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

2. Localisation and organisation of quantitative trait loci *

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Summary. Morphological variability was analysed in an F_2-generation derived from crosses between two ecotypes of *Plantago lanceolata* L. Six allozyme loci, localised in five linkage groups, were used as markers. For two marker loci, *Got-2* and *Gpi-1*, segregations did not fit monogenic ratios. In the linkage groups to which these two loci belonged, male sterility genes appeared to be present. In these crosses, male sterility (type 3, as described by Van Damme 1983) may be determined by two recessive loci located in the linkage groups of *Got-2* and of *Gpi-1*. Many correlations of morphological and life history characters with allozyme markers were observed. The quantitative trait loci did not appear to be concentrated in major gene complexes. Often many loci were involved, sometimes with effects opposite to those expected from the population values. Main effects of the linkage groups appeared to be more important than interaction effects in determining variability. It also appeared that there is a positive correlation between the number of heterozygous allozyme loci and generative growth.

Key words: *Plantago lanceolata* L. – Allozymes – Associations – Male sterilitiy – Heterosis

Introduction

The study of polygenic characters, many of which have an important contribution to fitness characters, has until now mainly been done using techniques from the field of biometrical genetics (e.g. Mather and Jinks 1977). Depending on the design of the crosses, the number of genes involved in each character can sometimes be estimated: the numbers found are often large. The localisation and organisation of the numerous polygenes, that is whether functionally related genes are clustered and how they cooperate, has been studied in only a few species. In view of the importance of quantitative traits for fitness, it is worthwhile to study their localisation and organisation.

The localisation of genes for quantitative traits (henceforth quantitative trait loci, QTL [Geldermann 1975]) can be done by using marker loci. The transmission of a marker gene allele is interpreted as the transmission of a chromosome segment. On this chromosome segment one or more QTL may be situated. The contribution of a particular chromosome segment depends on the crossover frequencies between the marker gene and QTL's, of the dosage of allele effects and of the allele combinations at QTL within the chromosome segment (Geldermann 1975). In the beginning of this study on the organisation of QTL, morphological markers were used. This gave problems due to dominance and the small number of marker genes available (Thompson and Thoday 1979). The techniques of electrophoresis and analysis of restriction length polymorphisms enlarged the possibilities for studying the organisation of QTL. These techniques can be used to improve agricultural varieties, although the economic importance has been questioned (Beckmann and Soller 1983; Soller and Beckmann 1983; Tanksley et al. 1981, 1982). Tanksley et al. (1982), for instance, used allozyme variation to detect QTL in an interspecific backcross of tomato. They mapped at least 21 QTL on 8 of the 12 chromosomes.

Van Dijk (1984) has used allozymes as markers in studying the genetic basis of quantitative traits in *Plantago major* subspecies. He showed that in this species QTL are concentrated in three linkage groups, of which the *Pgm-1* linkage group is the most important one. For each character, one to four, but mostly one or two, QTL could be located. In this inbreeding species, with different ecotypes in different habitats, the QTL concerned are concentrated in only a few gene complexes.

Plantago lanceolata is an obligate outbreeder and can be considered to be a generalist with plastic responses to environmental variation. It is to be found in a great variety of habitats (Haeck et al. 1982). Experiments have indicated rela-

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Table 1. Population means of several characters and significance of the difference between the two populations measured in a greenhouse experiment

Character	Population		Significance[a]
	Heteren	Westduinen	
Number of rosettes	2.4	4.0	*
Number of leaves	25	45	***
Leaf angle	39	126	***
Cotyledon length (mm)	51	32	***
Leaf length (mm)	261	166	***
Leaf width (mm)	29	25	**
Number of scapes	15	24	***
Scape length (cm)	54	39	***
Spike length (mm)	40	28	***
Flowering date	40	46	***
Leaft weight (g)	3.69	2.54	**
Generative weight (g)	5.75	4.59	**
Reproductive effort	1.68	2.18	ns
Seed weight (mg)	1.80	1.62	ns
Total seeds ($\times 10^3$)	1.80	2.08	ns
Total seed weight (g)	3.29	3.22	ns

[a] $P<0.001$; ** $P<0.01$; * $P<0.05$; ns $=P>0.05$ (Wolff and Van Delden 1986)

tively high heritabilities for some quantitative and life history characters and also a considerable differentiation between populations, although often much overlap and a high variability within populations occurs (van Groenendael 1985; Wolff and Van Delden 1986). In Table 1 results from a previous experiment in this series (Wolff and Van Delden 1986) are given, showing the differences between two populations for several characters. The two populations used are a hayfield population, Heteren (He), and a pasture population, Westduinen (Wd) (see for a description of the habitats Van der Toorn et al. 1980; Van Groenendael 1985). The habitats differ strongly in the selective forces operating. In He the emphasis lies on competition for light in a high vegetation. The vegetation in Wd is low and open but the plants suffer from severe unpredictable droughts.

Allozyme variation in *P. lanceolata* has been investigated by van Dijk et al. (in press). It appeared that the electrophoretic variation within populations was large and population differentiation, for electrophoretic variation, was relatively low, compared to other species. Furthermore, none of the alleles were specific for one habitat type, nor were there any other striking differences, such as a linkage disequilibrium: it seems safe to assume that allozymes can be used as neutral markers of linkage groups.

In this study the genetic organisation of quantitative variation is analysed to obtain a better understanding of quantitative variability: selection working on gene clusters may have a different impact than selection on separate genes. This has been done by analysing an F_2-generation from a cross between two different ecotypes of *P. lanceolata*. In view of the high plasticity and the high intrapopulational component of variability in *P. lanceolata*, correlations of allozyme genotypes and quantitative characters are lower than in *P. major*.

