

Genetic analysis of morphological variability in three *Plantago* species with different mating systems *

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Summary. Morphological variability in three *Plantago* species possessing different mating systems – *P. major*, *P. coronopus*, and *P. lanceolata* – was analyzed. From each species several populations were analyzed. In comparing the species, it appeared that both the inbreeding *P. major* and the outbreeding *P. lanceolata* showed a high degree of morphological differentiation between populations. It was hypothesized that the absence of population differentiation for the species with a mixed mating system, *P. coronopus*, was caused by high adaptive plasticity. Plasticity is probably the predominant mechanism by which this species copes with different environments. There were considerable differences between populations within each species for their levels of variability and their heritabilities. It appeared that besides the influence of the mating system, selection may diminish morphological variability in the case of strong directional selection, especially in *P. major* and *P. lanceolata*.

Key words: Morphological variability – *Plantago* – Differentiation – Adaptive plasticity – Mating system

Introduction

The evolutionary importance of the mating system is the subject of many studies on both plants and animals. Since in plants a diversity of mating systems occurs, often among closely related species or even within a single species, plants are particularly suitable for studies on the effects of the mating system on variability and genetic structure.

From theoretical and electrophoretic studies it has become clear that outbreeding species generally have high variability within populations and low heterogeneity among populations, whereas inbreeding species mostly possess low genetic variability within populations and significant population differentiation (e.g., Solbrig 1972; Brown 1979; Schoen 1982; Layton and Ganders 1984; Van Dijk et al. 1988). Populations of inbreeding species were sometimes even considered to possess hardly any genetic variability (cf. Jain 1976). Morphological studies in inbreeding species show a more detailed and diversified picture (Stebbins 1957; Jain 1976; Adams and Allard 1982). Often a much higher variability has been found within populations than expected from theoretical and electrophoretical studies. Still, in general, outcrossers have higher morphological intrapopulation variability, and inbreeders have a higher degree of population differentiation (see for reference Carey 1983), although in some studies no differences between outbreeding and inbreeding species were observed (e.g., Brown and Jain 1979) or even a reversed result was found (Hillel et al. 1973).

In the present study, three *Plantago* species with different mating systems – *P. major*, *P. coronopus*, and *P. lanceolata* – are used to study morphological differentiation. This was realized by measuring morphological variability in several populations within each species. Both subspecies of *P. major*, *major*, and *pleisneria*, as well as the different ecotypes present within each subspecies (Van Dijk 1985) were represented in the populations studied. For *P. coronopus* and *P. lanceolata*, populations from contrasting habitats were used. In this way, comparisons could be made within species and among species. The three species are closely related and have partly overlapping ecological demands, as the species may occur in the same habitat close to each other. Some sites with more than one *Plantago* species were used in the

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present analysis. In *P. lanceolata*, half-life times vary between 1.1 and 30.0 years, depending on the population (J. Van der Toorn, personal communication). *P. major* populations also have different life times, from annual on riverbanks up to perennial. Most *P. coronopus* plants, however, are biennials in nature. The *Plantago* species possess different mating systems: *P. lanceolata* is self-incompatible (outcrossing rate $t=1.0$), *P. coronopus* and *P. major* are self-compatible. From earlier studies it is known that *P. major* is highly self-pollinating ($t: 0.0-0.1$), whereas *P. coronopus* shows intermediate outcrossing rates ($t: 0.5-0.9$) (Van Dijk et al. 1988; Wolff et al. 1988). Van Dijk et al. (1988) described the genetic structure of the three species: it appeared that with respect to electrophoretic variability, *P. major* showed the highest population differentiation, *P. lanceolata* the lowest, whereas *P. coronopus* had an intermediate position, which is in accordance with what is generally found for species with comparable mating systems (Brown 1979). The effect of the mating system on the genetic structure of the *Plantago* species has clearly been shown (Van Dijk et al. 1988). In the present study the importance of differentiation, plasticity, and mating system for morphological variability is studied. In a succeeding study, I will compare the morphological and electrophoretic variability, both within and between species (Wolff 1991).

