Theor Appl Genet (1988) 75: 772–778

Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L.

3. Natural selection in an F_2 population *

K. Wolff

Department of Genetics, Centre of Biological Sciences, Kerklaan 30, NL-9751 NN Haren, The Netherlands

Received October 25, 1987; Accepted November 30, 1987
Communicated by P. M. A. Tigerstedt

Summary. Plants of an F_2 generation derived from crosses between two ecotypes of *Plantago lanceolata* L. had previously been studied in a greenhouse. In the present experiment, F_2 plants were transplanted into their original habitats (a hayfield and a pasture). Six allozyme loci were used as markers in the analysis of survival and performance of the segregating genotypes. Fitness differences between the plants were large enough to detect natural selection. In both transplantation sites selection appeared to operate, though in different ways. In the hayfield habitat directional selection was hypothesized and both survival and performance of the plants were related to genotype, with the genotypes originating from the hayfield almost always performing better. In the pasture habitat where the habitat is not uniform and unpredictable hazardous droughts occur, survival was nearly genotype independent and environmentally determined, whereas performance of the plants was genotype dependent. The expression of two morphological characteristics, number of leaves and leaf length, was often not in concordance with the greenhouse results and was contradictory in both sites. Expression of both characters in the field, therefore, appeared to be strongly dependent on the general performance and growth conditions of the plant and not on the genotype.

Key words: *Plantago lanceolata* – Life history variation – Allozyme markers – Fitness

Introduction

Many kinds of experiments have been done to demonstrate natural selection and to elucidate its causes (Endler 1986). In plants, a divergence in genetic composition of populations was often shown that was correlated with ecological factors (for review see Venable 1984; Bradshaw 1984). In these cases selection could be indirectly inferred from the differentiation observed. Experiments designed to establish the occurrence of selection in plants, in the wild and in the laboratory, have been done using the cyanogenesis polymorphism of, among others, *Lotus* and *Trifolium* species (see Endler 1986), metal tolerance polymorphisms (e.g. Hickey and McNeilly 1975) and the Adh polymorphism of *Bromus mollis* (Brown et al. 1976). In these cases the selecting agent could be directly linked with the outcome of selection. Short term experiments including only part of the different life stages of a plant may lead, however, to wrong conclusions as selection coefficients and the direction of selection may vary widely among life stages, while selection may occur only during a short period in one of the life stages (Clegg et al. 1978). Sexual selection is especially hard to demonstrate or to infer from experiments (Endler 1986).

Ecotypic differentiation in the species *Plantago lanceolata* has been described by several authors (Böcher 1943; Cavers et al. 1980; Teramura et al. 1981; Wolff and Van Delden 1987). Antonovics and Primack (1982) claimed that environmental differences were more important in determining differences in life-history characters than genetic differences. The reciprocal transplant experiments of Van der Toorn et al. (1984) showed, however, a better performance of the genotypes in their own environment in the adult phase, whereas Van Groenendael (1985) revealed genetic differences for life-history characteristics between two populations in almost all life history stages. Wolff (1987) analysed, in a greenhouse experiment, mor-

* Grassland Species Research Group Publication no. 143

phological variability in a pseudo F_2 generation derived from crosses between two ecotypes. In this experiment allozymes were used as markers for chromosome segments. Genes for quantitative traits, situated on the specific chromosome segments, could be localised by means of these markers. Many correlations of morphological and life history characters with the markers were observed. This experiment further showed that quantitative trait loci were scattered all over the genome, while a positive correlation between the number of heterozygous allozyme loci and generative growth was observed. As *Plantago lanceolata* shows relatively high levels of plasticity (Wolff and Van Delden 1987) the question arises as to whether the morphological differences found in the laboratory lead to measurable fitness differences in the field.

In the present study an answer to this question was sought by the analysis of two different populations originating from two contrasting habitats. One habitat, Heteren (He), was a hayfield with high competition for light in a high vegetation. Westduinen (Wd) was a pasture, extensively grazed by cows, leading to a low and open vegetation where unpredictable droughts may occur (for a description of the habitats see Van der Toorn et al. 1984 and Van Groenendael 1985). The populations had differentiated for many life history and morphological characters (Wolff and Van Delden 1987). Furthermore, it was posed by Wolff and Van Delden (1987) that selection in He is uniform and unidirectional, giving rise to a specific hayfield ecotype. Mortality and selection in Wd is thought, however, to be more dependent on coincidental conditions because growing conditions are not uniform in time and space in this habitat, leading to selection for high plastic genotypes with pasture ecotype characteristics.

