

# Gene flow and local adaptation in two endemic plant species

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## Abstract

In order to detect the evolutionary potential of two endangered species, *Brassica insularis* (Brassicaceae) and *Centaurea corymbosa* (Asteraceae), within and among-population genetic variation for both quantitative traits and allozymic markers was examined. Four populations of each species were studied, representing a large proportion of extant populations. High values of  $\theta_{ST}$  (0.213 and 0.364 for *B. insularis* and *C. corymbosa* respectively) suggested that low amounts of gene flow occur among the study populations. In each species, the genetic distance based on allozymes (estimated by the ratio  $(\theta_{ST}/1-\theta_{ST})$ ) was positively correlated with the geographical distance, indicating isolation by distance. In contrast to previous studies in either outcrossing or selfing plant species, and especially for *B. insularis*, population differentiation for quantitative traits ( $Q_{ST}$ ) was generally found lower than differentiation for allozymes ( $\theta_{ST}$ ), suggesting that the populations studied were experiencing similar selective forces acting upon the quantitative traits measured. Such forces would be strong enough to counteract local genetic drift. Interestingly, for both species  $Q_{ST}$ 's were statistically independent of geographical distance, in contrast to the marginally significant positive isolation by distance shown by  $\theta_{ST}$ . Altogether, these results suggest that  $\theta_{ST}$ 's might not always be used as conservative estimates of  $Q_{ST}$ 's, and might instead overestimate the evolutionary potential of endangered species. This would be especially expected in narrow-endemic species, whose ecological niche is often so restricted that indeed homogeneous selective forces are likely to occur, whereas small population sizes and restricted dispersal are likely to produce strong differentiation for neutral variation. In fact, knowledge of both neutral and quantitative diversity patterns allows identification of those traits undergoing natural selection, and could be useful in designing reinforcement or reintroduction programs. However, this approach might have limitations too, in the presence of outbreeding depression due to locally coevolved gene complexes.   2001 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

The major aim of conservation geneticists is to quantify and preserve or restore the evolutionary potential of endangered species, i.e. within and among population genetic variation in ecologically important traits (Hamrick et al., 1991; Storfer, 1996). Morphological, physiological and behavioral traits are often correlated with fitness (e.g. Kelly, 1992; Andersson, 1996; Petit and Thompson, 1998). The patterns of variation in quantitative traits are likely to be driven by environmental

variation, i.e. natural selection pressures (Lynch et al., 1999). In a conservation perspective, quantitative genetic studies may thus provide useful information on the ecologically important traits for which genetic variability is reduced in natural populations of endangered species (Storfer, 1996; Frankham, 1999).

Endangered species often show low levels of *genetic diversity within populations*, i.e. low population evolutionary potential. In this case, a major challenge of conservation biologists is to reinforce such diversity (Lande and Barrowclough, 1987; Bijlsma et al., 1994). Reinforcement of genetic diversity at the population level can be achieved by the introduction of new individuals from other, genetically differentiated, populations.

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This practice is possible if the species shows high levels of *among-population diversity*, i.e. high *species* evolutionary potential, and weak outbreeding depression (i.e. little loss of hybrid vigor occurring as a consequence of local adaptation or coadapted gene complexes). Then, from a conservation management perspective, it appears crucial to be able to quantify this evolutionary potential by studying the spatial distribution of genetic variation in putative adaptive traits, such as quantitative characters.

Quantitative genetic studies are in practice often difficult or impossible to conduct with endangered species because of the large sample sizes required for replicating genotypes, manipulation of individuals for morphological and physiological traits, etc. In contrast, the use of biochemical and molecular markers (e.g. allozymes, RFLPs, and microsatellites) is easier in such taxa because meaningful results can be obtained from relatively small sample sizes, using nondestructive sampling methods. The usefulness of knowledge of the distribution of genetic variability from such markers in conservation management, however, is controversial (Vrijenhoek, 1994). Molecular and biochemical markers may give misleading information on the amount of ecologically important genetic diversity (Gray, 1996; Storfer, 1996; but see Vrijenhoek, 1994) because they only provide insight into random evolutionary or demographic processes such as colonization history, effective size and past or present gene flow among extant populations (Barton, 2001; Lynch et al., 1999; Thompson, 1999). Most biochemical and molecular markers may indeed be considered selectively neutral (Watt, 1994, and Vrijenhoek, 1994, for alternative point of view). According to Frankham (1999, p. 240), ‘a major unresolved issue is the relationship between molecular measures of genetic diversity and quantitative genetic variation’. The comparison of patterns of variation for putative adaptive traits and for neutral markers is a good way to detect those traits for which population differentiation is due to selection (Schwaegerle et al., 1986; Spitze, 1993; Bonnin et al., 1996; see Lynch et al., 1999, for a review). In such comparisons, it is assumed that the pattern obtained for the majority of molecular markers indicates the neutral expectation. Any departure from the neutral pattern, for either individual molecular markers or individual quantitative traits, may provide evidence that forces other than gene flow and genetic drift, such as natural selection, shape the variation of these markers or traits, or that these markers or traits are in linkage disequilibrium with loci undergoing natural selection (Bonnin et al., 1996; see, however, Latta, 1998 for an analysis of linkage disequilibria). Note however that for quantitative traits, deriving such a conclusion is highly dependent on additivity of gene effects (Whitlock, 1999).

Several recent studies have dealt with the comparison of genetic variation in supposedly neutral markers and quantitative traits. For example, at the genus level, the patterns of interspecific variation in allozymes and

morphological traits have been shown to be similar in five widespread *Conyza* species (Thébaud and Abbott, 1995). At the species level, the comparison of genetic parameters such as  $F_{ST}$ ,  $Q_{ST}$ , or  $G_{ST}$  (all closely related estimates of population differentiation for molecular markers) with  $Q_{ST}$  (population differentiation for quantitative traits) has shown that population subdivision inferred through quantitative traits is often stronger than neutral population structure in predominantly outcrossing species (e.g. Spitze, 1993; Long and Singh, 1995; Podolsky and Holtsford, 1995; Yang et al., 1996). In highly inbred populations,  $Q_{ST}$ 's have been found at least equal to  $F_{ST}$ 's (e.g. Bonnin et al., 1996; Kuittinen et al., 1997; Hardy et al., 2000; see Lynch et al., 1999, for a review). Lynch et al. (1999) concluded that  $F_{ST}$ 's could be used as conservative estimates of  $Q_{ST}$ 's. However, one can question whether this holds true whatever the spatial scale of variation and the species considered. In particular, in endangered species with reduced population sizes and presumably limited dispersal ability, one can expect a large amount of genetic differentiation among populations for neutral traits, especially for molecular and biochemical markers (see Thompson, 1999, for a review). Conversely, because these species often have a restricted ecological niche, a different pattern of differentiation might be expected for growth or developmental traits, since these traits are likely to experience homogeneous selective pressures. It is unclear whether, in these species: (1) selection is indeed homogeneous among extant populations; and (2) random genetic drift is strong enough to counteract such putative homogeneous selection. The aim of the present study was to compare patterns of diversity among biochemical and quantitative traits ( $\theta_{ST}$  and  $Q_{ST}$ ) to assess the evolutionary potential of two endemic Mediterranean plant species, *Brassica insularis* Moris (Brassicaceae) and *Centaurea corymbosa* Pourret (Asteraceae). Populations of *B. insularis* are geographically separated by mountains (pairwise distances among populations vary from 9 to 64 km), while populations of *C. corymbosa* (Auzil, Peyral, E1 and E2) are confined to a restricted geographic area (3 km<sup>2</sup>; pairwise distances among populations vary from 0.3 to 2.3 km). The usefulness of the neutral markers to estimate the evolutionary potential of the two species is discussed in relation to: (1) the developmental stage of individuals; and (2) the effect of the spatial scale, i.e. the scale of geographic isolation among the different populations within species.