Materials and methods

Cultivation and electrophoresis

Cultivation circumstances, the way crosses were performed and electrophoresis techniques have been described earlier by Van Dijk (1985) and Van Dijk and Van Delden (1981). The pH of the staining buffer used for glutamate-oxaloacetate-transaminase was 8.1 instead of 7.5.

From seeds sampled in Heteren (He) and from adult plants sampled in the Westduinen (Wd) four plants were selected (two for each population), with the purpose of obtaining as many electrophoretic differences between parents as possible. This provided different alleles at six loci, distributed over five linkage groups. The distribution of variable enzyme loci over linkage groups is as follows (Van Dijk 1985): I: *Gpi-1 – Lap-1;* II: *Got-1 – Idh – Est-5;* III: *To-2 – Pgm-2 – Est-4;* IV: *6Pgd-3 – Got-2 – Lap-2;* V: *6Pgd-1/6Pgd-2 – Pgm-1/SI*, where *SI* is the self-incompatibility locus. 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). Allele denomination is according to Van Dijk (1985). Of these loci only *Pgm-2* and *To-2* appeared to be in the same linkage group (Van Dijk 1985).

An F_2 generation obtained by a cross within an F_1 would provide inbreeding effects (Van Damme 1983). It was therefore decided to select one plant from each of two F_1 crosses ($He_1 \times Wd_1$ and $Wd_2 \times He_2$) and make a reciprocal cross between these plants to obtain a pseudo-F_2. The genotypes of the plants and the crosses are shown in Table 2. Five hundred and eighty plants from the reciprocal pseudo-F_2 generation (290 of each cross) were grown in the greenhouse in a totally randomized design. The plant containers were placed in peat litter 17 days after germination. Almost all seeds germinated (99%), only one plant died during growth and seven plants did not start flowering before the end of the experiment.

Morphological characters

Cotyledon length (at the two leaf stage) and leaf angle (angle between third and fourth leaf at the nine leaf stage) were determined. Flowering date was recorded as the day after germination on which the first stamens were visible. Sex phenotype of the plants was determined as described by Van Damme and Van Delden (1982) and Van Damme (1983). The hairiness of the plants was determined as the number of hairs on the upper side of the ninth leaf, counted on a leaf punch with a radius of 75 mm, approximately 3 cm from the top of the leaf.

One or two times during growth some leaf material was used to determine the allozyme genotype of the plants.

Eleven weeks after germination the plants were harvested. The following characters were then measured: number of side rosettes, number of leaves, number of scapes, length of the longest leaf, width of the widest leave, length of the longest scape and length of the spike belonging to this scape. Weight of the leaves, generative biomass (scapes + spikes) and seed weight was determined after drying. From these measurements were calculated: leaf length/width ratio, reproductive effort (generative biomass/generative + vegetative biomass), seeds/mm spike, total seed number (seeds/mm spike × spike length × number of scapes) and total seed weight (total seed number × seed weight).

Statistics

The data were analysed using subprogram factor, *t*-test, anova and regression from the Statistical Package for the Social

Table 2. Genotypes of plants used in making the pseudo-F_2, characters in bold face are alleles from He

	Gpi1	*Got1*	*Got2*	*Pgm1*	*Pgm2*	*To2*	
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**	
						and	F_1 (HW) × F_1 (WH) → F_2^1
							F_1 (WH) × F_1 (HW) → F_2^2

Sciences (SPSS, Nie et al. 1975; Hull and Nie 1980). No transformation was applied to the data since a log transformation did not approve normality of the variables.

The effect of the five linkage groups and of interaction between them was surveyed using a five factor analysis of variance in which the genotypes of the five linkage groups were considered as fixed factors.

Relative dominance effects were calculated as follows (notation according to Falconer 1981): mean of both homozygotes = m, genotypic value of one homozygote is m + a, of the other homozygote is m − a, the heterozygote is m + d.

Relative dominance is d/a.

Significance of a dominance effect is calculated with a *t*-test, with H_0: d = 0. The same method is used to test the significance of an overdominance effect with H_0: d = | a | and H_1: d > | a | .

Results

Monogenic segregations and linkage

The segregation at each allozyme locus was first tested against the expected ratios (1 : 1 : 1 : 1 for *Gpi-1* and *Pgm-2* and 1 : 2 : 1 for the other loci). It appeared that for *Gpi-1* and *Got-2* there was a strong deviation from the expected proportions (Table 3). This deviation was equally large for both reciprocal crosses. The deviant segregation for *Gpi-1* is not easy to explain. In one F_2 cross (F_2^2) gametic selection against the I-gamete of both parents is probable, since an excess of SF genotypes exists. In the other F_2 cross (F_2^1), zygotic selection against the II zygote is more probable (no excess of the SF genotype), but gametic selection against both male and female gametes may also explain the numbers found in this cross (no significant χ^2 in a test for heterogeneity using realised gamete frequencies for expected genotype numbers). For *Got-2*, gametic selection against one parent (the male or the female) is sufficient to explain the distortion in the separate F_2 reciprocal crosses but zygotic selection against the II gamete can also be postulated to explain the ratios found.

Recombination frequencies between allozyme loci were calculated with the maximum likelihood method (Green 1981) and they were found to be in accordance with those of Van Dijk (1985). Only *Pgm-2* and *To-2* showed a linkage disequilibrium.

With a *t*-test maternal effects (found as differences between reciprocal $F_1 \times F_1$ crosses: F_2^1 and F_2^2) were detected for leaf weight, generative weight, reproductive effort, total seed number and weight, leaf length and leaf length/width ratio. The F_2^1 cross is superior over the F_2^2 cross in almost all characters.

