united nations
educational, scientific
and cultural
organization

international atomic
energy agency

the
abdus salam
international centre for theoretical physics

H4.SMR/1202-46

"Fifth Course on Mathematical Ecology
including and introduction to Ecological Economics"

28 February - 24 March 2000

PREDICTING ENDEMISM FROM POPULATION STRUCTURE OF A WIDESPREAD SPECIES: CASE STUDY IN *CENTAUREA MACULOSA* LAM (ASTERACEAE)

I. Olivieri

Institut des Sciences de l'Evolution
Université Montpellier 2
Montpellier, France

Predicting Endemism from Population Structure of a Widespread Species: Case Study in *Centaurea maculosa* Lam. (Asteraceae)

HÉLÈNE FRÉVILLE,* BRUNO COLAS,*†‡** JOËLLE RONFORT,* MIQUEL RIBA,§ AND ISABELLE OLIVIERI†‖

*Institut National de la Recherche Agronomique (INRA), Montpellier, Domaine de Melgueil, 34130 Mauguio, France

†Institut des Sciences de l'Evolution de Montpellier (ISEM), Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier, France

‡Conservatoire Botanique National Méditerranéen de Porquerolles, Castel Sainte-Claire, 83418 Hyères, France

§Centre de Recerca Ecològica i Aplicacions Forestals (CREAF), Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Abstract: *Amounts of genetic variability, genetic differentiation among taxa and populations, and population sizes were studied in five populations of* Centaurea maculosa *ssp.* maculosa *(a widespread taxon), all six populations of* C. corymbosa *(a narrowly endemic species), and the single population of* C. maculosa *ssp.* albida. *Seventeen isozyme loci were studied, of which nine were polymorphic. Results suggest that* C. corymbosa *and* C. maculosa *ssp.* albida *are likely derived from* C. maculosa *ssp.* maculosa *because the former represent a sample of the diversity of the latter. The percentage of polymorphic loci and Nei's genetic diversity were positively and significantly correlated with population size over all populations, but not within each taxon. Populations of both the widespread* C. maculosa *ssp.* maculosa *and the rare* C. corymbosa *were strongly differentiated: overall,* F_{ST} *values were 0.26 and 0.34, respectively. Differentiation among populations of different taxa was of the same order of magnitude as that observed among populations within taxa. Nevertheless, significant differentiation among the three taxa was found by a hierarchical analysis of variance on allele frequencies. We suggest that bottlenecks or founder effects associated with colonization events and ecological specialization in some populations of* C. maculosa *ssp.* maculosa *have led to new taxa such as* C. corymbosa *and* C. maculosa *ssp.* albida. *This may be a direct consequence of the particularly strong differentiation among populations of the widespread* C. maculosa *ssp.* maculosa. *Our study highlights the utility of considering closely related widespread taxa in order to understand the population biology and evolution of rare species, as well as to design proper management programs.*

Predicciones de Endemismo a Partir de la Estructura Poblacional de una Especie con Amplia Distribución: Estudio del Caso de *Centaurea maculosa* Lam. (Asteraceae)

Resumen: *Se determinó la variabilidad genética, la diferenciación genética entre taxones y entre poblaciones, y el tamaño de población en 5 poblaciones de* Centaurea maculosa *ssp.* maculosa *(un taxón de amplia distribución), las 6 únicas poblaciones de* C. corymbosa *(especie endémica), y la única población de* C. maculosa *ssp.* albida *existente. Se estudiaron diecisiete loci enzimáticos, de los cuales 9 fueron polimórficos. Los resultados sugieren que los dos taxones endémicos probablemente derivaron del taxón de amplia distribución, ya que los primeros representan una muestra de la diversidad de este último. Tanto el porcentaje de loci polimórficos como la diversidad genética (índice de Nei) mostraron una correlación positiva con el tamaño*

‖*Address correspondence to I. Olivieri, email olivieri@isem.univ-montp2.fr*

**Current address: Laboratoire d'Ecologie, Université Paris 6, 7 Quai Saint Bernard, 75252 Paris cedex 05, Paris, France.*

Paper submitted December 24, 1996; revised manuscript accepted November 11, 1997.

de la población. Se encontró una fuerte diferenciación entre las poblaciones tanto en el taxón endémico como en el de amplia distribución: los valores de F_{ST} globales fueron 0.34 para C. corymbosa *y 0.26 para* C. maculosa *ssp.* maculosa. *La diferenciación entre poblaciones de taxones diferentes fue del mismo orden de magnitud que la observada entre poblaciones dentro de un mismo taxón. Sin embargo, un análisis de la varianza jerárquico de las frecuencias génicas reveló una diferenciación significativa entre los tres taxones. Se sugiere que la aparición de taxones endémicos como* C. corymbosa *y* C. maculosa *ssp.* albida *se debe a efectos fundacionales, como consecuencia de "cuellos de botella" o procesos de colonización, y posterior especialización ecológica en algunas poblaciones de* C. maculosa *ssp.* maculosa. *Esto sería una consecuencia directa de la fuerte diferenciación entre poblaciones de* C. maculosa *ssp.* maculosa. *Este estudio remarca la utilidad del uso de especies de amplia distribución estrechamente emparentadas con especies endémicas para comprender la biología y evolución de especies raras, así como para diseñar programas de manejo adecuados.*

Introduction

Population structure and the maintenance of genetic variability depend on the balance between genetic drift, natural selection, and gene flow. If populations are spatially close enough to allow sufficient dispersal of pollen or seeds, gene flow will homogenize genetic variability among populations, a process preventing differentiation due to genetic drift and natural selection (Slatkin 1987). If populations are small and isolated, following habitat fragmentation, for example (Young et al. 1996), gene flow might not be sufficient to prevent genetic differentiation. In isolated populations, because of local adaptation and/or fixation of alleles through genetic drift, genetic diversity is expected to be lower at the population level compared to that of well-connected populations. At the species level, however, higher levels of genetic diversity might actually be maintained (Baskauf et al. 1994). Eventually, new species might arise as a result of geographic isolation and local differentiation of populations or metapopulations (Levin 1995). These new species, which would probably be rare, deriving from single populations or a few demes within metapopulations, may in turn be prone to extinction.