## 2. Materials and methods

### 2.1. Study species

*B. insularis* is a plant species endemic to Corsica, Sardinia and North Africa. Nine populations have been

described in Corsica (Widler and Bocquet, 1979), but only seven are actually known with certainty (I. Guyot, pers. commun.). *C. corymbosa* occurs over a much more restricted area. It is endemic to the “Massif de la Clape” (near Narbonne in southern France); only six populations are known in a 3 km<sup>2</sup>-area (Colas et al., 1996). Both species live on cliffs surrounded by unsuitable habitats (mattoral). This landscape structure is probably, at least in part, responsible for the low colonization ability and the rarity of the two species. *B. insularis* is a long-lived perennial (Snogerup et al., 1990), but only a small number of individuals flower in any given year, and there appears to be low levels of reproductive success in natural populations (Verlaque et al., 1993; I. Guyot, M. Virevaire, I. Olivieri, C. Petit and A. Vivat, personal observation). *C. corymbosa* is a monocarpic perennial with a mean flowering age of about 6 years (B. Colas, H. Fréville, M. Riba, A. Mignot and I. Olivieri, personal observation); its fruit-to-flower ratio (40–65%, Colas et al., 2000) is large compared to other hermaphroditic self-incompatible plants (mean 22%, Sutherland and Delph, 1984). Both species have been described as insect-pollinated outbreeders, at least partially self-incompatible (Hurtrez-Boussès, 1996; Colas et al., 1997; Fréville and Mignot, unpublished data).

## 2.2. Experimental design

Four populations of each species were studied (Fig. 1). This low sample size (four populations out of seven extant Corsican populations for *B. insularis* and four out of a total of six for *C. corymbosa*) was imposed by the demographic health of some populations (one

population of *B. insularis* and the two other populations of *C. corymbosa* produce too few seeds to allow destructive sampling), and by the inaccessibility of the remaining two Corsican populations of *B. insularis*. Seeds from 12 and 20 maternal plants were sampled in each natural population of *B. insularis* and *C. corymbosa*, respectively. Following germination (after removal of elaisomes in *C. corymbosa*) in Petri dishes, seedlings from each maternal family were transferred in spring 1998 to individual pots. Fourteen individuals per family in *B. insularis* and 10 in *C. corymbosa* were grown in a randomized block design (4 blocks in *B. insularis*, 10 blocks in *C. corymbosa*) in experimental greenhouses at the Conservatoire Botanique National Méditerranéen on Porquerolles Island in southern France. Each maternal family was represented one to four times per block. Total sample size of each experiment was 4 populations × 12 maternal plants × 14 seeds = 672 plants for *B. insularis*, and 4 populations × 20 maternal plants × 10 seeds = 800 plants for *C. corymbosa*. Various traits were measured for each species (Table 1) at the seedling stage, one month after germination (April 1998), and when plants either reached their adult size (June 1998 for *C. corymbosa*) or began to flower (March 1999 for *B. insularis*). In addition, one morphological and one reproductive qualitative trait were observed in *B. insularis*: pubescence at the seedling stage, and reproductive status (flowering/non flowering) of adult plants (Table 1).

## 2.3. Variance components estimates

Among- and within-population variance components for quantitative traits were estimated from expected

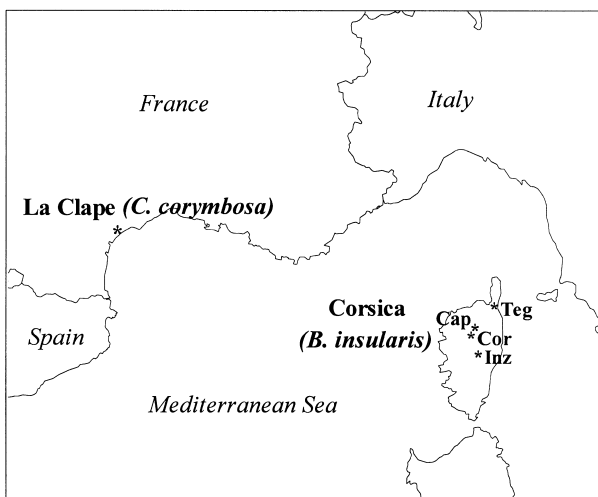


Fig. 1. Localization of natural populations of *Centaurea corymbosa* and *Brassica insularis* studied. The four populations of *C. corymbosa* are 300–2300 m apart (Colas et al., 1997). Populations of *B. insularis*: Teg, Teghime; Cap, Caporalino; Cor, Corbaghiola; Inz, Inzecca.

Table 1

Quantitative traits measured in seedlings and adults of *Brassica insularis* and *Centaurea corymbosa*<sup>a</sup>

Seedlings	Adults
<i>Brassica insularis</i>	
Leaf number [ $\sqrt{(x+1)}$ ]	Leaf number (log)
Rosette diameter	Rosette diameter (log)
Leaf length/leaf width [ $\log(x+1)$ ]	Number of secondary stems [ $\log(x+1)$ ]
Plant height [ $\log(x+1)$ ]	Inflorescence stem length (log)
Pubescence (3 classes)	Reproductive status (log) (2 classes)
<i>Centaurea corymbosa</i>	
Axis number (log)	Axis number (log)
Leaf number ( $\sqrt{\quad}$ )	Leaf number (log)
Leaf length/leaf width ( $\sqrt{\quad}$ )	Leaf length/leaf width ( $\sqrt{\quad}$ )
Greatest/smallest rosette diameter	Greatest/smallest rosette diameter
ratio (hereafter diameter ratio) (log)	ratio (hereafter diameter ratio) (log)

<sup>a</sup> In parentheses, the transformations performed to satisfy homoscedasticity and normality assumptions in single trait ANOVAs.

mean squares in successive nested analyses of variance with PROC GLM in SAS (1990), using the following general model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k(j)} + \varepsilon_{ijkl},$$

where  $y_{ijkl}$  = the phenotypic value on the  $l$ th progeny of the  $k$ th family nested in the  $j$ th population observed in the  $i$ th block;  $\mu$  = the overall mean;  $\alpha$  = the  $i$ th block effect;  $\beta_j$  = the  $j$ th population effect;  $\gamma_{k(j)}$  = the  $k$ th family effect nested in the  $j$ th population;  $\varepsilon_{ijkl}$  = the residual error representing the within-family variation. Block was considered as a fixed effect. Because a large proportion of extant populations was sampled (4/7 Corsican populations of *B. insularis* and 4/6 populations of *C. corymbosa*), the study populations were considered as representative of the Corsican populations of *B. insularis* and of *C. corymbosa*. Thus, population effect was considered as a random effect in the ANOVAs. This in turn allowed us to estimate the among population variance component:

$$V_{\text{POP}} = [\text{MS}_{\text{POP}} - V_\varepsilon - (n/n')(\text{MS}_{\text{FAM}} - V_\varepsilon)]/t$$

where  $\text{MS}_{\text{POP}}$  and  $\text{MS}_{\text{FAM}}$  are the mean squares of, respectively, the population and the family effects,  $V_\varepsilon$  is the residual term of the analysis of variance,  $n$  and  $n'$  are the weighted mean number of families per population and of progenies per family, respectively, and  $t$  is the total number of progenies used in the study (Podolsky and Holtsford, 1995; Yang et al., 1996).

