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

2. Quantitative characters and allozyme loci in *P. major**

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Summary. Genetic variation in leaf and inflorescence morphology and in generative development within the species *Plantago major* has been analysed by means of crosses between members of two different subspecies. The variable characters chosen are supposed to be important for determining the ecological differences between the subspecies and other ecotypes. The analyses of F_2's indicated that a substantial number of loci controlling the above mentioned characters are situated near the *Pgm*-1 locus, forming a gene complex. This gene complex can exist in at least three different forms in ssp. *pleiosperma,* ssp. *major* lawn type and ssp. *major* roadside type, respectively. In addition, some important factors for ecotypic differentiation are situated in the neighbourhood of the *Got*-1 locus and in a linkage group containing three other allozyme loci. These linkages between allozyme loci and fitness-affecting loci can explain the restriction of some enzyme alleles to a particular subspecies.

Key words: Allozyme genetics – Genecology – *Plantago*

Introduction

Two mechanisms allow a species to occur in a range of habitats. The first possibility is that any genotype of the species is "flexible" in the way it copes with environmental factors, and is thus able to survive in all those environments. The second mechanism is the division of the species into a number of specialized genotypes which are each adapted to a particular habitat. Whether a species belongs to the first category (the generalists) or to the latter (the specialists) depends strongly on the relative importance of gene flow and selection. A high level of gene flow will prevent the forming and maintenance of ecotypes, as on the other hand this will be favoured by high selective differences between habitats.

The existence of ecotypes and even subspecies, combined with the known high selfing rate which reduces gene flow, classifies *Plantago major* as a specialist. The two most important subspecies are ssp. *major* and ssp. *pleiosperma.* Both subspecies have been morphologically and ecologically well characterized by Mølgaard (1976). Their differences can largely be considered as a consequence of a difference in "strategy". In the terminology of Grime (1977), ssp. *major* is more "competitive", while ssp. *pleiosperma* is more "ruderal". This means that ssp. *major* invests more in the survival of the individual plant, while ssp. *pleiosperma* invests more in reproduction and is able to produce seeds within a relatively short time and in relatively large quantities. The best investigated ecotypes of the subspecies *major* are the lawn type and roadside type, as described by Warwick and Briggs (1979, 1980a, 1980b). The two types differ in growth form of leaves and inflorescences. The prostrate form of the lawn type is supposed to be a genetic adaptation to moving and grazing. The roadside type is adapted to trampling.

Allozyme differences between the subspecies have been described by Van Dijk and Van Delden (1981). At two loci, *Got*-1 and *Pgm*-1, they found alleles that appeared to be subspecies-specific. The alleles *Pgm*-1^{S_2} and *Got*-1^{F} are restricted to spp. *major,* and *Got*-1^{I} is an allele specific for spp. *pleiosperma.* The other variable enzyme loci show more or less identical allele frequencies in both subspecies. In this paper the relationship between allozyme loci, in particular *Got*-1 and *Pgm*-1, and the genes related with the differences for morphological and developmental characters between the subspecies is investigated. The rationale for this investigation is that linkage between allozyme loci and loci affecting environmental

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adaptability forms one of the possible explanations for the occurrence of specific enzyme alleles in the subspecies. In such a situation the allozyme loci themselves can be considered as neutral, but the fact that they are linked to loci that are highly subjected to selection can restrict the distribution of their alleles. In a predominantly selfing species like *P. major* such "hitch-hiking" effects may easily occur, even without close linkage (Hedrick 1980).

Knowledge about the way in which genetic specialization within the species *P. major* is realized will provide more insight into the way in which adaptation of plants to their environments is realized in general. The genetic basis of ecological differences is poorly understood thus far. A lot of information is available from experiments with economically important crop races, but the difference in aim allows only a limited understanding of what is happening in nature.

Materials and methods

Crosses were made between the plants G_1 and Z_2, H_{19} and H_{44} and between S_8 and A_2 (Table 1). The reciprocal crosses were also carried out. F_2's were obtained by selfing particular F_1 plants. The origin of the parent plants, the cultivation circumstances and the way of making the crosses as well as the electrophoresis methods have been described earlier (Van Dijk and Van Delden 1981). The enzyme loci mentioned in this paper refer to the following enzymes: *Pgm* = phosphoglucomutase; *Got* = glutamate-oxaloacetate-transaminase; *Me* = malic enzyme; *Shdh* = shikimate dehydrogenase; *6Pgd* = 6-phosphogluconate dehydrogenase; *Est* = *esterase.*

Leaf morphology

Adult leaves from all plants of F_2, F_1 and parental selfing progenies were measured simultaneously. This was done at the time when the first plants started flowering and leaf shape was relatively stable. Three to five leaves per plant were scored for petiole length, blade length and maximum blade width. Mean leaf length, mean petiole length/blade length ratio and mean blade length/blade width ratio were calculated (see figure in Mølgaard 1976).

