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Terrestrial growth and marine dispersal? Comparative phylogeography of five coastal plant species at a European scale

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The phylogeography of five flowering plant species (*Cakile maritima*, *Eryngium maritimum*, *Salsola kali*, *Halimione portulacoides*, *Crithmum maritimum*) widespread along the European coasts was investigated across their entire European range using AFLP evidence. Both similarities and dissimilarities were found. All species contain a distinct Black Sea/Aegean Sea cluster, and all except *E. maritimum* contain a distinct Adriatic Sea cluster or group of genetically very similar clusters. All species except *Cr. maritimum* contain a distinct Atlantic Ocean/North Sea/Baltic Sea cluster clearly separate from the Mediterranean material. In *Ca. maritima* a distinct Baltic Sea subcluster was found. In the western Mediterranean, two species groups can be recognized. Whereas in *S. kali* and *E. maritimum* material from this area falls into only one cluster or a group of genetically very similar clusters, it falls into two clusters or groups of clusters of either more Atlantic or more central Mediterranean similarity in *Ca. maritima*, *H. portulacoides* and *Cr. maritimum*. Similarities and dissimilarities in patterns found are discussed in terms of a combination of historical and extant abiotic and biotic factors. Thus, the distribution range of all species in the eastern Mediterranean area was not affected by Quaternary temperature changes, resulting in phylogeographic congruency here. The existence of distinct Black Sea/Aegean Sea and Adriatic Sea clusters or groups of clusters is the result of sea currents isolating these regions from each other. In the western Mediterranean basin the more cold-sensitive species (*H. portulacoides*, *Cr. maritimum*) but not the less cold-sensitive species (*S. kali*, *E. maritimum*) had to retreat from northern coasts. Re-colonization of these areas from two different directions is implied by their phylogeographic pattern. The existence of a distinct Gibraltar gap is explained in terms of extant sea currents. Comparison of phylogeographic patterns found with those observed in either marine or terrestrial organisms leads to the conclusion that marine dispersal is of overriding importance in these coastal plants.

KEYWORDS: coastal plant species, glacial distribution areas, phylogeography, sea water dispersal.

INTRODUCTION

In recent years, a steadily increasing number of phylogeographic studies of northern hemisphere plant and animal species have been published (reviewed in, e.g., Soltis & al., 1997; Comes & Kadereit, 1998, 2003; Taberlet & al., 1998; Hewitt, 2000, 2004; Abbott & Brochmann, 2003; Brochmann & al., 2003; Stehlik, 2003; Tribsch & Schönswetter, 2003; Lascoux & al., 2004). Major aims of many of these studies were to illuminate the distributional history of taxa on the background of the Quaternary climatic oscillations, and to contribute to the understanding of evolutionary process by trying to distinguish historical and extant factors as determinants of genetic structure. Their results potentially also can inform conservation biologists about the geographical location of populations particularly suitable for protection (Avise, 2000).

The distinction between historical and extant factors as determinants of intraspecific genetic structure is easiest when solid knowledge of the distributional history of species is available. Such knowledge can be obtained through the combined consideration of fossil and genetic evidence in those taxa that do have a good fossil record, such as the pollen record of common wind-pollinated tree species (e.g., Lange, 1994). Hypotheses on the location of refugial areas in insect-pollinated (or rare wind-pollinated) plants in most cases must rely on genetic evidence alone.

It is generally assumed that glacial refugia will contain higher amounts of genetic variation than inter- or postglacially colonized areas (Hewitt, 1996; Widmer & Lexer, 2001). Accordingly, geographical clines of decreasing genetic variation are interpreted as migration routes. This assumption is based on a particular model of dispersal (Ibrahim & al., 1996), where individual genotypes found new populations far removed from the source population. This model neglects the possibility that populations in presumed glacial refugia, now often located near the ecological limits of the species, may have suffered postglacial reduction of genetic variation (Coyer & al., 2003), or the possibility that areas of multiple colonization exhibit unexpectedly high levels of variation (Olsen & al., 2004). When, in consequence, the knowledge of the distributional history of species is uncertain, the differentiation between historical and extant factors as determinants of genetic structure, or the differentiation among different historical processes, often remains highly hypothetical even when analytical methods designed for the distinction of these factors are employed (Templeton, 1998, 2004; Knowles & Maddison, 2002).

