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The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size

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As part of a programme to determine the importance of the loss of genetic variation for the probability of population extinction, the amount of allozyme variation was determined in 14 populations of *Salvia pratensis* and in 12 populations of *Scabiosa columbaria*. Significant correlations were found between population size and the proportion of polymorphic loci (*Salvia*: $r=0.619$; *Scabiosa*: $r=0.713$) and between population size and mean observed number of alleles per locus (*Salvia*: $r=0.540$; *Scabiosa*: $r=0.819$). Genetic differentiation was substantially larger among small populations than among large populations: in *Salvia* G_{ST} was 0.181 and 0.115, respectively, and in *Scabiosa* 0.236 and 0.101, respectively. The results are discussed in relation to genetic drift, inbreeding and restricted gene flow.

Keywords: conservation biology, extinction, genetic erosion, population size, *Salvia pratensis*, *Scabiosa columbaria*.

Introduction

Due to the activities of man, populations of many plant and animal species have become small, fragmented and isolated. Research is needed to develop effective measures for conservation. Until the beginning of the last decade conservation was mainly the domain of ecologists, but recently much attention has been focused on the importance of the population genetic aspects (Soulé & Wilcox, 1980; Frankel & Soulé, 1981; Schonewald-Cox *et al.*, 1983; Soulé, 1986, 1987).

Population genetic theory predicts that, as a consequence of genetic drift and inbreeding, small populations will have decreased levels of genetic variation. Even favourable alleles may be lost and the potential to adapt to a changing environment may be seriously diminished (Vrijenhoek, 1985). Moreover, inbreeding results in increased levels of homozygosity which may cause inbreeding depression (Frankel R., 1983). Both processes may thus lead to 'genetic erosion', reduce the fitness of individuals in a population and increase the chance of the extinction of the population. Ultimately this may lead to the extinction of the species. The deleterious effects of genetic erosion in populations, however, can be counteracted by gene flow renewing the genetic variation.

Because experimental data about the significance of genetic erosion in extinction are virtually absent, we started a project to examine these processes in two plant species in The Netherlands: *Salvia pratensis* and *Scabiosa columbaria*. These species were selected because: (i) the number of populations has significantly declined in the last decades, (ii) they occur in both small and large populations, (iii) both are thought to be predominantly outbreeding. The research described in this paper is part of a comprehensive research project to determine the importance of genetic factors for population extinction and to develop effective management measures that could prevent species from becoming extinct. In this paper we present data on the amount of allozyme variation and the extent of genetic differentiation in relation to population size.

Materials and methods

Both *Salvia pratensis* and *Scabiosa columbaria* are gynodioecious, protandric perennials and are diploid with $2n=18$ (Tutin *et al.*, 1972) and $2n=16$ (Tutin *et*

al., 1976), respectively. *Salvia* occurs in dry, sunny, calcareous grassland on river dunes and dikes, *Scabiosa* in dry, grassy places on calcareous soils. The number of (1 × 1 km) grid squares in which *Salvia* and *Scabiosa* was observed, declined between 1950 and 1980 from 92 to 78 and from 82 to 52, respectively (Mennema *et al.*, 1985). In 1988 only 39 populations of *Salvia* and 24 populations of *Scabiosa* were recorded (Ouborg *et al.*, 1989).

The location of the populations examined is given in Fig. 1. The mean distance between the examined populations and their nearest neighbouring population was 4 km for *Salvia* and 7 km for *Scabiosa*. Because both species are pollinated mainly by bees, which are known to forage within considerably smaller distances (Levin & Kerster, 1974), gene flow between populations by means of pollen was expected to be restricted. Because seeds of both species have no special means of transport, seed dispersal may even be less important (Levin & Kerster, 1974).

In 1988, the size of small populations was determined by counting the total number of flowering individuals, whereas the size of large populations was estimated by stratified sampling of square metres and the subsequent extrapolation to total population area. Populations were grouped in two distinct size classes (small and large populations). This classification, however, was partly arbitrary. Criteria were such that the difference in population size between the largest small population and the smallest large population had to be substantial and that the difference in number of populations in each size class had to be as small as possible (Table 1).

