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Understanding the population genetic structure of coastal species (*Cakile maritima*): seed dispersal and the role of sea currents in determining population structure

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Summary

The nature and extent of long-distance seed dispersal are currently poorly understood, largely due to the inherent difficulty in detecting such a phenomenon. Genetic methods provide one of the few general approaches that offer the potential to accurately address this issue. Phenotypic and allozymic approaches were applied to characterize inter-population seed dispersal of the sea rocket (*Cakile maritima*, Brassicaceae), a glabrous and succulent annual herb. Genetic variation was assessed on 360 individuals sampled from nine populations. Genetic diversity across populations was high, 37% of which was represented by Qst and 16% by Fst. When genetic distances were used to construct the UPGMA dendrogram, populations were clustered into three groups at the 90% similarity level. The pattern of clustering can be explained by examining the direction of sea currents around Tunisian coasts. We have shown in this study that *C. maritima* seeds can survive up to 4 months immersion in sea water and up to 1 year of floating in sea water; therefore, seed dispersal between populations is possible both in terms of seed survival and current patterns.

1. Introduction

The historical abiotic factor (quaternary climatic change), the factor related to the specific biology of species and the modern abiotic factor (seed dispersal by sea currents) were identified to determine the phylogeographic structure (Clausing *et al.*, 2000; Kadereit & Westberg, 2007). Particularly, seed dispersal has a profound effect on the distribution and genetic structure of populations and species (Ouborg *et al.*, 1999; Cain *et al.*, 2000; Wang & Smith, 2002; Bond *et al.*, 2005), but it is hard to measure (Cain *et al.*, 2000). Indeed, the difficulties in understanding, measuring and analysing seed dispersal in coastal plant natural communities have been considered as an unavoidable limitation (Wheelwright & Orians, 1982). Consequently, empirical data are almost exclusively limited to short-distance dispersal (Ouborg *et al.*, 1999; Cain *et al.*, 2000). However, an understanding of long-distance dispersal is important since it influences many key aspects of plant biology (Hanski & Gilpin, 1997; Cheplick, 1998; Clark *et al.*, 1998; Hanski, 1998; Hovestadt *et al.*, 1999; Ouborg *et al.* 1999; Sork *et al.*, 1999; Cain *et al.*, 2000; Nathan & Muller-Landau, 2000; Nathan *et al.*, 2000; Ingvarsson, 2001; Wang & Smith, 2002).

Genetic markers, which track seed movement (Dow & Ashley, 1996; Petit *et al.*, 1996; Schnabel *et al.*, 1998), are useful to detect empirical evidence of long-distance dispersal events (Cain *et al.*, 2000). They provide a broadly applicable way to best quantify such a phenomenon (Ouborg *et al.*, 1999; Cain *et al.*, 2000). As a result, studies on dispersal (especially long-distance dispersal) using genetic markers have risen rapidly over the last 15 years (Nathan, 2003; Nathan *et al.*, 2003; He *et al.*, 2004; Bond *et al.*, 2005). To date, almost all the empirical studies using population allocation methods have used microsatellite DNA loci as the marker of choice (Paetkau *et al.*, 1995; Nielsen *et al.*, 1997). However, the allozyme and phenotypic markers are efficient not only for offering the benefits of minimal development time and cost but also for giving appropriate information.

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In this paper, we assess gene diversity, genetic structuring, population differentiation and the association between geographical and genetic distance using phenotypic and/or biochemical markers. Factors responsible for the structuring are also discussed.

2. Studied species

Cakile maritima, the sea rocket (Brassicaceae), is a glabrous and succulent annual herb. It has a short life cycle (3 months from seed to seed) and produces a large number of seeds with high germinative capacity even in the presence of salt. Its annual character is not strict, since some individuals may survive over two or three years. In such instances, the number of seeds produced may be almost 18-fold that produced by a single reproductive cycle (Thorne, 1967; Barbour & Rodman, 1970; Boyd, 1988; Boyd & Barbour, 1993). *C. maritima* is commonly found in ephemeral strandline communities on sandy shores in many parts of the world (Doing, 1985) and always close to the sea level, at or above the highest equinoctial spring tides. It tolerates climates ranging from Arctic to Mediterranean, reflecting its latitudinal distribution. Periodic appearance and loss of large strands of *C. maritima* may suggest that the tidal transport is the major factor in the population mobility. Dispersal, which depends on local dune and beach topography, can be realized by water (tides and sea currents) or more locally by wind. Once established, *C. maritima* may play a role in trapping blown sand, thus initiating fore dune formation and dune succession, especially on prograding coasts. The easily detached upper fruit segments are more suited to dispersal over long distances; however, the lower fruit segments that are retained by the maternal plants are more likely to disperse their seeds locally and exploit a proven habitat. Dune succession and predation by animals may also restrict the ability of *C. maritima* to persist on the same site in successive years. Fruit segments can remain buoyant in sea water for long periods thanks to spongy tissues in their corky pericarps (Guppy, 1917). *C. maritima* is tolerant to salt spray and transient seawater inundation; in particular, the vegetative growth of plants from Tunisian populations was stimulated by NaCl concentrations in the range 50–100 mM and plants were able to grow at up to 500 mM NaCl (Dabez *et al.*, 2004).