One goal of conservation biology is to preserve species from extinction. Species with low levels of genetic variability are thought to be more vulnerable to environmental changes and to be at greater risk of extinction than species with high genetic variability (Barrett & Kohn 1991; Huenneke 1991; Brussard & Ehrlich 1992; Barbault 1993; Dolan 1994). Knowledge about the magnitude and distribution patterns of genetic variability is often considered a necessary part of management of rare species (Hamrick et al. 1991, but see Lande 1988). We argue that, in order to understand how rare species arise and function, it might be useful to study the population structure of widespread relatives of narrowly endemic species. Indeed, when widespread species comprise geographically differentiated populations, they may be more likely to give rise to new, rare species by isolation and local adaptation. Management decisions can then be made that take into account not only the endangered species but also some populations of the related widespread progenitor. The latter can be considered either a source of new species or a source of genetic variability (through hybridization) for the endangered species (but for a review on the risks of extinction of rare species due to hybridization see Levin et al. 1996). Although the objective of conservation biology cannot be to preserve every nascent species, it could be to preserve the capacity of a genus (or a community) to evolve. Widespread species that are potential progenitors of new species are part of the community and should be considered useful in that respect.

Centaurea corymbosa Pourret (Asteraceae) is a narrowly endemic species that appears on the list of endangered species in France (Muséum National d'Histoire Naturelle 1995) and on the list of priority species of the European Habitat Directive (Wyse Jackson & Akeroyd 1994). With the aim of understanding the functioning of this rare species and improving our knowledge of mechanisms leading to endemism, we compared genetic and demographic parameters (population structure and population sizes) of *C. corymbosa* with those of two supposedly related taxa of the genus *Centaurea*: *C. maculosa* ssp. *albida*, endemic to southern France, and *C. maculosa* ssp. *maculosa*, a widespread taxon. The three taxa belong to subgenus *Acrolophus*, section *Maculosae*, but the relationships among species within *Centaurea* are unclear (Tutin et al. 1976; Susanna et al. 1995). We use the nomenclature suggested in *Flora Europea*, which is based on morphological and quantitative characters (Tutin et al. 1976). To our knowledge there have been no hybridization trials among the three studied taxa. In this paper the name *C. maculosa* will refer to both subspecies, *C. maculosa* ssp. *maculosa* and *C. maculosa* ssp. *albida*.

Using isozyme markers, we addressed the following questions: (1) Are there differences in the magnitude and partitioning of genetic variation within and among populations between widespread taxon and the two narrowly endemic taxa? (2) Can these differences be explained by different population sizes? (3) Are the taxa genetically differentiated? (4) Does the amount of isozymic differentiation among taxa reflect the taxonomic classification inferred from morphological traits?

Species Description and Methods

Geographic Distribution, Habitat, and Biology of the Three Taxa

Centaurea corymbosa is known from only six populations, distributed within a 3-km^2 area of the calcareous Massif de la Clape, near Narbonne, in southern France (Colas et al. 1996). Each population is isolated from the others by pine woods and scrubland (*garrigue*). In June 1995 only 495 flowering plants were observed out of an estimated total of 6500 plants (including seedlings) in the six populations.

Centaurea maculosa Lam. ssp. *albida* (Lecoq & Lamotte) Dostál is known from a single population (Anduze, southern France). Like *C. corymbosa*, it grows on calcareous cliffs. *C. maculosa* Lam. ssp. *maculosa*, the widespread taxon, occurs from southern France to central Europe. It was introduced to North America, where it is considered a weed (Harrod & Taylor 1995). In southern France it grows on calcareous rocky places, often on cliffs, and sometimes in open areas and along roadsides.

The three taxa are monocarpic, perennial, and insect-pollinated. The plants may persist as rosettes for 3–10 years before flowering. Pollination is mainly carried out by small Hymenoptera. *C. corymbosa* is self-incompatible (Colas et al. 1996). *C. maculosa* from North America has also been described as self-incompatible (Harrod & Taylor 1995).

Electrophoresis

Electrophoretic data on *C. corymbosa* are published elsewhere (Colas et al. 1997), along with data on pollination biology and seed dispersal.

During winter and spring of 1996, we sampled five populations of *C. maculosa* ssp. *maculosa* and the single population of *C. maculosa* ssp. *albida*. Their geographic locations along with those of the six *C. corymbosa* populations on the Massif de la Clape are shown in Fig. 1. In most cases, populations occurred as homogeneous, discrete units, so it was easy to determine what to call a population.

Young leaves from large rosettes (at least 6 cm in diameter, a minimum size for flowering) were collected haphazardly from each population. Leaves were kept frozen at −196° C until processing. Sample sizes ranged from 16 to 70 individuals (Table 1). In addition, a set of individuals of known genotypes of *C. corymbosa* was re-

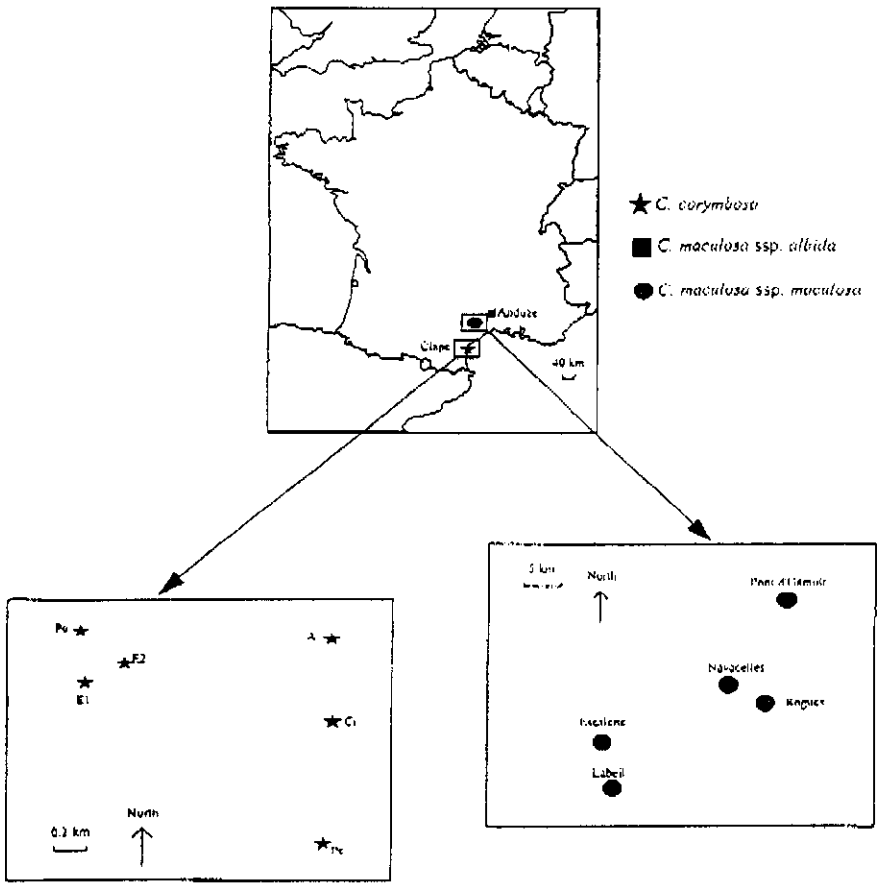

Figure 1. Geographic distribution of the sampled populations of C. corymbosa, Centaurea maculosa *ssp.* maculosa, *and* C. maculosa *ssp.* albida.