Individual plants were sampled in all populations in 1988 by cutting pieces of leaf material. In small populations as many plants as possible were sampled, whereas in large populations about 50 individuals were sampled with regular spacing, using the whole population area. The samples were put in plastic bags with a small amount of water and kept in a portable cooler. In the laboratory the samples were stored in a refrigerator (4°C) until the moment of electrophoretic analysis. The activity of the enzymes remained sufficiently high for at least 3 weeks. Of the 29 enzyme systems tested for electrophoresis, 10 showed a sufficiently clear pattern and were used for genetic analyses (Table 2).

Electrophoretic procedure

Buffer systems. Tris-citrate pH 7.0, LiOH-borate pH 8.3 and tris-borate EDTA pH 8.6. Apart from GPI, ICD and PGD for *Salvia*, which were performed on polyacrylamide, electrophoretic analyses were carried out on starch gels. Electrophoretic methods and recipes for buffers (except tris-borate EDTA pH 8.6) and staining solutions (except SORDH) are described

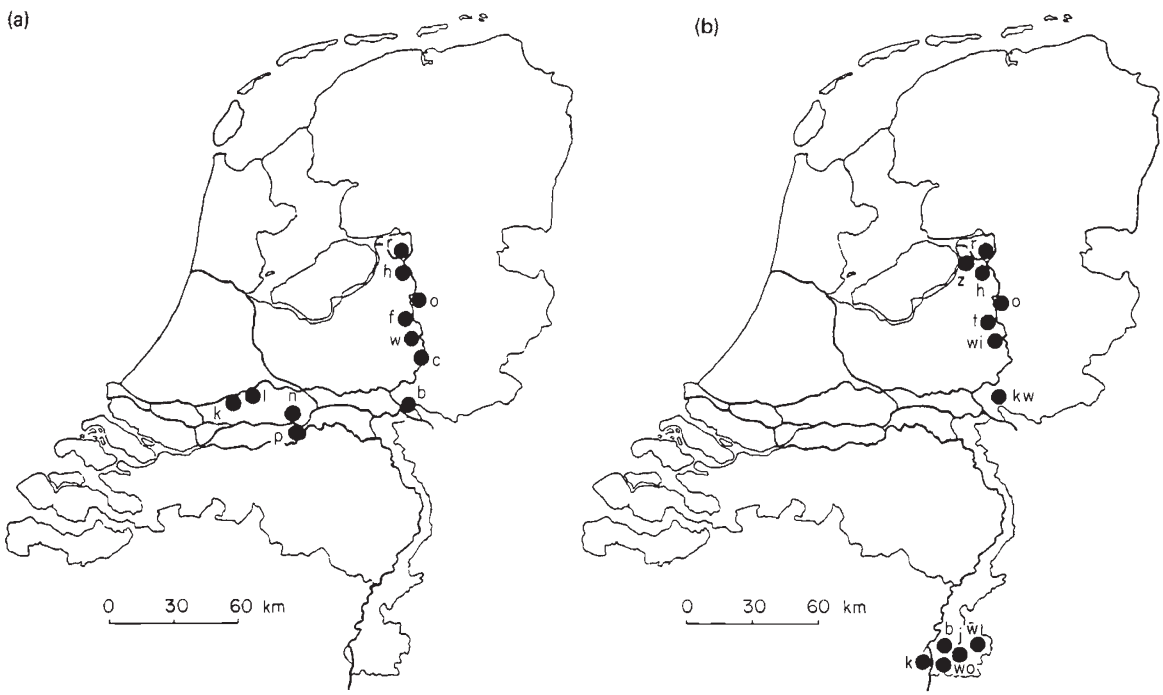

Fig. 1 Location of the examined populations of *Salvia pratensis* (a) and *Scabiosa columbaria* (b) in The Netherlands. For *Salvia*, the following three locations consisted of two (sub)populations (their interpopulational distances given between brackets): f (320 m), c (580 m) and b (160 m).

Table 1 Examined populations of *Salvia* and *Scabiosa* with their abbreviated name (A), number of flowering (N) and sampled (n) individuals. Populations are grouped according to size (see text)

Small populations	(A)	N	n	Large populations	(A)	N	n
Salvia							
Forten 1	f1	5	5	Olst	o	300	49
Forten 2	f2	14	14	Ruitenberg	r	300	50
Neerijnen	n	17	17	Cortenoever 2	c2	310	66
Lexmond	1	30	20	Wilpse klei	w	400	50
Hoenwaard	h	46	34	Bijland 2	b2	1000	36
Bijland 1	b_1	60	14	Koekoekswaard	k	1500	60
Cortenoever 1	c_1	60	10				
Piekenwaard	p	61	43				
Scabiosa							
Hoenwaard	h	14	6	Terwolde	t	200	50
Kwartierse dijk	kw	35	25	Wilpse klei	wi	200	50
Ruitenberg	r	90	42	Bemelerberg	b	300	50
Kannerhei	k	100	21	Wolfskop	wo	25000	48
Zalk	z	118	30	Olst	o	50000	50
				Julianagroeve	j	75000	50
				Wrakelberg	w	100000	50