3. Materials and methods

(i) *Seed collections and germination protocol*

In summer 2004, we collected fruits from plants of nine populations along Tunisian coasts (Table 1). The size of these populations varies from almost 60 individuals to much more than 500 individual plants.

Table 1. Cakile maritima *Tunisian populations. Their climate region and their alphanumeric code*

Code	Population locality	Climate region
*1 (TAB)	Tabarka	Humid
*2 (BIZ)	Bizerte	Sub-humid
*3 (RAD)	Raoued	Sub-humid
*4 (HAM)	Hamamet	Sub-humid
*5 (ENF)	Enfidha	Semi-arid
*6 (SOS)	Sousse	Semi-arid
*7 (BKH)	Bekalta	Semi-arid
*8 (CHF)	Chaffar	Arid
*9 (JRB)	Jerba	Arid

Ten seeds per family were sown in a Petri dish on moistened filter paper. Germination was carried out under laboratory conditions (in the dark at 20–25 °C). Four days later, four randomly chosen seedlings per family were planted in separate pots and randomized in an unheated greenhouse. Ten families per population (i.e. 40 seedlings per population) were cultivated under uniform environmental conditions and used for quantitative genetic study. Out of each group of 40 seedlings, 30 were used for allozyme analysis.

(ii) *Seed buoyancy and viability*

The trait buoyancy (floating capacity) indicates the potential of species to be dispersed by water. It is given as the proportion of seeds floating after a defined time period. For this purpose, 100 seeds of *C. maritima* were placed in a closed bottle containing 500 ml of sea water in October 2005. The number of seeds that sank was recorded over time. In February 2006, ten of the floating seeds were sown on filtered sand and irrigated with tap water to test their viability.

(iii) *Morphological measurements*

Two sets of morphological traits were considered discriminator traits between populations: vegetative traits (leaf morphotype (leaf form (LFF)) and leaf length (LFL)) and reproductive traits (flower date emergence (FED), petal length (PTLT), petal width (PTW), petal colour (PTC), pistil length (PSL), fruit type (FRT), fruit sizes (length of the upper segment (LUP) and length of the lower segment (LLW) and seed number (SN)). Leaf characteristics were graded from 1 to 10, with 1 being leaves with entire margins, 10 being leaves with the most deeply pinnatified margins, and intermediates classified by graduations between these extremes (Fig. 1). Leaves selected for morphotype determination were also used for length measurements. Fruit size was measured separately

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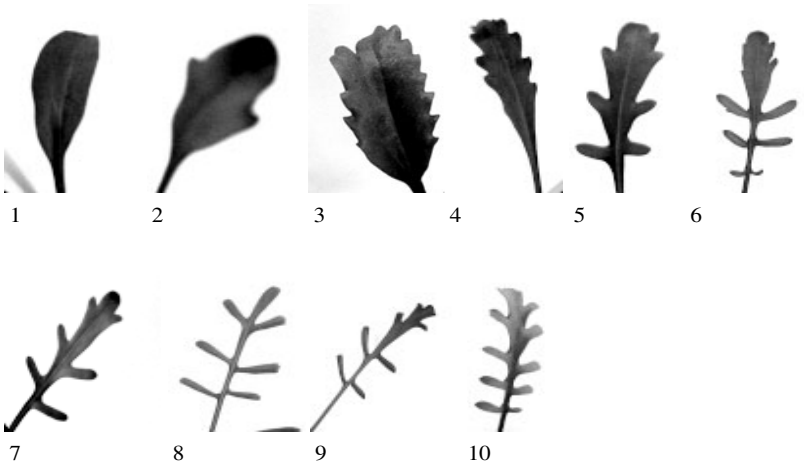

Fig. 1. Leaf morphotypes in *Cakile* arranged as a standard sequence, 1–10, for field assessment of leaf variation.

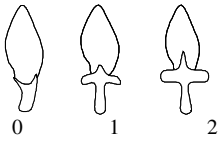

Fig. 2. Siliculas modal of *Cakile* (0: unhorned fruit; 1: intermediate fruit; 2: horned fruit).

for the upper and lower segments and was classified into three discriminate modals: unhorned, intermediate and horned quantified as a series of 0, 1 and 2, respectively (Fig. 2). Leaf length, petal length and width, pistil length and fruit size were measured by an electronic calliper. Colour and size measurements were performed on one petal chosen haphazardly from each individual.

(iv) *Data analysis*

A nested analysis of variance (ANOVA) including population and family (nested within population) as random effects was conducted for each quantitative trait. The level of population differentiation in the quantitative traits was measured with Qst (Spitze, 1993), which is analogous to Fst measured using allozyme marker loci. In order to estimate Qst, δ_b^2 is obtained directly from the population variance δ_p^2, that is, $(\delta_b^2 = \delta_p^2)$, whereas the family variance δ_f^2 has to be converted into δ_w^2 by multiplication with a coefficient (c) that depends on the relationship of individuals within families $(\delta_w^2 = c\delta_f^2)$. For half-sibs, full-sibs and cloned individuals, c is 4, 2 and 1 (under the assumption of no dominance and epistasis), respectively. When populations are in Hardy–Weinberg disequilibrium (Fis $\neq 0$), the level of differentiation in quantitative traits could be expressed as: $\frac{\text{Qst}}{1+\text{Fis}(1-\text{Qst})}$ (Yang *et al.*, 1996) where Qst has the expression $\text{Qst} = \frac{V_B}{V_B + 2V_W}$ (Spitze, 1993) and others (Lande, 1992; Latta, 2004).