Table 3. Genotype distribution of the six allozyme loci in the reciprocal crosses (see Table 2) and the significance of the segregation distortion as calculated with a χ^2-test (*** $P < 0.001$; ** $P < 0.01$; – $P > 0.05$)

Locus	Genotype	F_2^1	F_2^2	Total
Gpi-1	II	46	47	93
	SI	75	70	145
	IF	80	68	148
	SF	82	103	185
	sign.	***	***	***
Got-1	II	71	69	140
	SI	134	137	271
	SS	78	83	161
Got-2	II	42	32	74
	SI	134	137	271
	SS	78	83	161
	sign.	***	***	***
Pgm-1	NN	64	71	135
	SN	156	156	312
	SS	61	61	122
	sign.	–	–	–
Pgm-2	II	76	74	150
	SI	72	76	148
	IF	63	77	140
	SF	70	58	128
	sign.	–	–	–
To-2	FF	69	76	145
	NF	142	151	293
	NN	73	61	134
	sign.	–	–	–

Sex phenotypes

The parents involved in the crossings were all hermaphrodites. In the F_2 analysed, several sex phenotypes segregated (Table 4). Rather high proportions of male sterile type 3 (MS3, Van Damme 1983) appeared to be present. Male sterile type 1 (MS1) and intermediate type 1 (IN1) were present in smaller numbers. Sex phenotype numbers were similarly distributed over the two reciprocal crosses. Many hypotheses concerning the number and character of genes involved in the determination of the sex phenotypes are possible, of which some are described below. It is not yet known whether one or more of the same genes are involved in determining MS1 and MS3. The numbers found may indicate the determination by two recessive genes, giving an expected ratio of 1 : 15 for MS3 : rest ($\chi_1^2 = 1.57$, ns) or for MS1 + MS3 + IN1 : rest ($\chi_1^2 = 0.22$), ns). Both genes should be in a heterozygous state in the F_1 individuals used for making the pseudo-F_2. Another hypothesis is that two recessive and one dominant gene are involved in MS3, all being in a heterozygous state in both F_1 individuals, leading to an expected ratio of 3:64 ($\chi_1^2 = 1.50$, ns). When the same set of genes are responsible for both MS1 and MS3 (2 recessive and one dominant) a significant χ_1^2 value is found ($P < 0.05$).

The distribution of the sex phenotypes over the different genotypes was not random for *Gpi-1* and *Got-2* (Table 5). Linkage of male sterility genes with these allozyme loci was obvious (no recombination frequencies were calculated as numbers were low and selection for gametes or zygotes were disturbing ratios). There were no MS with genotype SF for *Gpi-1* (from Wd) and with genotype II for *Got-2* (from He).

Male sterile type 3 showed lower figures for a number of fitness traits, like flowering date and seeds/mm spike (Table 6). MS1 and IN1 appeared to be as fit as hermaphrodites.

Table 4. Distribution of sex phenotypes over the reciprocal crosses

Sex phenotype	F_2^1	F_2^2	Total
Hermaphrodite	267	262	529
Intermediate 1	3	3	6
Male sterile 1	2	2	4
Male sterile 3	12	21	33

Table 5. Allele frequencies of the alleles derived from Heteren plants for the six allozyme loci in two sex phenotype groups (hermaphrodites and all other sex phenotypes) and the significance of the difference between the two groups (χ^2-test, *** $P < 0.001$; – $P > 0.05$)

Locus	Hermaphrodites	Intermediates + male steriles	Significance of difference
Gpi-1	0.40	0.70	***
Got-1	0.48	0.52	–
Got-2	0.42	0.22	***
Pgm-1	0.50	0.60	–
Pgm-2	0.52	0.54	–
To-2	0.51	0.55	–

Table 6. Character means for hermaphrodites (H), male sterile types 3 (MS3) and 1 (MS1) and intermediate type 1 (IN1). The significance of the differences (Students t-test) between H and MS3 and H and MS1 + IN1 is shown (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$)

Sex type	No. of rosettes	No. of leaves	Leaf angle	Cotyledon length	Leaf length	Leaf width
H (n = 529)	1.84	36.7	86.4	35.9	181.6	35.7
MS3 (n = 33)	1.42**	32.3***	96.6–	32.8***	176.9–	38.4**
MS1 + IN1	1.45–	33.6–	93.3–	35.2–	200.3–	36.9–

Sex type	Hairiness	No. of scapes	Scape length	Spike length	Flowering date	Leaf wt
H	110.2	20.61	31.2	37.70	21.37	274.6
MS3	86.8–	20.33–	27.1***	41.41*	29.09***	284.0–
MS1 + IN1	143.1–	20.18–	33.4–	41.6–	24.45–	323.4–

Sex type	Generat. weight	Reproductive effort	Seed wt	Seeds/mm spike	Total seeds	Total seed wt
H	278.2	0.50	0.97	3.03	2,339	2,293
MS3	200.9***	0.41***	1.01–	0.76***	568***	509***
MS1 + IN1	313.9–	0.46–	1.12*	2.46–	2,399	2,702–

Correlations

Many significant correlations existed between characters. To obtain a better insight in the many correlations, principal components were extracted from the covariance matrix calculated over all individuals. In Appendix 1 the factors with their eigenvalue and the percentage of total variability are given. The first four components explain almost 70% of total variability: the first can be interpreted as an index of generative capability (positive with total number of seeds and negative with flowering date); the second as an index of growth with the accent on leaf width and length of the organs; the third as a contrast of leaf length and leaf angle (index for plant height) and the fourth as an index of vegetative growth, with the accent on number of units (leaves and rosettes) and late flowering. Each individual received a score (its loading) on the first four components. The scores were considered as characters of the individuals in some of the further analyses.