Table 1. Flowering population size (N_fl), sample size (n_s), proportion of polymorphic loci (P, 95% criterion), mean number of alleles per locus (A), observed heterozygosity (H_o), and gene diversity (expected heterozygosity H_e) within populations of *Centaurea corymbosa*, *C. maculosa* ssp. *maculosa*, and *C. maculosa* ssp. *albida*.

Population	N_{fl}	n_s	P	A	H_O	H_e
*C. corymbosa**						
A	75	55	11.8	1.2	0.056	0.054
Cr	5	16	17.6	1.2	0.077	0.078
E1	192	44	11.8	1.1	0.039	0.043
E2	151	57	11.8	1.1	0.027	0.028
Pe	61	32	11.8	1.1	0.057	0.054
Po	10	17	5.9	1.1	0.003	0.010
Mean per population	82.3	36.8	11.8	1.1	0.043	0.045
C. maculosa ssp. *maculosa*						
Escalette (Es)	300	40	17.6	1.2	0.050	0.062
P. d'Hérault (PH)	119	34	35.3	1.6	0.114	0.111
Labeil (L)	73	23	11.8	1.1	0.051	0.044
Navacelles (N)	2000	46	41.2	1.5	0.156	0.170
Rogues (R)	2000	40	29.4	1.4	0.101	0.101
Mean per population	898.4	36.6	27.1	1.4	0.094	0.098
C. maculosa ssp. *albida*						
Anduze (And)	120	70	23.5	1.5	0.071	0.076
C. maculosa (mean per population)	768.7	42.2	24	1.4	0.091	0.094

**Data for* C. corymbosa *are from Colas et al. (1996, 1997).*

sampled in the Massif de la Clape to serve as references for alleles at each marker locus.

Samples were ground at 4° C, in an extraction buffer (50 mL of tris-HCl 0.1 M at pH 7.6, along with 2 g of thioglycolic acid Na-salt, and 1 g of polyethylene glycol 20,000) and absorbed into wicks that were immediately frozen at −80° C for storage before gel runs. Five buffer systems were used for starch gels (modified from Soltis et al. 1983; Wendel & Weeden 1989; Kephart 1990; recipes available upon request from the first two authors). For each gel run, three *C. corymbosa* individuals were used as references. Of 19 loci resolved for *C. corymbosa* (Colas et al. 1997), two (*Aat-2* and *Per*) showed poor resolution for *C. maculosa*, and isozyme variation for these systems could not be reliably scored. Consequently, data analyses were conducted with the remaining 17 loci (*Aat-1*, *Ald*, *Cat*, *Dia*, *Est*, *Gdh*, *Idh*, *Lap*, *Mdh-1*, *Mdh-2*, *Me*, *Pgd-1*, *Pgd-2*, *Pgi*, *Pgm-1*, *Pgm-2*, and *Skdh*; recipes modified from Vallejos 1983; Soltis et al. 1983; Wendel & Weeden 1989), and electrophoretic data on *C. corymbosa* (Colas et al. 1997) were re-analyzed after removal of *Aat-2* and *Per* to allow direct comparisons.

Population Size and Data Analysis

During the summer of 1996, the flowering plants were counted in each sampled population of *C. maculosa* ssp. *maculosa* and *C. maculosa* ssp. *albida*. A one-way analysis of variance (ANOVA; with populations as replicates and taxon as main effect; SAS Institute 1992) was performed on log-transformed data at the taxon level to compare the average flowering population size of *C. corymbosa* (recorded in 1995, Colas et al. 1996) with that of *C. maculosa* ssp. *maculosa*.

The percentage of loci polymorphic per population (P, considering as polymorphic only those loci for which the frequency of the most frequent allele was below 95%), and gene diversity, (mean expected heterozygosity H_e, unbiased estimate [Nei 1978] averaged over loci and observed heterozygosity, H_o) were calculated with BIOSYS-1 (Swofford & Selander 1981) at both species and population levels. Differences in the amount of genetic variability between *C. maculosa* and *C. corymbosa* were tested by chi-square with a contingency table on the number of polymorphic and monomorphic loci in each species. One-way ANOVA (with loci as replicates and taxon as main effect) was performed for the within-taxon comparison of gene diversity (H_e).

To detect differences between *C. corymbosa* and *C. maculosa* ssp. *maculosa* in the amount of within-population genetic variability, a one-way ANOVA (with populations as replicates and taxon as main effect) was performed for both P and H_e. Because of the existence of a single population of *C. maculosa* ssp. *albida*, values of P and H_e obtained for this taxon were not compared with those of the two other taxa.

Finally, correlation coefficients were calculated between population size and both P and H_e at the subspecies and species levels, and over all populations, and tested for significance with a t test using Proc CORR (SAS Institute 1992).

Population genetic structure was analyzed with GENEPOP (Raymond & Rousset 1995*a*,1995*b*), which computes Fisher exact tests (Fisher 1954) for Hardy-Weinberg equilibrium (F_{IS}) and population differentia-

tion (F_{ST}). The F_{IS} values (for each locus separately and over all loci) were calculated and their significance was tested for each population. Global F_{ST} over populations of *C. maculosa* ssp. *maculosa* on the one hand and of *C. corymbosa* on the other hand, as well as F_{ST} between all pairs of populations, were calculated and tested with exact tests. Nei's genetic distances (Nei 1978) were calculated with BIOSYS-1 (Swofford & Selander 1981). As the two measures of genetic differentiation were correlated ($r = 0.82$, $p < 0.05$, Mantel test; Mantel & Li 1974), only F_{ST} values will be shown. A UPGMA cluster analysis based on pairwise F_{ST} values was performed.