Selection was studied in two contrasting habitats by transplanting a segregating F_2 population in the original habitats of the parental populations. The necessary conditions to demonstrate the presence of differential natural selection were present. Morphological variability was present in the F_2 population and appeared to be heritable (Wolff 1987). A relationship between the morphological characteristics of the ecotypes and fitness can be inferred from the correlation between differences in population and habitat. By scoring the genotypes of the surviving F_2 plants and their performance, it can be revealed whether the morphological differences observed in the greenhouse lead to differential survival in the field and whether selection in both sites is of the same nature.

Materials and methods

Crosses and electrophoresis

Cultivation circumstances, the way crosses were performed and electrophoresis techniques have been described earlier by Van Dijk (1985), Van Dijk and Van Delden (1981) and Wolff and Van Delden (1987). The enzyme loci used in this paper are Gpi-1 (glucose-phosphate-isomerase), Got-1 and -2 (glutamate-oxaloacetate-transaminase), Pgm-1 and -2 (phosphoglucomutase) and To-2 (tetrazolium-oxidase). Pgm-2 and To-2 are in the same linkage group (Van Dijk 1985). Two plants from Heteren (He) and two plants from Westduinen (Wd) were selected with the purpose of obtaining as many electrophoretic differences between parents as possible. An F_2 population obtained by making crosses within a single F_1 could possibly provide inbreeding effects (Van Damme 1983). Therefore, a reciprocal pseudo F_2 cross was made by crossing plants from two separate F_1 crosses. The genotypes of the plants and the crosses are shown in Table 1. In this experiment only the F_2^2 population (originating from Wd via the maternal line) was used. For further details on the material used see Wolff (1987).

Transplantation experiment

Seeds from the F_2 generation (F_2^2) were germinated in petri dishes on wet filter paper in the dark. Seeds that did not germinate spontaneously (5%) were cut open at the root tip end. The seedlings were transferred on large trays filled with a mixture of sand and normal soil. The trays were placed outdoors after 1 week in the greenhouse. Approximately 4 weeks after germination, 300 plants (with an average of 4 leaves) were transplanted into each of the original habitats of their grandparents, in Heteren and in Westduinen. The plants were all 15 cm apart in a grid, marked at the corners, of 4×75 plants (0.45×11.10 m). To be able to trace back the transplanted plants the plots were placed were *P. lanceolata* plants

Table 1. Genotypes of plants used in making the pseudo-F_2 (characters in *bold face* are alleles originating from He) and description of the crosses

	Gpi-1	Got-1	Got-2	Pgm-1	Pgm-2	To-2	Crosses
He1	**SI**	**II**	**SF**	**NN**	**II**	**NF**	
Wd1	SF	SI	SS	SN	SF_2	NN	He1 × Wd1 → F_1(HW)
He2	**SI**	**II**	**SF**	**NN**	**IF_1**	**NN**	
Wd2	SF	SI	SS	SN	IF_2	NN	Wd2 × He2 → F_1(WH)
F_1 (HW)	**SI**	**SI**	**SF**	**SN**	**SI**	**NF**	
F_1 (WH)	**IF**	**SI**	**SF**	**SN**	**IF_2**	**NF**	F_1(HW) × F_1(WH) → F_2^1 F_1(WH) × F_1(HW) → F_2^2

were present, though not abundant. After 2 weeks the plants in Wd, torn out by grazing cows, were replaced by new plants of the same origin. On all Wd plants the leaves were cut off to prevent the young plants from severe water loss and from being torn out by cows. In Wd only 182 plants survived the first 3 months. In He 5 plants died within 2 weeks. After 3 months only 20 plants could not be traced or were dead in He. After 1 year the surviving plants were gathered again, their place in the grid, the number of leaves, the length of the longest leaf and a score for their performance (on a scale of 1–9), hereafter referred to as the performance score, was noted. Plants with a performance score of 1 had no leaves. A score of 2 meant only brown leaves, 3 meant no growth observed in the field and greenish-brown leaves. From score 4–9 plants showed an increasing health and size, measured as the number and size of the leaves. In Wd only scores up to 6 were found; drought and a severe winter probably caused great losses and poor growth circumstances. Plants in He suffered mainly from grazing slugs and digging moles. The plants were transplanted into a greenhouse and their genotypes were determined. In all tables and for each locus the genotype homozygote for alleles originating from He is given first and the genotype possessing two alleles from Wd is given last; in Fig. 1 the same order is kept, from left to right.

