Theor Appl Genet (1994) 87:733–740

Population and species variation of minisatellite DNA in *Plantago*

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Received: 8 June 1993 / Accepted: 28 June 1993

Abstract. Three *Plantago* species were surveyed for within- and between-population variation at DNA sequences detected with the M13 minisatellite probe. The levels and patterns of variation detected by this probe correspond to those expected from the mating systems of the species. The highly-selfing species *P. major* has a relatively low variability of minisatellite sequences within populations and considerable differentiation between populations. The outcrossing species *P. lanceolata* exhibits higher minisatellite variability within populations and moderate differentiation between populations. *P. coronopus*, with a mixed mating system, has levels of variation intermediate between *P. major* and *P. lanceolata*. The levels of variation within and between populations corresponds, in general, to the levels of allozyme variation determined in an earlier study. Mating system and population structure appear to have a major influence on M13-detected fragment variation.

Key words: DNA fingerprinting – Minisatellites – *Plantago* – Population structure – Mating system

Introduction

In the last decade, moderately- to highly-variable sequences (minisatellites) have been discovered in many organisms, e.g., in humans (Wyman and White 1980; Jeffreys et al. 1985a, b), birds (Burke and Bruford 1987; Wetton et al. 1987), protozoans (Rogstad et al. 1989) and in several angiosperms and gymnosperms (Dallas 1988; Rogstad et al. 1988; Ryskov et al. 1988). For some species individual specific patterns, termed "DNA fingerprints", have been detected with several minisatellite probes (Jeffreys et al. 1985a, b).

The M13 repeat probe reveals hypervariable sequences in humans and other animals (Vassart et al. 1987) and in plants (Rogstad et al. 1988; Ryskov et al. 1988). In plants this probe has been used to detect genetic variation in species of Rosaceae (Nybom 1990; Nybom et al. 1990; Nybom and Hall 1991) and to determine clonal growth in *Rubus* (Nybom and Schaal 1990), *Asimina triloba* (Rogstad et al. 1991), and *Populus tremuloides* (Rogstad et al. 1991). DNA fingerprinting has become a powerful tool in both ecological and evolutionary studies. In spite of the widespread use of DNA fingerprinting, the application of the technique, particularly in humans, has been controversial (Lewontin and Hartl 1991; Chakraborty and Kidd 1991). Much of the controversy surrounds the issue of whether or not population structure influences the level of variation, and hence the probability that two individuals will share identical fragments. Our purpose here is to examine variability at minisatellite loci in three species of *Plantago* which vary widely in breeding system, allozyme variation, and population structure. If minisatellite DNA variation paralleles allozyme variability, then a strong case can be made for population structure influencing fingerprinting loci. In addition, this study will calibrate minisatellite variation with allozyme variation.

The *Plantago* species studied here have a range of mating systems. *P. major* is predominantly selfing with

Communicated by P. M. A. Tigerstedt

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very low outcrossing rates (0–5%, Van Dijk et al. 1988; Wolff 1988). *P. coronopus* is self compatible and has a broad range of outcrossing rates in different populations (34–100%) due in part to variable plant density (Wolff et al. 1988). *P. lanceolata* is self-incompatible and completely outcrossing. Both theoretical work and empirical allozyme studies indicate that outbreeding species generally have high genetic variability within populations and low heterogeneity between populations, whereas inbreeding species mostly possess low genetic variability within populations and significant population differentiation (e.g., Brown 1979; Layton and Ganders 1984). A previous study of population variation in three species of *Plantago* showed that the mating system and the history of a population was of prime importance in establishing the amount and distribution of allozyme variation (Wolff 1991a, b).