To estimate genetic differentiation among taxa, and in light of the hierarchical structure of the data set (several taxa, several populations within taxa), a hierarchical analysis of variance on F_{ST} was performed for each polymorphic locus, following Weir (1996). Three random factors were declared: individuals within populations, populations within taxa, and taxa. The component of variance associated with each factor was calculated and used to compute *F* statistics according to Weir and Cockerham (1984). Pairwise F_{ST} values between taxa were tested by jackknifing over loci to obtain a standard error (Weir 1996).

To compare genetic and geographic distances among populations, a Mantel test was performed over populations within the two taxa that contained more than one population. A Mantel test was also done at the species level by grouping *C. maculosa* ssp. *maculosa* and *C. maculosa* ssp. *albida*, and over all studied populations. The software FSTAT (Goudet 1995) was used for all Mantel tests.

Results

Population Sizes and Levels of Isozyme Variation

Population sizes ranged from 5 to 2000 flowering individuals and were on average significantly smaller in rare *C. corymbosa* than in widespread *C. maculosa* ssp. *maculosa* (on average 82 and 898 flowering individuals, respectively; $t = 2.41$; $p = 0.04$). The population of *C. maculosa* ssp. *albida* had an intermediate size (120) (Table 1). At the species level, flowering population size was larger in *C. maculosa* than in *C. corymbosa* (768 and 82, respectively; $t = 2.37$; $p = 0.04$).

Considering as polymorphic any locus with at least two alleles, whatever their frequency at the population level, 10, 8, and 4 loci were found polymorphic in *C. maculosa* ssp. *maculosa*, *C. maculosa* ssp. *albida*, and *C. corymbosa*, respectively (Table 2). Loci *Gdh*, *Idh*, *Mdh-1*, *Mdh-2*, *Pgm-1*, *Pgm-2*, and *Skdh* were fixed for the same allele in the three taxa. The difference in the level of polymorphism between *C. maculosa* and *C. corymbosa* was significant ($\chi^2 = 4.37$, $p = 0.04$). Considering as polymorphic at the taxon level only those loci for which the frequency of the predominant allele was below 95% in at

Table 2. Allele-frequency data for polymorphic loci from populations of *C. corymbosa*, *C. maculosa* ssp. *maculosa*, and *C. maculosa* ssp. *albida*).*

		Centaurea corymbosa						Centaurea maculosa *ssp.* maculosa					Centaurea maculosa *ssp.* albida
Locus	*Allele*	*A*	*Cr*	*E1*	*E2*	*Pe*	*Po*	*Es*	*PH*	*L*	*N*	*R*	*And*
Aat	a	1.000	1.000	1.000	1.000	1.000	1.000	0.813	0.853	1.000	0.717	0.950	0.793
	b							0.187	0.147		0.283	0.050	0.207
Ald	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.985	1.000	1.000	1.000	1.000
	b								0.015				
Cat	a	0.282	0.625	0.193	0.053				0.044		0.348	0.175	0.014
	b	0.718	0.375	0.807	0.947	1.000	1.000	1.000	0.956	1.000	0.652	0.825	0.986
Dia	a	0.600	0.219			0.297		0.188	0.029		0.293	0.213	0.071
	b	0.400	0.781	1.000	1.000	0.703	1.000	0.813	0.971	1.000	0.707	0.787	0.929
Est	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.897	1.000	0.598	0.962	0.629
	b								0.103		0.370	0.038	0.371
	c										0.033		
Lap	a	0.009	0.375			0.594		0.325	0.338	0.870	0.402	0.300	0.100
	b	0.991	0.625	1.000	1.000	0.406	1.000	0.675	0.662	0.130	0.598	0.700	0.900
Me	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.971	1.000	0.772	0.688	0.993
	b								0.029		0.228	0.312	0.007
Pgd-1	a								0.029				0.007
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.103	1.000	0.880	0.975	0.964
	c								0.868		0.120	0.025	0.029
Pgd-2	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.868	0.522	0.978	1.000	0.971
	b								0.132	0.478	0.022		0.029
Pgi	a								0.176				
	b	1.000	1.000	0.705	0.754	1.000	0.912	1.000	0.824	1.000	1.000	1.000	1.000
	c			0.295	0.246		0.088						

*Abbreviations for populations are given in Table 1.

least one population of that taxon, *C. maculosa* ssp. *maculosa* exhibited polymorphism at nine loci, whereas the two narrowly endemic taxa were polymorphic at only four loci (Table 2). All loci that were polymorphic in either *C. maculosa* ssp. *albida* or *C. corymbosa* were also polymorphic in *C. maculosa* ssp. *maculosa* (at both 95% and two alleles criteria). The difference in the amount of polymorphism between *C. maculosa* and *C. corymbosa* was not significant at the 5% level ($\chi^2 = 3.11$, $p = 0.08$). Considering only those alleles whose frequency was above 5%, four alleles were specific to *C. maculosa* ssp. *maculosa* (*Me-b*, *Pgi-a*, *Pgd-1c*, and *Pgd-2b*), and one allele (*Pgi-c*) was specific to *C. corymbosa* (occurring in three of six populations of *C. corymbosa* and in no other taxon). Within-species gene diversity was significantly higher in *C. maculosa* ($H_e = 0.123$, averaged over loci) than in *C. corymbosa* ($H_e = 0.061$) ($p < 0.05$).

All populations were polymorphic, with P and H_e ranging from 5.9% to 41.2% and from 0.010 to 0.170, respectively (Table 1). P and H_e were significantly greater in *C. maculosa* ssp. *maculosa* populations (on average, $P = 27.1\%$ and $H_e = 0.098$) than in *C. corymbosa* ($P = 11.8\%$ and $H_e = 0.045$; $p < 0.05$). The *C. maculosa* ssp. *albida* population had intermediate P and H_e values (Table 1).

When all populations were considered independently of taxon, both P and H_e were significantly and positively correlated with population size ($r = 0.70$, $p < 0.05$; $r = 0.73$, $p < 0.01$, respectively). But there was no significant correlation between population size and either P or H_e when the analysis was conducted within each taxon. Within *C. maculosa*, the correlation coefficients were of the same order of magnitude as when all populations were included, but they were no longer significantly different from zero (P: $r = 0.61$, $p = 0.196$; H_e: $r = 0.71$, $p = 0.111$), whereas within *C. corymbosa* the correlation coefficients were close to zero (P: $r = -0.02$, $p = 0.977$; H_e: $r = 0.19$, $p = 0.720$; Fig. 2).