Results

The genotype numbers of the surviving plants in He and Wd were determined and compared to the numbers expected from genotype proportions found in the greenhouse experiment (Wolff 1987) (Table 2). Although the number of plants was low, some significant differences between expected and observed genotype numbers were found. For the individual loci significant differences were found for Got-2 in He and for Pgm-1 in both Wd and He; in these cases the genotypes, possessing alleles originating from the specific transplant site, were more frequent than expected. In most other cases the same trend (genotypes being over-represented in their own environment), although not significantly, can be seen, often more strongly in He plants than in Wd plants. For Gpi-1 and Pgm-2 two heterozygote genotype classes could be distinguished; in He their relative presence is especially different.

The genotypes of the surviving plants were analysed with respect to their performance score. The plants from each population were split into two groups, one with a low and one with a high performance score. For Wd scores 1, 2 and 3–6 were taken together, whereas for He scores 1–3 and 4–9 were grouped to get groups of comparable sizes. In Table 3 the genotype numbers of each group are given and tested for independence (Sokal and Rohlf 1981). In each population a significant χ^2 was observed for one locus only, Got-2 for the He plants and Pgm-1 for the Wd plants. In general, the genotypes were over-represented in the higher performance score class in their own environment. In Fig. 1 the mean performance score is given for each genotype class. The difference in performance scores between genotypes possessing both alleles originating from one population was tested with a *t*-test. A significant trend was shown, again with genotypes doing best in their own environment, with only one exception: the FF genotype of Got-2 (originating from He) was superior in both sites.

All individual plants were classified according to the number of He-alleles and were divided into two groups having 1–6 and 7–12 He-alleles and into two groups according to their performance score (Table 4). A contingency test showed a significantly higher proportion of He-alleles in the He plants with a high performance score ($P=0.0043$), whereas the He-alleles had a significantly lower proportion in the Wd plants with a high performance score ($P=0.0288$). In the same way the number of heterozygous loci of the plants in both performance score groups was analysed (Table 5). Al-

Table 2. Genotype numbers as found in the F_2 in a greenhouse experiment (Wolff and Van Delden 1987) and in transplantation sites He and Wd. For the field experiments the deviations from expected (calculated from the greenhouse results) are given (between brackets) as well as the significance of the difference between numbers observed and expected, calculated with a χ^2 test

Locus	Genotype	Greenhouse	He	Wd
Gpi-1	II	47	9 (−3.1)	7 (+0.3)
	SI	70	22 (+4.0)	11 (+1.0)
	IF	68	14 (−3.5)	10 (+0.3)
	SF	103	29 (+2.5)	13 (−1.7)
			$P=0.46$	$P=0.96$
Got-1	II	69	23 (+5.3)	9 (−0.8)
	SI	137	35 (0)	22 (+2.6)
	SS	83	16 (−5.2)	10 (−1.8)
			$P=0.23$	$P=0.72$
Got-2	FF	32	14 (+5.8)	7 (+2.5)
	SF	154	47 (+7.6)	23 (+1.2)
	SS	103	13 (−13.4)	11 (−3.6)
			$P=0.002$**	$P=0.32$
Pgm-1	NN	71	28 (+9.8)	11 (+0.9)
	SN	156	32 (−8.1)	15 (−7.2)
	SS	61	14 (−1.7)	15 (+6.3)
			$P=0.03$*	$P=0.03$*
Pgm-2	II	74	18 (−1.2)	14 (+3.4)
	SI	76	16 (−3.7)	6 (−4.9)
	IF	77	29 (+9.0)	12 (+0.9)
	SF	58	11 (−4.1)	9 (+0.7)
			$P=0.12$	$P=0.33$
To-2	FF	76	22 (+2.5)	13 (+2.2)
	NF	151	35 (−3.0)	22 (+0.5)
	NN	61	17 (+1.3)	6 (−2.7)
			$P=0.23$	$P=0.53$