Inflorescence morphology

Inflorescence position was determined prior to collection of the inflorescences. A value 1 to 5 was given according to Mølgaard (1976): 1 = erect, 3 = bent, 5 = double bent, 2 and 4 being intermediate positions. After ripening, the inflorescences were collected for measuring spike length and scape length and counting seeds per capsule. Mean inflorescence length and mean spike/scape ratio were calculated from the values of three to five inflorescences. Five capsules were taken from the lower part of each spike, to count seeds, after which the mean seed number per capsule was calculated.

Generative devepolment

The time in days between germination and first flowering was noted for each plant. Because of disturbances of the normal distribution of these intervals in the F_2 by weather influences, the data were transformed into flowering time classes. The first 20% ($A_2 \times S_8$) or 25% ($G_1 \times Z_2$) of the F_2 plants that reached flowering got number 1, the second portion number 2, etc. A few weeks after the beginning of flowering all plants were scored on the same day for the number of inflorescences produced thus far.

Table 1. The parent plants: relative differences in quantitative characters; allozyme genotypes

Plant	Subspecies	Origin	Leaf shape	Petiole	Inflorescence position	Spike/scape ratio	Seed no. per capsule	Flowering time	*Pgm-1*	*Got-1*	*Shdh*	*Me-1*	*6Pgd-2*	*Got-2*	*Est-4*
G_1	*major*	lawn	round	short	bent	high	low	late	S_2S_2	SS	NN	NN	OO	NN	NN
H_{19}	*major*	path	round	long	erect	low	low	late	S_2S_2	FF	S_2S_2	NN	NN	NN	NN
S_8	*major*	ruderal	round	long	erect	low	low	late	S_1S_1	FF	NN	NN	NN	NN	NN
Z_2	*pleiosperma*	river bank	oval	long	double bent	low	high	early	NN	II	S_2S_2	SS	NN	FF	FF
H_{44}	*pleiosperma*	wet ruderal	round	long	double bent	low	high	early	NN	II	NN	NN	NN	FF	FF
A_2	*pleiosperma*	ditch side	oval	long	double bent	low	high	early	NN	SS	NN	NN	NN	NN	NN

Table 2. The F_2's $G_1 \times Z_2$: mean and standard deviation of nine characters for each genotype. Eventual significant differences between homozygotes are indicated per locus under the values concerned

Linkage group	Locus	Genotype	No. (max.)	Leaf length (mm)		Leaf blade length/width		Petiole length/blade length		Inflorescence length (mm)		Inflorescence position		Spike/scape		Seed no. per capsule		Flowering time class		No. of inflorescences	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	*Pgm*-1	S_2S_2	59	120.0	25.4	1.633	0.178	0.495	0.077	132,9	35.9	4.20	0.65	1.633	0.358	13.44	2.39	3.37	0.79	0.64	1.37
		S_2N	93	130.8	28.7	1.794	0.205	0.530	0.098	146.4	39.7	4.03	0.60	1.533	0.330	15.69	2.86	2.33	0.95	3.02	2.50
		NN	33	144.3	37.3	1.875	0.203	0.635	0.087	154.4	36.2	3.90	0.70	1.292	0.255	17.54	3.09	1.22	0.49	4.94	2.00
				***		***		***		*				***		***		***		***	
2	*Got*-1	SS	50	128.7	29.6	1.597	0.135	0.531	0.093	151.3	42.8	4.22	0.64	1.569	0.331	15.42	2.92	2.28	1.09	2.66	2.70
		SI	99	130.1	32.3	1.758	0.171	0.539	0.109	142.5	35.8	4.01	0.65	1.506	0.364	15.52	3.25	2.44	1.11	2.70	2.61
		II	36	128.7	26.0	1.981	0.218	0.543	0.093	134.0	37.9	3.92	0.58	1.506	0.316	14.45	2.82	2.83	1.08	2.19	2.40
						***						*						*			
3	*Me*-1	NN	54	126.4	30.7	1.753	0.219	0.545	0.107	141.9	42.0	4.07	0.63	1.504	0.346	15.49	3.19	2.45	1.17	2.23	2.40
		SN	93	131.3	30.0	1.745	0.211	0.539	0.100	145.4	38.3	4.07	0.67	1.563	0.368	15.25	3.16	2.55	1.04	2.73	2.70
		SS	38	129.4	30.7	1.796	0.222	0.523	0.098	140.1	34.0	3.97	0.56	1.452	0.279	15.07	2.87	2.32	1.21	2.76	2.57
3	*Shdh*	NN	48	122.2	28.8	1.755	0.228	0.535	0.100	137.6	40.7	4.08	0.62	1.492	0.333	15.06	3.18	2.45	1.19	2.21	2.37
		S_2N	106	132.5	30.1	1.755	0.215	0.542	0.102	146.5	38.3	4.06	0.67	1.553	0.365	15.47	3.09	2.54	1.03	2.73	2.71
		S_2S_2	31	130.1	31.9	1.769	0.202	0.525	0.105	140.9	35.7	3.96	0.56	1.469	0.295	14.99	3.05	2.29	1.27	2.71	2.52
4	*6Pgd*-2	OO	51	135.8	30.0	1.739	0.202	0.543	0.103	155.8	38.4	3.89	0.64	1.632	0.341	14.65	3.06	2.59	1.08	2.37	2.32
		NO	85	134.4	29.2	1.752	0.216	0.538	0.105	147.0	37.2	4.04	0.62	1.551	0.344	15.50	3.25	2.48	1.16	2.52	2.59
		NN	49	112.0	26.5	1.787	0.228	0.531	0.100	119.9	30.8	4.23	0.64	1.354	0.295	15.57	2.81	2.33	1.06	2.96	2.87
				***						***		*		***							
4	*Got*-2	NN	48	136.0	30.2	1.752	0.217	0.551	0.105	157.2	37.9	3.81	0.67	1.607	0.333	14.86	2.45	2.48	1.13	2.50	2.30
		NF	77	133.3	29.2	1.746	0.223	0.534	0.101	146.1	37.6	4.04	0.56	1.571	0.353	15.45	3.23	2.49	1.11	2.53	2.52
		FF	60	117.6	29.1	1.776	0.207	0.531	0.100	125.4	33.9	4.25	0.63	1.389	0.311	15.41	3.41	2.45	1.11	2.75	2.92
				**						***		**		***							
4	*Est*-4	NN	52	132.9	27.0	1.792	0.238	0.548	0.092	148.4	34.6	3.90	0.65	1.588	0.309	15.24	2.83	2.42	1.11	2.73	2.48
		NF	75	135.9	31.7	1.749	0.209	0.541	0.121	148.3	39.2	4.03	0.62	1.542	0.389	15.55	3.40	2.46	1.10	2.57	2.67
		FF	58	116.5	27.8	1.738	0.202	0.524	0.081	130.1	39.0	4.20	0.62	1.437	0.307	14.98	2.96	2.53	1.14	2.50	2.61
				**						*		*		*							
Entire F_2			185	129.4	27.0	1.758	0.215	0.537	0.102	143.2	38.4	4.05	0.64	1.522	0.346	15.28	3.10	2.47	1.11	2.59	2.59
F_1's $G_1 \times Z_2$			9	149		1.78		0.55		156		4.5		1.12		20.3				4.1	
$G_1 \times G_1$			9	121		1.395		0.44		122		3.5		2.45		8.4		4.0		1.6	
$Z_2 \times Z_2$			10	166		2.41		0.71		114		5.0		0.84		34.5		1.0		3.3	