Population Genetic Structure

Of 51 tests for Hardy-Weinberg equilibrium at individual loci, only three F_{IS} values were significantly different from zero at the $p < 0.05$ level. But these three values were no longer significantly different from zero when a table-wide significance level (Holm 1979) was used to set an upper bound on the family-wise error rate, as suggested by Rice (1989). Over all loci, no significant departure from Hardy-Weinberg expectation was detected in any population.

The F_{ST} over all populations of *C. maculosa* ssp. *maculosa* was 0.255, ranging from 0.080 (*Aat*) to 0.715 (*Pgd-1*) (bootstrap 95% confidence interval: 0.134–0.440), and F_{ST} for *C. corymbosa* was 0.339, ranging from 0.169 (*Pgi*) to 0.520 (*Lap*) (bootstrap 95% confidence interval: 0.169–0.520) (Table 3). Both values were significantly different from zero (exact test, $p < 0.0001$) but not different from each other, as shown by bootstrap confidence intervals.

All pairwise F_{ST} values but one (between populations E1 and E2 of *C. corymbosa*, which were in fact different when *Aat-2* and *Per* were included; see Colas et al. 1997) were significantly different from zero ($p < 0.05$). The phenogram based on UPGMA clustering of F_{ST} between pairs of populations shows a topology in which the 12 populations are hierarchically organized independently of their taxonomic status (Fig. 3).

With a hierarchical analysis of variance, however, significant differentiation was found between all taxa. Pair-

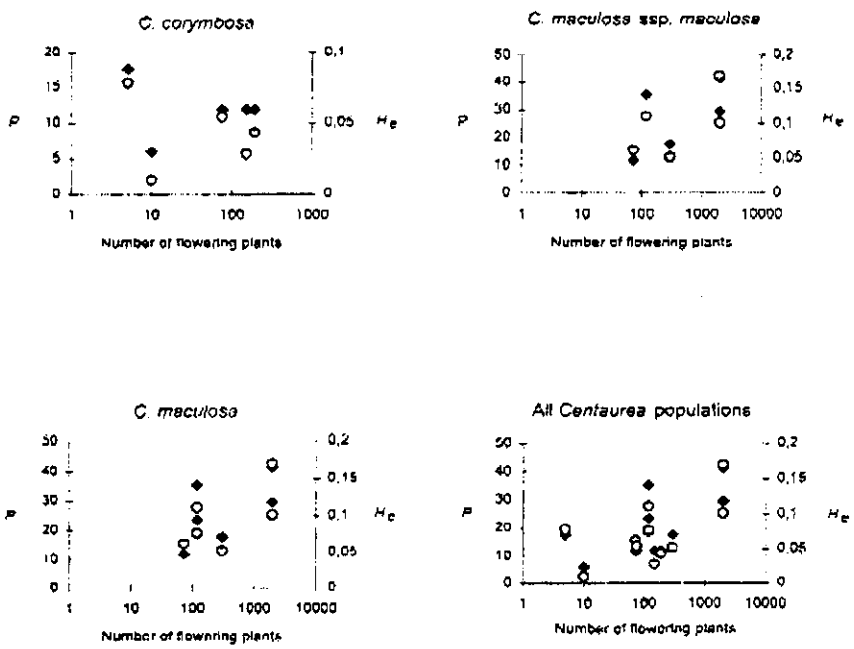

Figure 2. Correlation between number of flowering plants and percentage of polymorphic loci (P, ◆) and expected heterozygosity (H_e, ○) for four data sets: Centaurea corymbosa *(P: r = −0.02, p = 0.977; H: r = −0.19, p = 0.720),* C. maculosa *ssp.* maculosa *(P: r = 0.61, p = 0.276; H: r = 0.70, p = 0.186),* C. maculosa *(P: r = 0.61, p = 0.196; H: r = 0.71, p = 0.111), and all populations (P: r = 0.70, p = 0.011; H: r = 0.73, p = 0.007).*

Table 3. The F_{ST} per polymorphic locus over all populations of *Centaurea corymbosa* and of *C. maculosa* ssp. *maculosa*

	Taxon	
Locus	*C. corymbosa*	*C. maculosa* ssp. *maculosa*
Aat	—	0.080
Cat	0.228	0.194
Dia	0.426	0.091
Est	—	0.247
Lap	0.520	0.148
Me	—	0.172
Pgd-1	—	0.715
Pgd-2	—	0.336
Pgi	0.169	0.199
Total	0.339	0.255
Confidence interval*	0.169–0.520	0.134–0.440

*The 95% confidence intervals were obtained by bootstrapping over loci (FSTAT, Goudet 1995).

wise F_{ST} values were 0.075 (±0.030) between *C. maculosa* ssp. *maculosa* and *C. corymbosa*, 0.040 (±0.020) between *C. maculosa* ssp. *maculosa* and *C. maculosa* ssp. *albida*, and 0.126 (±0.088) between *C. corymbosa* and *C. maculosa* ssp. *albida*. Pairwise F_{ST} between the two species *C. maculosa* and *C. corymbosa* was also significantly different from zero (0.065 ± 0.018).

A significant correlation coefficient ($r = 0.83$; $p = 0.008$) between pairwise geographic distances and F_{ST} values was found only over *C. corymbosa* populations for which the mean pairwise geographic distance was 1.3 km (Fig. 4). The correlation within *C. maculosa* ssp. *maculosa* was marginally significant ($r = 0.48$, $p = 0.052$). The greater the mean distance between populations, the smaller the correlation and its associated significance probability (Fig. 4).

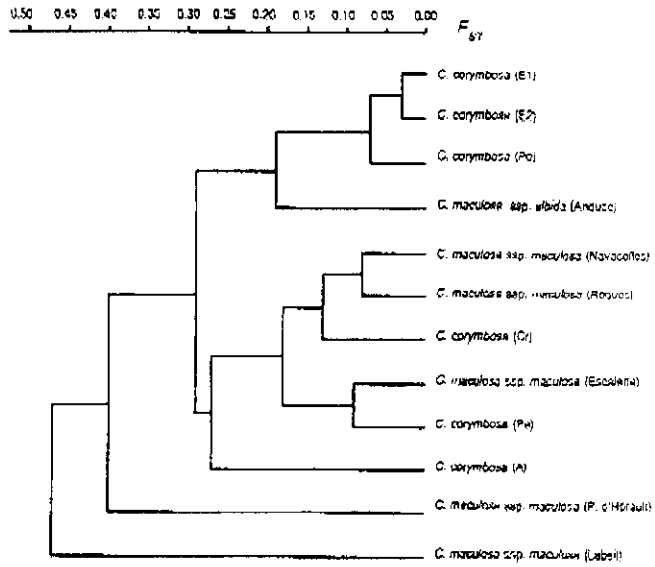

Figure 3. Phenogram based on unweighted pair group method with aritmetic averages (UPGMA) clustering of pairwise F_{ST} values for populations of Centaurea corymbosa, C. maculosa *ssp.* maculosa, *and* C. maculosa *ssp.* albida.