Table 2 Enzymes studied and buffer-systems used for *Salvia* and *Scabiosa*

			Buffer pH	
Enzyme	Abbreviation	E.C. number	*Salvia*	*Scabiosa*
Sorbitol dehydrogenase	SORDH	1.1.1.14.	8.3	—
Malic enzyme	ME	1.1.1.40.	8.3	—
Isocitrate dehydrogenase	ICD	1.1.1.42.	7.0	—
Phosphogluconate dehydrogenase	PGD	1.1.1.44.	7.0	7.0
Peroxidase	PEROX	1.11.1.17.	8.6	—
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1.	8.3	8.3
Phosphoglucomutase	PGM	2.7.5.1.	8.6	8.6
Aconitase	ACN	4.2.1.3.	—	8.6
Triose phosphate isomerase	TPI	5.3.1.1.	8.6	8.3
Glucose phosphate isomerase	GPI	5.3.1.9.	7.0	7.0/8.6

in Hofman (1988). The recipe of the tris-borate EDTA buffer-system pH 8.6 was: 12.11 g Tris/l aquadest (0.1 M), 1.86 g EDTA/l aquadest (0.005 M), adjust to pH 8.6 with boric acid. The staining solution of SORDH was: 50 ml 0.06 M Tris-HCl pH 8.1 (7.27 g Tris/l aquadest, adjust to pH 8.1 with 25 per cent HCl), 125 mg sorbitol, 20 mg NAD^+, 5 mg MTT, 1 mg PMS, 50 mg pyrazol and 50 mg sodium pyruvate. Because of the complexity of some enzyme-activity patterns, due to the presence of a gene duplication (R. Van Treuren & R. Bijlsma, in preparation), electrophoresis of *Scabiosa* for GPI was performed on two buffer systems. On tris-citrate pH 7.0 electrophoretic mobility was higher (better separated bands), whereas tris-borate EDTA pH 8.6 gave a higher resolution (sharper bands).

Estimation of genetic variation

To estimate the amount of genetic variation, the following measures were calculated: (i) the proportion of polymorphic loci (P), (ii) the mean observed number of alleles per locus (A_0) and (iii) gene diversity (H_e). A locus was considered polymorphic if the frequency of the most frequent allele was less than 0.99. Gene diversity and standard genetic distance were computed

according to Nei (1987) in which corrections were made for small sample sizes.

Results

Enzyme variability is given in Table 3. Because the allelic variation and number of subunits of *Pgd-2* for *Scabiosa* was only recently established, the results for this locus were omitted from the genetic analyses. In order to determine if the inheritance of the allozymes was Mendelian, crosses were performed with plants grown from seeds collected in 1987. Because not all genotypes were represented in the seed samples, only the relatively frequent variants could be tested (Table 4). No significant deviation from Mendelian inheritance was found for any of the loci examined. Independent segregation was observed for most loci, strong linkage was only found between *Pgd-1* and *Gpi-2* of *Scabiosa*. Recombination between these loci was estimated to occur at a frequency of about 6 per cent (R. Van Treuren & R. Bijlsma, in preparation).

To establish the relationship between population size and the amount of genetic variation, corrections for differences in sample size were calculated in two different ways. Firstly, Spearman's rank correlation

Table 3 Enzyme variability of *Salvia* and *Scabiosa*. Loci coding for similar enzymes were numbered in ascendence according to the electrophoretic mobility of their gene products. Alleles were named according to their relative position to the N (normal) allele, which was present in every population and which was usually the most frequent. I_1 was intermediate between S (slow) and N, I_2 between N and F (fast)