Materials and methods

All *Plantago* populations were collected in the Netherlands. Plants were grown from seeds generated from greenhouse crosses. Plants were originally collected from the field populations listed in Wolff (1991a). The geographic location of populations, except for the Weever's duin and Oostvoornse meer populations, is given in Van Dijk et al. (1988). Five populations of *P. major* were sampled by randomly collecting eight plants from each population. These individuals were then selfed to produce seeds. Two populations, Westduinen (Wd) and Weever's duin (Wv), belong to the subspecies *major* and the other three populations, Angeren (An), Kwade Hoek (Kh) and Oostvoornse meer (Om), belong to the subspecies *pleiosperma* (Wolff 1991a). *P. major* subspecies are differentiated by several morphological characters and a few diagnostic allozyme alleles (Van Dijk and Van Delden 1981; Wolff 1991a). Wv, Kh and Om are within 15 km of each other, while An and Kh are 130 and 30 km from these three populations, respectively. An and Kh are approximately 140 km apart. Four populations each of *P. coronopus* and *P. lanceolata* were sampled (see Wolff 1991 a for site discriptions). *P. coronopus* was also sampled by selfing eight plants in each population. The populations are Uddel (Ud), Schiermonnikoog dijk (Sd), Kwade Hoek (Kh) and Westduinen (Wd). Seeds were generated for *P. lanceolata* by crossing pairwise 16 plants per population, which resulted in eight full-sib families per population. The *P. lanceolata* locations are Heteren (He), Westduinen (Wd), Schiermonnikoog pad (Sp) and Uddel (Ud). One offspring was used for analysis from each plant family; therefore 40 *P. major*, 32 *P. coronopus* and 32 *P. lanceolata* plants were analyzed. Additionally, 24 offspring of one selfed family of *P. major* were used to study levels of homozygosity and to survey the variability within a family.

Plants were grown in the greenhouse and their leaves were ground in liquid nitrogen, after which the tissue was stored at − 80 °C. DNA was extracted from tthe nitrogen-powdered tissue according to Saghai-Maroof et al. (1984) with the modification of CTAB extraction time to 1 min at room temperature. The DNA was digested with a five-fold excess of *Taq*I at 65 °C for *P. lanceolata* (overnight) and *P. major* (4 h) and at 55 °C for *P. coronopus* (4 h). DNA of the three species was digested at different temperatures and for different time periods in order to reach complete digestion and to prevent degradation of the more-heat-sensitive DNA from *P. major* and *P. coronopus*. Digested DNA was electrophoresed for 28–32 h in a submerged 0.9% agarose gel, 25 cm long, with approximately 1.5 V/cm using a TPE buffer (Sambrook et al. 1989). The gels were run until the marker dye, bromophenol blue, reached the end of the gel. Lambda DNA cut with *Hin*dIII was used as a molecular weight marker. Afterwards the gel was Southern blotted onto MSI or Nytran (Schleicher and Schuell) nylon filter. The 780-bp repeat probe, cut out from M13 using *Bsm*I and *Cla*I, was hexamer-labeled with ^{32}P according to Rogstad et al. (1988). Filter hybridizations and washing conditions were according to Westneat et al. (1988). Autoradiography was at − 80 °C for 1–14 days with intensifying screens. Several autoradiograms with different exposure times were used to analyse fragment profiles.

For each species every fragment with a different molecular weight was numbered sequentially and all plants were scored for the presence or absence of the numbered fragments. Three individual plants were run on every gel as internal standards for comparisons among filters. In addition, plants were run two-to-three times on each gel to assure internal consistency. Differences in band intensity were not scored. A similarity coefficient (D) was calculated to express the variation within populations (Wetton et al. 1987), where

$$D = \frac{2N_{AB}}{N_A + N_B}$$

in which N_A and N_B are the number of fragments in individuals A and B and N_{AB} is the number of fragments shared by both [instead of D other authors may use the symbol S (Lynch 1990)]. D was calculated for all pairwise comparisons within a population and its variance was determined for each population. These measures were used to test differences between populations for the level of variation by employing the multiple-comparisons-among-means test (Sokal and Rohlf 1981). The similarity coefficient was also used to give an estimate of the probability that two randomly-chosen individuals would have an identical fragment pattern. This probability is $\bar{D}^{\bar{N}}$, where $\bar{N}$ is the average number of fragments per individual (Wetton et al. 1987; Nybom et al. 1990). This measure assumes the independence of fragments.

Some populations of a highly-selfing plant species may consist of only a limited number of different homozygous, multilocus genotypes among which the genomes do not mix, like pure inbred lines. In this case the fragments are not independent and the use of $\bar{D}$ and $\bar{D}^{\bar{N}}$ for calculating similarity and the probability of fragment profile sharing will be underestimates. It is then better to use numbers and frequencies of multilocus genotypes and calculate the probability of sharing an identical fragment pattern with Σp_i, where p is the frequency of multilocus genotype i in the population. This method is not used here as eight individuals per population is not sufficient to estimate the numbers of multilocus genotypes and their frequencies in a population.