*, **, *** Significant differences between homozygotes, $P < 0.05$, 0.01 and 0.001, respectively

Results

To detect any linkage between allozyme loci and quantitative characters three different crosses and their reciprocals were made between a ssp. *major* individual and a ssp. *pleiosperma* individual. From each of these six F_1's, one individual was used to obtain an F_2 by means of selfing. The original parents (like most *P. major* plants) appeared to be highly homozygous, for when selfed their progenies were very homogeneous, and further inbreeding by selfing could not perceptibly increase homogeneity. The F_1's of the crosses also looked perfectly homogeneous. These observations are in agreement with the combination of a high selfing rate of the species and the fact that infrequent outcrossings occur mostly between genetically related neighbours which are not distinguishable morphologically.

The three pairs of F_2's were grown at different times, so they are not fully comparable. But F_1 plants and plants obtained by selfing the parents were grown simultaneously with their F_2's. Both subspecies differ in a set of morphological and developmental characters, of which leaf and inflorescence morphology (Mølgaard 1976) and generative development are the most relevant ones. In Table 1 a survey of the relative differences between the parent plants is given. Not all characters appeared to be diagnostic for the subspecies: G_1, a lawn type of ssp. *major*, had short petioles and bent inflorescences with short scapes. H_{44}, a *pleiosperma* plant from a mixed population, possessed a ssp. *major*-like leaf shape. General plant size is not included in Table 1 because this character is variable in both subspecies.

The allozyme genotypes are also given in Table 1. The plants chosen for the crosses were at least differently homozygous for the loci *Pgm*-1 and *Got*-1, and as different as possible for the other enzyme loci.

The crosses $G_1 \times Z_2$

Seven enzyme loci belonging to four linkage groups (Van Dijk and Van Delden 1981) varied in the F_2. Each F_2 individual was scored for allozyme genotype and for nine morphological and developmental characters. For each of the three genotypes per enzyme locus a mean value was calculated for all characters. In Table 2 a survey of these mean values and their standard deviations is given. Some plants could not be measured for all characters, n is then somewhat lower than the maximum value of Table 2. The mean values of the entire F_2 are also shown in Table 2, together with the mean values of the F_1 and plants obtained by selfing the parents. No significant differences between the reciprocal F_2's were found, so only the combined values are given. A Student's *t*-test was applied on the differences between homozygotes and between the two homozygotes and the heterozygote. Flowering time class was not normally distributed; a chi-square test was used here. For reasons of conciseness only the significance classes of differences between homozygotes are given in Table 2. When no significant differences between homozygotes were