On this background, we conducted a comparative study of seven widely and largely co-distributed European coastal plant species of partly contrasting biology. We believe that coastal plant taxa offer several advantages when trying to infer distributional history and recognize the effects of species-specific biology on geographical patterns of genetic variation. First, they often are azonally distributed and have both latitudinally and longitudinally large geographical ranges. These ranges, more than in narrowly distributed taxa, are likely to still contain both refugial and recolonized areas. Second, and in contrast to inland species, coastal species show a linear distribution range. On the one hand, this limits the spatial options for migration, which is likely to have taken place along the coasts (or across the sea where suitable dispersal units are formed) but not across extensive land masses. This drastically reduces the number of potentially possible migration routes into and out of refugial areas. On the other hand, limitation to coastal habitats easily allows reconstruction of distributional limits in glacial times when, in a first approach, the extant northern distributional limits are assumed to be determined primarily by temperature or close correlates of temperature (Beerling, 1993), and when glacial isotherms are available. Accordingly, fairly safe assumptions can be made about the location of potential refugial areas without fossil evidence, and also without complex modelling. An example for the interpretation of extant geographical distribution of genetic variation in combination with modelled glacial refugia recently was presented by Hugall & al. (2002).

The seven plant taxa investigated are *Cakila maritima* Scop. (Brassicaceae), *Calystegia* (= *Convolvulus*) *soldanella* (L.) R. et Sch. (Convolvulaceae), *Crithmum maritimum* L., *Eryngium maritimum* L. (both Apiaceae), *Halimione portulacoides* (L.) Aellen, *Salsola kali* L. (both Chenopodiaceae) and *Triglochin maritimum* L. (Juncaginaceae). Results relating to *C. soldanella* and *T. maritimum* will be published elsewhere. In *C. soldanella*, the almost complete absence of phylogeographic pattern is interpreted as the combined result of extensive clonal growth resulting in high genet age and highly dispersable seeds (Arafeh & Kadereit, unpubl.). The history of *T. maritimum* (Lambracht & Kadereit, unpubl.) differs from that of the remaining species through its periglacial survival as documented by fossil evidence (Bell, 1969).

Cakile maritima. — *Cakile maritima* is an annual species often growing in the drift-line of sandy beaches. Its insect- or self-pollinated flowers are self-compatible, but selfing apparently results in comparatively low seed set (Rodman, 1974; Thrall & al., 2000). The ovary develops into a two-segmented siliqua. Of the two segments, the upper breaks off at maturity. Its distinctly spongy pericarp is responsible for its buoyancy, and mericarps have been reported to float in sea water for between seven and 10 (Ridley, 1930; Rodman 1974), and for more than 100 days in tap water (Maun & Payne, 1989). They remain viable for up to 10 weeks, but germination is inhibited by salt (Rodman, 1974). The overall geographical distribution of *Ca. maritima* in the study area is shown in Fig. 1. The species has been subdivided into three to four subspecies (Rodman, 1974; Ball, 1993).

Salsola kali. — *Salsola kali* subsp. *kali* [as one of two (Greuter & al., 1984) or three commonly recognized subspecies (Aellen, 1961)] is an annual that shares its habitat with *Ca. maritima*. The species is wind-pollinated, but nothing is known about its compatibility system. Either the entire plant is dispersed as a tumbleweed, or the persistent winged calyx results in wind dispersal of the attached fruits (Ridley, 1930). Fruits have been reported to float in sea water between five days and four weeks where they remain viable for at least 40 days (Ridley, 1930). The European range of *S. kali* subsp. *kali* is shown in Fig. 1.

Eryngium maritimum. — Like the preceding species, the perennial *E. maritimum* grows on sand, but often in more stabilized habitats such as dunes. Nothing is known about its reproductive biology, although its showy flowers are likely to be insect-pollinated. Although the spiny sepals of the fruit may indicate epizoochorous dispersal (Thellung, 1926), they do not adhere efficiently to animal surfaces (pers. observ.). The fruits contain a spongy tissue restricted to the commissural ridges of the mericarps and can float in sea water for between two and four days (Ridley, 1930), and remain viable for up to 40 days (Ridley, 1930; Thellung, 1926). Germination is inhibited by sea water (Ridley, 1930). The species has never been subdivided taxonomically. Its European distribution is shown in Fig. 1.

Halimione portulacoides. — *Halimione portulacoides* (partly included in *Atriplex*; Kühn, 1993) is a perennial subshrub found in estuaries and salt marshes of the European coasts (Fig. 1). Its either perfect or unisexual and monoecious flowers presumably are wind-pollinated (Chapman, 1950). Its fruits have a papery pericarp enclosing the one seed. They normally are enclosed by the bracteoles which contribute to their floating ability (Aellen, 1961). Fruits have been reported to float for more than one month in sea water, and a proportion of seeds remain viable for this period (Koutstaal & al., 1987). Sometimes the fruits open and release the seeds, which have been reported to show almost no germination in sea water (Aellen, 1961). Apart from the description of three varieties of the species in the British Isles (Chapman, 1950), and the recognition of two varieties in Spain (Castroviejo & al., 1990), no taxonomic subdivision of the species has been proposed.