V_W is estimated to be four times the between-family component of variance V_{fam} under the assumption that maternal effects were weak and that within open-pollinated families were related as half-sibs (Yang *et al.*, 1996), while V_B is simply the among-population variance component. Approximate standard error (SE) values of Qst were obtained by the 'delta' technique (Stuart & Ord, 1987). This method, used by Podolsky & Holtsford (1995), provides reliable SE estimates of genetic variance components for setting confidence limits (CLs) to genetic parameters (Hohls, 1996).

(v) *Allozyme experiment*

For the allozyme analysis, an electrophoresis survey was used to estimate genetic variability within and among *C. maritima* populations. Approximately 200 mg leaf tissue were collected from each plant (1 month old), ground under liquid nitrogen and mixed with 100 μl of extraction buffer (PVP–potassium phosphate grinding buffer, pH 7), as described by Thrall *et al.* (2000), and were centrifuged at 19 000 ***g*** for 20 min. Ground material was absorbed on Whatman 3MM filter paper wicks and stored in an ultra-cold freezer (-70 °C) until analysis. Horizontal starch-gel electrophoresis was performed for seven enzyme systems revealing a minimum of 13 loci: peroxidase (Px; EC 1.11.1.7), isocitrate dehydrogenase (IDH; EC 1.1.1.42), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1), shikimate dehydrogenase (SDH; EC 1.1.1.25), leucine aminopeptidase (LAP; EC 3.4.11.1), 6-phosphogluconate dehydrogenase (6-PGD; EC 1.1.1.44) and malate dehydrogenase (MDH; EC 1.1.1.37). The compositions of gel and electrode buffers were described in Soltis *et al.* (1983) and the methods used to stain allozyme bands were described in Michaud *et al.* (1992) for Px and in Cardy *et al.* (1980) for IDH, GOT, LAP, SDH, 6-PGD and

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MDH. For phosphatase acid (ACPH; EC 3.1.3.2), vertical zoned polyacrylamide gels were prepared following Laemmli (1970) and were stained according to Selander *et al.* (1971). Loci were numbered sequentially with the most anodally migrating locus designated as locus 1. Genetic interpretation for all loci was straightforward.

(vi) *Data analysis*

At each of the 13 loci studied in *C. maritima*, genotypic and allelic frequencies were assessed from a survey of gel phenograms. Three coefficients, measuring genetic variation, were computed using BIOSYS-1: (i) the percentage of polymorphic loci (P) using 0·95 criterion (a locus is considered polymorphic if the most common allele has a frequency of less than 95% in all the populations analysed); (ii) the mean number of alleles per locus (A); and (iii) the expected heterozygosity (He) under Hardy–Weinberg equilibrium (Nei, 1978). These three coefficients were estimated for each of the sampled populations. The mean and standard deviation of the previously mentioned coefficients were then calculated over all populations sampled. The genetic structure within and among populations was also evaluated using Wright's (1965) F-statistics Fit, Fis and Fst. Heterozygote frequency for each polymorphic locus in each population was tested for significant deviation from the Hardy–Weinberg expectations with a χ^2 analysis (Li & Horvitz, 1953) (BIOSYS-1). Estimate of Nm (the number of migrants per generation) was based on Fst (Wright, 1951) (Genetix 4.02). To test if populations' genetic differences followed the model of isolation by distance, we established the relationship between the genetic difference Fst values between pairs of populations and their geographical distance using the Mantel test (Genetix 4.02). In order to examine the genetic distance and the geographic distance, the UPGMA clustering analyses were conducted (BIOSYS-1).

(vii) *Matrix correlation*

The association between morphological, genetic, sea current speed and geographical distance matrices was examined with the Mantel test (1967). The Mantel test uses random permutations of matrix rows and columns to test if correlations between distance matrices are greater than expected by chance (Sokal & Rohlf, 1995). We compared genetic and morphological distance matrices to each other with geographical matrices individually and then we compared Nm estimated from Fst and Qst to sea current speed. Pairwise population of sea current speed was measured by using ship-drift. Ship-drift measurement of surface current velocity consists of the vector difference between the velocity of a ship determined from two position fixes and the average estimated velocity of the ship through the water during the same time interval, usually 12–24 h. The vector difference is considered to be due to a surface current. The absence of connectedness was marked as 0.