Discussion

Genetic Variability

Hamrick and Godt (1989) have shown that the amount of genetic variability, measured both at population and species levels, is significantly dependent on geographic range. In our study, populations of the narrowly endemic

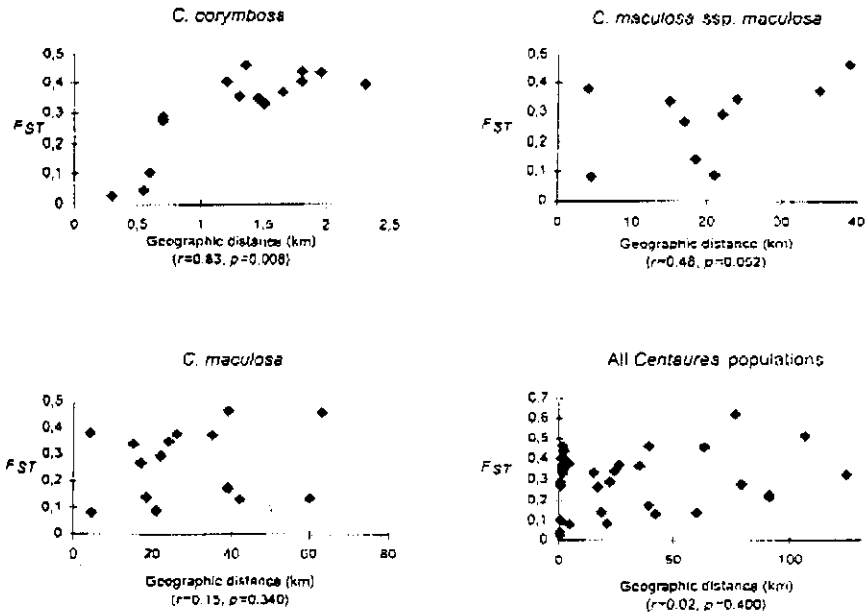

Figure 4. Correlation between F_{ST} and geographic distances for four data sets: C. corymbosa *(n = 6, $\bar{d}$ = 1.3 km),* C. maculosa *ssp.* maculosa *(n = 5, $\bar{d}$ = 20.0 km),* C. maculosa *(n = 6, $\bar{d}$ = 28.7 km), and all populations (n = 12, $\bar{d}$ = 58.3 km); n, number of populations; $\bar{d}$, average geographic distance between populations.*

taxa (*C. corymbosa* and *C. maculosa* ssp. *albida*) were less variable for isozyme markers than populations of the widespread taxon (*C. maculosa* ssp. *maculosa*). Although in some cases the rare species have been found to be more variable than the widespread congeners (Ranker 1994; Lewis & Crawford 1995; Purdy & Bayer 1996), our results are consistent with those of most studies of widespread versus rare congeneric species (Babbel & Selander 1975; Karron 1987; Loveless & Hamrick 1988; Baskauf et al. 1994; Edwards & Wyatt 1994). The observed differences in the amount of genetic variability at the population and species levels cannot, in our case, be explained by differences in mating system (neither species shows evidence of departure from random mating). They may instead reflect the observed differences in average population size and differences in the total number of individuals within each taxon (whereas only six populations of *C. corymbosa* are known, *C. maculosa* ssp. *maculosa* comprises many populations across southern and central Europe). The large positive correlation between genetic variability (P and H_e) and the number of flowering plants over all populations suggests that there is some scale at which the latter correlates well with the effective population size. Such a correlation has also been observed in other plant species (reviewed by Young et al. 1996). Within either *C. maculosa* or *C. corymbosa*, however, no significant correlation was found; this could be due to a small sample size (only six populations were studied in each species). In *C. corymbosa* the correlation coefficient was very close to zero, suggesting that some factors other than population size (e.g., migration, as suggested by the observed isolation by distance pattern) might influence genetic variability at the population level.

All polymorphic loci in either *C. corymbosa* or *C. maculosa* ssp. *albida* were also polymorphic in *C. maculosa* ssp. *maculosa*, whereas the reverse was not true. One allele (*Pgi-c*) was found in some populations of *C. corymbosa* only. The fact that it was not recorded in *C. maculosa* ssp. *maculosa* could result from our limited sampling of populations of this taxon. With the exception of this allele, the isozymic variability of the two narrowly endemic taxa, *C. corymbosa* and *C. maculosa* ssp. *albida*, appears to represent a subset of the total isozymic variability present in the widespread *C. maculosa* ssp. *maculosa*. The reduced genetic variability of the narrowly endemic taxa suggests that *C. corymbosa* and *C. maculosa* ssp. *albida* may be derived from *C. maculosa* ssp. *maculosa* from a bottleneck or by a founder event following colonization. This result could illustrate the progenitor-derivative model already found in other plant species (Gottlieb 1974; Crawford & Smith 1982; Warwick & Gottlieb 1985; Loveless & Hamrick 1988). Such geographic speciation is likely to have occurred several times in section *Maculosae* of subgenus *Acrophalus*, as suggested by the present-day distribution of western European species belonging to this group (Tutin et al. 1976; Pignatti 1982).

Population Structure

WITHIN-POPULATION STRUCTURE

None of the 12 sampled populations deviated significantly from Hardy-Weinberg expectations. These results are surprising given that both pollen and seeds show low dispersal ability in *C. corymbosa* (Colas et al. 1997 have shown that mean seed dispersal distance is 32 cm). Because of the great similarity of seed morphology (personal observation) and of pollination (Hymenoptera) among the three studied taxa, their dispersal distances of pollen and seeds are probably quite similar.

Given the large area sampled within each population (several hectares in some cases) and because of this low ability to disperse, we might have expected to find Wahlund effects (i.e., F_{IS} significantly different from zero due to among-subpopulation differentiation). This was not the case, suggesting that some mechanisms (e.g., self-incompatibility or inbreeding depression) might counterbalance the limited dispersal distances by precluding or reducing the success of crosses between spatially close individuals.