Salvia			*Scabiosa*		
Locus	Isozyme structure	Alleles	Locus	Isozyme structure	Alleles
Sordh	Monomeric	S, N	*Pgd-1*	Dimeric	S, N, F
Me-1	Dimeric	S, I_1, N	*Pgd-2*	Dimeric	S, N
Icd-1	?	N	*Pgd-3*	Monomeric	S, N
Pgd-1	Dimeric	S, N, F	*Got-1*	Dimeric	S, N
Pgd-2	Dimeric	S, N, F	*Pgm-1*	Monomeric	S, N, F
Perox	Monomeric	S, I_1, N, F	*Pgm-2*	?	N
Got-1	Dimeric	S, I_1, N, F	*Acn-1*	Monomeric	S, N, F
Got-2	Dimeric	S, N, F	*Acn-2*	Monomeric	S, N
Pgm-1	Monomeric	S, N, F	*Tpi-1*	Dimeric	S, N, F
Pgm-2	Monomeric	S, I_1, N, I_2, F	*Tpi-2*	Dimeric	N, F
Tpi-1	Dimeric	S, I_1, N	*Gpi-1*	Dimeric	S, N, F
Tpi-2	Dimeric	N, F	*Gpi-2*	Dimeric	S, I_1, N, I_2, F
Gpi-1	?	N	*Gpi-3*	?	N

Table 4 Parental genotypes (P) and genotype numbers in the F_1-generation with Chi-square values for the deviation from Mendelian segregation

Salvia								*Scabiosa*				
Locus	*Pgd-2*	*Perox*	*Got-1*	*Got-2*	*Pgm-1*	*Pgm-2*	*Tpi-1*	*Pgd-1*	*Got-1*	*Tpi-1*	*Gpi-1*	*Gpi-2*
P	NF	NF	SN	SN	NF	NN	I_1N	SN	SN	SN	SN	NF
	SN	NF	SN	SN	NF	NI_2	NN	SN	SN	SN	SN	NF
F_1	SN 7	NN 10	SS 6	SS 5	NN 9	NN 22	I_1N 19	SS 22	SS 23	SS 16	SS 8	NN 17
	SF 14	NF 11	SN 20	SN 15	NF 13	NI_2 15	NN 18	SN 61	SN 58	SN 41	SN 16	NF 40
	NN 9	FF 8	NN 16	NN 9	FF 7			NN 24	NN 26	NN 12	NN 10	FF 15
	NF 7											
χ^2	3.541	1.966	4.857	1.138	0.586	1.324	0.027	2.178	0.925	2.913	0.353	1.000

coefficients were calculated between population size, sample size and the amount of genetic variation. Subsequently, by keeping sample size constant, the partial correlation coefficients (Sokal & Rohlf, 1981) between population size and the amount of genetic variation were computed. Significant correlations were found between population size and the proportion of polymorphic loci (*Salvia*: $r=0.807$, $t=4.534$, $P<0.0005$; *Scabiosa*: $r=0.622$, $t=2.386$, $0.01<P<0.025$) and between population size and the mean observed number of alleles (*Salvia*: $r=0.570$, $t=2.303$, $0.01<P<0.025$; *Scabiosa*: $r=0.653$, $t=2.584$, $0.01<P<0.025$), although it should be stressed that P and A_0 are not independent measures. However, no significant correlation was found between population size and gene diversity (*Salvia*: $r=0.051$, $t=0.169$, $0.4<P$; *Scabiosa*: $r=0.135$, $t=0.410$, $0.3<P<0.4$). Secondly, repeated samples of equal size were taken from the master file (*Salvia*: sample size = 5; *Scabiosa*: sample size = 6), the number of repetitions in each population were determined by the original sample size. Subsequently, the mean values of P, A_0 and H_e were used in a weighted regression (Fig. 2). Again, significant positive correlations were found between the population size and proportion of polymorphic loci (*Salvia*: $r=0.619$, $t=2.73$, $0.005<P<0.01$; *Scabiosa*: $r=0.713$, $t=3.21$, $0.001<P<0.005$) and between population size and the mean observed number of alleles (*Salvia*: $r=0.540$, $t=2.22$, $0.01<P<0.025$; *Scabiosa*: $r=0.819$, $t=4.51$, $0.0005<P<0.001$), but not between the population size and gene diversity (*Salvia*: $r=0.309$, $t=1.12$, $0.1<P<0.15$; *Scabiosa*: $r=0.490$, $t=1.78$, $0.05<P<0.075$). The lack of correlation between the population size and H_e in both species was mainly due to the smallest population showing a relatively high value and the largest population showing a relatively low value.