The genetic identities within populations (ID_w) and between populations (ID_b) and an index of population subdivision were calculated. A resampling program, kindly provided by Alan Templeton of Washington University, derives these measures and their confidence intervals. This program is based on a calculation of N_{AB}/N_A. This calculation is iterated over all individuals A, while B is drawn at random. The identity within and between populations is calculated as N_{AB}/N_A with B being drawn from the same and another population, respectively (see also Davis et al. 1990). A thousand resampling episodes were run for all of the analyses.

The index of population subdivision F'_{ST} was estimated according to Davis et al. (1990) as:

$$F'_{ST} \approx \frac{ID_w - ID_b}{1 - ID_b}.$$

F_{ST} is a measure for population subdivision: it gives the correlation between random gametes, drawn from the same subpopulation, relative to the total (Wright 1965). Lynch (1990) describes an alternative way to estimate F_{ST}, namely:

$$F''_{ST} \approx \frac{1 - ID_b}{(1 - ID_w) + (1 - ID_b)}.$$

Both within- and between-population identity are biased but in the same direction, therefore they may (partly) cancel out and the equation will be a good approximation (Lynch 1990). Both F'_{ST} and F''_{ST} are calculated and presented in the results.

The genetic distance between individual pairs of populations as determined by Nei (1972) is ln (the normalized identity between populations). The normalized identity between populations 1 and 2 is calculated according to Lynch (1990) as:

$$\frac{ID_b(1,2)}{\sqrt{ID_w(1) * ID_w(2)}}.$$

Results

The DNA fragment profiles, detected by the M13 probe, were distinct for the three species (Figs. 1 and 2 show representative plants of *P. lanceolata* and *P. major*, respectively). The two subspecies of *P. major* had very different fragment patterns. Individuals from subspecies *major* (populations Wv and Wd) showed an almost constant fragment pattern, whereas the individuals from subspecies *pleiosperma* (populations Kh, An and Om) had more variable fragment patterns (Fig. 2). For *P. major* 42 fragments were distinguished; only one was present in all plants. Ten fragments were present in all individuals of subspecies *P. coronopus* while 32 fragments, ranging from 4.4 to 12 kb, were distinguishable (data not shown). None of these fragments was present in all individuals.

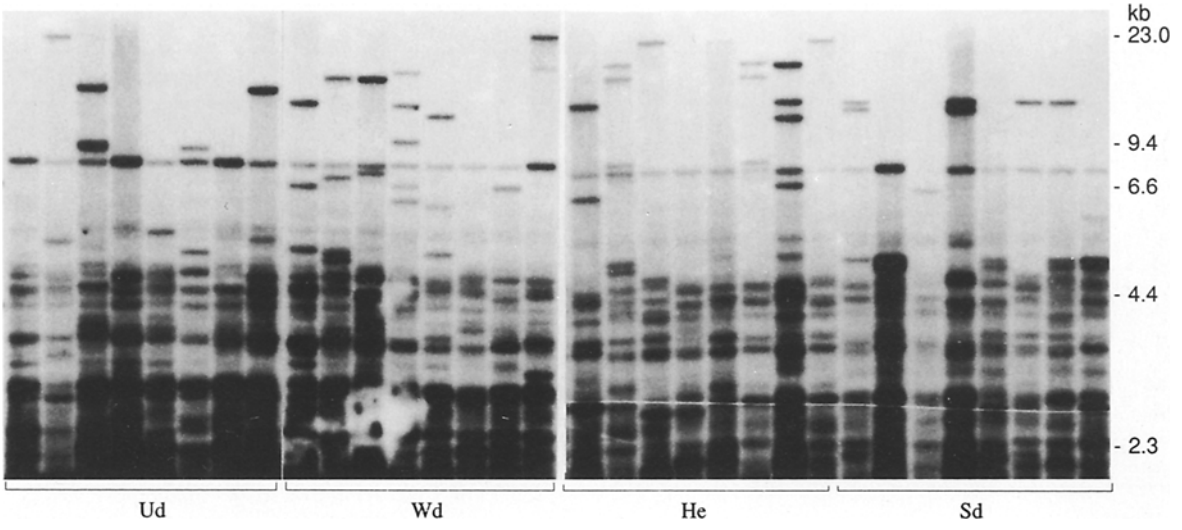

Fig. 1. Autoradiograph of *Taq*I-digested DNA from *P. lanceolata* plants probed with the M13 repeat probe. The populations are designated at the bottom. Size markers are from Lamda DNA digested with *Hin*dIII