Table 3. The F_2's $G_1 \times Z_2$: correlations between characters. Correlation coefficients (above diagonal) and levels of significance (below diagonal). *n* is between 139 and 185

	Leaf length	Leaf blade length/ width	Petiole length/ blade length	Inflorescence length	Inflorescence position	Spike/ scape	Seed no. per capsule	Flowering time class	No. of inflorescences
Leaf length	×	0.133	0.345	0.807	−0.328	0.166	0.502	−0.104	0.099
Leaf blade length/width	–	×	0.108	−0.088	−0.303	−0.334	0.124	−0.127	0.174
Petiole length/ blade length	***	–	×	0.279	−0.285	−0.314	0.191	−0.243	0.163
Inflorescence length	***	–	***	×	−0.195	0.329	0.436	−0.110	0.087
Inflorescence position	***	***	***	*	×	−0.032	−0.060	0.176	−0.055
Spike/scape	*	***	***	***	–	×	−0.117	0.175	−0.059
Seed no. per capsule	***	–	*	***	–	–	×	−0.301	0.255
Flowering time class	–	–	***	–	*	*	***	×	−0.729
No. of inflorescences	–	*	*	–	–	–	***	***	×

– Not significantly correlated: $P \geqq 0.05$
* $P < 0.05$; *** $P < 0.001$

found, no differences between heterozygote and one or both homozygotes existed.

As can be seen in Table 2, the effects of linkage groups 1 and 4, and to a lesser extent, linkage group 2, are considerable for a number of characters, whereas linkage group 3 shows no effect in any case. In linkage group 4 the strongest effects are noticeable at the *6-Pgd-2* locus for leaf and inflorescence length and for spike/scape ratio. Inflorescence position is more associated with *Got-2*.

For two characters, leaf length and spike/scape ratio, a strong relation exists with both linkage groups 1 and 4. Leaf blade length/width ratio is related with both linkage groups 1 and 2. In those cases where two linkage groups were involved, the independence of their effects could be tested by means of analysis of variance. No interaction effects were found: the interaction F-values were for leaf length (*Pgm*-1 and *6Pgd*-2): F (4,157) = 1.35; leaf blade length/width ratio (*Pgm*-1 and *Got*-1): F (4,176) = 1.88; spike scape ratio (*Pgm*-1 and *6Pgd*-2); F (4,172) = 0.63.

Correlations between the quantitative characters were frequent, as can be seen in Table 3. Very strong correlations exist between leaf length and inflorescence

Table 4. The F_2's $H_{19} \times H_{44}$: mean and standard deviation of four characters for each enzyme genotype

Linkage group	Locus	Genotype	No. (max.)	Leaf length		Leaf blade length/width		Petiole length/ blade length		No. of inflorescences	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	*Pgm*-1	S_2S_2	58	135.2	28.1	1.493	0.117	0.433	0.108	2.52	1.49
		S_2N	94	134.8	23.9	1.462	0.089	0.451	0.112	3.78	1.39
		NN	43	128.6	25.7	1.510 (*)	0.128	0.499 **	0.099	4.06 ***	1.78
2	*Got*-1	FF	46	136.3	24.5	1.469	0.096	0.465	0.097	3.18	1.78
		IF	107	132.1	25.5	1.473	0.096	0.454	0.113	3.50	1.40
		II	42	134.3	27.2	1.515 (*)	0.139	0.454	0.115	3.82	1.91
3	*Shdh*	S_2S_2	54	136.1	24.6	1.453	0.095	0.459	0.091	3.14	1.59
		S_2N	105	130.9	25.3	1.488	0.114	0.445	0.112	3.53	1.63
		NN	36	137.3	27.8	1.508 *	0.104	0.486	0.126	3.94 *	1.52
4	*Got*-2	NN	53	137.5	26.3	1.489	0.113	0.476	0.103	3.36	1.71
		NF	100	134.2	25.1	1.470	0.098	0.450	0.111	3.56	1.64
		FF	42	127.0	25.4	1.503	0.133	0.453	0.117	3.42	1.21
4	*Est*-4	NN	57	136.8	27.1	1.476	0.110	0.475	0.106	3.33	1.82
		NF	102	133.5	26.0	1.478	0.105	0.451	0.113	3.54	1.47
		FF	32	125.8	21.1	1.499	0.117	0.445	0.106	3.65	1.70
Entire F_2			195	133.5	25.6	1.482	0.109	0.456	0.110	3.50	1.61
F_1's $H_{19} \times H_{44}$			43	157.2	22.6	1.470	0.071	0.548	0.101	5.20	1.46
$H_{19} \times H_{19}$			27	125.3	24.5	1.335	0.074	0.497	0.108	0.10	0.44
$H_{44} \times H_{44}$			17	129.8	27.0	1.675	0.090	0.516	0.079	4.83	1.03

*. **. *** Significant differences between homozygotes, $P < 0.05$, 0.01 and 0.001, respectively
(*) Significant difference only between the heterozygote and one homozygote; $P < 0.05$

Table 5. The F_2's $H_{19} \times H_{44}$: correlations between characters. Correlation coefficients (above diagonal) and levels of significance (below diagonal). *n* is between 147 and 195

	Leaf length	Leaf blade length/width	Petiole length/ blade length	No. of inflorescences
Leaf length	×	−0.017	0.195	0.103
Leaf blade length/width	–	×	0.216	0.093
Petiole length/ blade length	**	**	×	0.102
No. of inflorescences	–	–	–	×

– Not significantly correlated: $P \geqq 0.05$ ** $P < 0.01$

length and also between flowering time and number of inflorescences.