Table 1. Description of the sites where the populations of *P. lanceolata* (*P.l.*), *P. coronopus* (*P.c.*), and *P. major* ssp. *major* (*P.m.m.*) and ssp. *pleiosperma* (*P.m.p.*) occurred

Site	<i>Plantago</i> spp. occurring	Soil type	Habitat and management	Height of details	Vegetation
Angeren (An)	<i>P.m.p.</i> in strongly variable numbers over years	Sandy	Riverbank; grazed moderately	10-30 cm	Flooded during winter
Heteren (He)	<i>P.l.</i> in a homogeneous, stable habitat	Heavy clay	Hayfield; mown end of July	42-73 cm	High and dense vegetation
Kwade Hoek (Kh)	<i>P.c.</i> in high densities <i>P.m.p.</i> in low densities	Low parts: sandy High parts: clay	Salt meadow; extensively grazed by cattle	low: 5 cm high: 20 cm	Occasionally flooded by sea Seldom flooded
Oostvoornse meer (Om)	<i>P.m.p.</i> from harsh part	Sand	Former beach plain; grazed by rabbits	1-15 cm	In winter frequently inundated by rain water, low biomass
Schiermonnikoog dike (Sd)	<i>P.c.</i> in low densities	Sandy clay	Sea dike; mown	15 cm	Relatively high grassland vegetation
Schiermonnikoog path (Sp)	<i>P.l.</i> isolated population among dunes	Sandy clay	Pathside	12 cm	Poor grassland
Uddel (Ud)	<i>P.c.</i> , <i>P.l.</i> isolated, in heath fields	Sand	Roadside	2-4 cm	Harsh, dry habitat
Westduinen (Wd)	<i>P.m.m.</i> along cow path, <i>P.c.</i> and <i>P.l.</i> in grass vegetation	Dune sand	Pasture; extensively grazed	4-7 cm	Old coastal grassland, severe unpredictable dryness (summer)
Weevers' Duin (Wv)	<i>P.m.m.</i> plants	Sand	Gravel path	1-5 cm	Harsh habitat, only some <i>P.m.m.</i> plants

Materials and methods

Populations sampled

Adult *P. major* plants have been sampled from five populations, and *P. coronopus* and *P. lanceolata* plants from four populations. Two of the *P. major* populations belong to subspecies *major*, whereas the other three populations are subspecies *pleiosperma*. These subspecies differ in allozyme composition and have some discernable morphological and life history features (Mølgaard 1976; Van Dijk and Van Delden 1981; Van Dijk 1984; Van Delden 1985). For these characters significant population differentiation is expected a priori. The populations of the three plantain species chosen are representative for the morphological and life history variation present in these species in The Netherlands (Wolff and Van Delden 1987; Lotz 1989; K. Wolff, this study). Some of the *P. major* populations have been studied by Lotz and Blom (1986) for plasticity in life history traits, while all populations were used in a transplantation experiment by Lotz (1989). Two of the *P. lanceolata* populations, He and Wd, were also represented in the morphological study of Wolff and Van Delden (1987), who found that differences between the populations were present for means, variability, heritability, and plasticity of the characters studied. A short description of the populations is given in Table 1.

Morphological measurements

From each *P. coronopus* and *P. major* population, eight randomly chosen plants were selfed, giving eight selfed families per population (S_1 families). Sixteen *P. lanceolata* plants were pairwise crossed, giving eight full-sib families per population (F_1 families).

The S_1 and F_1 seeds were germinated in petri dishes on wet filter paper – *P. lanceolata* seeds in the dark, *P. coronopus* seeds in the light – while *P. major* seeds were germinated in pots. Eight members of each family were grown in the greenhouse in the same period, each species in a randomized design. All three species could not be grown in one randomized design, because each species needed specific watering and the smaller *P. coronopus* would have been shaded by the taller *P. lanceolata* plants. The plants were kept in pots at 20–25°C with 16 h light daily.