* $P<0.005$; ** $P<0.01$

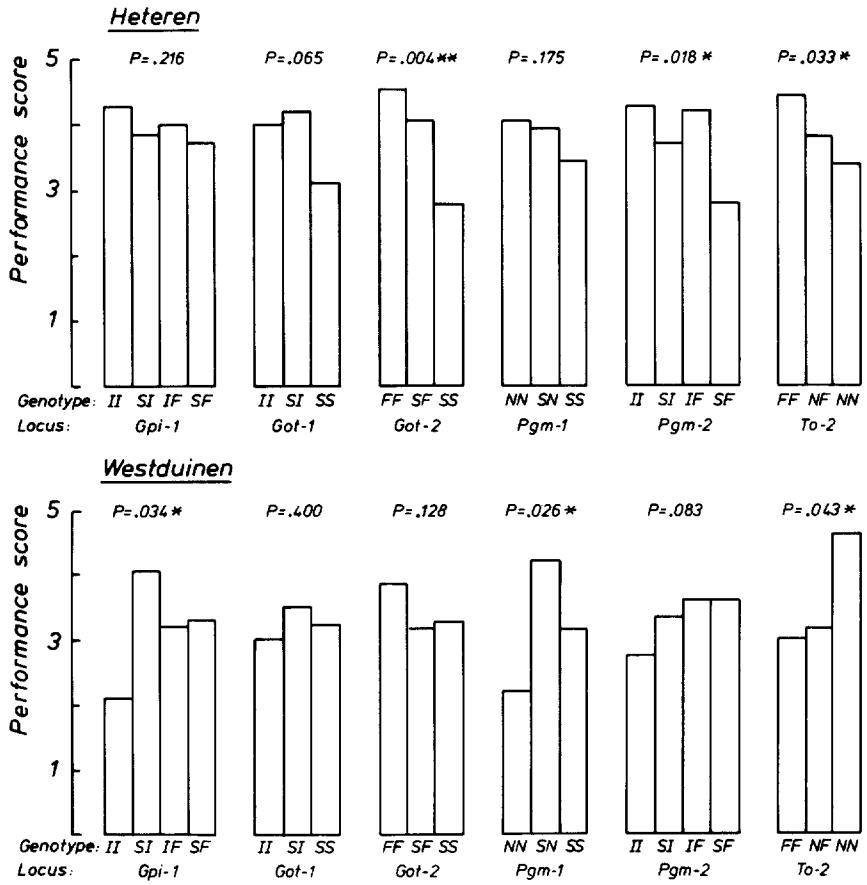

Fig. 1. Mean performance score in both transplantation sites for each genotype; the significance of the difference between the genotypes possessing two alleles from either the He or the Wd population (t-test) is given

Table 3. Genotype numbers in each transplantation site as distributed over two performance score classes and the results of a test of independence of both genotype number sets as calculated with a χ^2 test

Locus	Genotype	Site				
		He			Wd	
		Score 1–3	Score 4–9		Score 1–2	Score 3–6	
Gpi-1	II	4 (+0.1)	5 (−0.1)		5 (+2.3)	2 (−2.3)	
	SI	10 (+0.5)	12 (−0.5)		1 (−3.3)	10 (+3.3)	
	IF	4 (−2.0)	10 (+2.0)		5 (+1.1)	5 (−1.1)	
	SF	14 (+1.5)	15 (−1.5)	$P=0.66$	5 (+0.1)	8 (−0.1)	$P=0.05$
Got-1	II	9 (−1.0)	14 (+1.0)		4 (+0.5)	5 (−0.5)	
	SI	13 (−2.1)	22 (+2.1)		7 (−1.6)	15 (+1.6)	
	SS	10 (+3.1)	6 (−3.1)	$P=0.21$	5 (+1.1)	5 (−1.1)	$P=0.58$
Got-2	FF	3 (−3.0)	11 (+3.0)		1 (−1.7)	6 (+1.7)	
	SF	20 (−0.3)	27 (−0.3)		11 (+2.0)	12 (−2.0)	
	SS	9 (+3.4)	4 (−3.4)	$P=0.04$*	4 (−0.3)	7 (+0.3)	$P=0.28$
Pgm-1	NN	12 (−0.1)	16 (+0.1)		8 (+4.1)	2 (−4.1)	
	SN	12 (−1.8)	20 (+1.8)		2 (−3.8)	13 (+3.8)	
	SS	8 (+2.0)	6 (−2.0)	$P=0.46$	6 (−0.2)	10 (+0.2)	$P=0.004$**
Pgm-2	II	5 (−2.8)	13 (+2.8)		8 (+2.5)	6 (−2.5)	
	SI	9 (+2.1)	7 (−2.1)		2 (−0.3)	4 (+0.3)	
	IF	11 (−1.5)	18 (+1.5)		4 (−0.7)	8 (+0.7)	
	SF	7 (+2.2)	4 (−2.2)	$P=0.17$	2 (−1.5)	7 (+1.5)	$P=0.36$
To-2	FF	6 (−3.5)	16 (+3.5)		6 (+0.9)	7 (−0.9)	
	NF	18 (+2.9)	17 (−2.9)		9 (+0.4)	13 (−0.4)	
	NN	8 (+0.6)	9 (−0.6)	$P=0.19$	1 (−1.3)	5 (+1.3)	$P=0.46$