Linkage of allozyme loci and quantitative trait loci

An analysis of variance revealed significant differences between the genotypes of each allozyme locus for the different characters (Table 7). The first genotype of each locus contains alleles derived from the He population, the last genotype alleles from the Wd population, and the intermediate genotype one allele from He and one from Wd. The directions of the effects could be predicted from population means (Table 1). In Table 7 + indicates a difference in the expected direction and – a difference in the unexpected direction; * indicates no difference between homozygotes or no expectance of the direction of the difference.

All characters were associated with two to five linkage groups. The + and – signs showed some contradictions. Only for some characters did all influences have the same (expected) direction. Influences on flowering date, for example, were three times in the unexpected direction and three times (in two linkage groups) in the expected direction.

Linkage of a character with *Pgm-2* often coincided with a linkage with *To-2*, which was expected from the linkage between *To-2* and *Pgm-2*. Differences in the strength of the correlation of a character and both allozyme loci give an indication of the localisation of the gene or genes for that quantitative trait on the chromosome segment.

Relative dominance effects were calculated as described and only those with a significant dominance or overdominance effects were given (Table 8). For *Gpi-1* and *Pgm-2* dominance of the two different alleles from Wd were calculated separately. It appears that dominance effects were present in many cases, even overdominance occurred several times. The differential dominance effects of the distinguishable alleles from Wd for *Pgm-2* and *Gpi-1* (Tables 7 and 8) was striking. The influence of *Pgm-2* on, for example, flowering date, showed a dominance of the S-allele from Wd in the SI-genotype, but in the IF genotype the I from He showed dominance over the F-allele.

Interaction components in determining morphological variation

Influences of single additive or dominance effects of the loci have been shown (Tables 7 and 8). By applying a five factor analysis of variance, with the five linkage groups as treatments, main effects and interaction effects could be distinguished. Percentages of total variation caused by each component separately is presented for the characters measured and for the factor scores (Tables 9 and 10, respectively). It showed that the most important main effects were in linkage groups *Got-2* and *Got-1*, followed by *To-2* and *Pgm-1*. The group with *Gpi-1* had the least effect.

The number of significant interaction effects was relatively low. Only in some cases did interaction cause a considerable part of total variability (e.g. hairiness). Hairiness was the only character which was not normally distributed. About half of the plants had no or almost no hairs. *Pgm-1* and *Got-1* had a strong effect on hairiness, *Gpi-1* had less effect. Still the exact determination of hairiness could not be ascertained, there are too many types of interaction present. The handling of this character in an anova, conforming to the other characters is, in fact, improper.

Heterozygosity

To detect differences, if any, in the means or in variances between individuals varying in the number of heterozygous loci, plants were ordered into seven groups: from having zero to six heterozygous allozyme loci. Means and variances of the groups were compared for all characters except hairiness. Both coefficients of variance (CV) and means of the groups were ranked from one to seven for each character. Friedmans test (Sokal and Rohlf 1981) showed a significant heterogeneity in CV over the groups ($\chi^2_6 = 23.77$, $P < 0.005$). The more heterozygous individuals are less variable.

The other comparison between the groups is in means of the characters. Friedmans test is significant when all characters were involved ($\chi^2_6 = 28.08$, $P < 0.001$) but an even higher χ^2 is obtained when vegetative characters were left out ($\chi^2_6 = 42.73$, $P < 0.001$). In the set of vegetative characters no heterogeneity was present ($\chi^2_6 = 6.26$, $P > 0.05$). The generative characters had the highest mean in the groups with the most heterozygous individuals (group with 5 heterozygote loci > group with $6 > 2 > 4 > 3 > 1 > 0$). Generative

Table 7. Means of characters measured in the segregating F_2 generation for the different genotypes of each locus and the significance of the difference between the genotypes as calculated with an analysis of variance. Further explanation in the text. + + +, − − −, *** $P<0.001$; + +, − −, ** $P<0.01$; +, −, * $P<0.05$; ns = $P>0.05$)

Locus	Character						
	Genotype	No. of rosettes	No. of leaves	Leaf angle	Cotyledon length	Leaf length	Leaf width
Gpi-1	II	1.68	34.6	90.3	36.1	190	36.5
	SI	1.99	36.8	83.8	35.1	184	36.3
	IF	1.76	36.2	89.7	36.2	188	35.9
	SF	1.78	37.0	86.3	35.6	172	35.3
		ns	ns	ns	ns	+ +	ns
Got-1	II	2.36	38.8	75.2	37.2	192	38.4
	SI	1.70	35.8	84.9	36.3	184	35.9
	SS	1.45	34.9	101.0 +	33.5	169	33.8
		− − −	− −	+ + +	+ + +	+ + +	+ + +
Got-2	FF	2.49	41.5	64.8	35.1	223	38.2
	SF	1.76	36.6	85.5	37.1	187	36.7
	SS	1.61	34.0	98.4	33.8	156	33.7
		− − −	− − −	+ + +	+ + +	+ + +	+ + +
Pgm-1	NN	1.47	32.9	85.4	36.1	188	37.3
	SN	1.84	36.8	89.6	35.7	179	35.7
	SS	1.99	38.2	83.9	35.3	179	34.8
		+ +	+ + +	ns	ns	ns	+ + +
Pgm-2	II	1.56	33.7	81.8	36.8	191	36.7
	SI	1.96	36.6	86.0	36.2	184	36.2
	IF	1.56	35.2	90.8	34.6	174	35.3
	SF	2.09	39.5	91.8	35.1	175	35.3
		+ + +	+ + +	ns	+	+ +	ns
To-2	FF	1.43	32.3	78.9	37.1	187	36.4
	NF	1.82	36.5	88.5	35.6	179	35.7
	NN	2.19	40.4	93.4	34.5	182	36.0
		+ + +	+ + +	+ +	+ +	ns	ns