During growth of the plants, cotyledon length, leaf angle, leaf lamina length and width, and petiole length of the three longest leaves at the moment of first flowering were determined for *P. major* (presented as a mean). Flowering date was recorded as the first day after germination on which the stamens were visible. The plants were harvested after 14 weeks (*P. coronopus* and *major*) or after 16 weeks (*P. lanceolata*). The following characters were also measured: number of rosettes, number of leaves, length of the longest leaf, width of the widest leaf, number of scapes, length of the longest scape, length of the spike belonging to this scape, and a measure for the degree of toothing was taken by estimation for *P. coronopus* (on a scale of 0 to 100). For *P. major*, four characters characterizing the subspecies were measured: scape position, toothing of the leaves (both on a scale of 0 to 6), number of nerves, and number of seeds per capsule (cf. Mølgaard 1976). The aboveground biomass was separated in leaf material (leaf weight) and in scapes, spikes, and seeds (generative weight). For weight determination the material was dried at 80°C for several days. The weight of the leaves, the generative organs, and the roots, as well as the weight of the dry seeds, in ripe spikes only, was determined.

From these data were calculated leaf length/width ratio, reproductive effort measured as the ratio generative weight/(generative + leaf weight), shoot/root ratio, in which the shoot is the sum of leaf and generative weight, spike/scape ratio, petiole/lamina length, seeds/mm spike, total number of seeds (number of spikes \times spike length \times seeds/mm spike), and total seed weight (total number of seeds \times seed weight).

Calculation of morphological variability

In the statistical analyses no transformations were used, as this did not improve normality and most variables were close to a normal distribution. Most calculations on the morphological data were carried out using the Statistical Package for the Social Sciences (SPSSx) (SPSS 1986). For *P. coronopus*, variability within populations was measured using selfed families, although outcrossing frequently occurs in nature. By using selfed families, the within-population variability is magnified by a factor that is maximally 1.5 in the case of a population that is normally random mating. A mean outcrossing rate of 0.61 (Wolff et al. 1988) is taken to calculate the appropriate correction factor. This factor is

$$\frac{1+F_1}{1+F_0}, \quad \text{or} \quad \frac{1+(1/2+1/2 F_0)}{1+F_0},$$

in which

$$F_0 = \frac{1-t_m}{1+t_m}.$$

For $t=0.61$ this factor equals 1.305. Within-population variability is corrected by 1/1.305 before calculating total variability and partitioning variability over populations and families (Falconer 1982; P. Stam personal communication).

Heritability estimates were calculated as

$$h^2 = r \left(\frac{s_B^2}{s_B^2 + s_W^2} \right),$$

where s_B^2 and s_W^2 are the estimates of between- and within-family components of variance, respectively. For full-sib families in a randomly mating population, r is the reciprocal of the efficient of the additive variance in the covariances of sibs (1/2) (Falconer 1982). For *P. lanceolata*, this coefficient r was thus approximated by two (full sibs in a randomly breeding species). For progenies derived by selfing, r has another derivation. The within-line variance σ_W^2 is $(1-F) V_G$ and the between-line variance σ_B^2 is $2-F V_G$ (Falconer 1982). For one generation of selfing in a normally random mating population ($F=1/2$), σ_B^2 is V_G and σ_W^2 is $1/2 V_G$. For *P. coronopus*, r is best approximated by the factor, as mentioned above (selfed progenies in a mixed breeding species with t as the outcrossing rate):

$$\frac{1+F_1}{1+F_0}$$

For each population this factor was calculated with the appropriate t . For the species as a whole and the population Ud, the mean outcrossing of the species was used ($t=0.61$). For *P. major*, r is best approximated by 1 (self progenies in a selfing species) as proposed by Lawrence (1984). For *P. lanceolata* and *P. coronopus* the heritability estimates are maximum estimates, as the dominance and maternal effects components of variability unfortunately cannot be separated; for *P. major* these are minimum estimates (Lawrence 1984). Consequently, the heritability estimates of *P. lanceolata* and of *P. coronopus* must not be regarded as pure narrow heritabilities. In a diallel analysis with *P. lanceolata* it was found that the maternal effects component is only significant for early life characters like germination rate and cotyledon length (Wolff 1990). The 95% confidence intervals were calculated as given in Wolff and Van Delden (1987) using the appropriate r .