(viii) *Assignment tests*

To test whether the marine currents, as described in Fig. 5, drive the direction of gene flow, it will be interesting to use the Assignment tests. These methods are strong tools to detect recent immigration events even when the overall population differentiation is low (Rannala & Mountain, 1997; Waser & Strobeck, 1998; Castric & Bernatchez, 2004). In fact, assignment tests are particularly useful to trace the contemporary dynamics of natural populations without requiring equilibrium assumptions based on long-term genetic processes (Manel *et al.*, 2005). As the pattern of misclassification of individuals within a population can be used to determine the direction of migration, assignment tests might be a useful method to examine the influence of sea currents on gene flow. The probability that an individual sampled within a given population is a migrant originating from another population was determined by using a Bayesian method (Geneclass2 software) (Piry *et al.*, 2004).

4. Results

(i) *Quantitative genetic variation*

Phenotypic values were compared by region using a nested ANOVA. ANOVA showed a significant variation among populations in all the traits except LFL (Table 2). Significant differences among families within populations were also found for all the traits. Accordingly, with the exception of the population variance component for leaf length, variance components among populations and among families were significant as the respective lower CLs by bootstrapping were greater than zero. Values of Qst varied considerably from 39·6% for LFL to 72·2% for LFF with an average value of 0·55 among all populations (Table 2). Pairwise Qst estimates between populations show that comparisons involving population in different regions often had large (and significant) Qst values (Table 5). They ranged from 0·53 (between populations Bekalta and Chaffar; geographical distance = 196) to 0·858 (between Bekalta and Enfidha; distance = 89 km). Despite the relatively high degree of differentiation, matrix randomization tests revealed a statistically non-significant correlation of Qst with both Fst and geographical distance separating pairs of populations ($r = 0{\cdot}03$; the Mantel test, $P = 0{\cdot}412$) and ($r = -0{\cdot}07$; the Mantel test, $P = 0{\cdot}638$).

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Table 2. *Descriptive statistics of characters measured in nine populations of Cakile maritima* (*the means, between-population variance* (V_B)*, within population variance* (V_W)*,* F*-statistics for characters that gave a significant result using ANOVA and the level of population differentiation in quantitative characters as determined by Qst and its 95% lower confidence limit* (*CL*)*). Standard errors* (*SE*) *of Qst are given in parentheses*

Variable	Means	V_B	V_W	ddl	*F*	*P*	Qst	CL
LFF	7·27	86·74	7·081	8	12·207	0	0·722 (0·13)	0·5
FED	31·55	363·74	90·23	8	4·036	0·000	0·534 (0·115)	0·34
PTLT	8·26	46·04	11·35	8	4·041	0·000	0·534 (0·115)	0·34
PTW	2·74	6·44	1·39	8	4·597	0·000	0·555 (0·117)	0·36
PSL	4·71	17·17	3·99	8	4·325	0·000	0·545 (0·116)	0·35
PTC	1·8	2·38	0·813	8	2·940	0·003	0·488 (0·11)	0·3
LFL	4·56	11·76	9·39	8	1·281	0·251	0·396 (0·099)	0·23
FRT	1·1	3·59	0·55	8	6·395	0	0·61 (0·123)	0·4
LUP	10·18	126·42	19·06	8	6·607	0	0·616 (0·124)	0·41
LLW	6·67	75·95	31·56	8	2·38	0·016	0·46 (0·107)	0·28
SN	54·45	11203·8	1167·4	8	9·501	0	0·68 (0·13)	0·46

$CL = Qst - (1645 \times SE)$.

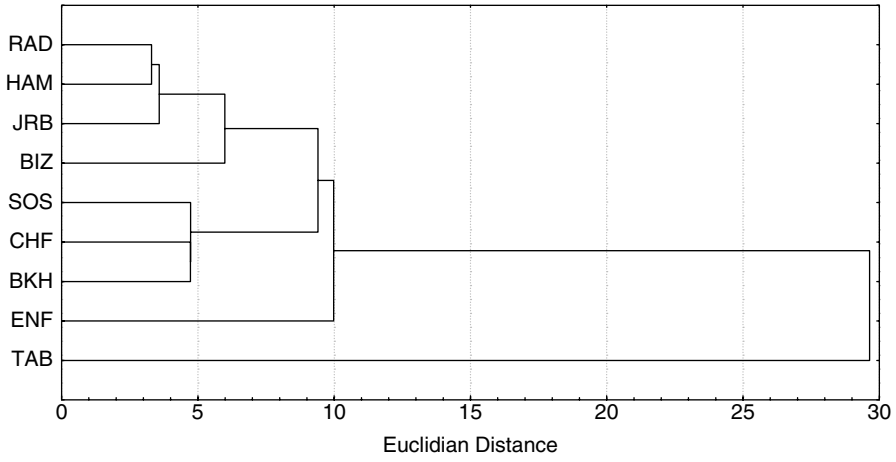

Fig. 3. Phenogram showing the relationships of sampled populations of *Cakile maritima* based on genetic distances among populations calculated from quantitative traits.

A matrix of genetic distance values using the Euclidian distances method was built to generate a dendrogram showing quantitative trait relationships between Tunisian populations of *C. maritima*. In this dendrogram, two main groups were identified (Fig. 3). The first group was represented with a single population (Tabarka) and the second one enclosed all the remaining populations structured as two subgroups, namely Jerba–Hammamet–Raoued–Bizerte and Sousse–Chaffar–Bekalta.