Locus	Character						
	Genotype	Hairiness	No. of scapes	Scape length	Spike length	Flowering date	Leaf wt
Gpi-1	II	109	20.0	298	38.9	23.2	2.92
	SI	124	20.6	313	38.1	22.4	2.85
	IF	81	21.1	316	38.8	22.2	2.85
	SF	121	20.5	308	37.0	20.6	2.53
		−	ns	ns	ns	−	+ +
Got-1	II	90	19.9	315	42.5	25.3	3.21
	SI	105	20.5	314	37.4	21.3	2.73
	SS	134	21.3	300	35.3	19.8	2.39
		−	+	ns	+ + +	− − −	+ + +
Got-2	FF	111	18.7	380	38.8	24.6	3.65
	SF	101	20.8	322	39.1	20.9	2.89
	SS	122	21.0	261	36.1	22.4	2.19
		ns	+ +	+ +	+ +	− − −	+ + +
Pgm-1	NN	140	19.8	335	37.2	20.1	2.69
	SN	115	21.0	305	38.4	21.6	2.76
	SS	62	20.5	299	38.2	23.9	2.76
		+ + +	ns	+ +	ns	+ + +	ns
Pgm-2	II	104	19.8	310	40.5	19.5	2.81
	SI	113	20.2	314	39.8	24.1	2.87
	IF	106	21.4	302	36.2	19.4	2.48
	SF	117	21.2	313	35.1	24.0	2.81
		ns	+ +	ns	+ + +	+ + +	+ +
To-2	FF	103	19.6	304	40.6	18.7	2.60
	NF	105	21.0	306	38.3	21.9	2.75
	NN	127	20.6	326	34.6	25.3	2.95
		ns	+ +	− −	+ + +	+ + +	−

Table 7 (continued)

Locus	Character						
	Genotype	Generative wt	Reproductive effort	Seed wt	Seeds/mm scapes	Total seeds	Total seed wt
Gpi-1	II	2.57	0.46	1.02	3.14	2.38	2.40
	SI	2.87	0.50	1.01	3.20	2.61	2.61
	IF	2.77	0.50	1.04	2.88	2.38	2.49
	SF	2.72	0.52	1.00	3.10	2.35	2.37
		ns	***	ns	ns	ns	ns
Got-1	II	2.75	0.46	1.03	2.65	2.28	2.39
	SI	2.75	0.50	1.04	3.06	2.40	2.48
	SS	2.72	0.53	0.97	3.40	2.59	2.49
		ns	***	+	+ + +	ns	ns
Got-2	FF	3.23	0.47	1.20	3.28	2.36	2.81
	SF	2.94	0.51	1.02	3.15	2.58	2.62
	SS	2.19	0.50	0.90	2.73	2.08	1.83
		+ + +	*	+ + +	− − −	***	***
Pgm-1	NN	2.96	0.52	1.03	3.35	2.54	2.58
	SN	2.77	0.50	1.01	3.07	2.47	2.49
	SS	2.45	0.47	1.03	2.73	2.20	2.25
		+ + +	***	ns	− − −	*	ns
Pgm-2	II	2.78	0.50	1.07	2.87	2.31	2.48
	SI	2.74	0.49	1.01	3.15	2.56	2.59
	IF	2.74	0.52	1.01	3.06	2.40	2.39
	SF	2.74	0.49	0.96	3.35	2.54	2.43
		ns	**	+ + +	+ +	ns	ns
To-2	FF	2.70	0.51	1.06	2.84	2.23	2.38
	NF	2.75	0.50	1.00	3.06	2.47	2.46
	NN	2.76	0.48	1.00	3.43	2.60	2.57
		ns	*	+	+ + +	*	ns

characters included: number of scapes, scape length, spike length, flowering date, generative weight, reproductive effort, seed weight, total seeds and total seed weight.

The correlation of heterozygosity and character means has also been computed for individual factor scores on the first four principal components (Table 11). The use of principal components avoids getting a significant trend due to multiple comparisons. A significant correlation existed between heterozygosity and factor scores on PC1, which accounted for generative growth. No correlation with the other three components was observed.

Discussion

Deviating segregations

The deviations at allozyme loci from the expected segregation for *Gpi-1* and *Got-2* cannot be attributed to lethality during germination or later life stages. Selection must be attributed to the zygotic and or the gametic phase. It is known that in perennials about 50% of the ovules set seed (Wiens 1984). In the crossings of this experiment the percentage of seed set is not known. Van Damme (1984) found a seed set in *P. lanceolata* of 50–80%; this reduced seed set was not caused by a shortage of pollen. It could be caused by random abortion but as the results of this experiment indicate, possibly also by zygotic selection. This has also been suggested by Wiens (1984) who stated that crossbreeding in perennials maintains variability but also maintains relatively high frequencies of lethal and sublethal allele combinations causing a lower seed/ovule ratio compared to inbreeding species, due to the occurrence of detrimental homozygotes.