To make comparisons between species or between populations within species, it was not feasible to calculate means of CV or heritabilities over all characters, as a mean has no biological meaning. Two solutions were found for this problem. A non-parametric test, Friedman's test, was used for this purpose (Sokal and Rohlf 1981). In this test the species or the populations within a species were ranked, for each character, from one to five for *P. major* populations, from one to four for *P. coronopus* and *P. lanceolata* populations, and from one to three for the species comparison. The rank numbers were summed up over characters ($\sum R$) and, with a Friedman's test, the populations (or species) were tested for the presence of heterogeneity. Differences between pairs of populations were tested using a Wilcoxon signed rank test, as there is to my knowledge no nonparametric test for multiple comparisons among means available. The Wilcoxon signed rank test may give a slight overestimation of the significance of the differences. In the text and in the tables, mean rank (\bar{R}) over characters is given.

Many of the characters mentioned above are more or less correlated; therefore, multivariate analysis was appropriate. Consequently, the second solution was a multivariate analysis of variance. In order to obtain independent characters, a principal component analysis on the correlation matrix was applied to the data. The individual component scores on the principal component axes were further used as characters of the individuals (Chatfield and Collins 1980). Only those principal components (PC) were used with an eigenvalue larger than 1.0. Means over PC are in all cases weighted means, in which the components are weighted to their relative contribution to the total variance explained. In the within-species comparisons, all characters measured in that specific species are used for the analysis, whereas in the between-species comparison, a subset of the characters measured was used in the principal component analysis, namely, those characters measured in all three species (number of

Table 2. Between-subspecies, between-population, and between-family components of variance of quantitative characters and of weighted means of PC of *P. major*, presented as percentages of total variance and calculated from variance components derived from a nested analysis of variance

Character	Factor		
	Subspecies	Population	Family
\$ Scape position	60*	8**	16***
\$ No. of teeths	74**	2	12***
\$ Nerves	84***	0	8***
\$ Seeds/capsule	39	41***	7***
No. of rosettes	0	32***	14***
No. of leaves	18	27***	23***
Lamina length	20	18***	18***
Lamina width	0	16**	31***
Petiole length	16	33***	12***
Lamina length/width	11	30***	19***
Petiole/lamina length	1	44***	16***
No. of scapes	33	6	24***
Scape length	52***	8**	12***
Spike length	0	0	52***
Spike/scape ratio	47*	8*	20***
Flowering date	0	10	42***
Leaf weight	0	30***	21***
Generative weight	44*	9*	12***
Root weight	2	4	12***
Reproductive effort	8	43***	32***
Shoot/root ratio	24	4*	10***
Mean PC (all char.)	17	17	22
Mean PC (subset)	19	20	25

\$ Subspecies characteristic traits (Mølggaard 1976)

*, **, *** = $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

Table 3. Between-population and between-family components of variance of quantitative characters and weighted means of PC of *P. coronopus*, presented as percentages of total variance and calculated from variance components derived from a nested analysis of variance

Character	Factor		
	Population	Family	
Leaf angle	27**	18***	
No. of rosettes	0	44***	
Leaf length	10	38***	
Leaf width	46***	18***	
Toothing	54***	8***	
No. of scapes	26***	2	
Scape length	0	50***	
Spike length	15	42***	
Spike/scape ratio	25**	22***	
Flowering date	10	32***	
Leaf weight	1	39***	
Generative weight	0	22***	
Root weight	2	28***	
Reproductive effort	0	44***	
Shoot/root ratio	6*	12***	
Mean PC (all char.)	6	39	
Mean PC (subset)	17	35	