AMONG-POPULATION STRUCTURE

Global F_{ST} values in each taxon were large compared to those found in other studies of narrowly endemic or widespread, perennial, self-incompatible, and insect-pollinated plant species (Baskauf et al. 1994; Edwards & Wyatt 1994). Although populations of *C. corymbosa* were geographically much closer to each other ($\bar{d}$ = 1.3 km) than populations of *C. maculosa* ssp. *maculosa* ($\bar{d}$ = 20.0 km), overall F_{ST} values were similar in both taxa. Because of their larger sizes, populations of *C. maculosa* ssp. *maculosa* are likely to experience less genetic drift and thus be slightly less differentiated from each other for a given geographic distance. The positive and significant correlation between geographic distance and genetic distance (F_{ST}) found only in *C. corymbosa* (whose populations are very close, less than 2.5 km from each other), suggests that gene flow occurs mostly among close or adjacent populations, as described by the stepping-stone model (Wright 1943). It seems that when populations are isolated by more than a few kilometers, gene flow is much reduced and insufficient to counterbalance genetic drift; the larger the distance between populations, the smaller the correlation between geographic distance and F_{ST} and the larger the correlation between population size and genetic diversity. This can be related to the "threshold isolation effect" described by Young et al. (1996). In *C. maculosa* ssp. *maculosa*, both the correlations between geographic distance and F_{ST} and between population size and gene diversity are strong (although not significant). This suggests a continuum of the converse effects of maintenance of diversity by a small amount of recurrent gene flow and loss of diversity by drift, rather than a threshold

per se. Clearly, some modeling is needed here, along the lines developed by Slatkin (1993).

If we consider that the two narrowly endemic taxa are derived from the widespread one and thus share a common gene pool, reduced migration and strong genetic drift within each population of each taxon could explain why genetic distances between populations (F_{ST} values) are not consistent with taxonomic relationships. Nevertheless, using a hierarchical F_{ST} between taxa, significant, although small differentiation was detected between all pairs of taxa, suggesting that genetic drift occurs at the level of the whole taxon or occurred in the formation of the species.

Following a founder event from *C. maculosa* ssp. *maculosa*, each of the two rare taxa, *C. corymbosa* and *C. maculosa* ssp. *albida*, could have become morphologically differentiated under local drift and/or selective pressures. Compared to inland locations, selective pressures are likely to be different in the Massif de la Clape, whose proximity to the Mediterranean Sea leads to particular climatological conditions—for example, mean annual precipitation about three times lower (570 mm) than in other places where populations of *C. maculosa* ssp. *maculosa* were sampled. Moreover, it is likely that plants of *C. corymbosa* are exposed to stronger and more regular winds than those in the studied sites of *C. maculosa* ssp. *maculosa*. The low genetic differentiation for presumably neutral markers among the three taxa suggests that the populations of the two rare taxa were founded too recently to differentiate strongly under genetic drift. The fact that these taxa are nevertheless easily distinguished from *C. maculosa* ssp. *maculosa* by means of morphological and quantitative characters (Tutin et al. 1976; personal observation) suggests that these characters are not neutral because they do not behave in the same way as neutral markers (Bonnin et al. 1996) and that unknown, but strong, divergent selective pressures have allowed the development of ecological specialization.

Because of geographic isolation (no gene flow) and genetic drift (small population sizes), it could be assumed that every population of the widespread subspecies *C. maculosa* ssp. *maculosa* is potentially a rare species that could differentiate under divergent selective pressures. As has already been shown in other genera (e.g., in the genus *Layia*; Warwick & Gottlieb 1985), geographic isolation and adaptation to local environments can give rise to species with different morphological, cytological, ecological, and life-history traits. *C. corymbosa*, *C. maculosa* ssp. *albida*, and *C. maculosa* ssp. *maculosa* could thus be another example of on-going geographic speciation in plants.

Conclusion

From the perspective of conservation biology, opposite views, either pessimistic or optimistic, may exist about the fate of the *Maculosae* group of *Centaurea* in southwestern Europe. This group comprises a single widespread taxon plus seven other taxa with restricted geographic ranges (Tutin et al. 1976). Most of the rare taxa (not studied here) are probably as endangered as *C. maculosa* ssp. *albida* or *C. corymbosa*. Nevertheless, if the mechanisms leading to endemism as proposed in this paper—geographic isolation followed by ecological specialization and finally speciation—are operating in the widespread *C. maculosa* ssp. *maculosa*, one can predict that they will give rise to additional new taxa in the future. Thus, several new taxa may emerge while others go extinct. Because we studied only a small group of taxa, we cannot generalize this prediction to the entire genus *Centaurea*. But this mechanism might well explain, in part, the large species richness in *Centaurea* as a whole (about 500 species, Bremer 1994).

C. maculosa ssp. *maculosa* can therefore be seen as a source of both new rare species and as a source of variability for existing narrowly endemic species through rare hybridization events, so its protection might actually be more useful than that of rare species that it has generated in the past. We thus suggest that it is important in a conservation program to include along with the protection of endangered species a survey (and possibly protection, if necessary) of a large variety of populations of related widespread species with different habitats and geographic distributions. Clearly, hybridization events with widespread taxa might drive a rare species to extinction (Levin et al. 1996) through genetic "pollution." But the risk of extinction through the loss of genetic variation could well be large too. One might prefer a polluted extant species to a pure extinct one.

Acknowledgments

J. Molina and H. Michaux helped with locating and identifying populations of *C. maculosa*. We thank E. Jenczewski and J. M. Prosperi for useful discussions. D. J. Schoen and R. Vitalis made useful comments on a late draft of the manuscript. We thank P. Soltis and an anonymous reviewer for their comments and extensive copy editing. Financial support was provided by the Conservatoire Botanique National Méditerranéen de Porquerolles. Logistic support was provided by the Genetics and Plant Breeding Research Unit, INRA Montpellier. This is contribution no. 97077 of the Institut des Sciences de l'Evolution, Université de Montpellier 2.

Literature Cited

Babbel, G. R., and R. K. Selander. 1973. Genetic variability in edaphically restricted and widespread plant species. Evolution 28:619–630.

Barbault, R. 1993. Une approche écologique de la biodiversité. Natures-Sciences-Sociétés 1:322–329.

Barrett, S. C. H., and J. R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conserva-

tion. Pages 3–30 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Oxford, United Kingdom.

Baskauf, C. J., D. E. McCauley, and W. J. Eickmeier. 1994. Genetic analysis of a rare and a widespread species of *Echinacea* (Asteraceae). Evolution **48**:180–188.