Similarly, we assumed that the families were a representative sample of each population they were sampled from, so that we could estimate the within population, among family variance component by  $V_{\text{FAM}} = [\text{MS}_{\text{FAM}} - V_\varepsilon]/n'$ .

Since the number of progenies per family was not homogeneous, type III sums of squares were calculated (Shaw and Mitchell-Olds, 1993). To satisfy homoscedasticity and normality assumptions, the traits were transformed as described in Table 1. Since all seeds did not germinate simultaneously in *B. insularis*, the date of germination was added as a covariate in the nested ANOVA performed on seedlings to remove the variance due to age differences from the error term.

#### 2.4. Quantitative variation of study populations: narrow sense heritability

The genetic diversity of each population was assessed by estimating narrow-sense heritabilities ( $h^2$ ). Narrow-sense heritability represents the part of the total variance that can be explained by additive genetic differences among individuals, i.e. the ratio of additive genetic variance to total phenotypic variance. This quantity is often used to describe the evolutionary potential of current

populations to respond to future environmental changes (but see Houle, 1992, for an alternative point of view). Since the two study species are outbreeders, it is likely that within an individual maternal plant, ovules were sired by many different fathers, so that we have been manipulating half-sib families. On-going microsatellite analyses of the *B. insularis* families suggest that indeed this is the case (F. Alberto and M. Lourmas, pers. commun.). In half-sib experimental designs, the variance among families ( $V_{\text{FAM}}$ ) represents a quarter of the additive genetic variance ( $V_A$ ). Thus,  $h^2$  was calculated for each trait and each population, as the following ratio:

$$h^2 = V_A/(V_A + V_\varepsilon) = 4V_{\text{FAM}}/(4V_{\text{FAM}} + V_\varepsilon),$$

where  $V_\varepsilon$  is the residual variance.  $V_{\text{FAM}}$  was extracted from analyses of variance performed within each population, using the above-described analysis after removing the population effect from the ANOVAs.  $h^2$  was estimated for each trait using the Jackknife procedure by resampling families, i.e. deleting iteratively one family and performing a new ANOVA with the new data set obtained (Bonnin et al., 1996, 1997; Lynch and Walsh, 1998, pp. 569–70). Jackknifing provides an estimation of  $h^2$  and its standard deviation, from which, assuming a normal distribution of these estimates, we derived a 95% confidence interval.

#### 2.5. Quantitative population structure analyses: $Q_{ST}$ estimates.

Quantitative population structure was estimated over the four populations and for each pairwise combination of populations within each species. To compare global variation in quantitative traits at a particular developmental stage, principal component analyses were performed on the five quantitative traits studied to create a new variable taking into account the relative contribution of each trait to the total phenotypic variance. For each pair of populations,  $Q_{ST}$  estimates were computed following Wright (1951) as the ratio:

$$Q_{ST} = V_{\text{POP}}/(2V_{\text{GEN}} + V_{\text{POP}}),$$

where  $V_{\text{POP}}$  is the between-population variance and  $V_{\text{GEN}}$  the average genetic variance within populations.

Assuming again, as seems to be the case in at least *B. insularis*, that the progenies of the same maternal plant were half-sibs, the within-population genetic variance, was estimated by  $4V_{\text{FAM}}$ .  $Q_{ST}$  were thus estimated using the following term:

$$Q_{ST} = V_{\text{POP}}/(8V_{\text{FAM}} + V_{\text{POP}}).$$

Standard deviations of  $Q_{ST}$  were estimated using the Jackknife procedure over families.

Table 2

Mean values (standard deviation) of several quantitative traits in four populations of *Brassica insularis* and *Centaurea corymbosa* grown in experimental conditions

Population	Juvenile leaf number	Juvenile rosette diameter (mm)	Juvenile leaf length/width	Juvenile plant height (mm)	Juvenile pubescence	Adult leaf number	Adult rosette diameter (cm)	Adult number of secondary stems	Adult inflorescence stem length (cm)	Reproductive status
(a) <i>Brassica insularis</i>										
Caporalino	5.3 (1.0)	104.8 (22.3)	1.1 (0.1)	23.9 (8.6)	1.4 (0.5)	9.2 (4.9)	8.3 (3.2)	0.3 (0.9)	1.3 (4.0)	1.2 (0.5)
Inzecca	5.8 (1.0)	98.7 (20.4)	1.2 (0.1)	9.4 (5.3)	1.3 (0.5)	12.3 (5.4)	8.7 (2.6)	0.4 (0.9)	1.7 (3.7)	1.4 (0.4)
Teghime	6.2 (1.2)	86.7 (18.9)	1.2 (0.2)	27.4 (11.5)	1.4 (0.5)	12.9 (3.6)	6.8 (2.5)	0.6 (1.2)	0.1 (0.3)	1.2 (0.4)
Corbaghiola	5.4 (1.4)	90.3 (28.2)	1.2 (0.9)	17.6 (6.8)	1.0 (0.3)	11.3 (3.9)	7.6 (1.8)	0.4 (1.1)	1.0 (2.3)	1.4 (0.5)
	Juvenile axis number	Juvenile leaf number	Juvenile leaf length/width	Juvenile diameter ratio	Adult axis number	Adult leaf number	Adult leaf length/width	Adult diameter ratio		
(b) <i>Centaurea colymbosa</i>										
Auzil	1.0 (0.1)	8.8 (1.2)	4.3 (1.2)	1.5 (0.5)	4.4 (2.8)	47.6 (16.9)	2.3 (0.3)	1.1 (0.1)		
Peyral	1.0 (0.2)	9.4 (1.9)	3.8 (1.1)	1.4 (0.7)	3.2 (2.6)	42.8 (17.3)	2.6 (0.3)	1.1 (0.2)		
E1	1.0 (0.0)	8.8 (1.4)	3.5 (1.0)	1.3 (0.4)	1.2 (0.9)	35.2 (6.7)	2.3 (0.3)	1.1 (0.1)		
E2	1.0 (0.1)	7.5 (1.2)	4.3 (0.8)	1.4 (0.5)	1.3 (0.8)	30.2 (4.5)	2.2 (0.3)	1.1 (0.1)		

## 2.6. Enzyme polymorphism

For *B. insularis*, we used data from Hurtrez-Boussès (1996) for three populations (Teghime, Inzecca, Corbaghiola), and new data on the same loci for the Caporalino population. Analyses were based on the five polymorphic enzymatic loci (a total of nine loci were previously tested): ACP — acid phosphatase, EST — esterase, LAP — leucine amino-peptidase, PRX — peroxidase, and PGM — phosphoglucumutase (Hurtrez-Boussès, 1996). Sample sizes varied from 15 to 49 individuals per population.