*, **, *** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

Table 4. Between-population and between-family components of variance of quantitative characters and weighted mean PC of *P. lanceolata*, presented as percentages of total variance and calculated from variance components derived from a nested analysis of variance

Character	Factor	
	Population	Family
Leaf angle	36***	16***
No. of rosettes	17**	27***
No. of leaves	25***	26***
Cotyledon length	18**	31***
Leaf length	57***	7***
Leaf width	52***	10***
Leaf length/width	24**	24***
No. of scapes	36***	13***
Scape length	43***	14***
Spike length	4	26***
Spike/scape ratio	10*	37***
Flowering date	4	25***
Seed weight	8**	4
Seeds/mm spike	6*	14**
Total seed no.	24***	14***
Total seed weight	11**	10**
Leaf weight	34***	17***
Generative weight	8*	10**
Root weight	32***	14***
Reproductive effort	21**	19***
Shoot/root ratio	10*	20***
Mean PC (all char.)	23	19
Mean PC (subset)	25	18

*, **, *** = $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

rosettes, flowering date, leaf length, leaf width, scape length, spike length, leaf weight, generative weight, and reproductive effort). Heritabilities of PC cannot be calculated as they have no biological meaning. It was desirable, however, to have a relative measure of the additive component of variability of PC. Therefore, this measure, henceforth denoted as V_A/V_P PC, was calculated in the same way as described for morphological characters, but instead of the character of the individual, the individual component score on the PC was used. Differences between populations for V_A/V_P PC and standard deviations of PC were tested with a Student-Newman-Keul's test (SPSS 1986), in which each PC was weighted as described above. It must be noted that, although the principal component analysis in the three species was based on the same set of characters, PC for the species are likely to represent different combinations of traits. Therefore PC1 is an entirely different trait in one species than in another species.

Results

Differences in morphological characters between populations

Means of morphological characters were calculated (data not presented). In Tables 2 to 4 the partitioning of variability over populations and families is given as calculat-

ed with a nested analysis of variance. In these tables, the significance of the factor *populations* is given and, for *P. major*, also the factor *subspecies* (double nested anova). In *P. major* the factor *subspecies* was found to be significant for three of the first four characteristics that distinguish the subspecies (Mølgaard 1976). In this analysis the factor *subspecies* was not significant for the number of seeds per capsule. This was caused by a large variance in the number of seeds per capsule between the *P. pleiosperma* populations, which is a part of the within-subspecies variance over which the factor *subspecies* is tested. A Student-Newman-Keul's test showed significant differences among populations for all four characteristics. The two *P. major* populations from habitats with a higher vegetation (An and Kh) had the longest leaves and the highest leaf weight. The reproductive effort and the shoot/root ratio was highest in the Om population. Wv had a high spike/scape ratio, a feature of a *P. major* ssp. *major* lawn type. For *P. coronopus*, only a relatively small number of characters were significantly different between populations. No consistent ecotype differences seemed to be present. The narrow leaves and high degree of toothing of the Ud population was extreme. The differences between the *P. lanceolata* populations were predominantly as described by Wolff and Van Delden (1987). For almost all characters differentiation was found. The He population, the hayfield ecotype, was clearly different from the other populations, with Wd at the other extreme. The He population had a low number of long leaves, a small leaf angle, long scapes and spikes, heavy seeds, and a high flowering date (late flowering). It must be kept in mind that due to the high number of tests, a number of tests (5% at a significance level of 5%) is significant by chance alone. The multivariate analysis of

variance showed highly significant ($P < 0.001$) population and family effects for all three species.

In the principal component analyses (PC), axes could generally easily be biologically interpreted, often representing height of the plants (e.g., leaf and scape length), vegetative growth, and generative growth. Exact interpretation of each component is not given for reasons of space and because it is not a prerequisite for further use of PC in the analysis (Chatfield and Collins 1980). In Fig. 1 the family means of individual component scores of each population and each species are presented. From this figure it can easily be seen that populations of *P. major* and *P. lanceolata* have more or less different positions in the figure, whereas the populations of *P. coronopus* are to a great deal overlapping.