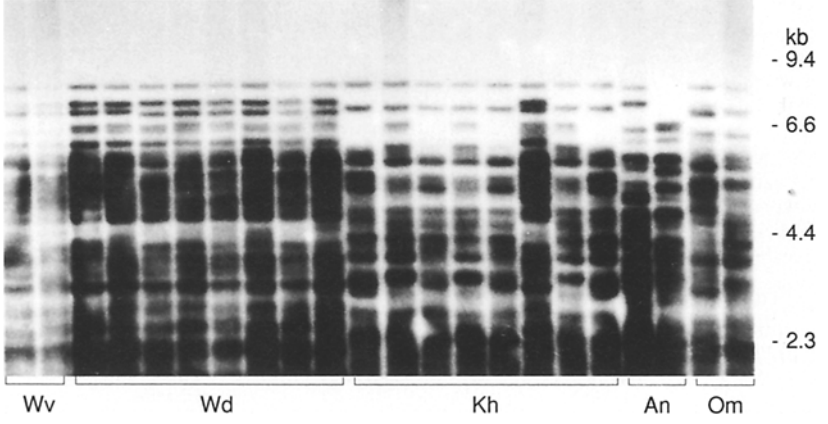

Fig. 2. Autoradiograph of *Taq*I-digested DNA from a subsample of the *P. major* plants, probed with the M13 repeat probe. The populations are designated at the bottom. See text for further explanation

Table 1 gives the mean number of fragments per individual and the mean similarity coefficient for each population. The probability of two randomly-chosen individuals sharing identical fragment profiles is very different for the three species and even for the populations within a given species. In *P. major* populations the mean probability of two individuals having the same fragment profiles ranges from 5.4×10^{-3} to 0.28. For *P. coronopus* this range is from 3.4×10^{-4} to 1.3×10^{-2}, whereas in *P. lanceolata* populations the mean probability range is from 1.1×10^{-6} to 3.9×10^{-4}. Within *P. coronopus* there are no significant differences in D between populations, which is at least partially the result of a high variance of D within populations (see Table 2). In *P. major* the similarity coefficients of populations An and Kh are not significantly different from each other but are significantly different from the other three populations of the species. These latter populations (Wd, Wv and Om) are, in turn, not significantly different from each other. In *P. lanceolata* the similarity coefficient of Wd is significantly lower than that of Ud. It must, however, be noted that the probability of sampling two identical individuals is underestimated in most populations of *P. major* and in the Ud population of *P. coronopus*. This is because the assumed independence of fragments is clearly not valid: the eight Ud individuals of *P. coronopus* consisted of only two different fragment phenotypes sharing only one fragment (data not shown). These two fragment phenotypes should be

Table 1. Results from DNA fingerprinting of different populations of three *Plantago* species. Eight individuals per population were sampled using *Taq*I as an endonuclease and the 780-bp M13 repeat probe. The mean number of bands per individual ($\bar{N}$) and the mean similarity coefficient ($\bar{D}$), with the standard deviation in parenthesis, and the probability of exact similarity by chance, $\bar{D}^{\bar{N}}$, are given

Species	Population	$\bar{N}$	$\bar{D}$	$\bar{D}^{\bar{N}}$
P. major	Kh	12.0 (1.22)	0.655 (0.23)	6.8×10^{-3}
	An	11.7 (2.31)	0.638 (0.10)	5.4×10^{-3}
	Wd	16.6 (0.69)	0.886 (0.07)	0.14
	Wv	16.1 (1.17)	0.908 (0.05)	0.22
	Om	11.0 (0)	0.886 (0.20)	0.28
P. coronopus	Wd	8.6 (1.51)	0.451 (0.20)	1.0×10^{-3}
	Sd	9.2 (1.28)	0.549 (0.16)	4.1×10^{-3}
	Kh	7.6 (1.90)	0.349 (0.13)	3.4×10^{-4}
	Ud	8.2 (0.46)	0.590 (0.44)	1.3×10^{-2}
P. lanceolata	He	20.2 (2.66)	0.566 (0.08)	9.8×10^{-6}
	Wd	21.1 (1.13)	0.522 (0.09)	1.1×10^{-6}
	Sp	20.4 (1.68)	0.640 (0.10)	1.1×10^{-4}
	Ud	19.1 (2.70)	0.663 (0.10)	3.9×10^{-4}