Sex phenotypes and their fitness

Sex determination of *P. lanceolata* has been analysed thoroughly by Van Damme (1983) and by Van Damme and Van Delden (1982). They showed that in MS1 at least two recessive and three dominant nuclear genes were involved in addition to a particular, cytoplasmic

Table 8. Relative dominance and overdominance effects of the six loci for the characters with significant dominance effects, $ = a significant overdominance effect is observed (*** $P<0.001$; ** $P<0.01$; * $P<0.05$)

Locus	No. of rosettes	No. of leaves	Leaf angle	Cotyledon length	Leaf length	Leaf width
Gpi-1 SI	5.20*	–	–	–	–	–
Gpi-1 IF	–	–	–	–	–	–
Got-1	0.45**	–	–	0.51*	–	–
Got-2	0.66**	0.31*	–	4.08***$	–	0.33*
Pgm-1	–	0.47*	6.59*$	–	–	–
Pgm-2 SI	–	–	–	–	–	–
Pgm-2 IF	1.00***	–	–	1.59*	1.12*	–
To-2	–	–	–	–	2.20*	–

Locus	Hairiness	No. of scapes	Scape length	Spike length	Flowering date	Leaf wt
Gpi-1 SI	–	–	–	–	–	–
Gpi-1 IF	5.67***$	3.40*	2.60*	–	–	–
Got-1	–	–	–	0.46***	0.45**	–
Got-2	2.82*	0.83***	–*	1.22***	2.36***$	–
Pgm-1	–	1.67***	0.67**	–	–	–
Pgm-2 SI	–	–	–	0.74**	1.04**	–
Pgm-2 IF	–	1.28*	–	0.59*	.04***	high***$
To-2	–	1.80**	0.82*	–	–	–

Locus	Generative weight	Reproductive effort	Seed wt	Seeds/mm spike	Total seeds	Total seed wt
Gpi-1 SI	2.30*	–	–	–	16.00**$	15.00*$
Gpi-1 IF	–	–	3.00*	12.0***$	–	–
Got-1	–	–	1.33***	–	–	–
Got-2	0.44***	1.17***	0.20**	0.53**	2.57***$	0.61***
Pgm-1	–	–	–	–	–	–
Pgm-2 SI	–	–	–	–	–	–
Pgm-2 IF	–	high***$	–	–	–	–
To-2	–	–	1.00*	–	–	–

factor, called R. Male steriles of type 3 were not abundant in his crosses but appeared occasionally in low numbers; apparently two recessive genes were involved. In the particular cross analysed in our study it can be argued that two recessive genes were involved in the determination of the sex phenotypes, as the ratio MS/hermaphrodites is close to 1 : 15. The two loci coincide possibly with the recessive loci Van Damme (1981, 1983) proposes. It cannot be concluded from these experiments whether or not both MS types are determined (partly) by the same genes. The differing allozyme frequencies for MS and hermaphrodites for *Gpi-1* and *Got-2* suggests that the two recessive male sterility genes are located near *Gpi-1* and *Got-2*. Van Dijk (1985) concluded from his experiments that for the determination of MS1 at least two recessive male sterility loci and one dominant locus (respectively in the *Pgm-1*, the *Pgm-2* and the *Got-2* linkage group) were present. The male sterility locus near the *SI* locus (*Pgm-1* group) (Van Dijk 1985) shows in our study some but insignificant, influence and is possibly the same as the locus Van Dijk found near *Pgm-1*. This hypothesis is also applicable for the type 1 males steriles in this experiment: two recessive and three dominant genes, all in a heterozygous state in one F_1 individual, and in the other F_1 individual the recessive genes are heterozygous and at the dominant loci wild type alleles are present (1 : 127, with a $\chi_1^2 = 0.05$, ns). Numbers are too small, however, for firm conclusions, and further investigations are necessary.

Since the male steriles appeared in both reciprocal crosses, the cytoplasm coming from Heteren (via F_1^1) and from Westduinen (via F_1^2) both must have the R plasmon.

The loci deviating from normal segregation were the same as the ones linked with a male sterility gene but the direction of the deviation, in correlation with the MS-alleles, was not the same for both allozyme loci. When gametic selection is postulated it appears that in the case of the selection against the *Gpi-1* I allele (from He), male sterility is coupled with the same allele. With *Got-2* the least viable is the I allele (from He) while MS

Table 9. Results of a five factor analysis of variance on several characters of the individuals of a F_2 generation. The significance of the separate main effects and interactions is given as well as the part of the total SS caused by each main effect and interactions (*** $P<0.001$; ** $P<0.01$; * $P<0.05$; – $P>0.05$)

Linkage group	Character					
	No. of rosettes	No. of leaves	Leaf angle	Cotyledon length	Leaf length	Leaf width
Gpi-1	– 0	– 0	– 0	– 0	*** 4	* 1
Got-1	*** 7	*** 2	*** 2	*** 5	*** 4	*** 10
Got-2	*** 3	*** 4	*** 6	*** 5	*** 22	*** 9
Pgm-1	*** 2	*** 4	– 0	– 0	– 0	*** 2
To-2	*** 4	*** 8	*** 7	* 1	– 0	– 0
Interaction:						
2-way	* 9	– 8	– 5	– 5	– 6	– 5
3-way	– 11	– 13	– 9	– 9	– 9	– 9
4-way	– 4	– 5	– 4	– 7	– 6	– 6

Linkage group	Character					
	Hairiness	No. of scapes	Scape length	Spike length	Flowering date	Leaf wt
Gpi-1	– 0	– 0	– 0	* 1	** 1	*** 3
Got-1	** 1	* 1	– 0	*** 12	*** 7	*** 8
Got-2	– 0	*** 3	*** 30	*** 4	** 2	*** 18
Pgm-1	*** 4	– 0	*** 4	– 0	*** 3	– 0
To-2	– 0	* 1	* 1	* 7	*** 9	* 1
Interaction:						
2-way	– 7	– 7	– 1	– 7	– 5	– 5
3-way	* 16	– 14	– 6	– 1	– 9	– 9
4-way	** 10	– 8	– 4	– 1	– 1	– 5