The crosses $H_{19} \times H_{44}$

Allozyme loci from the same linkage groups were segregating in these crosses but the quantitative characters studied were restricted to leaf morphology and number of inflorescences produced. The parents had a much more similar morphology in comparison with the parents of the previous crosses. The developmental characters of H_{19} and H_{44} were quite different however: H_{44} grew faster and flowered earlier.

As in crosses $G_1 \times Z_2$, the reciprocals were not significantly different. The mean values and their standard deviations are in a similar way summarized in Table 4. Significant effects of enzyme loci at the 1% level are only present for *Pgm*-1 for petiole length/blade length ratio and for the number of inflorescences produced. The latter character was also related to the *Shdh* locus at the 5% level. The effects of both linkage groups were independent: the F-value for interaction $F(4,147) = 0.31$.

The correlations between the four measured characters are given in Table 5.

The crosses $A_2 \times S_8$

Only *Pgm*-1 and *Got*-1 segregated in the F_2. The nine measured characters were the same as in crosses $G_1 \times Z_2$. No significant differences were found between reciprocals. Almost all morphological characters appeared to be associated with *Pgm*-1 or with both enzyme loci. Generative development was related with *Pgm*-1 only (Table 6).

No interactions between *Pgm*-1 and *Got*-1 were found for leaf blade length/width: $F(4,182) = 0.41$; for petiole length/blade length: $F(4,182) = 2.03$ and for spike/scape ratio: $F(4,178) = 1.44$. Correlations between the characters were very frequent in the F_2 (Table 7). Sufficient F_1 plants (46) were examined to calculate correlations between the same characters (Table 8). Less significant correlations were found here, both caused by lower r-values and by lower numbers of plants. In one case, however, a correlation in the $F_1$was significant at the 1% level and not at all significant in the F_2 (inflorescence position-spike/scape ratio).

Discussion

Relations between the quantitative characters

A first question in measuring a series of quantitative characters on the same object is whether they are independent from each other or not. The results from the F_2's show that they are dependent in most cases (Tables 3, 5 and 7). Three mechanisms are possible by which

Table 6. The F_2's $A_2 \times S_8$: mean and standard deviation of nine characters for each enzyme genotype

Linkage group	Locus	genotype	No.	Leaf length (max.) (mm)		Leaf blade length/width		Petiole length/blade length		Inflorescence length (mm)		Inflorescence position		Spike/scape		Seed no. per capsule		Flowering time class		No. of inflorescences	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	*Pgm*-1	S_1S_1	38	160.1	26.7	1.533	0.139	0.600	0.082	106.7	20.2	3.49	0.58	0.982	0.158	9.51	1.47	3.94	1.16	4.47	2.24
		S_1N	99	159.5	28.6	1.582	0.119	0.581	0.083	102.1	19.9	3.91	0.55	0.854	0.142	10.65	1.80	2.83	1.40	6.02	1.43
		NN	54	151.8	27.6	1.610	0.127	0.553	0.079	96.8	18.8	4.30	0.57	0.816	0.131	10.69	1.87	2.74	1.45	6.41	1.61
						**		**		*		***		***		**		***		***	
2	*Got*-1	FF	42	148.5	30.0	1.503	0.108	0.584	0.079	99.6	21.9	3.99	0.51	0.841	0.133	10.28	1.69	3.39	1.52	5.48	1.94
		SF	102	161.6	25.6	1.575	0.119	0.597	0.076	105.4	17.8	3.84	0.64	0.838	0.140	10.61	1.90	2.96	1.41	5.87	1.70
		SS	47	156.3	29.9	1.661	0.115	0.525	0.081	95.0	20.5	4.09	0.66	0.963	0.162	10.20	1.72	2.86	1.41	6.00	1.89
						***		**		(**)				***							
Entire F_2			191	157.4	28.0	1.580	0.127	0.577	0.083	101.5	19.8	3.93	0.63	0.870	0.154	10.43	1.81	3.02	1.44	5.83	1.80
F_1's $A_2 \times S_8$			46	172.5	19.8	1.617	0.044	0.677	0.065	104.6	15.0	3.45	0.38	0.739	0.100	9.98	0.91	2.93	1.14	5.69	1.33
$S_8 \times S_8$			9	118		1.39		0.58		106		2.0		0.77		6.0		5.0		2.1	
$A_2 \times A_2$			10	175		1.83		0.585		107		5.0		0.96		16.5		1.0		6.2	