For *C. corymbosa* populations, we reanalyzed published data (Colas et al., 1997) on five polymorphic enzymatic loci (a total of 35 loci were previously tested), using only data from the four populations studied in our common garden experiment: PGI — phosphogluco isomerase, DIA — diaphorase, LAP — leucine amino-peptidase, CAT — catalase, and PRX — peroxydase. Sample sizes varied from 32 to 57 individuals per population.

Among population differentiation based on genotype frequencies was estimated by  $\theta_{ST}$  (Weir and Cockerham, 1984), for all combinations of pairwise populations, using TFPGA-1.3 computer software (Miller, 1997). Statistical significance of differentiation was tested using exact tests for population differentiation (Raymond and Rousset, 1995).

## 2.7. Comparison of population structure for quantitative traits and neutral markers

To determine whether the geographic scale might influence the extent to which the amounts of variation among populations for quantitative traits and for biochemical markers were related,  $Q_{ST}$  were compared to  $\theta_{ST}$  for each species.

Differences in population variation in quantitative traits and markers were visualized by drawing genetic distance trees with UPGMA clustering analysis, using the computer software PHYLIP Version 3.5c of Felsenstein (1995).  $\theta_{ST}$  and overall (from PCA)  $Q_{ST}$  were used as genetic distances. A Mantel test (Sokal and Rohlf, 1995, pp. 813–819) was used to test the hypothesis that the matrix of pairwise  $Q_{ST}$ 's was independent from the matrix of pairwise  $\theta_{ST}$ 's. A Mann–Whitney U-test was used to test the hypothesis that the ranks of  $Q_{ST}$ 's and  $\theta_{ST}$ 's were similar. We used the same methods to compare  $Q_{ST}$ 's on juvenile and adult traits.

## 2.8. Isolation by distance patterns

Following Rousset (1997), the comparison between genetic and geographical distances was assessed using the “ $F_{ST}/(1-F_{ST})$  method”, i.e. a regression of the ratio [genetic distance/(1–genetic distance)] on the logarithmic transformation of the geographical distance. This method may provide more easily interpretable information on the relation of the genetic parameters and geographical distance between pairs of populations presumably isolated by distance. Independence of genetic and geographic distance matrices was tested using a Mantel test.

## 3. Results

### 3.1. Within-population genetic diversity and narrow-sense heritability

Mean values for each trait or markers (allelic frequencies) and each population are given in Tables 2 and 3. Within-population genetic diversity (family effect, with estimated variance component  $V_{FAM}$ ) was significantly

positive for most quantitative traits of both species. Narrow-sense heritability estimates appeared larger for many seedling traits (range values 0–0.84) compared to adult (range values 0–0.69), especially in *B. insularis* (Fig. 2).

There was high variation among characters for heritability estimates, as well as among enzyme loci for expected heterozygosities (Fig. 2). For instance, in *B. insularis*, the ratio of leaf length/width had a very low heritability in three out of four populations, and the number of secondary stems at the adult stage had a very low heritability in all populations. In *C. corymbosa*, the number of axes of juveniles and the rosette diameter

ratio of both juveniles and adults had very low heritability in all four populations. In contrast, some traits were highly heritable, e.g. the number of leaves at both stages and for both species.

For a given trait or locus, there was also high variation among populations. Populations from Teghime and Corbaghiola were the least variable for enzymes (average expected heterozygosity,  $H_e=0.26$  and 0.31), whereas populations from Caporalino and Inzecca were more variable ( $H_e=0.47$  and 0.43).

Heritability estimates for quantitative traits, especially when measured at the juvenile stage, also widely varied among populations. In *B. insularis*, average heritability

Table 3  
Allelic frequencies for five polymorphic enzymatic loci in each study population ( $n$  = sample size)

Population	Locus	$n$	Allelic frequencies				Population	Locus	$n$	Allelic frequencies			
			1	2	3	4				1	2	3	4
(a) <i>Brassica insularis</i>						(b) <i>Centaurea corymbosa</i>							
Caporalino	ACP	49	0.47	0.10	0.43		Auzil	PGI	55	1.00	0.00		
	PGM	59	0.58	0.12	0.27	0.03		DIA	55	0.60	0.40		
	EST	56	0.69	0.27	0.04			LAP	55	0.01	0.99		
	PRX	62	0.73	0.11	0.16			CAT	55	0.28	0.72		
	LAP	50	0.80	0.15	0.05	0		PRX	55	0.00	1.00		
Inzecca	ACP	45	0.59	0.01	0.40		Peyral	PGI	32	1.00	0.00		
	PGM	20	0.72	0.05	0.23	0		DIA	32	0.30	0.70		
	EST	45	0.84	0.13	0.03			LAP	32	0.59	0.41		
	PRX	46	0.79	0.00	0.21			GAT	32	0.00	1.00		
	LAP	45	0.14	0.48	0.36	0.02		PRX	32	0.00	1.00		
Teghime	ACP	32	0.89	0.02	0.09		E1	PGI	44	0.70	0.30		
	PGM	31	0.06	0.00	0.11	0.83		DIA	44	0.00	1.00		
	EST	42	0.75	0.25	0.00			LAP	44	0.00	1.00		
	PRX	45	0.89	0.07	0.04			CAT	44	0.19	0.81		
	LAP	49	0.89	0.05	0.06	0		PRX	44	0.37	0.63		
Corbaghiola	ACP	38	0.76	0.03	0.21		E2	PGI	57	0.75	0.25		
	PGM	17	0.76	0.12	0.12	0		DIA	57	0.00	1.00		
	EST	43	0.94	0.06	0.00			LAP	57	0.00	1.00		
	PRX	41	0.85	0.13	0.01			CAT	57	0.05	0.95		
	LAP	44	0.72	0.07	0.21	0		PRX	57	0.59	0.41		

Table 4  
Overall and pairwise populations genetic differentiation for quantitative traits ( $Q_{ST}$ ) and allozymes ( $\theta_{ST}$ )<sup>a</sup>

Population	<i>B. insularis</i>			Population	<i>C. corymbosa</i>		
	Inzecca	Teghime	Corbaghiola		Peyral	E1	E2
Seedlings ( $Q_{ST}$ )							
Caporalino	0	0.018	0.188	Auzil	0.056	0.001	0.088
Inzecca		0	0.037	Peyral		0	0.329
Teghime			0.085	E1		0.214	
Overall	0.023			Overall	0.105		
(b) Adults ( $Q_{ST}$ )							
Caporalino	0.174	0	0.054	Auzil	0.063	0.014	0.468
Inzecca		0.193	0.043	Peyral		0.398	0.523
Teghime			0.162	E1			0.207
Overall	0.087			Overall	0.341		
(c) Allozymes ( $\theta_{ST}$ )							
Caporalino	0.127	0.243	0.071	Auzil	0.355	0.359	0.463
Inzecca		0.380	0.140	Peyral		0.388	0.469
Teghime			0.309	E1			0.049
Overall	0.213			Overall	0.364		

<sup>a</sup> Population genetic variation on quantitative traits was quantified for the whole phenotype between pairs of populations at two developmental stages, juveniles and adults (see text). Population differentiation for allozymes was estimated by  $\theta_{ST}$  from genotypic data. When the population effect was not significant,  $Q_{ST}$  was set to zero. All non-zero quantities are thus significant (based on 95% confidence intervals).

estimate at the juvenile stage varied from 0.30 (Inzecca population) to 0.68 (Corbaghiola population). Average heritability at the adult stage was lower, varying from 0.08 (Caporalino population) to 0.22 (Corbaghiola population). In *C. corymbosa*, average heritability was overall lower than *B. insularis* at the juvenile stage, ranging from 0.20 (Peyral population) to 0.29 (Auzils population), whereas it was similar or slightly larger at the adult stage, ranging from 0.16 (El population) to 0.33 (E2 population).