(ii) *Patterns of allozyme differentiation*

A total of 46 alleles were found at the 13 polymorphic loci, with an average of 3·54 alleles per polymorphic loci. Allelic frequencies per locus, observed heterozygosity (Ho) and expected heterozygosity (He) are shown in Table 3. The level of Ho ranged from $0{\cdot}152 \pm 0{\cdot}076$ for the Sousse population to $0{\cdot}311 \pm 0{\cdot}089$ for the Tabarka population. This population also exhibited the highest mean number of alleles ($A = 2{\cdot}4 \pm 0{\cdot}3$). Most of the alleles were well spread out over the population, and a few of them were found only in one, two or a few populations. Tabarka was the population with the greatest number of unique genotypes (two). All other populations had only one or no unique genotype. Percentage of polymorphic stains ranged from a low of 53·8 in the Sousse and Raoued populations to a high of 84·6 in the Tabarka, Bekalta, Bizerte and Jerba populations. The mean Ho for the species is 0·2 and a significant excess of heterozygotes was detected in IDH2, LAP and ACPH1 (Table 4).

Genetic variation based on allozyme marker genes was estimated by the average (across loci) of He under Hardy–Weinberg equilibrium. He-values ranged from

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Table 3. *Mean number of alleles per locus, observed heterozygosity, expected heterozygosity, percentage of polymorphic loci and Fis (correlations between uniting gametes within subpopulations). Values are given as the means for 13 enzyme loci*

Population	Mean number of alleles per locus (*A*)	Percentage of polymorphic loci[a] (*P*)	Mean heterozygosity		Fis
			Observed heterozygosity (Ho)	Expected heterozygosity[b] (He)	
Tabarka	2·4 (0·3)	84·6	0·311 (0·089)	0·385 (0·067)	0·194
Raoued	1·9 (0·3)	53·8	0·182 (0·076)	0·255 (0·08)	0·287
Hammamet	1·9 (0·2)	69·2	0·194 (0·085)	0·244 (0·064)	0·207
Bekalta	2·2 (0·2)	84·6	0·204 (0·063)	0·349 (0·06)	0·419
Chaffar	2·2 (0·3)	76·9	0·194 (0·073)	0·33 (0·072)	0·415
Sousse	1·6 (0·2)	53·8	0·152 (0·076)	0·193 (0·061)	0·213
Enfidha	2·1 (0·3)	69·2	0·197 (0·084)	0·286 (0·066)	0·315
Bizerte	2·2 (0·2)	84·6	0·196 (0·078)	0·29 (0·058)	0·328
Jerba	2·3 (0·2)	84·6	0·174 (0·068)	0·287 (0·054)	0·396

[a] A locus is considered polymorphic if more than one allele was detected.
[b] Unbiased estimate (see Nei, 1978).

Table 4. *Summary of* F-*statistics at all loci: Fis (correlations between uniting gametes within subpopulations or inbreeding in individuals relative to subpopulations to which they belong), Fit (correlations between uniting gametes for the total population or inbreeding in subpopulations relative to the total population) and Fst (the average population differentiation in allozyme marker loci)*

Locus	Fis	Fit	Fst
POX1	0·354	0·417	0·097
POX2	0·824	0·876	0·298
POX3	0·914	0·928	0·156
POX4	0·459	0·683	0·414
POX5	0·1	0·188	0·098
IDH2	−0·33	−0·292	0·092
ACPH1	−0·017	−0·004	0·013
MDH	0·431	0·506	0·133
SDH	0·972	0·974	0·098
GOT	0·474	0·582	0·206
LAP	−0·567	−0·507	0·038
6PGDH1	0·63	0·695	0·176
6PGDH2	0·741	0·781	0·152
Mean	0·298	0·410	0·155

$0{\cdot}193 \pm 0{\cdot}061$ for the Sousse population to $0{\cdot}385 \pm 0{\cdot}067$ for the Tabarka population (Table 3) and indicated that *C. maritima* exhibits within population levels of genetic diversity concordant with predominantly outcrossed species (Hamrick & Godt, 1989).

Overall, our data indicate a deficiency of heterozygotes both for within populations and for the total sample relative to the Hardy–Weinberg expectations (Table 4). For the complete set of nine populations, the mean and range of Fis (correlations between uniting gametes within subpopulations or inbreeding in individuals relative to subpopulations to which they belong), Fit (correlations between uniting gametes for the total population or inbreeding in subpopulations relative to the total population) and Fst values over the loci were 0·3, 0·41 and 0·155, respectively (Table 4). A gene flow rate estimated from Fst values was 1·63 migrants among the complete set of nine populations.

Pairwise Fst values were computed for all possible pairs of populations ranging from 0·05 to 0·285. The overall average was 0·155. The pair of populations of Enfidha–Tabarka displayed the highest genetic divergence (Fst = 0·285), followed by Sousse–Enfidha (Fst = 0·282). The lowest degree of differentiation was observed between the populations of Hammamet and Raoued (Fst = 0·057), followed by the pair of populations Bizerte and Raoued (Fst = 0·072). Based on the Mantel test, no correlation was found between pairwise Fst and geographic distances measured as a straight line, indicating that the genetic distance did not necessarily correlate with the geographical distance since the genetic distance among geographically distant populations was large and those among close populations were small ($r = 0{\cdot}07$; $P = 0{\cdot}26$). But Fst was significantly correlated with pairwise sea current speed measured by the use of ship–drift, indicating that sea current can be one of the major factors affecting population structure in this species ($r = 0{\cdot}23$; $P = 0{\cdot}042$).