* $P<0.05$; ** $P<0.01$

Table 4. No. of plants subdivided according to the no. of He-alleles and their performance score together with the results of a χ^2 test of independence

No. of He-alleles	Site			
	He Performance score		Wd Performance score	
	1–3	4–9	1–2	3–6
0–6	25 (+6.0)	19 (−6.0)	6 (−3.4)	18 (+3.4)
7–12	7 (−6.0)	23 (+6.0)	10 (+3.4)	7 (−3.4)
		$\chi^2=8.15$ $P=0.004$**		$\chi^2=4.78$ $P=0.029$*

* $P<0.05$; ** $P<0.01$

Table 5. No. of plants subdivided according to the no. of heterozygous loci and their performance score together with the results of a contingency test

No. of heterozygous loci	Site			
	He Performance score		Wd Performance score	
	1–3	4–9	1–2	3–6
0–3	18 (−0.2)	24 (+6.0)	13 (+2.1)	15 (−2.1)
4–6	14 (+0.2)	18 (−0.2)	3 (−2.1)	10 (+2.1)
		$\chi^2=0.006$ $P=0.94$		$\chi^2=2.03$ $P=0.15$

though the Wd plants in the group with a low performance score had a lower heterozygosity ($P=0.154$), no significant difference was found in both populations.

In the greenhouse experiment several significant correlations between genotypes and morphological characters were found. To analyse whether similar correlations were present in the field, means for the number of leaves and the length of the longest leaf were calculated for each genotype (Table 6). Differences between plants possessing two alleles per locus from He or possessing two alleles from Wd were tested with a Student's *t*-test. Often results were not as expected and the plants from transplantation sites He and Wd showed contradictiory effects of the genotypes. In the He plants the number of leaves and leaf length had higher values for the homozygous genotypes originating from He in all cases, although this was only expected for the number of leaves (from the greenhouse results) for Got-1 and Got-2. In the Wd plants the relationships were miscellaneous.

Discussion

The genotype numbers found in both transplantation sites were different from those of the greenhouse experiment. Selection can be inferred from this result.

Table 6. Mean performance score, leaf length (in mm) and no. of leaves for each genotype as measured in the sites He and Wd as well as leaf length and no. of leaves in the greenhouse experiment

Locus	Genotype	He			Wd			Greenhouse	
		Perf. score	No. of leaves	Leaf length	Perf. score	No. of leaves	Leaf length	No. of leaves	Leaf length
Gpi-1	II	4.3	5.1	159	2.1	3.4	29.1	34.6	190
	SI	3.9	3.8	135	4.1	5.3	32.8	36.8	184
	IF	4.0	5.0	152	3.2	4.9	30.8	36.2	188
	SF	3.8	4.5	153	3.3	4.4	28.2	37.0	172
Got-1	II	4.0	4.6	149	3.0	4.1	31.0	38.8	192
	SI	4.2	4.5	162	3.5	4.5	30.6	35.8	184
	SS	3.1	4.2	116	3.2	5.3	28.7	34.9	169
Got-2	FF	4.6	4.9	187	3.9	4.7	32.7	41.5	223
	SF	4.1	4.5	145	3.2	4.7	29.4	36.6	187
	SS	2.8	3.9	118	3.2	4.2	30.3	34.0	156
Pgm-1	NN	4.1	4.6	144	2.2	3.7	23.7	32.9	188
	SN	4.0	4.5	161	4.3	5.5	31.8	36.8	179
	SS	3.4	4.1	125	3.1	4.3	33.4	38.2	179
Pgm-2	II	4.3	4.6	153	2.7	3.9	28.6	33.7	191
	SI	3.7	4.3	149	3.3	4.2	29.3	36.6	184
	IF	4.2	4.7	157	3.7	5.2	32.2	35.2	174
	SF	2.8	3.7	115	3.7	5.1	30.7	39.5	175
To-2	FF	4.5	4.6	156	3.0	4.0	32.3	32.3	187
	NF	3.8	4.5	147	3.2	4.5	29.3	36.5	179
	NN	3.4	4.2	140	4.3	6.2	29.0	40.4	182