Analyses of morphological variability

Within species, the variability for each character within each population was expressed as the coefficient of variation (CV_p) (data not presented) and also as the weighted mean of the standard deviation of PC (Table 5). It appeared that length and width measures, such as leaf length, leaf width, and spike length, had a relatively low variability within the populations. Flowering date also had a low CV_p , especially in *P. major* and *P. coronopus*. To compare the species for total variability, the CV_s has been calculated for each character of the common character set, using the plants from all populations. The mean rank of CV_s over these characters was taken as a within-species measure of total morphological variability (the variability of PC cannot be used, as this is 1 by definition). *P. lanceolata* had the highest total morphological variability ($\bar{R} = 2.64$), *P. coronopus* took an intermediate posi-

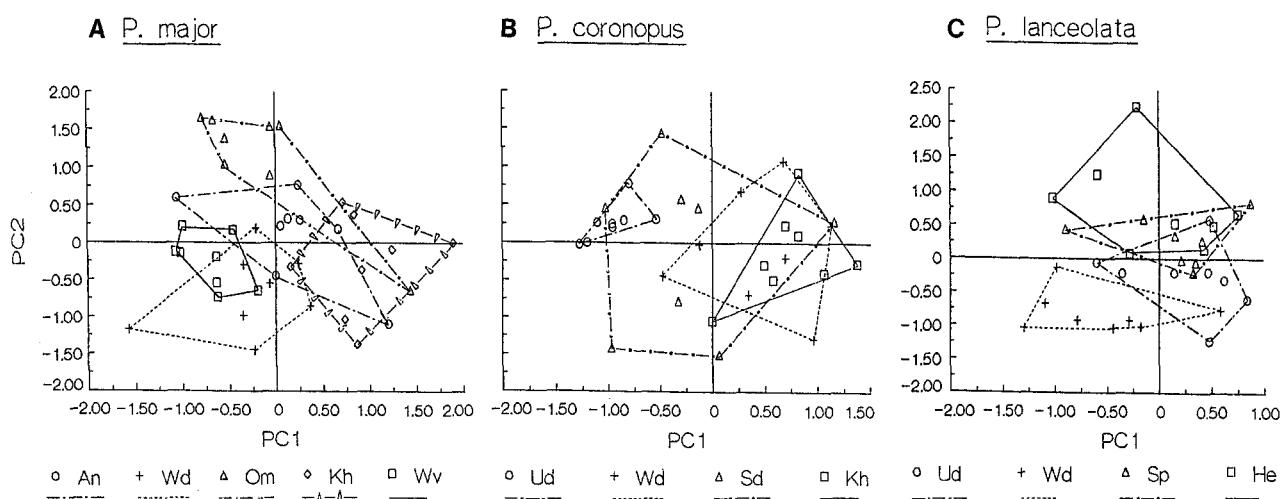


Fig. 1A–C. Family means of individual component scores on the first two axes derived from a principal component analysis. In each population each family mean is designated with a different sign and the most extreme family means of each population are connected by lines; for each population a distinct line type is used. A *P. major*; B *P. coronopus*; C *P. lanceolata*

Table 5. The populations of the three *Plantago* species studied and their morphological variability. In (A) the populations are ordered in descending order to mean rank number (\bar{R}) for CV_p and the weighted mean standard deviation of PC. In (B) populations are ranked to mean rank number of their h^2 and the additive component of the variance of PC in each population. Populations connected by a common line are not significantly different ($P > 0.05$). See text for further explanation

