Discussion

Alternatives in strategy

The results indicate that a wide array of ecotypes occur within the species *P. major*. Within both subspecies, which are readily recognizable from these experiments, a lot of varieties appear to be distinctly adapted genotypes. A detailed descriptive subdivision of the species into numerous varieties makes no sense, because each type is slightly different in an apparently continuous multidimensional space. A better approach is the analysis of the various points in which alternative possibilities can be realized – controlled by the genome – concerning the production and allocation of biomass and other developmental processes. In Table 11, a list of the various alternative possibilities in several developmental stages is shown with the 12 types arranged in the appropriate positions. The alternatives are:

Germination versus staying dormant. These alternatives are included for reason of completeness, but have not been studied here. In *P. lanceolata,* genetic differences in germination behaviour have been described earlier (Van Dijk 1985).

High versus low juvenile growth rate. A bimodal distribution of growth rates has been observed, coinciding with the subdivision into the two subspecies *major* and *pleiosperma*. Growth rate will be associated with environ-

Table 11. Classification of the 12 parental types according to their strategy components

Alternatives	G_1	G_7	H_{19}	S_8	W_9	L_6	K_1	V_{13}	A_2	H_{44}	Z_2	A_4
1. Germination behaviour (no observations)	–	–	–	–	–	–	–	–	–	–	–	–
2. Juvenile growth rate (l – low; h – high)	l	l	l	l	l	h	h	h	h	h	h	h
3. Leaves (t – tall; c – closed rosette)	c	t	t	t	t	t	t	c	t	t	t	t
4. Switch to generative growth (e – early; m – medium; l – late)	l	m	l	m	m	m	m	l	e	e	e	e
5. Secondary rosettes (f – few; m – many)	f	f	f	f	f	m	m	m	f	m	f	f
6. Leaf growth during reproductive period (m – much; l – little)	m	l	l	l	l	l	l	m	l	l	l	l
7. Survival at the end of the reproductive period (h – high; l – low)	h	h	h	h	h	h	h	h	h	h	h	l
8. Seed size (S – very small; s – small; l – large; L – very large)	l	l	l	L	l	s	s	s	s	s	S	l

mental circumstances. In ruderal conditions, mostly species with high growth rates are met while in a competitive situation, low growth rates are more frequent (Grime and Hunt 1975). A low growth rate may be the consequence of a 'better quality' of the organs produced, e.g. tougher leaves and spikes in order to achieve trampling-resistance.

Forming tall leaves versus a closed rosette. The preference for more leaves with short petioles instead of few, but longer erect leaves is easily understood from the occurrence in nature of the respective types. This difference is also described by Warwick and Briggs (1980), who observed that erect types in a lawn regime were severely defoliated by mowing. On the other hand, prostrate types produced less seeds in a higher vegetation. It is remarkable that trampling resistance needs an erect growth-form in this species.

The time of switch-over to the generative phase. A counterbalance of different advantages can be noticed here. On the one hand an early switch-over to seed production will ensure progeny when the season is short and the probability for surviving in the winter is low. On the other hand, a longer period of vegetative growth will lead to larger quantities of seeds produced in a longer season, or will be of importance for better winter survival and the subsequent production of larger quantities of seeds in the next year(s) (Hawthorn and Cavers 1978). This perennial strategy is represented by ssp. *major;* flowering in the year of germination is unusual under natural conditions.

The initiation of secondary rosettes. Coastal *pleiosperma* types do clearly invest more in secondary rosettes than all other types. The adaptive value could be that the same plant size is realized in a more prostrate growth habit if a plant possesses many side rosettes instead of one large rosette with tall leaves and spikes. A prostrate growth habit may be favourable in coastal vegetations.

The production of new leaves during the flowering period. In a situation without competition, the existing leaves at the beginning of the flowering period will be sufficient to complete the season as regards the provision of energy and nutrients necessary for seed set and eventually for saving storage materials for a next season. A further investment in leaves would happen at the cost of seed production. A continuity in making new leaves is supposed to be important, however, in a competitive situation like a lawn in which the maintenance of a closed rosette is of decisive importance for the ensurance of a place in the vegetation on the long term. This strategy is represented by G_1 and V_{13}; their seed production is, indeed, consistently lower.

Winter survival. P. major is supposed to be a perennial species. But there is a tendency towards annual behaviour in the inland *pleiosperma* types. The most extreme is A_4, which often dies after having flowered abundantly. Although it is possible to keep these plants alive at the end of the season, especially when kept under sub-optimal conditions, clearly little investment in survival is made under optimal development. Also the other inland *pleiosperma* types look less prosperous at the end of the growth season. This is undoubtedly related to the habitat in which these types occur. Survival probability in the winter season is low at a riverbank because of the thorough disturbance of the soil by the river. But in some winters, survival is possible and surviving plants will have a relatively large seed production in the next year. Ploughed arable land is a second example of a habitat which favours annuality.

Seed size. If the alternatives are: producing many small seeds or fewer large seeds, the highest fitness will be reached at the point at which a further increase in seed number is compensated by a loss in seedling success. This compensation point is related to the conditions of germination and juvenile growth in the pertinent habitat. The ssp. *major* types are dealing with dense vegetation or with physical threat, whereas ssp. *pleiosperma* types germinate in favourable circumstances. Seeds can thus be smaller in the latter subspecies. An exception must be made for the larger-seeded A_4, which is supposed to be adapted to arable land conditions in which there exists a competition with crop plants.

Crosses between ecotypes

A first generation hybrid obtained by crossing two members of different ecotypes (which is often observed in nature) does still contain two complete sets of coadapted genes. In the F_2, these complete sets are lost. The best investigated F_1s and F_2s, $G_1 \times Z_2$, $H_{19} \times H_{44}$ and $A_2 \times S_8$ (crosses between members of different subspecies), show what happens in natural situations. The F_1s seem to be reasonable well-adapted to physically severe conditions but are also early seed-producing under favourable circumstances. In all cases, the F_2s are less well adapted to physically severe conditions and also have a lower reproductive output than the related F_1s.

An occasional intermingling of the genomes of different ecotypes is important for the evolutionary potential of this species. New combinations of traits allow adaptations to new environmental conditions. For instance, the occurrence of the lawn-type characteristics in the coastal *pleiosperma* V_{13} could have resulted from the introgression of genes from one ecotype into the other.

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