When the allozyme loci are assumed to be neutral, selection must have favoured alleles of genes coding for characters related to fitness, which were present in the same linkage group as the allozyme locus. For the Got-2 locus some further explanation is needed. In the greenhouse a strong deviation from expectance in genotype numbers was observed. The numbers found in the field showed a weaker deviation from a Mendelian segregation. The genotypes of Got-2, however, not only differed in survival but also in mean performance score (Table 6). This was highest for the FF genotype in both populations and is discussed later. The difference in genotype numbers for Got-2 in the field and in the greenhouse is probably a real fitness difference and not an artefact. The survival in He seems more strongly related with genotype than in Wd, as the trend of a higher frequency of the genotype in their own environment is more prominent in He (Table 2). This does not necessarily mean that in Wd no relation between survival and genotype exists but a relation may, if present, have been dominated by environmentally-induced mortality. This can also be concluded from the distribution of the surviving plants over the plot. In He the surviving plants were equally distributed over the whole plot and mortality seemed to be independent of microhabitat. Apparently the habitat was uniform, with the exception of mole-hills into which some plants disappeared. In Wd a strong concentration of the surviving plants was found in one end of the plot and the surrounding vegetation was slightly higher (3–5 cm). Apparently growing condition (water, nutrients) were strongly different over the plot. Whether a plant survived in Wd was strongly dependent on the microhabitat more than on the genotype of the plant.

For both populations a difference in performance score was present for the different genotype classes: in all cases but one, the plants with a genotype originating from that population had a better mean performance score. Even in Wd when a plant survived (probably genotype independent in Wd) the genotype of the plant was important in how the plant performed and grew. This is in concordance with the results from a previous study (Wolff and Van Delden 1987) in which the inhomogeneous and hazardous microclimate in Wd (Van Damme and Van Delden 1984) was found to be highly important for the type of selection. Survival is for the greater part environment-dependent, whereas growth and fecundity are genotype dependent. In He a more uniform and unidirectional selection is present in which survival and growth are both, for a great part, genotype dependent. Only for Got-2 did one genotype (FF) have a better performance in both populations; a strong general fitness character must be correlated to this locus. This may be related to the sex phenotype of the plants, which was not determined in this study (for determination of sex phenotypes see Van Damme and Van Delden 1982; Van Damme 1983). From the greenhouse experiment it is known that male sterile plants are present in this cross in a frequency of approximately 10%. Alleles for male sterility are linked with the Got-2 S allele in this cross. The male steriles present are, for the greater part, type 3, which means that in addition to aberrant sex expression in those plants vegetative growth is also strongly inhibited. This may partially explain the better performance of the Got-2 FF genotype in both sites.

In the situation where genotypes perform best in their own environment, over-dominance is not likely. In both populations the performance of plants, measured as vegetative growth, was not related to heterozygosity. This is in agreement with the greenhouse experiment in which a positive relation between heterozygosity and generative characters, but not with vegetative characters, was found. The relatively large difference, in numbers and in performance, between the two heterozygote genotype classes of Gpi-1 and Pgm-2 shows that the heterozygous state may be less important than the specific allele combination.

The correlation between genotype and the morphological characters measured often differs between two habitats and from the greenhouse results. This is in concordance with results of a transplantation experiment of Van Groenendael (1985). Although genotypes did best in their own environment, there was only a weak correlation between morphological characteristics in the greenhouse and performance in the field. The expression of characters, such as number of leaves and leaf length in the field, seems to be more strongly influenced by the general fitness and growth conditions of the plant than by the genotype of the plant, as is the case under greenhouse conditions. As stated by Antonovics and Primack (1982), field experiments, in addition to greenhouse and common garden experiments, are a necessity in showing ecological adaptations. An experiment over a prolonged period would give more information on several other morphological and life history characters like fecundity. But, although in this experiment only a small part of the life history phases of the plants was investigated, it is known from other experiments (Mook et al. 1981) that for *P. lanceolata* the size of a plant at a juvenile life stage is positively correlated with survival and reproductive effort in later stages. Therefore, it seems safe to relate the fitness of the plant with the performance score, and for He both survival and performance score, with fitness of the plant. It can be concluded that fitness differences between individuals of the F_2 generation are large enough for measuring natural selection and for ascribing different selection characteristics to different habitats.