(A)		\bar{R} CV	Om (3.74)	Wd (3.62)	An (3.38)	Kh (2.88)	Wv (1.3)
<i>P. major</i>		sd PC	Om (0.873)	Wd (0.836)	Kh (0.832)	An (0.820)	Wv (0.591)
<i>P. coronopus</i>		\bar{R} CV	Sd (3.36)	Kh (2.97)	Wd (2.68)	Ud (1.00)	
		sd PC	Sd (1.244)	Wd (0.943)	Kh (0.871)	Ud (0.566)	
<i>P. lanceolata</i>		\bar{R} CV	He (2.98)	Sp (2.79)	Wd (2.33)	Ud (1.90)	
		sd PC	He (0.944)	Wd (0.921)	Ud (0.839)	Sp (0.799)	
(B)		\bar{R} h^2	Kh (3.11)	Om (3.29)	An (3.14)	Wd (3.00)	Wv (1.88)
<i>P. major</i>		V_A/V_p PC	Kh (0.416)	An (0.332)	Om (0.286)	Wd (0.259)	Wv (0.258)
<i>P. coronopus</i>		\bar{R} h^2	Wd (3.11)	Kh (2.75)	Sd (2.61)	Ud (1.54)	
		V_A/V_p PC	Wd (0.660)	Sd (0.499)	Kh (0.478)	Ud (0.314)	
<i>P. lanceolata</i>		\bar{R} h^2	Wd (2.95)	Sp (2.60)	Ud (2.48)	He (1.98)	
		V_A/V_p PC	Wd (0.633)	Sp (0.507)	Ud (0.482)	He (0.456)	

Table 6. Distribution of morphological variability between populations, between and within families, presented as a percentage of total within-species variability. Both the mean of the distribution of the common characterset (CHAR) and the distribution of the variability of the PC are given

Species	<i>P. major</i>		<i>P. coronopus</i>		<i>P. lanceolata</i>	
	CHAR	PC	CHAR	PC	CHAR	PC
Between populations	30	36	15	17	25	23
Between families	26	27	33	35	18	21
Within families	44	37	52	48	57	56

tion ($\bar{R}=1.82$), and *P. major* showed the lowest overall variability ($\bar{R}=1.55$). There was no significant heterogeneity in variability over species (Friedman's test). Within each species the populations were compared, firstly for their variability (mean rank of CV_p) using the same test, and secondly for the standard deviations of their PC. In all three species heterogeneity in CV_p over populations was present using Friedman's test ($P \ll 0.001$ for *P. major* and *coronopus*; $P=0.03$ for *P. lanceolata*). The populations, ordered to mean rank number (\bar{R}), are given in Table 5, together with the weighted mean standard deviation of PC in each population. It appeared that the rank order showed only minor differences using the nonparametric and the PC method in all three species.

In order to compare the species for their distribution of morphological variability, means were calculated over

the common characters (Table 2 to 4) and a weighted mean of the distribution of variability for the PC (based on the common character set) was calculated (Table 6). In this case, the factor subspecies was not included in the analysis of variance of *P. major*. For *coronopus* the appropriate correction factor was attributed to the within-population variability, as mentioned in the 'Materials and methods' section. It appeared that *P. major* had the highest between-populations component, followed by *P. lanceolata*, whereas *P. coronopus* showed a low between-populations component of variance and a relatively high between-family component of variance.

Heritability estimates

For each species, the heritabilities were estimated from an analysis of variance within each population (data not presented). Spike/scape ratios were left out of the analysis for *P. coronopus* and *P. lanceolata* because of a low variability. For *P. lanceolata*, total seed weight and total number of seeds were left out as well, because too much data was missing. In all three species, heritabilities significantly different from zero were found, although with a lot of variability between characters and between populations. To compare the level of genetic variance in morphological characters of the three species, mean rank-orders of heritabilities of the common character set and weighted means over V_A/V_p PC were calculated. *P. major* and *P. coronopus* had the lowest mean rank number of heritabilities ($\bar{R}=1.80$), whereas *P. lanceolata* had the highest rank ($\bar{R}=2.40$). The weighted mean V_A/V_p PC

was highest for *P. lanceolata* (0.51), *P. coronopus* had a mean of 0.46, and *P. major* had the lowest mean (0.38). Within each species, the populations were compared for their heritabilities, also using rank numbers and weighted means of V_A/V_P PC (Table 5). For two of the species, a heterogeneity in rank numbers over populations for heritabilities of characters was present: *P. major* ($P=0.004$), *P. coronopus* ($P=0.009$) (*P. lanceolata*: $P=0.12$). Rank orders of populations using heritabilities of the subset of characters or using V_A/V_P PC showed no or only minor differences.