### 3.2. Population structure

As shown in Table 4, the *B. insularis* populations were significantly differentiated for quantitative traits, for either juvenile traits or adult traits, or both. The same pattern was observed in *C. corymbosa*. However, there was no clear relationship between population differentiation of quantitative traits measured on seedlings and on adults (Mantel tests:  $r = -0.29$ ,  $P = 0.24$  and

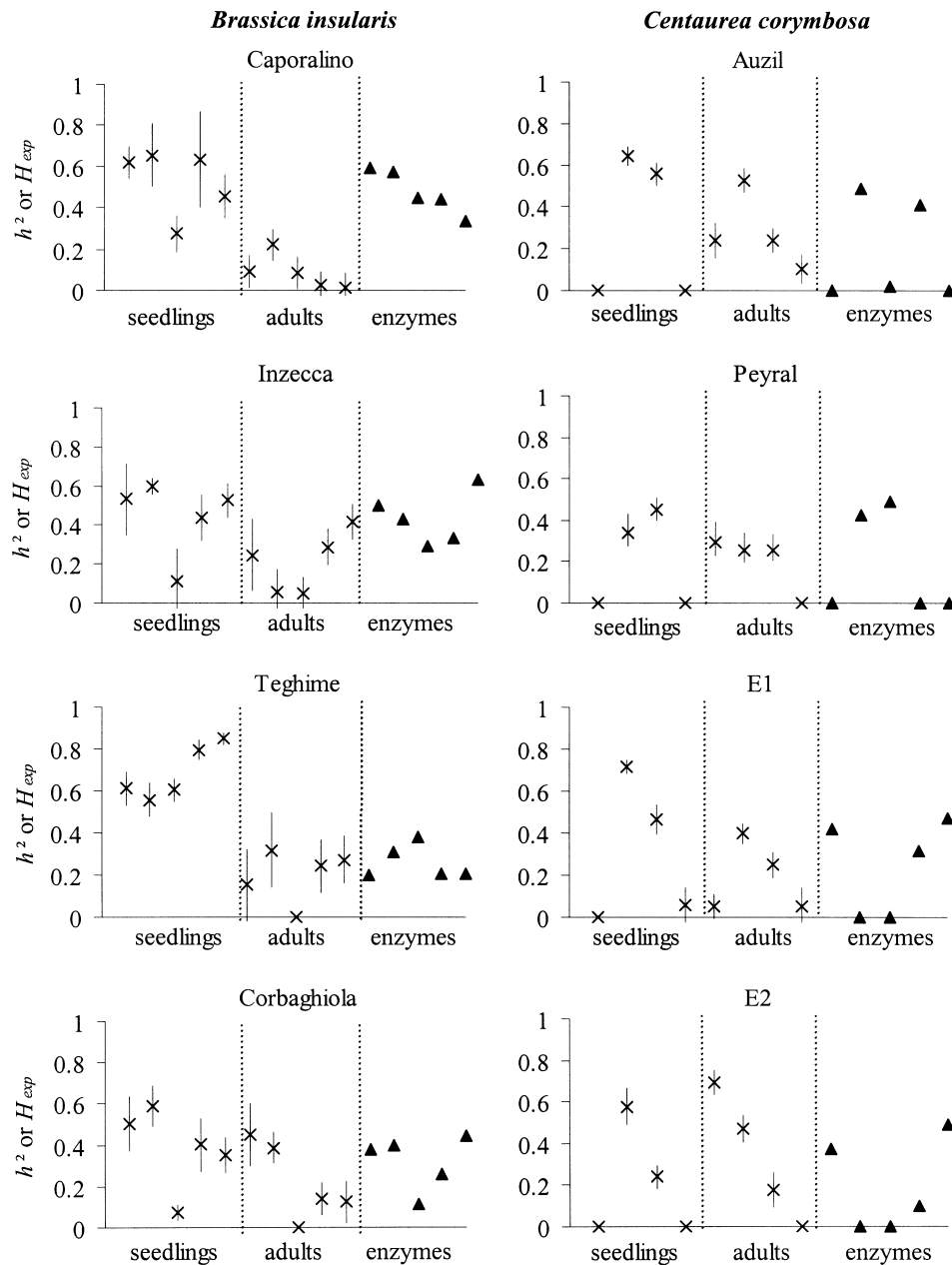


Fig. 2. Narrow-sense heritabilities ( $h^2$ ) using maternal progenies as half sibs for seedling and adult quantitative traits studied, and expected heterozygosity in five enzymatic loci in four populations of *Brassica insularis* and *Centaurea corymbosa*. For *B. insularis*, quantitative traits and loci are (from left to right): leaf number, rosette diameter, leaf length/width ratio, plant height and pubescence (seedling traits), leaf number, rosette diameter, number of secondary stems, inflorescence stem height and reproductive status (adult traits), and ACP, PGM, EST, PRX and LAP (enzymatic loci). For *C. corymbosa*, quantitative traits and loci are (from left to right): axis number, leaf number, leaf length/width ratio and diameter ratio (seedling and adult traits), and PGI, DIA, LAP, CAT and PRX (enzymatic locus).

$r=0.49$ ,  $P=0.17$  in *B. insularis* and *C. corymbosa*, respectively).  $Q_{ST}$  were thus strongly variable depending on the developmental stage. In both species, populations were significantly differentiated for allelic frequencies (exact Fisher tests,  $P < 0.01$ ).

Overall, levels of genetic differentiation for biochemical markers ( $\theta_{ST}$ ) were, in the two species, significantly larger compared to juvenile quantitative traits ( $Q_{ST}$ ; Fig. 3). In *B. insularis* only, the same trend was observed for adult traits, although not significantly. The correlation between  $Q_{ST}$  and  $\theta_{ST}$  was never significant in any species, whether juvenile or adult traits were considered (Fig. 4).

In both species, there was no significant correlation between  $Q_{ST}$  and geographical distance among populations (Fig. 5). In contrast,  $\theta_{ST}$ 's were marginally correlated with geographical distances among populations (Fig. 5). In *C. corymbosa*, the observation of a marginally significant correlation between markers and geographical distance was due to a single point (with a low value of both  $\theta_{ST}$  and geographical distance). However, Colas et al. (1997) observed a highly significant correlation between differentiation for genetic markers and geographical distance when using all six populations of *C. corymbosa*. We cannot rule out the possibility that, had we studied all populations of *C. corymbosa* in the

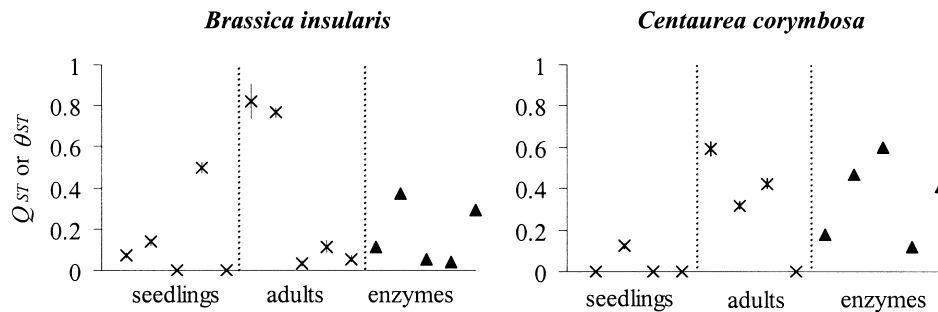


Fig. 3. Overall population structure for each quantitative traits and enzymatic locus. Error bars represent 95% confidence intervals estimated by 2SD.