To establish the extent of genetic differentiation

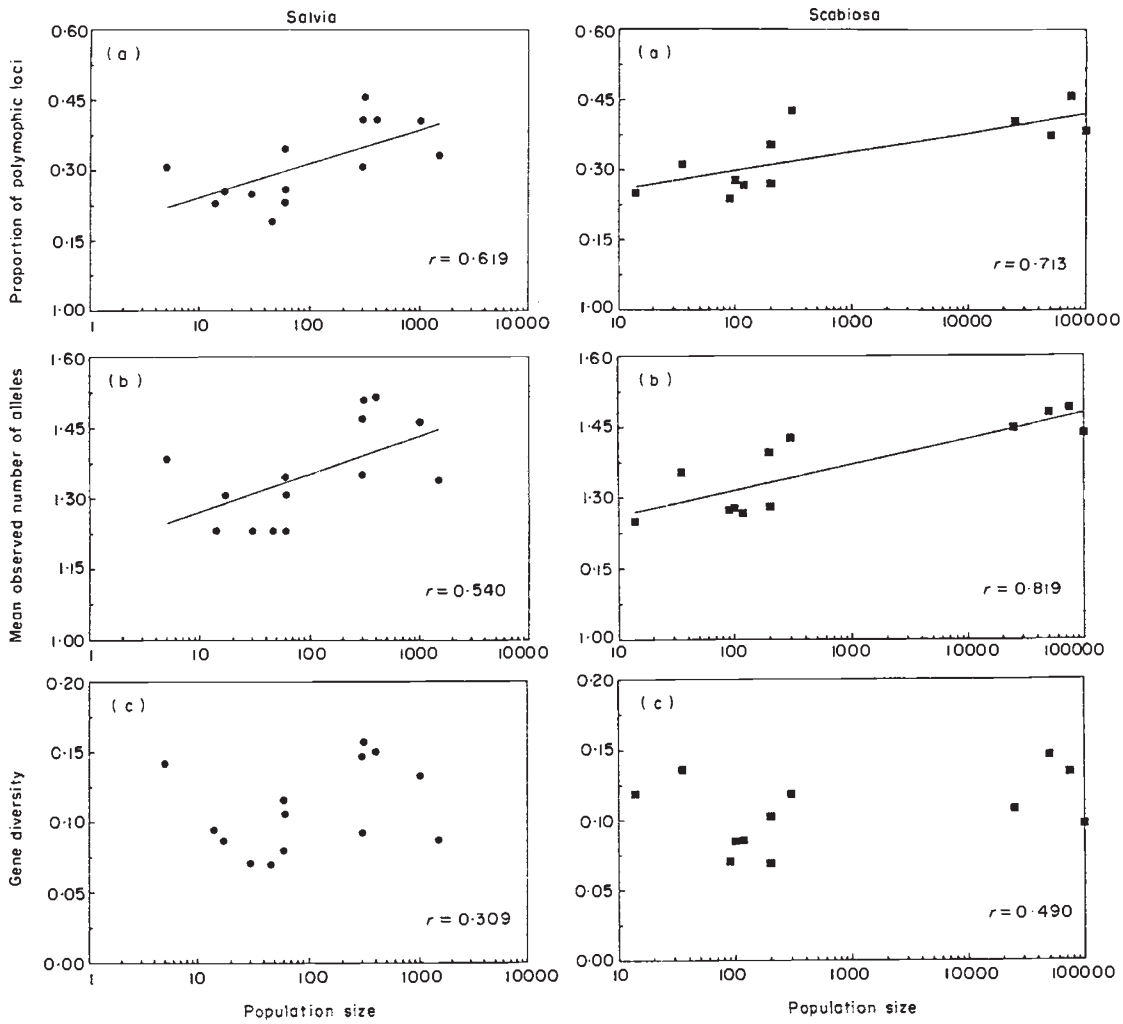

Fig. 2 Weighted regression of proportion of polymorphic loci (a), mean observed number of alleles (b) and gene diversity (c) on population size (log scale) for both *Salvia* and *Scabiosa*. r is the correlation coefficient.