The overall pattern of between-population allozyme differentiation is summarized in the cluster diagram (Fig. 4). The major division of the populations into two clusters contains little geographic structure. At the three-cluster level, the top cluster in

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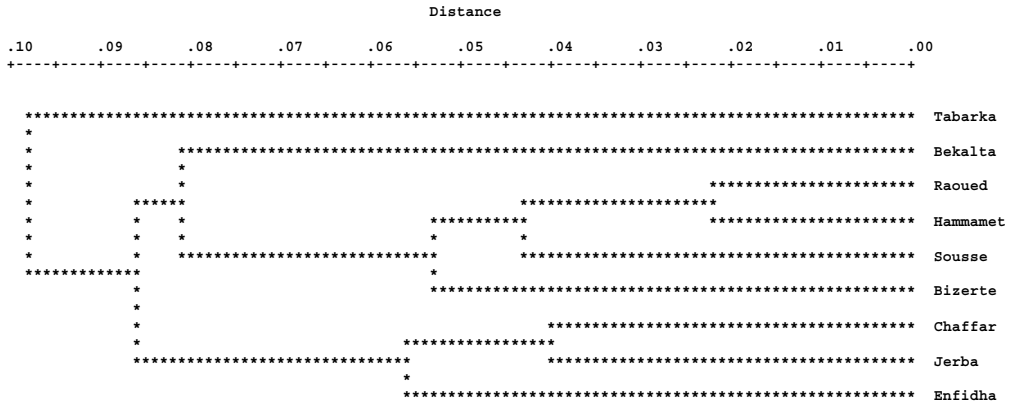

Fig. 4. Phenogram showing the relationships of sampled populations of *Cakile maritima* based on Nei's (1978) genetic distances among populations calculated from allele frequencies.

the diagram contains only the Tabarka population. The second cluster contains a mixture of populations from north and central populations (Bizerte, Raoued, Hammamet, Sousse and Bekalta). The third cluster contains mainly southern (Jerba and Chaffar) and central populations (Enfidha). Pairwise Fst values agreed with the phylogeny in indicating that the Tabarka population was genetically isolated from the remaining populations (Table 5). Large and significant values in pairwise Fst statistics indicated that haplotype distribution was not random with respect to geographic location (Table 5). Most of this variation, however, was due to differences between major sea currents in the region.

(iii) *Comparison of Qst with Fst*

The average population differentiation in allozyme marker loci (Fst) across 13 loci was 0·155, which was significantly larger than zero at $P = 0{\cdot}01$. Estimates of population differentiation in quantitative traits (Qst) varied widely among the 11 quantitative traits (Table 2). If allozymes can be considered effectively neutral, the estimate of proportion of diversity among populations for the electrophoretic data (0·155) can be used as the null hypothesis for testing among population variance in quantitative traits. Qst values of each trait were compared with the corresponding Fst values. Qst was generally greater than Fst for all studied variables. Across the entire transect, the average pairwise estimate of Qst was 3·5-fold larger than that of Fst (0·558 vs. 0·155). For average quantitative trait sizes (mean Qst values across 11 instar-specific measures), the Qst value of 0·558 is significantly greater than 0·155 ($P = 0{\cdot}005$). No significant correlation was detected between the observed patterns of molecular variation and morphological variation. There may be several reasons for the discrepancy between results based on morphology and allozymes. First, allozymes are considered to be neutral and thus to provide no direct assessment of fitness. The forces that cause differentiation for these markers would be the result of mutation, genetic drift and no selection. Conversely, morphological traits are generally believed to be subject to natural selection, and their expression is partially under the influence of environmental factors. Second, in contrast with morphological traits, allozymes variation is directly based on DNA sequence variation. A change in allozymes can result from a change in nucleotide sequence. Despite these basic differences, low gene flow would allow accumulation of small or higher adaptive differences, explaining some concordance between morphological and molecular traits.

A significant correlation was detected between the pairwise population sea current speed and the observed patterns of molecular variation and morphological variation.

(iv) *Gene flow*

Average Nm was estimated to be 1·65 individuals using Fst index and 0·125 individuals using Qst index. Estimates for pairwise Nm values (Table 5) were lowest for Enfidha–Tabarka (Nm = 0·62; distance = 527 km; sea current speed = 0 knots) and highest for Hammamet–Raoued (Nm = 4·09; distance = 244 km; sea current speed = 0·5 knots). Intermediate Nm values were obtained for a number of populations separated by shorter distances and low current speed, for example, Jerba–Enfidha or Sousse–Raoued.