Table 2. *Plantago* populations ordered for their level of allozyme variation (Wolff 1991 a) and the levels of variation for hypervariable loci, expressed as the mean expected heterozygosity ($\bar{H}_e$) and the similarity coefficient within populations ($\bar{D}$), respectively. Populations connected by a line are not significantly different at the 5% level as tested with the T-method (Sokal and Rohlf 1981)

P. major		An	Kh	Wd	Wv	Om	Rank order
	$\bar{H}_e$	0.052	0.039	0.028	0.023	0.010	An Kh Wd Wv Om
	$\bar{D}$	0.638	0.655	0.886	0.908	0.886	An Kh Wd Om Wv
P. coronopus		Wd	Kh	Sd	Ud		
	$\bar{H}_e$	0.093	0.088	0.024	0.00		Wd Kh Sd Ud
	$\bar{D}$	0.451	0.349	0.549	0.590		Kh Wd Sd Ud
P. lanceolata		He	Wd	Ud	Sp		
	$\bar{H}_e$	0.131	0.124	0.104	0.102		He Wd Ud Sp
	$\bar{D}$	0.566	0.522	0.663	0.640		Wd He Sp Ud

Table 3. Population structure estimates (with their 95% confidence interval) for hypervariable loci and the F_{ST} value for allozyme loci (Wolff 1991a)

Species	Identity within pop.	Identity between pop.	F'_{ST} (Davis 1990)	F''_{ST} (Lynch 1990)	F_{ST} allozymes
P. major	0.81 (0.77–0.84)	0.46 (0.41–0.52)	0.65 (0.58–0.71)	0.74	0.216
P. coronopus	0.50 (0.43–0.58)	0.36 (0.32–0.41)	0.22 (0.08–0.35)	0.56	0.070
P. lanceolata	0.60 (0.57–0.64)	0.54 (0.51–0.57)	0.14 (0.04–0.23)	0.53	0.037

considered as two independent multilocus genotypes. For *P. major*, population An consisted of eight individuals each with a different fragment pattern (data not shown). Population Om had two phenotypes, population Kh had five phenotypes, and populations Wd and Wv each had seven different fragment phenotypes.

The species showed different levels of variation and genetic differentiation by the resampling analysis (Table 3). F'_{ST} and F''_{ST}, calculated according to the equations given in Materials and methods, give rather different figures. The outcrossing species *P. lanceolata* had high levels of variation and low differentiation (low F'_{ST} and F''_{ST}) between populations, whereas the inbreeding *P. major* showed the opposite: low levels of variation within populations and a high population differentiation. *P. coronopus* had an intermediate level of genetic differentiation between populations and a high diversity within populations.

The similarity coefficient is the same as the genetic identity within populations and is the inverse of the variability within populations. The similarity coefficient was highly variable among *P. major* and *P. coronopus* populations, ranging from 0.638 to 0.908 and from 0.349 to 0.590, respectively (Table 2). Although the number of populations studied is small, we ordered populations within species for their levels of variability of these presumed minisatellite loci and also for allozyme loci. The allozyme data are from Wolff (1991a). In that study 20 to 149 plants per population were scored for variable allozyme loci (11 loci in *P. lanceolata*, eight loci in *P. major* and seven loci in *P. coronopus*). Allozyme variability within the populations was expressed as $\bar{H}_e$, the mean expected heterozygosity over all loci (Nei 1975). The rank orders for minisatellite and allozyme variability showed good concordance. The ranking for *P. major* is nearly the same for both kinds of data, except that the order of the Wv and Om populations is reversed. In *P. lanceolata* the rank orders of allozyme and minisatellite variability are less concordant but the two populations with a high $\bar{H}_e$ (mean expected heterozygosity) have the lowest $\bar{D}$ values. In *P. coronopus* the two populations with the highest allozyme variability have been switched in their relative positions for levels of minisatellite variation. A non-parametric test (Spearman rank test) for the correlation of variability between presumed minisatellite loci and allozyme loci gave positive correlations for all three species, but this correlation was significant only for *P. major* ($P = 0.04$). *P. coronopus* and *P. lanceolata* did not show significant correlations between minisatellite and allozyme variability ($P = 0.10$ and $P = 0.20$, respectively). The high P values may partly be due to the low number of populations.