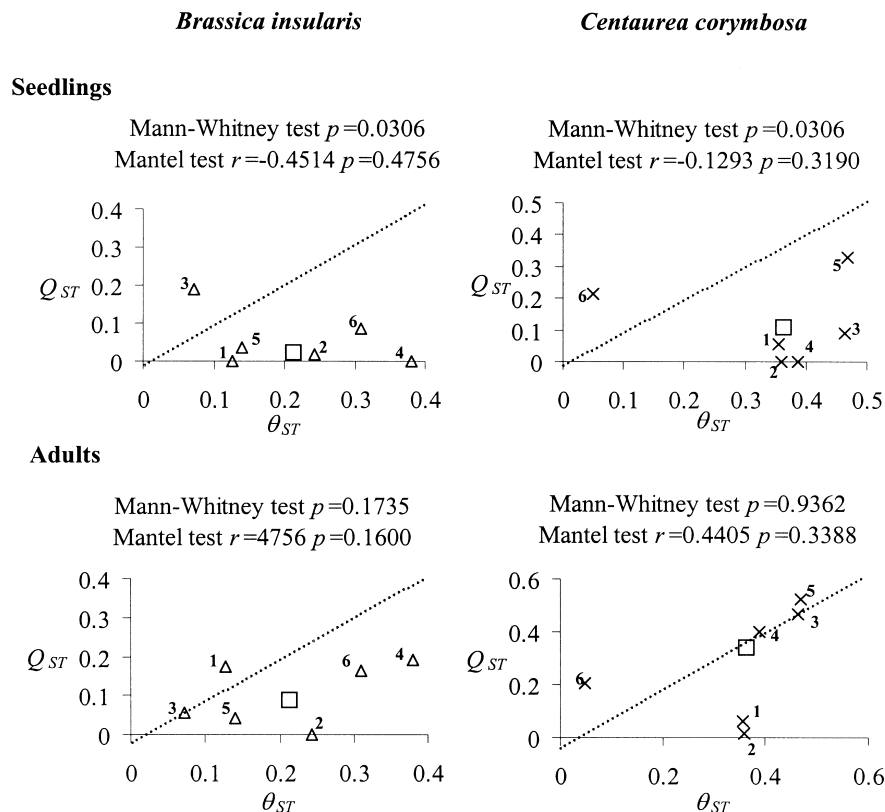


Fig. 4. Comparison of population structure for neutral markers and quantitative trait at two developmental stages for each combination of pairs of populations. Squares represent overall genetic structure on the four populations. Numbers associated with each point represent each comparison by pair of populations. In *Brassica insularis*: <sup>1</sup>Caporalino–Inzecca; <sup>2</sup>Caporalino–Teghime; <sup>3</sup>Caporalino–Corbaghiola; <sup>4</sup>Inzecca–Teghime; <sup>5</sup>Inzecca–

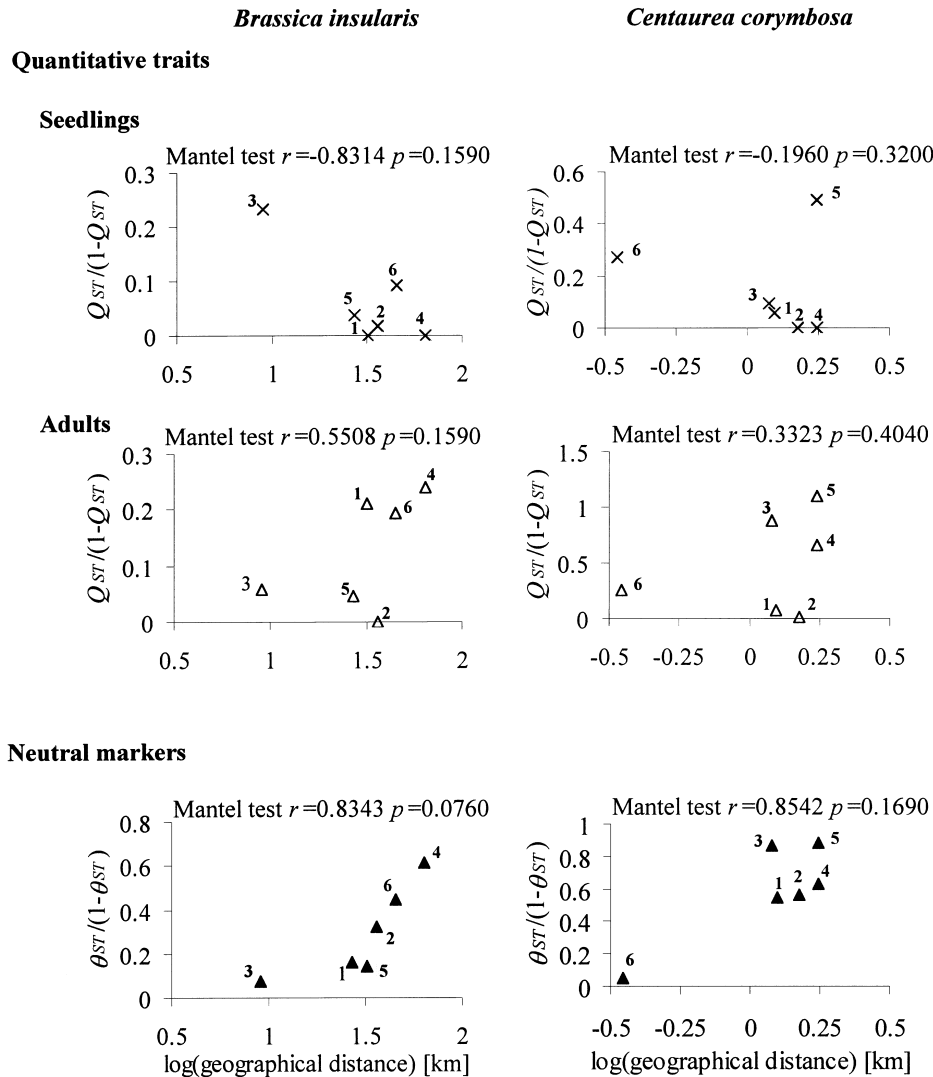


Fig. 5. Relationship between genetic structure parameters and geographical distance among populations in *Brassica insularis* and *Centaurea corymbosa*.  $Q_{ST}$  and  $\theta_{ST}$  are transformed following Rousset (1997).

common garden experiment, a pattern of isolation by distance would also have been found for quantitative traits variation.