(v) *Assignments tests*

The number of individuals assigned to a population that differed from the ones they were sampled in varied considerably between populations (mean = 46·33 %, min = 10·6 %, max = 79 % for Tabarka and Raoued, respectively) but the Chaffar, Bekalta and Sousse populations featured similar proportions of

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Table 5. *Matrices of* (*a*) *straight-line separation distances* (*km*), (*b*) *Qst*, (*c*) *Fst for each pairwise combination of populations and* (*d and e*) *Nm estimated from Fst and Qst respectively*

	TAB	RAD	HAM	BKH	CHF	SOS	ENF	BIZ
(a)								
RAD	245							
HAM	489	244						
BKH	616	371	127					
CHF	812	567	323	196				
SOS	562	317	73	54	250			
ENF	527	282	38	89	285	35		
BIZ	134	111	355	482	678	428	393	
JRB	1045	800	557	430	234	484	519	912
(b)								
RAD	0·121							
HAM	0·142	0·057						
BKH	0·097	0·133	0·127					
CHF	0·137	0·113	0·179	0·145				
SOS	0·229	0·096	0·154	0·236	0·148			
ENF	0·194	0·246	0·219	0·149	0·128	0·282		
BIZ	0·102	0·072	0·121	0·113	0·101	0·177	0·211	
JRB	0·181	0·209	0·228	0·148	0·074	0·23	0·088	0·122
(c)								
RAD	0·532							
HAM	0·759	0·489						
BKH	0·77	0·455	0·442					
CHF	0·494	0·488	0·407	0·331				
SOS	0·589	0·47	0·618	0·46	0·508			
ENF	0·41	0·644	0·568	0·798	0·774	0·774		
BIZ	0·76	0·458	0·342	0·52	0·562	0·562	0·52	
JRB	0·593	0·484	0·401	0·418	0·489	0·472	0·623	0·579
(d)								
RAD	1·811							
HAM	1·501	4·09						
BKH	2·308	1·621	1·712					
CHF	1·569	1·958	1·140	1·464				
SOS	0·837	2·354	1·373	0·805	1·430			
ENF	0·627	0·763	0·888	1·421	1·694	0·634		
BIZ	2·193	3·217	1·805	1·956	2·203	1·16	0·934	
JRB	1·128	0·942	0·845	1·435	3·119	0·836	2·587	1·787
(e)								
RAD	0·121							
HAM	0·050	0·139						
BKH	0·047	0·154	0·160					
CHF	0·137	0·139	0·177	0·220				
SOS	0·100	0·147	0·091	0·151	0·131			
ENF	0·175	0·082	0·108	0·041	0·066	0·046		
BIZ	0·050	0·152	0·213	0·126	0·184	0·110	0·126	
JRB	0·099	0·141	0·180	0·171	0·139	0·146	0·089	0·104

within-population misclassified individuals. Indeed, for these three populations, more than 75% of individuals were well assigned to the site they were sampled from.

(vi) *Seed buoyancy and viability*

C. maritima is a coastal species that benefits from sea water in its dispersal; in order to ensure the hypothesis of seed dispersal ability for this species, it will be interesting to prove its ability to float for a long time while maintaining viability. Results show that after 1 year of immersion in sea water, seven of the 90 seeds left after the germination experiment had sunk and the remaining 83 were still afloat. Of the ten seeds tested for viability, eight germinated within 10 days.

5. Discussion

The advantage of the approach based on Qst vs. Fst comparison is that results can be interpreted within the framework of the neutral theory of phenotypic

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evolution (Lande, 1976, 1977, 1992; Chakraborty & Nei, 1982; Rogers & Harpending, 1983; Lynch & Hill, 1986; Lynch, 1988, 1994; Whitlock, 1999). This approach provides a means of testing long-standing hypotheses about the ecological causes of population differentiation (modern abiotic factor) and the role of selection in maintaining clinal variation. This study showed that *C. maritima* populations have significant levels of phenotypic and allozymic differentiation as indicated by their morphological traits and enzymatic systems. The second main finding is that there is a general deficiency of heterozygote individuals and high levels of genetic divergence between samples from the same population. This was indicated by the fact that Fis is positive (0·3) and significantly different from zero in the majority of the studied loci. More than 15% of the total genetic variation is among populations (Fst = 0·155), which indicates high differentiation. This is also reflected in Qst values (another estimate of differentiation degree) of 0·558. Several deviations from Hardy–Weinberg equilibrium were found in all the populations analysed. Given that *C. maritima* is an outbreeder, observed deviation from Hardy–Weinberg equilibrium cannot be explained by inbreeding effect, but it can be explained by assuming that such long-distance dispersal events have occurred and have led to diversification. A study of another species whose seeds are dispersed by water, *Hibiscus moscheatus*, showed that over short distances the relationship between populations and their level of differentiation was based not on geographical distances between populations but on their accessibility to the main stream of water (Kudoh & Whigham, 1997). Indeed, dispersal mode is an important factor that affects the ability of a plant species to colonize a new area, especially those at long distances from the seed source. The presumed modes of seed dispersal in the genus *Cakile* are over short distances by fruits being blown along the beach, and over longer distances via tidal currents (Clausing *et al*., 2000). In addition, *C. maritima* is an allogamous often insect-pollinated species; therefore, most pollen should be transferred between plants in the same or adjacent population. Long-distance pollen transfer, because of extended foraging or by insects being blown along the coast, is rare. However, if gene flow in *C. maritima* was only through pollen transfer we would expect to find isolation by distance. In our case, we show that pairs of populations that are geographically close generate a high genetic distance in comparison with others that are more distinct geographically. Analyses of tidal maps (Fig. 5) show that there are three major currents in the coast of Tunisia, one flowing northwards into Algeria, the second flowing into the Gulf of Hammamet and the third flowing into south of Tunisia. These current patterns make it virtually impossible for seeds to be exchanged between Tabarka and the remaining populations (Fig. 5), thus backing up our results, which showed that the greatest genetic differentiation occurred between the Tabarka population and all others. Thus, the hypothesis of a random distribution of lineage between those habitats was rejected. The tidal patterns may also explain the low levels of genetic diversity between populations around the Cap Bon region. Estimates of Nm between populations and its high correlation with sea current speed supported this assumption. In addition, results of assignment tests revealed that a population preferentially exchanged migrants with other populations located within the area of the Cap Bon region. We also found a lower proportion of misclassified individuals within the Sahel region (a region characterized by lower sea current). Dispersal by sea currents can lead to exchange of genes between related populations. Thus, the dispersal pattern of seeds contributes to the genetic structure of populations. However, seeds that travel long distances are inherently difficult to observe. We have shown in this paper that genetic methods can be used to provide data on the tail of the dispersal curve (Waser & Strobeck, 1998; Bohonak, 1999; Ouborg *et al*., 1999). Molecular markers characteristically reveal more complex and diffuse patterns of geographic variation and show a lower variation between-population component of genetic diversity than maternally inherited, seed-dispersed, markers (Ennos, 2001).