Table 5 Analysis of gene diversity (Nei, 1987) in populations of *Salvia* and *Scabiosa*, together with the Chi-square value of G_{ST} for the deviation from zero. The coefficient of gene differentiation (G_{ST}) is the proportion of the total gene diversity (H_T) that can be attributed to the average gene diversity between populations ($H_T - H_S$). Deviation of G_{ST} from 0 (no differentiation) was tested by using the Chi-square test of heterogeneity of gene frequencies (Workman & Niswander, 1970)

	Salvia					*Scabiosa*				
	Locus	H_T	H_S	G_{ST}	χ^2	Locus	H_T	H_S	G_{ST}	χ^2
All populations	*Sordh*	0.034	0.030	0.122	58.6**	*Pgd-1*	0.237	0.206	0.131	190.9**
	Me-1	0.077	0.061	0.203	202.9**	*Pgd-3*	0.022	0.021	0.055	34.8**
	Icd-1	0.000	0.000	—	—	*Got-1*	0.271	0.213	0.212	170.4**
	Pgd-1	0.094	0.072	0.237	75.5**	*Pgm-1*	0.228	0.178	0.220	252.0**
	Pgd-2	0.214	0.197	0.081	131.1**	*Pgm-2*	0.000	0.000	—	—
	Perox	0.562	0.436	0.224	434.9**	*Acn-1*	0.008	0.007	0.035	38.4*
	Got-1	0.198	0.167	0.155	294.4**	*Acn-2*	0.095	0.083	0.122	92.5**
	Got-2	0.128	0.118	0.080	100.6**	*Tpi-1*	0.257	0.231	0.103	122.8**
	Pgm-1	0.115	0.101	0.120	94.4**	*Tpi-2*	0.044	0.041	0.078	54.9**
	Pgm-2	0.190	0.165	0.130	209.2**	*Gpi-1*	0.130	0.102	0.216	260.6**
	Tpi-1	0.140	0.129	0.073	124.0**	*Gpi-2*	0.262	0.199	0.238	417.5**
	Tpi-2	0.015	0.014	0.076	41.1**	*Gpi-3*	0.000	0.000	—	—
	Gpi-1	0.000	0.000	—	—	—	—	—	—	—
Populations (all loci)										
All		0.136	0.115	0.156			0.129	0.107	0.175	
Small		0.128	0.105	0.181			0.131	0.100	0.236	
Large		0.144	0.127	0.115			0.124	0.112	0.101	

*$0.01 < P < 0.025$.
**$P < 0.0005$.

among populations, an analysis of gene diversity was performed (Table 5). Significant differentiation was found for all loci of both species. For all loci combined, G_{ST} was 0.156 and 0.175 for *Salvia* and *Scabiosa*, respectively. A subsequent analysis of gene diversity in the group of small populations and in the group of large populations showed that the coefficient of gene differentiation was substantially higher in the group of small populations (*Salvia*: $G_{ST} = 0.181$ and 0.115, respectively; *Scabiosa*: $G_{ST} = 0.236$ and 0.101, respectively).

Discussion

The basic assumption underlying our research project was the hypothesis that, as a consequence of genetic drift, inbreeding and restricted gene flow, small and isolated populations show decreased levels of genetic variation. The smaller number of variable loci and the smaller number of alleles found in the small populations are in agreement with this prediction. Similar results have been reported by Hamrick *et al.* (1979), Levin *et al.* (1979), Schmidtke & Engel (1980), Moran & Hopper (1983) and Karron (1987). No significant correlation was found between population size and gene diversity. However, 'rare' alleles reach high frequencies (possibly due to genetic drift) only in small populations. This effect will inflate H_e in small populations and consequently weaken the correlations between H_e and the population size. Therefore, gene diversity appears not to be a useful comparative measure of genetic variation in small populations. Moreover, many other population characteristics, such as population structure (neighbourhood size, relative plant density, etc.), effective population size and breeding system might affect not only H_e but also the other measures of genetic variation. Varvio-Aho (1981) for example showed that gene diversity in the Finnish waterstrider was not correlated with population size but clearly with *effective* population size. The impact of these other population characteristics is currently being investigated.

If genetic drift predominantly affects allelic frequencies in populations, and levels of gene flow between populations are low, population genetic theory predicts genetic differentiation between populations. The results of the analysis of gene diversity agree with this prediction. Loveless & Hamrick (1984) analysed the available data that describes genetic differentiation from a large number of studies and

computed the mean G_{ST} values for a number of variables. They found that G_{ST} was 0.118, 0.109 and 0.077 for predominantly outcrossing species ($n=76$), dioecious species ($n=3$) and long-lived perennials ($n=48$), respectively. The mean G_{ST} of 43 predominantly outcrossing, long-lived perennials was 0.068. Compared to these values, *Salvia* ($G_{ST}=0.156$) and *Scabiosa* ($G_{ST}=0.175$) show substantial genetic differentiation, probably resulting from a significant amount of genetic drift in the small populations. This explanation is supported by the finding that small populations are more differentiated from each other than are large populations. Together with the observation that average gene diversity did not differ substantially between the group of small and large populations (Table 5), our results are similar to those of Brakefield (1989). Rich *et al.* (1979) also found a significant increase in the variance of allelic frequencies among populations due to genetic drift and showed that this increase was inversely proportional to population size. This agrees with our observation that the rare alleles are found in high frequency only in small populations, resulting in larger variances of allelic frequencies and subsequently larger G_{ST} values in the group of small populations.