Linkage group	Character					
	Generative wt	Reproductive effort	Seed wt	Seeds/mm spike	Total seeds	Total seed wt
Gpi-1	– 0	*** 4	– 0	– 0	– 0	– 0
Got-1	– 0	*** 8	* 1	*** 4	– 0	* 2
Got-2	*** 15	– 0	*** 21	*** 9	*** 12	*** 6
Pgm-1	*** 3	*** 4	– 0	*** 5	* 2	* 2
To-2	– 0	* 1	*** 2	*** 4	– 0	* 2
Interaction:						
2-way	– 7	– 5	– 7	– 8	– 9	– 8
3-way	– 10	– 12	– 17	– 14	– 14	– 14
4-way	– 5	– 8	– 5	– 15	– 4	– 6

is coupled with the S allele (from Wd). There may be an advantage for a gamete having a linkage group (*Got-2*) with a male sterility allele and, in the other case, a disadvantage for a gamete having another linkage group (*Gpi-1*) with a male sterility allele. When, on the other hand, zygote selection is postulated for *Gpi-1* II zygotes, this might be a selection against male sterile zygotes.

Male steriles of type 1 are known to be at least as fit as hermaphrodites or to have an even higher survival and reproductive output (Van Damme 1984; Van Damme and Van Delden 1984). The numbers of MS1 are too low in this experiment for statistical tests but the same trend is observed. In an experiment with transplants from seven populations, male steriles flowered earlier than hermaphrodites, however only MS1 and MS2, and IN1 and IN2 and not MS3 were involved (Wolff, unpublished). In the present experiment hermaphrodites flowered somewhat earlier than MS3. MS3 appeared to be totally different from MS1 in having a lower reproductive output.

Quantitative characters and their genetic determination

The linkage of allozyme loci and QTL is clearly shown. In most cases two to five of the five linkage groups have a pronounced effect on morphological variation. Concentration of QTL for related characters in a specific linkage group is not found; no major gene complexes

Table 10. Results of a five factor analysis of variance on factor scores of the individuals on the principal component axis. This significance of the effects is given as well as the part of total SS caused by the effects. Significant interactions are given: *** $P<0.001$; ** $P<0.01$; * $P<0.05$; – $P>0.05$

	PC1	PC2	PC3	PC4
Gpi1	–	* 1	* 2	–
Got1	*** 3	*** 7	** 2	*** 4
Got2	*** 4	*** 11	*** 10	*** 3
Pgm1	*** 3	*** 3	–	*** 6
To2	–	–	** 2	*** 11
2-way interactions	–	–	– *Gpi-To** 2	– *To-Got1** 2
3-way interactions	–	– *To-Got1-Got2** 3	–	–
4-way interactions	–	– *To-Got1-Got2-Pgm1** 2	–	** 8 *Gpi-To-Got1-Got2** 3 *Gpi-To-Got1-Pgm1*** 4 *To-Got1-Got2-Pgm1*** 2

Table 11. Means of individual factor scores on first four principal components of groups determined by their number of heterozygous loci (HL) and significance of regression HL on individual factor scores (** $P<0.01$; – $P>0.05$)

	Heterozygous loci							Sign.
	0	1	2	3	4	5	6	
Means of PC								
PC1	−0.337	−0.345	−0.025	−0.038	0.084	0.260	0.271	**
PC2	−0.423	−0.245	0.152	−0.017	0.008	0.91	−0.281	–
PC3	−0.119	0.066	−0.059	0.039	−0.038	−0.008	0.230	–
PC4	0.054	0.222	0.064	−0.111	0.076	−0.114	−0.215	–
No. of plants	10	44	108	158	121	50	15	

have been detected. This can also be concluded from the fact that specific linkage group influences on the four principal components are not reflected in the patterns of linkage group influences on the characters highly correlated with the same specific principal component.

Selective interaction among alleles at different loci is very common in nature (Hamrick and Holden 1979). It may lead to the formation of gene complexes if these are not broken down by randomisation forces. Further forces influencing the conservation of gene associations are, amongst others, close linkage, chromosome inversions, selfing and drift in small populations whereas by outbreeding and recombination the associations may decay (Hedrick et al. 1978). Selection for the clustering of functionally related genes on a chromosome is plausible: the best allele combinations at the clustered genes will not easily be broken down by recombination. In procaryotes evidence exists for this process (Elston and Glassman 1967). There is hardly any strong evidence that clustering happens in eucaryotes (Elston and Glassman 1967; Hedrick et al. 1978), except for some exceptional cases as, for instance, can happen with the occurrence of inversions (Van Delden and Kamping 1987) and with supergenes in *Cepaea, Papilio* and *Primula.* In these cases the genes forming the supergene are all involved in the determination of facets of the same character. In other organisms no strong evidence exists for clustering of genes except possibly in the case of *Plantago major* (Van Dijk 1984), which will be discussed later. Apparently the forces breaking down gene complexes are stronger than the opposing forces in eucaryotes. Some care must be taken in concluding that selective forces are the cause of the existence of gene complexes; nonselective historical causes must first be eliminated (Hedrick et al. 1978).

In a review Brown (1979) expects that inbreeding species will show a greater degree of multilocus associations compared to outbreeding species. Inbreeders show less variation per population but more markedly differentiation between populations (Brown 1979): often a few specialised types are formed (Hamrick and Holden 1979) as, for example, in *Plantago major* (Van Dijk 1985) and in *Avena barbata* (Allard et al. 1972). In these cases selection forces keeping complexes together may be stronger than the randomisation forces. The distribution of QTL over the genome in the outbreeding species *Plantago lanceolata* and in the inbreeding species *Plantago major* is in accordance with what might have been expected (Brown 1979; Hedrick et al. 1978). It seems that in *Plantago lanceolata* the randomisation forces are stronger than the complex-forming forces, while in *P. major* gene complexes are possibly present. The results of Van Dijk (1985) point to clustering in *Plantago*

major. In some crosses, however, only a few loci were used. Some doubts are also created by the fact that in the complex situated in the linkage group of *Pgm-1* the allele combination is not always the correct one. Maybe historical causes at the onset of subspecies formation have had an influence.