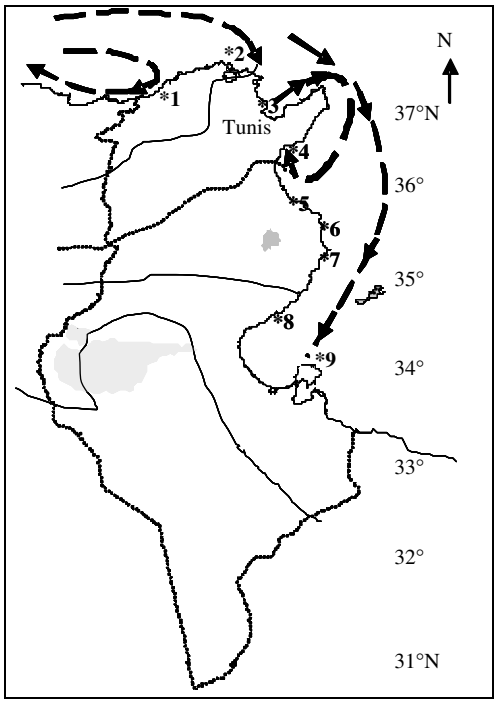

Fig. 5. *Cakile maritima* Tunisian populations, their climate region and the direction of sea currents.

Our data fit the general patterns of current flow in the region and suggest that there is gene flow between

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C. maritima Tunisian populations. However, it is crucial to ensure the ability of this species to float for a long time while maintaining viability. Results showed that there are 92% buoyancy after 1 year (October 2005 to October 2006) and 80% seed germination after 4 months of immersion in sea water. These values for seed buoyancy and viability are relatively similar to those obtained in *Calystegia soldanella* (Arafeh & Kadereit, 2006) and are much higher than those reported for the other coastal species investigated by Kadereit *et al.* (2005). The result suggests that the floating ability of seeds is important for the creation of plant distribution patterns. Similarly, Schneider & Sharitz (1988) and Boedeltje *et al.* (2003) demonstrated the significance of buoyancy for effective hydrochorous dispersal. Nilsson *et al.* (1994) proposed that water dispersal of seeds is effective in the Vindel River, and that inability to float for a long time does not disqualify species from being dispersed by water over considerable distances. In *C. maritima*, we suggest that long time buoyancy was a crucial factor for seed dispersion since the movement of seed along 100 km takes 1 year.

One of the limitations of this study is that the genetic variance within populations V_G was estimated as four times the variance component among families. Since seeds were taken from natural habitats of *C. maritima*, we assumed that there are no factors affecting the mating system in this species; consequently, seeds from a single individual were considered as half-sibs. If the open-pollinated progeny from a single mother were all full-sibs, V_G should be estimated to be twice the variance among families. This decrease in V_G would effect an increase in our Qst estimates.

6. Conclusion

The results of this study indicate that *C. maritima* is characterized by a relatively high within-population genetic diversity. Indeed, the estimates of genetic differentiation and gene flow suggest that the species is primarily outcrossing. In addition, we have shown that *C. maritima* seeds can survive up to 4 months immersion in sea water and up to 1 year of floating in sea water and the genetic distances were more correlated with sea current speed than with geographic distances. Therefore seed dispersal between populations is possible both in terms of seed survival and current patterns. Consequently, tides are probably the main cause of long-distance gene flow via seeds. In this context, some populations are most likely to be a source of gene (probably northern populations). Long-distance dispersal of floating seeds and micro-scale eco-geographic factors may play important roles in the genetic diversity of the populations and the genetic structure of this species.

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