*, **, *** Significant differences between homozygotes, $P < 0.05$, 0.01 and 0.001, respectively
(**) Significant differences only between the heterozygote and one homozygote; $P < 0.01$

Table 7. The F_2's $A_2 \times S_8$: correlations between characters. Correlation coefficients (above diagonal) and levels of significance (below diagonal). *n* is between 161 and 191

	Leaf length	Leaf blade length/ width	Petiole length/ blade length	Inflores-cence length	Inflores-cence position	Spike/ scape	Seed no. per capsule	Flowering time class	No. of inflores-cences
Leaf length	×	0.305	0.626	0.800	−0.263	−0.290	0.240	−0.285	0.379
Leaf blade length/width	***	×	0.077	0.102	0.158	−0.258	0.175	−0.375	0.434
Petiole length/ blade length	***	–	×	0.634	−0.383	−0.382	0.043	−0.056	0.113
Inflorescence length	***	–	***	×	−0.367	−0.188	0.188	−0.154	0.171
Inflorescence position	***	*	***	***	×	−0.064	0.350	−0.098	0.201
Spike/scape	***	***	***	*	–	×	−0.257	0.349	−0.418
Seed no. per capsule	***	*	–	*	***	***	×	−0.217	0.330
Flowering time class	***	***	–	–	–	***	**	×	−0.761
No. of inflorescences	***	***	–	*	*	***	***	***	×

– Not significantly correlated: $P \geqq 0.05$
*, **, *** $P < 0.05$, 0.01 and 0.001, respectively

Table 8. The F_1's $A_2 \times S_8$: correlations between characters. Correlation coefficients (above diagonal) and levels of significance (below diagonal). *n* is between 42 and 46

	Leaf length	Leaf blade length/ width	Petiole length/ blade length	Inflores-cence length	Inflores-cence position	Spike/ scape	Seed no. per capsule	Flowering time class	No. of inflores-cences
Leaf length	×	0.172	0.462	0.799	−0.243	−0.208	−0.176	−0.149	0.240
Leaf blade Length/width	–	×	0.180	0.069	−0.130	−0.311	−0.330	−0.180	0.239
Petiole length/ Blade length	**	–	×	0.232	−0.529	−0.670	−0.102	−0.083	0.002
Inflorescence Length	***	–	–	×	−0.082	−0.010	0.105	0.250	0.017
Inflorescence position	–	–	***	–	×	0.444	0.211	0.099	0.068
Spike/scape	–	*	***	–	**	×	0.261	0.310	−0.372
Seed no. per capsule	–	*	–	–	–	–	×	−0.101	−0.013
Flowering time class	–	–	–	–	–	*	–	×	−0.643
No. of inflorescences	–	–	–	–	–	*	–	***	×

– Not significantly correlated: $P \geqq 0.05$
*, **, *** $P < 0.05$, 0.01 and 0.001, respectively

correlations between two characters could be brought about:

1) Environmental differences affect both characters in the same or opposite direction, e.g. because both characters are "plastic" in the same way or because both characters have one or more factors in common.
2) Variable loci occur which affect both characters in the same or opposite direction.
3) A variable locus affecting one character is chromosomally linked with a variable locus affecting an other character; in addition both loci could be linked with a particular enzyme locus.

In the F_1's, assuming that they are genetically uniform, only mechanism 1 is bringing about correlations. The way in which characters are affected by the environment (their plasticity) may in turn be genetically defined, and can, therefore, be different for each cross.

Two correlation coefficients are very high, both in the F_2's and in the F_1 $A_2 \times S_8$ (Table 8): those for the couples leaf length – inflorescence length and flowering time – number of inflorescences. Mechanism 1 may be the main responsible factor. For all other correlations the extent of which the possible mechanisms contribute to the r-values is less clear. The observed high number of correlations between characters is not uncommon in F_2 progenies of interracial crosses when chromosome numbers are low. In Grant (1975) experiments with interracial crosses in *Gilia capitata* (Grant) and *Potentilla glandulosa* (Clausen and Hiesey) and with crosses between interfertile *Mimulus* species (Hiesey et al.) have been described, leading to results which are very well comparable with the results in the present study.

Relations between allozyme loci and quantitative characters

In several cases a significant relation between an allozyme locus and a quantitative character is found in the F_2's (Tables 2, 4 and 6). The most probable explanation is linkage between the allozyme locus and one or more loci that control the character. A pleiotropic effect of the enzyme locus itself is less likely, as the enzymes involved are not known to be key enzymes in morphogenesis. Thus, the enzyme loci can most probably be considered as "marker loci" only.

The tightness of a relation between an allozyme locus and a quantitative character depends on the chromosomal distance between the allozyme locus and the locus (or loci) that controls the character, and also on the differential contribution to the character by the alleles of the latter loci. It is not possible to separate both effects by the analysis of F_2's, nor to determine the number of linked loci: this would need analyses of further generations.