Table 4. Differentiation between *P. major* populations for hypervariable DNA fragment patterns, expressed as the identity between populations (ID_b, upper triangle) and as the genetic distance between populations (lower triangle). The 95% confidence intervals of the distances are given

	Wd	Wv	Kh	An	Om
Wd		0.812	0.340	0.484	0.421
Wv	0.108 (0.07–0.14)		0.361	0.442	0.451
Kh	0.827 (0.59–1.02)	0.779 (0.54–0.99)		0.356	0.562
An	0.461 (0.39–0.54)	0.561 (0.46–0.67)	0.628 (0.46–0.80)		0.404
Om	0.756 (0.66–0.84)	0.698 (0.60–0.78)	0.323 (0.19–0.43)	0.642 (0.19–0.74)	

Distances between pairs of populations within *P. lanceolata* were similar to each other, ranging from 0.064 to 0.147. The Ud population of *P. coronopus* is more strongly differentiated, with distances to the other populations of *P. coronopus* being 0.335, 0.369 and 0.267. The distances between the other three populations of *P. coronopus* were 0.186, 0.131 and 0.043. For *P. major* the distance between the two subspecies *major* populations (Wv and Wd, distance = 0.108) was significantly lower than between any other pair within the species (Table 4). Populations Om and Kh also had similar fragment patterns (distance = 0.323). The genetic distances between populations were generally largest between a subspecies *major* population and a subspecies *pleiosperma* population.

The DNA fragment patterns of 24 members of a selfed family of *P. major* did not show any observable variation.

Discussion

In this study we tried to relate variability at minisatellite (fingerprinting) loci to variability at allozyme loci. Fingerprint data are often difficult to interpret. One of the obvious difficulties is that fragments with the same mobility are not necessarily identical and small changes in fragment size are difficult to detect. In spite of these limitations, conclusions about the relative variation of minisatellite loci both within and between species can be drawn.

Variation at minisatellite loci in the three *Plantago* species ranged from moderate to high compared to variation in other plant species. The positive relationship between outcrossing rates and the degree of variability in the three species suggests that the mating system in plants is an important factor in determining levels of minisatellite variability. Our results show that, in plant species with high selfing rates, population structure may have a major impact on fragment phenotype frequencies, and thereby on the similarity coefficient D (see discussion in next paragraph). Furthermore, the relationship between allozyme variation and the distribution of variation at hypervariable loci within species suggests that both kinds of variability are influenced by the same factors. The experimental breeding design used to obtain plants of *P. lanceolata* and *P. major* simulated their breeding system in the field, namely outcrossing and high selfing, respectively. The similarity by chance (Table 1) should be generally higher in populations with a history of selfing; the data suggest that lower levels of similarity are indeed found in outcrossed *P. lanceolata* than are present in *P. major*. The resampling analysis of population structure estimates (Table 3) provides strong evidence that the within-population genetic identity is higher for the highly-selfed *P. major* than the outcrossed *P. lanceolata*, as expected from the breeding system. Populations within species have different levels of variability at hypervariable sequences. Clearly the breeding system is not the only factor affecting minisatellite variation in *Plantago*. In *Plantago* the processes of founder effects, migration, and drift, are influential in establishing allozyme variation (e. g., Van Dijk et al., 1988) and influence minisatellite loci as well.

P. coronopus plants were also selfed in this study whereas the natural breeding system is from partly selfing to outcrossing. Comparisons with *P. lanceolata*, therefore, need to be made with caution. However, comparing individuals produced by selfing for both the highly-selfed *P. major* and the mixed-mating *P. coronopus* shows that *P. major* has both a higher mean coefficient of similarity values (Table 1) and a higher level of identity within populations, as is expected from their mating systems. A similar comparison between *P. coronopus* and the highly-outcrossed *P. lanceolata*, reveals that, even though selfed, a relatively high degree of minisatellite variability exists in *P. coronopus*. The fragment size ranges of the three species is not identical. Fragments with a higher molecular weight are usually more variable than the lower-molecular-weight fragments. In *P. coronopus* only fragments in the higher-molecular-weight region could be scored and, therefore, the variability in *P. coronopus* may be overestimated compared to *P. major*. In *P. lanceolata* fragments with both a higher and a lower molecular weight than *P. coronopus* could be scored and this may equal out variability differences between fragment size classes.