The UPGMA trees (Fig. 6) confirmed that patterns of population structure vary with the developmental stage (seedling vs. adult plants) and the nature of study traits (quantitative traits vs. biochemical markers).

## 4. Discussion

### 4.1. Comparative population structure for quantitative traits in seedlings and adults

The estimation of the evolutionary potential of a species requires determination of the extent to which populations are genetically differentiated for quantitative traits and are locally adapted to different environments.

The computation of  $Q_{ST}$  may be a useful method to quantify the degree of genetic differences among populations, but our study shows that  $Q_{ST}$  estimates may vary greatly across developmental stages. There was no significant positive relationship between  $Q_{ST}$  values measured in seedlings and those estimated in adult plants in both species. These differences in  $Q_{ST}$  values through time may have three main causes. First, selection might be age-dependent. Assuming some degree of independence in the genetic determinism and/or the expression of juvenile and adult traits, this would translate into various amounts of genetic variance within populations, and thus possibly different  $Q_{ST}$  between juvenile and adult suites of traits. Second, narrow-sense heritability estimates appeared larger for seedling traits than for adult traits, which would be expected if maternal effects occur, since those are likely to be stronger at the juvenile stage (see Roach

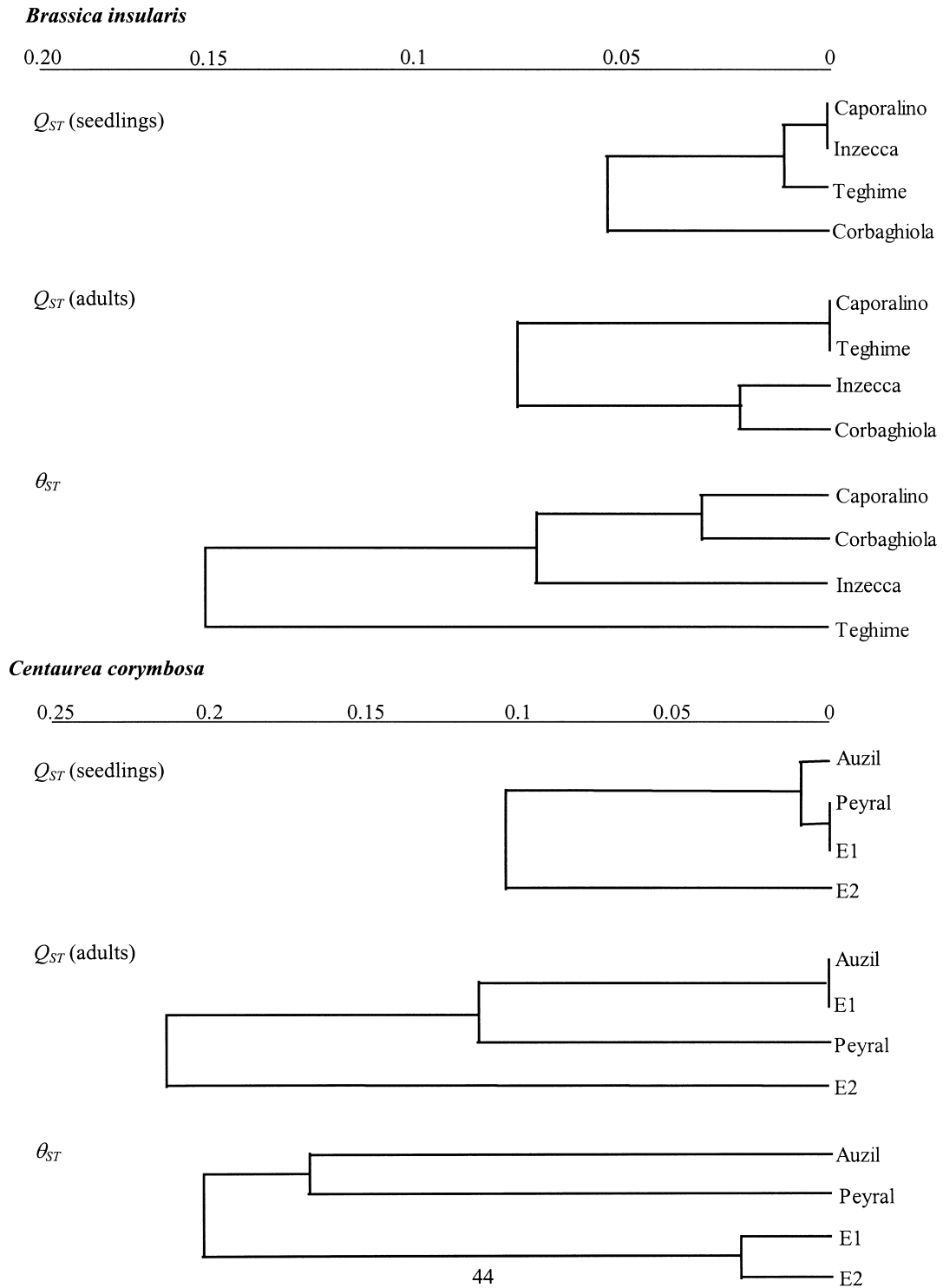


Fig. 6. UPGMA trees using population structure parameters as genetic distance measures, calculated for each combination of pairs of populations in *Brassica insularis* and *Centaurea corymbosa*.

and Wulff, 1987, for review). Such maternal effects may increase the genetic variance ( $V_{GEN}$ ) and thus bias the estimation of  $Q_{ST}$  in seedlings. Third, there

could have been selection during the course of the experiment, leading to a lower amount of genetic variation at later stages. This is unlikely, as the mortality

rate from seedling to adults was extremely low (less than 1 and 6% in *C. corymbosa* and *B. insularis*, respectively).

#### 4.2. Comparative population structure for biochemical markers and quantitative traits suggests that homogeneous selection occurs on quantitative traits

In outbreeding species,  $\theta_{ST}$  estimates for neutral markers are usually lower than 0.15 (Loveless and Hamrick, 1984). In the two species studied in this paper, high levels of population structure for enzymatic loci suggested low levels of gene flow among populations (Table 4).  $Q_{ST}$  were expected to be higher than  $\theta_{ST}$  since population differentiation in selected traits may be due not only to genetic drift, gene flow, and mutation rate but also to heterogeneous directional selection (Lynch et al., 1999). However, this pattern was not observed in our study. Populations appeared more differentiated for biochemical markers than for quantitative variation, as  $Q_{ST}$  were usually lower than  $\theta_{ST}$ , suggesting that populations experienced homogeneous, strong selection pressures decreasing the phenotypic variability within and among populations (Bonnin et al., 1996). This confirms our earlier expectation, namely that populations of rare species might be experiencing both strong genetic drift and homogeneous selective forces upon phenotypic variation. Surprisingly, this seems to be particularly the case in *B. insularis*, whose populations were geographically more distant from one another than in *C. corymbosa*. The fact that genetic differentiation for both allozymes and quantitative traits was smaller in *B. insularis* than in *C. corymbosa* could be due to larger population sizes in the former. Another factor that could increase effective size in the former species might be a lower turnover in *B. insularis*, in which seed production and juvenile recruitment appear very limited (C. Petit et al., unpublished demographic data).