Of the large numbers of reported relations in Tables 2, 4, and 6, only those at a significance level of 1% will be taken into account for statistical reasons. The significance levels are usually given for differences between marker homozygotes only. Dominance effects, however, are noticed frequently. These effects are probably not of much interest for natural situations because they are only expressed in hybrids, which are infrequent in nature. To some extent the effects could allow F_1 hybrids to survive and reproduce in one of the parental habitats, but in later generations this advantage will be lost.

Leaf morphology

Variability has been found in total leaf length and in both leaf dimension ratio's: leaf blade length/width ratio and petiole length/blade length ratio. From these results the existence of four sets of genes can be derived which determine together the values of the measured leaf characters under the chosen experimental conditions: genes for petiole length, for blade length, for blade width and genes which determine total plant size, leaving the leaf shape ratio's unaffected. When variable loci are present for all categories, a number of correlations are to be expected: a positive one between total leaf length and blade length/width ratio, a positive or negative one between total leaf length and petiole length/blade length ratio and a negative one between blade length/width ratio and petiole length/blade length ratio. In the data the latter correlation is never negative and the correlation between total leaf length and petiole length/blade length ratio is always positive. Furthermore, total leaf length is in only one case (F_2's $A_2 \times S_8$) correlated with blade length/width ratio. All these results can be most simply explained by an absence of variable loci which affect blade length specifically while variable loci of the other groups do certainly exist. This enables us to consider blade length/width ratio and petiole length/ blade length ratio henceforth as measures of blade width and petiole length respectively which are independent of non-specific plant size.

That total leaf length is determined for the largest part by non-specific plant growth genes is suggested by the very strong correlation with inflorescence length (r = about 0.8 in all cases). Only in the F_2's $G_1 \times Z_2$ has a significant effect of marker genes on total leaf length been found. The effect of *Pgm*-1 can be explained by the locus or loci in this linkage group that affect petiole length. In linkage group 4, near the *6Pgd*-2 locus, one or more loci are probably situated that control non-specific plant size, for inflorescence length is behaving in the same way in this linkage group.

Petiole length is quite different in the parents G_1 and Z_2. This difference can be ascribed to loci in linkage group 1 to an extent of about 50%. Although the

parents of the other crosses possess almost equal petiole lengths, the effect of linkage group 1 can be established here also.

Leaf blade width is related to both *Pgm*-1 and *Got*-1. The effect connected with *Got*-1 is somewhat stronger. In the crosses $H_{19} \times H_{44}$, where leaf shape is less different between the parents in comparison with the other crosses, the effect of both *Pgm*-1 and *Got*-1 does not reach the 1% significance level.

Inflorescence morphology

Inflorescence position, the way in which the inflorescence is growing: prostrate or erect, is influenced weakly by linkage group 4 in the crosses $G_1 \times Z_2$ and rather strongly by *Pgm*-1 in the cross $A_2 \times S_8$. The ssp. *major* parent of the first cross, G_1, is of the lawn type with prostrate inflorescences and is therefore closer to the spp. *pleiosperma* appearance than the erect form of ssp. *major*. A possible conclusion from these data is that in the lawn type the locus (loci) for inflorescence position in linkage group 1 is in the "*pleiosperma* state", so that no segregation will be noticed in the F_2.

Spike/scape ratio may be positively or negatively correlated with inflorescence length depending on whether the spike length changes with constant scape length or the reverse situation is true. A large difference in spike/scape ratio exists between G_1 and Z_2: the lawn type plant G_1 has relatively short scapes. Genes for this difference in ratio are traced in the linkage groups 1 and 4. The differences in spike/scape ratio between A_2 and S_8 is only small. Genes that affect this ratio are, however, distinctly found in the linkage groups 1 and 2, with about equal effects but in opposite directions. Apparently there is no segregation for the linkage group 2 genes in the crosses $G_1 \times Z_2$.

Seed number per capsule is related with linkage group 1 (*Pgm*-1) only, both in the crosses $G_1 \times Z_2$ and $A_2 \times S_8$.

Generative development

The production of inflorescences after the juvenile stage can be described by the two parameters precocity and quantity, measured respectively by noting the time of appearance of the first inflorescence and by counting the number of inflorescence produced in a certain period after that first appearance. In the experiments, however, the number of inflorescences produced in a certain period after germination is counted, so a combination of precocity and quantity is measured. The high correlation coefficients between flowering time and number of inflorescences, also found in the investigated F_1, illustrate this dependency.

Flowering time class is strongly related with *Pgm*-1 in both the crosses $G_1 \times Z_2$ and $A_2 \times S_8$. In the first cross the correlation between the genotypic state of *Pgm*-1 and flowering time class is almost complete: almost all *Pgm*-1^{NN} plants are already flowering before the other *Pgm*-1 genotypes start to do so. The number of inflorescences, which could be measured in all three crosses, is also always strongly related with *Pgm*-1 only.