In the study of Van Dijk et al. (1988) it was shown that in a sample of 70 plants from one population of *P. coronopus*, Ud, no allozyme variation was present. This lack of variation has been attributed to a small founder population and isolation of this inland population from other (coastal) populations (Van Dijk et al. 1988). The present DNA analysis showed that, in the eight individuals tested, two extremely different fragment phenotypes were present, indicating that probably at least two individuals founded the population and its genetic variability is indeed lower than in the other populations.

Genetic differentiation expressed as F'_{ST} (Davis et al. 1990) is in good concordance with the F_{ST} values found for allozymes: figures of F'_{ST} for each species seem to be approximately three-fold larger than F_{ST} values, but the relative differences between species is very similar. Therefore, F'_{ST} seems to give a better estimate of the differentiation between populations, compared to the total variation, than does F''_{ST} (Lynch 1990). Genetic distances between populations calculated with the DNA fingerprint data are low for *P. lanceolata*, intermediate for *P. coronopus*, and high for *P. major*, and this is in agreement with results from allozyme data (Van Dijk et al. 1988; this study). *P. major* subspecies are strongly differentiated from each other for many characters. Their morphology and ecological distribution is distinctly different (Lotz and Blom 1986; Lotz 1989) as is their allozyme variation, with some alleles occurring in only one of the two subspecies (Van Dijk and Van Delden 1981). The observed differences in fragment patterns for hypervariable loci between the two subspecies, as expressed in distances between populations, are in concordance with these results. The high similarity of the fragment patterns of Kh and Om within the subspecies *pleiosperma* can be explained by the close geographical distance between the populations (approximately 10 km) and the possibly recent founding of Om (a former beach plain) by seeds from surrounding populations near Kh. In Kh one individual was present (lane indicated with * in Fig. 2) with a fragment pattern closely resembling the subspecies *major* fragment pattern. Migration of a *major* individual into this population is most likely. A chloroplast

DNA study showed that Kh is a population where the two subspecies seem to coexist (Wolff and Schaal 1992). Therefore, this *major* individual in Kh is not excluded from the analysis. In the species *P. coronopus* the only inland population, Ud, has a high genetic distance from the other populations. As mentioned above Ud may have been founded by few plants with genotypes deviating from those in the coastal populations.

Plants of *P. major* are highly homozygous. The offspring of a selfed family shows no variation at hypervariable loci, indicating that the parent was homozygous for all minisatellite loci detected. This homozygosity is in concordance with the high homozygosity for allozyme loci in this species. The fixation index found by Wolff (1991b) ranged from 0.75 to 0.95 in *P. major*. Heterozygosity at hypervariable loci in humans may be up to 96% (Jeffreys et al. 1985 a). The homozygosity determined by the selfing experiment and the high similarity (D = 1) between many pairs of plants in some populations of *P. major* and *P. coronopus* also indicates a low mutation rate. In humans, high mutation rates, upto 5% per gamete (Jeffreys et al. 1988), were found. The moderate variability of presumed minisatellite loci observed in the present study, combined with the inference of a low mutation rate in *Plantago*, promises a profitable use of minisatellite analysis for population genetic studies, and, therefore this approach complements other useful techniques such as allozyme electrophoresis and RAPD analysis.

The present study clearly documents both within-species and between-species variation. These populations and species of *Plantago* differ in many parameters, such as mating system, migration, and history. Levels of minisatellite variation correspond closely to levels of allozyme variation, which in turn appear to be most strongly influenced by breeding system, a component of population structure. This study presents strong evidence that population structure affects levels of minisatellite variability. Moreover the comparison of allozyme and minisatellite variability indicates that in general, the two measures vary in content, and that minisatellite variation tends to be higher than allozyme variation.

Acknowledgements. We are grateful to Alan R. Templeton for the use of his resampling program. We thank Hilde Nybom for practical advice. This research was supported by a fellowship from NWO (Netherlands Organization for Scientific Research) to K.W., by NSF grant BSR 8714397 to B.S., and NSF grant BSR 8700214 to S.H.R.

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