Our study thus suggests no clear relationship between levels of genetic variation within and among populations based on enzymatic and quantitative data, whatever the geographical distance among populations. Preliminary results of a complementary study using microsatellites in *C. corymbosa* confirm the lack of relationship between population structure of neutral markers and of quantitative traits (H. Fréville et al., unpublished data).

Whereas previous studies have shown that population structure parameters on neutral markers are conservative estimators of population structure in quantitative traits (see Lynch et al., 1999, for review), our results suggest that for endangered species with low levels of among-population gene flow, i.e. high values of  $\theta_{ST}$ , the use of neutral markers may lead to an overestimation of the evolutionary potential of the species considered.

Alternative explanations for low  $Q_{ST}$  include the possibility that the differentiation for quantitative traits might not reflect the underlying genetic variation. Indeed, the expectation that for neutral quantitative traits  $Q_{ST} = \theta_{ST}$  was derived assuming additive gene action (Wright, 1951). Epistatic interactions among loci determining phenotypic variation (QTL) have been shown to increase both within and between population variance (Whitlock, 1999), and might bias among-population differentiation (remember that  $Q_{ST}$  can be thought of as the part of total variance that occurs among populations) with a resulting lower  $Q_{ST}$  (Whitlock, 1999). Moreover, Latta (1998) recently showed theoretically that under stabilizing, homogeneous selection and restricted gene flow, populations are likely to evolve towards the same phenotype, but that the underlying multi-locus genotype is likely to differ from one population to another. Such among-population polymorphism could be revealed when analyzing either QTL or neutral markers polymorphism ( $F_{ST}$ ), but not from analysis of quantitative traits polymorphism ( $Q_{ST}$ ). It could thus well be that the low  $Q_{ST}$  found in this study corresponds indeed to homogeneous selection forces, but that this does not preclude the existence of genetic diversity at the underlying loci.

#### 4.3. The effect of the spatial scale to quantify the evolutionary potential of an endangered species

The positive correlation between geographical distance and population structure in genetic markers was marginally significant in both species (but highly significant in *C. corymbosa* when all six populations were taken into account — see Colas et al., 1997). Although the same pattern was observed for adult quantitative traits in both *B. insularis* and *C. corymbosa*, the population differentiation for quantitative traits ( $Q_{ST}$ ) was statistically independent from the geographical distance among populations. These results suggest that evolutionary processes other than limited gene flow determine the degree of genetic differentiation among populations for the quantitative traits studied. The selective pressures that the study populations experience may play a more important role than random processes such as drift or mutation in the genetic differentiation among populations. These results also suggest that selective factors do not vary along a spatial gradient. Whatever the level of gene flow and the geographical distance, the effect of strong selection pressures will tend to homogenize the expression of the phenotypes among populations.

However, the use of quantitative genetics to detect adaptive variation among populations assumes that quantitative traits are or were selected, and that the environmental variance in experimental designs was controlled. The use of inappropriate environmental

conditions to show genetic differences in ecologically important traits among populations may be an alternative explanation.  $Q_{ST}$  in the two species may be independent of geographic distance among populations because the experimental conditions may have, for example, favored the “canalization” of the phenotypes within and between populations. In this case, the expression of similar phenotypes due to phenotypic plasticity or strong environmental constraints may be the cause of the observed lack of genetic variation among populations. Moreover, physiological traits might be more relevant to future adaptation than the traits studied here.

Other questions are, however, unresolved. From a conservation management perspective, the next step is the estimation of levels of outbreeding depression, i.e. the comparison of levels of inviability of offspring from different populations and from the same population (H. Fréville and A. Mignot, unpublished data). From a theoretical viewpoint, more investigations are needed to clarify the relation between population genetic parameters and adaptive variation. What is the biological meaning of a particular value of  $Q_{ST}$  and a fortiori of  $\theta_{ST}$  in a conservation management perspective? Can the estimation of  $Q_{ST}$  really replace quantitative studies of local adaptation, i.e. without inferring the environmental causes of adaptive differentiation among populations?

#### 4.4. Implications for conservation

The most important result of our study is the suggestion that  $\theta_{ST}$  cannot always be used as a conservative estimate of  $Q_{ST}$  to estimate between population genetic structure for selected traits, contrary to what has been suggested (Lynch et al., 1999). We now suggest ways in which combining both types of information can actually help design conservation actions.

One promising use of a combination of data from molecular markers and from quantitative traits, is the following: if  $Q_{ST}$ 's are small and  $\theta_{ST}$ 's are large, it probably means that selective pressures are homogeneous and gene flow is limited. Note, however, that this interpretation assumes additive gene effects on the quantitative traits. Because of drift and random fixation of mildly deleterious mutations, it is likely that, in this context, different populations will have found different solutions to the same problem, that is the same phenotype will be produced by different genotypes in genetically distant populations (Latta, 1998, and see above). We would then expect to find a large amount of heterosis among populations, suggesting that extant natural populations could largely benefit from reinforcement using genes from other populations. Notice that this is the opposite of usual recommendations based on molecular markers, to keep differentiated gene

pools well separated. By so doing, it is implicitly assumed that populations are locally adapted to different environments.

However, assuming indeed a homogeneous environment, one cannot reject the hypothesis that local adaptation, even to similar conditions, might lead to the existence of coadapted gene complexes, so that introducing new genes would not be recommended. Certainly, it would be necessary to study offspring resulting from crosses among populations before taking any reinforcement action. But if, conversely, one finds large  $Q_{ST}$  compared to  $\theta_{ST}$ , it is probably worthless conducting such experiments: most likely, selection is heterogeneous among populations, and gene flow already large enough to bring new variation enabling each population to locally adapt.

With regard to practical implications for our study species, further experiments are needed before any recommendation can be made. In *C. corymbosa*, considering average values of  $Q_{ST}$ , our study on quantitative traits seems to confirm the apparent homogeneity of the environment of the Massif de la Clape. Therefore, new populations established from a mixture of seeds from all populations would probably be a good strategy, especially since seedling establishment using introduced seeds was successful in several other sites (Colas et al., 1997). However, it is striking that some  $Q_{ST}$  values among populations were actually higher than  $\theta_{ST}$ 's; for instance E1 and E2 were much more differentiated for quantitative traits than for allozymes, suggesting the action of heterogeneous selection. We are presently performing within and among populations crossing experiments to document the extent of outbreeding depression.

In *B. insularis*, we would also need further studies before we can suggest reinforcement actions. Indeed the patterns of differentiation obtained for markers and for quantitative traits were very different. In contrast to the case of *C. corymbosa*, pairwise  $Q_{ST}$ 's were generally lower than pairwise  $\theta_{ST}$ 's. Such results were unexpected, as there is no obvious homogeneity of the habitat among the study populations. Given that the study populations of *B. insularis* are separated by several dozens of kilometers, gene flow among them seems unlikely. The isolation by distance pattern observed for allozymes might simply reflect a common ancestry, with foundations in a stepping stone manner. The among-population similarity of quantitative traits in this species is quite puzzling, and it could be that epistatic interactions among loci decrease  $Q_{ST}$  in *B. insularis*. It could also be that the traits we studied are not those involved in local adaptation. Finally, because the species is long-lived, it could be that potential maternal effects last longer than in *C. corymbosa*. Such maternal effects would artificially increase the additive genetic variation within populations, thus decreasing the among-population part of the genetic variance.

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