The genetic basis of ecological differences

The two subspecies of *P. major*, ssp. *major* and ssp. *pleiosperma*, occur in distinct habitats. Ssp. *major* is a perennial growing in environments which are exposed to physical stress. Ssp. *pleiosperma* is often forced to behave like an annual species due to high winter mortality. There is considerable competition to reach reproduction within a limited season length. In short it can be supposed that ssp. *major* individuals cannot keep pace with ssp. *pleiosperma* individuals in reproducing under ssp. *pleiosperma* conditions and on the other hand ssp. *pleiosperma* individuals are not sufficiently equipped to cope with ssp. *major*'s stress situations. The genes responsible for these differences between the subspecies have been noted partly in the experiments of this paper. Also, differences within the subspecies major have been put forward in these experiments. As concluded by Warwick and Briggs (1980a) the lawn type of *P. major* is genetically adapted to avoid the harmful effect of mowing and grazing by producing short leaves and prostrate inflorescences. These features are recovered in the parameters petiole length/blade length ratio, inflorescence position, spike/scape ratio and plant size (leaf length and inflorescence length). The relatively strong differences in these parameters in the crosses between G_1 and Z_2 when compared with the other crosses are for a substantial part determined by genes in the linkage groups 1 and 4.

The genetic constitution of ssp. *pleiosperma* distinguishes itself from that of ssp. *major* by bringing about earlier development of flowers and production of more inflorescences with more seeds per capsule. Leaf width is distinctly less in ssp. *pleiosperma*, what could be explained by the necessity of producing leaves of sufficient length to catch light, but with a smaller investment. More energy or nutrients are so remaining for generative development, as measured by flowering time, number of inflorescences produced and seed number per capsule. As spike length and number of capsules per cm spike are about the same in both subspecies, total seed number produced by ssp. *pleiosperma* exceeds that of ssp. *major* several times. Although ssp. *pleiosperma* seeds are smaller (by about a factor 2 in weight) the investment as measured by the production of seed biomass is still higher than in ssp. *major*.

Genes for generative development are strongly associated with *Pgm*-1 (linkage group 1) in all cases.

Genes for leaf width are found in the linkage groups 2 and 1. Apparently linkage group 1 plays an important role in subspecies differentiation.

Gene complexes

The genes affecting the quantitative characters which are important for both subspecies and ecotype differentiation are certainly not randomly distributed over the *P. major* genome. A striking quantity of them is associated with the *Pgm*-1 locus, suggesting a cluster of loci situated in the neighbourhood of this enzyme locus. At least three different important states of this gene complex are met: one for ssp. *pleiosperma,* one for the lawn type of ssp. *major* and one for the erect type of ssp. *major*. The ssp. *pleiosperma* complex is always accompanied by the *Pgm*-1^N allele, the ssp. *major* complexes can contain all three known alleles of *Pgm*-1. If in the complex any loci affecting fitness are situated on both sides of the *Pgm*-1 locus, then it seems almost impossible to exchange a *Pgm*-1 gene among gene complexes in different states, for the complex has to be broken down to accomplish this. This would result in plants which are poorly adapted to each of the parental habitats. Only F_2 individuals possessing an integrated gene complex can function adequately in the appropriate environment. The chance that the complex is transferred as a whole is evidently greater when the complex is more compact.

A gene complex is not stable when recombination within the complex is frequent – unless the selection coefficients against incomplete complexes are very high. A predominantly selfing species, however, is able to maintain such complexes rather easily, as a high degree of selfing has a similar effect on recombination frequency as tight linkage (Hedrick 1980).

The presence of different alleles of *Got*-1 in both subspecies suggests a similar gene complex around the *Got*-1 locus. In the set of investigated characters, however, only leaf shape is strongly associated with the *Got*-1 locus, but of course other important loci for characters not included in this study may be linked to it. The more relaxed relationship between the occurrence of the *Got*-1 alleles I and F and seed number per capsule in natural populations compared with the *Pgm*-1 locus (Van Dijk and Van Delden 1981) can be explained now because no variable loci for seed number per capsule are associated with *Got*-1. The finding of a relationship between *Got*-1 and seed number per capsule has to be the result of having both gene complexes simultaneously in the ssp. *major* or *pleiosperma* state. *Pgm*-1, on the contrary, is clearly linked with seed number, so the reported absolute relationship in this case is no longer suprising.

Of course, other gene complexes may exist that do not comprise any marker loci, but certainly the *Pgm*-1 complex is a very important one, for it contributes for a large part to the relevant differences between the subspecies.

Concluding remarks

The ecological differences between the subspecies and other ecotypes of *P. major* have been partially analysed genetically in this paper. The presence of suitable marker loci admidst loci which affect the ecologically important characters allows further analyses of the relevant gene complexes in an efficient way. To determine the relative importance of loci in the complex accompanying fitness measurements are necessary. The set of morphological and developmental characters could be supplemented with physiological characters. Of great interest would be the analysis of the genetic basis of trampling resistance which is supposed to be the adaptive feature of the roadside type of ssp. *major*. Whether morphological or physiological parameters are the most important for this adaptation is till uncertain.

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