Acknowledgements. I wish to thank Dr. W. Van Delden for his comments on the manuscript, Henk Mulder for drawing the figure and Arthur Leewis and Jan Van der Toorn for assistance during the field work.

These investigations were supported by the Foundation for Fundamental Biological Research (BION), which is subsidised by the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

Antonovics J, Primack RB (1982) Experimental ecological genetics in *Plantago*. VI. The demography of seedling transplants in *Plantago lanceolata*. J Ecol 70:55–75

Böcher TW (1943) Studies on variation and biology in *Plantago lanceolata* L. Dan Bot Ark 11:1–18

Bradshaw AD (1984) The importance of evolutionary ideas in ecology and vice versa. In: Shorrocks B (ed) Evolutionary ecology. Blackwell, Oxford

Brown ADH, Marshall DR, Munday J (1976) Adaptedness of variants at an alcohol dehydrogenase locus in *Bromus mollis* L. (soft brome grass). Aust J Biol Sci 29:389–396

Cavers PB, Bassett IJ, Crompton CS (1980) The biology of Canadian weeds. 47. *Plantago lanceolata* L. Can J Plant Sci 60:1269–1282

Clegg MT, Kahler AL, Allard RW (1978) Estimation of life cycle components of selection in an experimental plant population. Genetics 89:765–792

Endler JA (1986) Natural selection in the wild. Princeton University Press, New Jersey

Hickey PA, McNeilly T (1975) Competition between metal tolerant and normal plant populations: a field experiment on normal soil. Evolution 29:458–464

Mook JW, Haeck J, Van der Toorn J (1981) Survival of *Plantago lanceolata* in vegetation of varying structure. Proc K Ned Akad Wet, Afd Nat Tweede Reeks 77:32–35

Sokal RR, Rohlf FJ (1981) Biometry. Freemann, San Francisco

Teramura AH, Antonovics J, Strain BR (1981) Experimental ecological genetics in *Plantago*. IV. Effects of temperature on growth rate and reproduction in three populations of *Plantago lanceolata* L. (*Plantaginaceae*). Am J Bot 68: 425–434

Van Damme JMM (1983) Gynodioecy in *Plantago lanceolata* L. 2. Inheritance of three male sterility types. Heredity 52:77–93

Van Damme JMM, Van Delden W (1982) Gynodioecy in *Plantago lanceolata* L. I. Polymorphism for plasmon type. Heredity 49:303–318

Van Damme JMM, Van Delden W (1984) Gynodioecy in *Plantago lanceolata* L. IV. Fitness components of sex types in different life cycle stages. Evolution 38:1326–1336

Van Dijk H (1985) Allozyme genetics, self-incompatability and male sterility in *Plantago lanceolata*. Heredity 54:53–63

Van Dijk H, Van Delden W (1981) Genetic variability in *Plantago* species in relation to their ecology. Part 1. Genetic analysis of the allozyme variation in *Plantago major* subspecies. Theor Appl Genet 60:285–290

Van Groenendael J (1985) Selection for different life histories in *Plantago lanceolata*. PhD thesis, University of Nijmegen

Van Der Toorn J, Ten Hove HJ, Van Eldijk F (1984) Transplant experiments with *Plantago lanceolata* Proc K Ned Akad Wet Afd Nat Tweede Reeks 82:39–44

Venable DL (1984) Using intraspecific variation to study the ecological significance and evolution of plant life-histories. In: Dirzo R, Sarukhan J (eds) Perspectives on plant population ecology. Sunderland, Massachusetts

Wolff K (1987) Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L. II. Localisation and organisation of quantitative trait loci. Theor Appl Genet 73:903–914

Wolff K, Van Delden W (1987) Genetic analysis of ecological relevant morphological variability in *Plantago lanceolata* L. I. Population characteristics. Heredity 58:183–192