Discussion

The two methods used to express morphological variability and to give the partitioning of variability both have their theoretical drawbacks, as the non-parametric method uses dependent variables and the PC analysis uses artificial characters. The results and the conclusions drawn from the results are very similar, however, and both methods seem to give satisfactory answers to the questions posed.

The outcrossing species (*P. lanceolata*) has the highest total variability and the highest within-population variability. The selfing species *P. major*, on the other hand, has the lowest within-population variability and the highest between-population differentiation. Differences in mating system seem to be associated with the level and the partitioning of variability at the species level, as was also predicted from the literature (e.g., Solbrig 1972; Jain 1976). The general higher heritabilities in *P. lanceolata* would predict a greater response to (local) selection, though this may be counterbalanced by greater gene flow between populations. From other publications, it was expected that morphological differentiation in *P. major* would be higher than in *P. lanceolata* (Van Dijk 1985). In the present experiment, this difference is only weakly present, which may be brought about by the populations chosen or the use of the common character set: *P. major* populations may be more strongly differentiated for other characters not in the set, such as seeds/capsule, scape position. When all characters were used, a mean population differentiation of 38% of total variability was present. The morphological differentiation in *P. major* measured with the common character set is, however, still considerable (30–36%) and ecotypes can clearly be distinguished (Fig. 1).

Although populations of *P. coronopus*, observed in situ, showed considerable differences between populations for many morphological characters (personal observation), these differences were not maintained when grown in the greenhouse (Fig. 1). (The exception to this is the population from Ud, which will be discussed later on.) In contrast to *P. coronopus*, the differences between pop-

ulations of *P. lanceolata* and *P. major* found in the field are (partly) expressed in the greenhouse and have a genetic basis (Fig. 1). The low morphological differentiation and the absence of ecotypic differentiation in *P. coronopus* could be caused by high plasticity for morphological characters. Plasticity in *P. coronopus* was also suggested by other studies, in which reproductive effort appeared to be a character with especially high levels of plasticity. Waite and Hutchings (1982) found different patterns of energy allocation in different habitats, which could be reproduced under controlled environments by different levels of nutrients. Schat et al. (1984) could also influence reproductive effort by nutrient stress. Schat (1981) found that the forma pygmaea, described by Dodds (1953), was not genetically determined. Morphological plasticity, as opposed to genetic diversity, was also described for other species, e.g., Marshall and Jain (1968), Wu and Jain (1978), and Carey (1983), and can be seen as a special way of adaptation (Bradshaw 1965). In *P. coronopus* there is no shortage of genetic variation for morphological characters within populations, as heritabilities are significantly different from zero in most populations for many characters. In a comparison of *P. lanceolata* and *P. major* with another *Plantago* species, *P. maritima*, Blom and Lotz (1985) found that the first two species are specialists in comparison to *P. maritima*, which is considered a plastic species. The level of plasticity of *P. coronopus*, in relation to *P. maritima* is, at present, not known.

A test for the hypothesis that *P. coronopus* is adapted through high plasticity is to carry out reciprocal transplantation experiments. This has already been done for *P. lanceolata* by Van Groenendael (1985), Van der Toorn et al. (1984), and Van Tienderen (1989), and for *P. major* by Lotz (1989). Both species revealed population-specific interactions for life history characteristics such as survival and reproductive effort, while generally the genotypes performed best in their own environments. Since a high level of plasticity is hypothesized for *P. coronopus*, fewer or less pronounced genotype-environment interactions are expected in a reciprocal transplantation experiment with this species.

From this study it can be inferred that selection may, both in selfers and in outcrossers, cause pronounced morphological differentiation between (sub)populations in those cases where the species copes with different habitats through genetic differentiation instead of adaptive plasticity.

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