If allelic frequencies in populations are predominantly affected by genetic drift and gene flow between populations is restricted, it is also to be expected that genetic differentiation occurs even within relatively short distances. No significant correlations are found between geographic and genetic distances (*Salvia*: $r=-0.022$, $t=-0.206$, $P<0.4$; *Scabiosa*: $r=0.167$, $t=1.357$, $0.075<P<0.1$), which suggests that gene flow is indeed restricted. The genetic structure of both species resembles an island model of population structure, as found for *Desmodium nudiflorum* (Schaal & Smith, 1980) and *Sarracenia purpurea* (Schwaegerle & Schaal, 1979), where populations are geographically isolated from each other with the chance of gene flow between populations being greatly reduced.

Estimates of the average level of gene flow between natural populations can be derived from the G_{ST} values (Slatkin & Barton, 1989). G_{ST} values of 0.156 (*Salvia*) and 0.175 (*Scabiosa*) are equivalent to $N_m=1.166$ and $N_m=0.990$ respectively, which means about only one migrant every generation. It has been suggested that the exchange of a single individual per generation among small endangered (sub)populations is sufficient to have them behave almost as one panmictic population (e.g. Franklin, 1980; Frankel & Soulé, 1981; Allendorf, 1983; and Frankel, O. H., 1983). This general guideline for the management of small populations has been deduced from the equilibrium theory of Wright's infinite island model (Wright, 1931). However, populations are generally not in equilibrium and the number of subpopulations is often small. Therefore, Varvio *et al.* (1986) studied the dynamics of genetic differentiation by using the finite island model and showed that the values of H_S, H_T and G_{ST} in transient populations depend on the pattern of population subdivision and that it may take time for them to approximate the equilibrium values. Furthermore, the problem is complicated, among others, by fluctuating population sizes, subdivision and extinction of subpopulations. They therefore concluded that a single guideline, e.g. the 'one migrant per generation' rule, is not theoretically well justified.

In practice it is difficult to separate the role of selective and non-selective forces in genetic differentiation (Varvio-Aho, 1983). A way to examine the problem is to investigate whether the gene frequency variation is homogeneous over loci. If so, genetic differentiation is most likely to be the result of random processes because they affect all loci simultaneously and to the same extent (Lewontin & Krakauer, 1973; Schaal, 1975; Varvio-Aho, 1983). F_{ST} values for individual alleles range from 0.031 to 0.243 (*Salvia*) and from 0.018 to 0.450 (*Scabiosa*). The ratio observed/expected variance in F_{ST} which is Chi-square/d.f. distributed (Lewontin & Krakauer, 1973) is 2.264 ($0.0005<P<0.001$) and 3.332 ($P<0.0005$) for *Salvia* and *Scabiosa* respectively, indicating that the differentiation of gene frequencies is clearly non-random. It seems unlikely, therefore, that the genetic constitution of both species can be entirely accounted for by random processes.

The amount of allozyme variation is clearly correlated with population size, the small populations being less variable. The genetic structure of *Salvia* and *Scabiosa* in The Netherlands can most satisfactorily be explained by an island model of population structure, with restricted gene flow between populations. This may imply that: (i) small populations are also less variable with respect to favourable alleles and/or fixed for deleterious ones, as a result of genetic drift and inbreeding, and/or (ii) small populations have reduced evolutionary potential but are well adapted to their current environment, as a result of selective forces. Experiments are planned to reveal whether: (i) plants in small populations show decreased fitness due to inbreeding depression, and/or (ii) plants in small populations experience different environmental conditions to which they have become adapted. The outcome of these experiments will be used to evaluate the effectiveness of measures proposed to restore the level of genetic variation. Hybridization of populations, for example, will increase genetic variability and fitness when populations are suffering from inbreeding

depression but will result in outbreeding depression (Templeton, 1986) when populations have been adapted to differential local conditions.

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