Bonnin, I., J.-M. Prosperi, and I. Olivieri. 1996. Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminosae): a comparative analysis of population structure. Genetics **143**:1795–1805.

Bremer, K. 1994. Asteraceae-Cladistics and classification. Timber Press, Portland, Oregon.

Brussard, P. F., and P. R. Ehrlich. 1992. The challenge of conservation biology. Ecological Applications **2**:1–2.

Colas, B., M. Riba, and J. Molina. 1996. Statut démographique de *Centaurea corymbosa* Pourret (Asteraceae), *Hormatophylla pyrenaica* (Lapeyr.) Cullen & Dudley (Brassicaceae) et *Marsilea strigosa* Willd. (Marsileaceae-Pteridophyta), trois plantes rares dans le sud de la France. Acta botanica Gallica **143**:191–198.

Colas, B., I. Olivieri, and M. Riba. 1997. *Centaurea corymbosa*, a cliff-dwelling species tottering on the brink of extinction: a demographic and genetic study. Proceedings of the National Academy of Sciences USA **94**: 3471–3476.

Crawford, D. J., and E. B. Smith. 1982. Allozyme variation in *Coreopsis nuecensoides* and *C. nuecensis* (Compositae), a progenitor-derivative species pair. Evolution **36**:379–386.

Dolan, R. W. 1991. Patterns of isozyme variation in relation to population size, isolation, and phytogeographic history in royal catchfly (*Silene regia*; Caryophyllaceae). American Journal of Botany **81**:965–972.

Edwards, A. L., and R. Wyatt. 1994. Population genetics of the rare *Asclepias texana* and its widespread sister species, *A. perennis*. Systematic Botany **19**:291–307.

Fisher, R. 1954. Statistical methods for research workers. 12th edition. Oliver and Boyd, Edinburgh, United Kingdom.

Gottlieb, L. D. 1974. Genetic confirmation of the origin of *Clarkia lingulata*. Evolution **28**:244–250.

Goudet, J. 1995. A computer program to calculate F-statistics. Journal of Heredity **86**:485–486.

Hamrick, J. L., and M. J. W. Godt. 1989. Allozyme diversity in plant species. Pages 43–63 in A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, editors. Plant population genetics, breeding, and genetic resources. Sinauer, Associates, Sunderland, Massachusetts.

Hamrick, J. L., M. J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlation between species traits and allozyme diversity: implications for conservation biology. Pages 75–86 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Oxford, United Kingdom.

Harrod, R. J., and R. J. Taylor. 1995. Reproduction and pollination biology of *Centaurea* and *Acroptilon* species, with emphasis on *C. diffusa*. Northwest Science **69**:97–105.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics **6**:65–70.

Huenneke, L. F. 1991. Ecological implications of genetic variation in plant populations. Pages 31–44 in D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Oxford, United Kingdom.

Karron, J. D. 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. Evolutionary Ecology **1**:47–58.

Kephart, S. R. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. American Journal of Botany **77**:693–712.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455–1460.

Levin, D. A. 1995. Metapopulations: an area for local speciation. Journal of Evolutionary Biology **8**:635–644.

Levin, D. A., J. Francisco-Ortega, and R. K. Jansen. 1996. Hybridization and the extinction of rare plant species. Conservation Biology **10**:10–16.

Lewis P. O., and D. J. Crawford. 1995. Pleistocene refugium endemics exhibit greater allozymic diversity than widespread congeners in the genus *Polygonella* (Polygonaceae). American Journal of Botany **82**:141–149.

Loveless, M. D., and J. L. Hamrick. 1988. Genetic organization and evolutionary history in two North American species of *Cirsium*. Evolution **42**:254–265.

Mantel, N., and C. C. Li. 1974. Estimation and testing of a measure of non-random mating. Annals of Human Genetics **37**:445–454.

Muséum National d'Histoire Naturelle. 1995. Livre Rouge de la Flore menacée de France. Tome I. Espèces prioritaires. Muséum National d'Histoire Naturelle, Paris.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583–590.

Pignatti, S. 1982. Flora d'Italia 3. Edagricole, Bologne, Italy.

Purdy, B. G., and R. J. Bayer. 1996. Genetic variation in populations of the endemic *Achillea millefolium* ssp. *megacephala* (from the Athabasca sand dunes and the widespread ssp. *lanulosa* in western North America. Canadian Journal of Botany **74**:1138–1146.

Ranker, T. A. 1994. Evolution of high genetic variability in the rare Hawaiian fern *Adenophorus periens* and implications for conservation management. Biological Conservation **70**:19–24.

Raymond, M., and F. Rousset. 1995*a*. An exact test for population differentiation. Evolution **49**:1280–1283.

Raymond, M., and F. Rousset. 1995*b*. GENEPOP, Version 1.2. A population genetics software for exact tests and eucumenism. Journal of Heredity **86**:248–249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**: 223–225.

SAS Institute. 1992. SAS/STAT User's guide. Version 6, 4th edition. Cary, North Carolina.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science **236**:787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution **47**:264–279.

Soltis, D. E., C. H. Haufler, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns; a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal **73**:9–27.

Susanna, A., N. Garcia Jacas, D. E. Soltis, and P. S. Soltis. 1995. Phylogenetic relationships in tribe Cardueae (Asteraceae) based on ITS sequences. American Journal of Botany **82**:1056–1068.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity **72**:281–283.

Tutin, T. G., V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb. 1976. Flora Europaea 4. Cambridge University Press, Cambridge, United Kingdom.

Vallejos, C. E. 1983. Enzyme activity staining. Pages 469–516 in S. D. Tanksley and T. J. Orton, editors. Isozymes in plant genetic and breeding. Part A. Elsevier Science Publishers, Amsterdam.

Warwick, S. I., and L. D. Gottlieb. 1985. Genetic divergence and geographic speciation in *Layia* (Compositae). Evolution **39**:1236–1241.

Weir, B. S. 1996. Genetic data analysis II. Sinauer Associates, Sunderland, Massachusetts.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38**:1358–1370.

Wendel, J. F., and N. F. Weeden. 1989. Visualization and interpretation of plant isozymes. Pages 5–45 in D. E. Soltis and P. S. Soltis, editors. Isozymes in plant biology. Chapman and Hall, London.

Wright, S. 1943. Isolation by distance. Genetics **28**:114–138.

Wyse Jackson, P. S., and J. R. Akeroyd. 1994. Guidelines to be followed in the design of plant conservation or recovery plans. Council of Europe, Strasbourg, France.

Young, A., T. Boyle, and T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution **11**:413–418.