Plantago lanceolata experiences a higher environmental variability than *Plantago major* by its higher gene flow (Van Dijk et al., in press). Gene complexes, with low recombination rates and fixed allele combinations, may be less favorable in a situation where gene flow is considerable and the environment experienced by the successive generations is more variable as in *Plantago lanceolata* (Maynard Smith 1977).

Correlations between characters can, among others, be caused by pleiotropic effects or linkage of QTL. Probably both effects will be present in our crosses. To be able to decide between both possibilities it will be necessary to study other crosses and other generations, for example, F_1 generations.

Opposing effects of different linkage groups on the characters have also been found for *P. major* (Van Dijk 1984) and for tomato (Tanksley et al. 1982). For *P. lanceolata*, it was to be expected from the high intrapopulational component of variability. Tanskley et al. (1982) ascribe the + and – effects to the many interaction effects present in their experiment, probably partly because in this experiment a backcross between two different species was used. Interaction effects are, however, unimportant in *Plantago lanceolata* (Tables 9 and 10).

The importance of QTL linked to *Got-1* and *Got-2* in determining total variation in our crosses is clearly shown. The accordance with one of the important linkage groups of *P. major* must be accidental since the distribution of the allozyme loci in the coupling groups is totally different (Van Dijk et al., in press).

Heterosis

During the last few years a vast amount of work on the differences in character means and stability (variance) of individuals or populations with differing levels of heterozygosity has been published (Mitton and Grant 1984; Livshits and Kobyliansky 1985). The ideas are based on the work of Lerner (1954) about homeostasis. In some cases relations between level of heterozygosity and character means or variability have been observed (Mitton 1978) and in other cases no relation was present (Zink et al. 1985). The criticism that allozyme heterozygosity in most experiments does not imply genome heterozygosity does not hold for our experiment. Correlations of quantitative traits with allozyme loci have been observed in great numbers so linkage disequilibria between QTL and allozyme loci in this cross are ascertained, meaning that a high allozyme heterozygosity also implicates a high genome heterozygosity.

The lower variability in the more heterozygous groups could be brought about by the nature of the material used. The individuals in these groups have combinations of alleles from two extreme ecotypes, giving a combination of + and – alleles with a less variable morphology.

Heterozygote advantage for morphological characters has also been found by Ledig et al. (1983) in pitch pine and by Mitchell-Olds and Waller (1985) in *Impatiens capensis*. It is striking that Kahler and Wehrhahn (1986) also found a heterosis effect, but only for seed yield characteristics, in a F_2 population of a maize hybrid, as was also found in this experiment. It can theoretically be expected in crosses between individuals from populations which are slightly or highly inbred. Whether this is the case here cannot be decided, although it is not probable because of the self incompatibility system. Absence of this effect in vegetative characters and the presence in generative characters cannot be explained by a general outbreeding effect.

Selection for important fitness characters leads to the loss of additive genetic variability (Falconer 1981). For *Plantago lanceolata* this was shown in a study where four populations are compared. It was found that especially in the hayfield population (He) a small additive genetic component of variability was present (Wolff and Van Delden, in press). It is possible that for the generative characters a high dominance component of genetic variability is left since a high reproductive output is important in general, but especially in Wd. It is probable that in highly heterozygous plants those dominance effects are more prominent.

Concluding remarks

In this paper it has been demonstrated that strong correlations exist between morphological characters and allozyme markers. It must be noted that correlations of specific allozyme alleles with a specific morphology is coincidental and specific for this cross; the alleles are considered neutral.

Hypotheses about the importance of some morphological or life history characters can be formulated in view of the general ecological demands of both habitats (hayfield and pasture), or in connection with demographic data, as has been done by Van Groenendael (1985), or by using data on the existence of additive genetic variability (Wolff and Van Delden 1986). In a reciprocal transplant of members of the pseudo-F_2 generation in both habitats, it can be expected that selection will be against plants that have much "strange" genetic material. This will show us the meaning of selection on morphological characters in both habitats.

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Appendix 1. Results from a principal component analysis. The first four factors and the factor score index of the characters with a significant correlation with the components ($P<0.001$) are given

Factor	Eigenvalue	Pct. of var.	Cum. pct.
1	5.765	30.3	30.3
2	3.424	18.0	48.4
3	2.060	10.8	59.2
4	1.805	9.5	68.7

Character/factor	1	2	3	4
No. of rosettes			+0.137	+0.829
No. of leaves	+0.247			+0.904
Leaf angle			−0.760	−0.155
Cotyledon length	+0.183	+0.225	+0.398	
Leaf length		+0.501	+0.737	+0.167
Leaf width		+0.931		
No. of scapes	+0.628	−0.267	−0.259	+0.310
Scape length	+0.230	+0.643	+0.382	
Spike length	+0.145	+0.619	+0.300	
Flowering date	−0.475	+0.380		+0.545
Leaf wt	+0.194	+0.630	+0.326	+0.548
Generative wt	+0.739	+0.463	+0.155	
Reproductive effort	+0.569	−0.264	−0.217	−0.535
Seed wt		+0.286	+0.302	
Seeds/mm spike	+0.626			
Total seeds	+0.935	+0.138		
Total seed wt	+0.883	+0.254	+0.221	
l length/width ratio			+0.878	
Scape/spike ratio				