Crithmum maritimum. — This perennial species grows on rocky shores along the coasts of Europe (Fig. 1). Nothing is known about its reproductive biology. Its strongly spongy fruits (Thellung, 1926) can float in sea water for up to 1 year (Ridley, 1930). However, nothing is known about the duration of viability of seeds. No attempts have been made to subdivide the species taxonomically.

Although different in habitat requirements by growing on sand, in salt marshes or on rocks, the distribution of all five species is essentially continuous as judged from their European-scale distribution maps (Fig. 1).

■ MATERIAL AND METHODS

Plant material. — Leaf material of each species was collected approximately every 100 to 200 km along the coast from Eregli/Turkey to Åhus/Sweden and dried in silica gel. A variable number of individuals was selected for the AFLP analysis (see Results). Apart from between three and five individuals from two populations in *Ca. maritima*, three populations in *S. kali*, one population in *E. maritimum*, four populations in *H. portulacoides* and four populations in *Cr. maritimum*, each sampling locality was represented by one individual. Sampling localities are shown in Fig. 1 and listed in the Appendix. This sampling strategy was employed to detect large scale patterns but essentially precludes any type of inference on the population level.

DNA isolation. — Approximately 100 mg of dried leaf material was ground with autoclaved sand (Roth). Total genomic DNA was extracted using the DNeasy™ plant minikit (Qiagen) following the manufacturer's instructions. DNA concentration was measured spectrophotometrically with a GeneQuant RNA/DNA calculator (Pharmacia), or estimated visually by ethidium bromide stained agarose gels. After dilution to 30 ng/μl, DNA was stored at -20°C.

AFLP analysis. — The AFLP protocol follows the modifications to the protocol of Vos & al. (1995) by Kropf & al. (2003). All reactions were performed simultaneously for all samples of each species. Approximately 150 ng total genomic DNA was simultaneously digested and ligated using 2 U *Eco*RI (GeneCraft), 0.8 U *Mse*I (NEB) as well as 0.5 U T4-ligase (GeneCraft) in a volume of 10 μl containing 2.5 pmol *Eco*RI adapter, 25 pmol *Mse*I adapter, 0.1 μl bovine serum albumin (10μg/ml; GeneCraft), 1.0 μl 0.5 m NaCl, 1.0 μl 10x T4-ligasebuffer (Genecraft) and PCR-grade water. Reactions were incubated for 14 hours at 23°C to insure complete digestion and ligation.

Products of the restriction-ligation reaction were diluted 1:3, and 5 μl were used as template in the preselective PCRs performed in 25 μl total volume supplemented with 12.5 ng each of primers *Eco*RI+1 and *Mse*I+1, 2.5 μl BioTherm 10× PCR-buffer (GeneCraft), 0.25 μl 20mM dNTP, 1.25 μl 50mM MgCl$_2$, 0.5 U BioTherm Taq-polymerase (GeneCraft) and PCR-grade water. The thermocycling profile consisted of 2 min at 72 °C, followed by 20 cycles of 10 s at 94°C, 30 s at 56°C, and 2 min at 72°C, and a final incubation of 30 min at 60 °C.

Selective PCRs were performed in 15 μl total volume containing 5 μl of 1:16 diluted product from the preselective PCR as template, 12.5 ng *Mse*I+3 primer, 9 ng labeled *Eco*RI+3 primer, 0.15 μl 20mM dNTP, 0.75 μl 50mM MgCl$_2$, 1.5 μl BioTherm 10× PCR-buffer and 0.25 U BioTherm Taq-polymerase (GeneCraft). The PCR protocol consisted of 2 min at 94°C, followed by eight cycles of 10 s at 94°C, 30 s at 64°C, and 2 min at 72°C. For each of these cycles the annealing temperature was reduced by 1°C to reach a final temperature of 56 °C. Under these conditions, the reaction was continued for 24 cycles, followed by a final post-treatment of 30

Table 1. Primers and adapters used in the AFLP analysis.

		Sequence 5′- 3′
Adapters	*Eco*RI	5′-CTCGTAGACTGCGTACC-3′
		3′-CATCTGACGCATGGTTAA-5′
	*Mse*I	5′-GACGATGAGTCCTGAG-3′
		3′-TACTCAGGACTCAT-5′
Primers	**Preselective (*Eco*+1)**	
	Eco+A	GACTGCGTACCAATTCA
	Mse+C	GATGAGTCCTGAGTAAC
	Selective (*Eco*+3)	
Ca. maritima	NED	*Eco*+ACG - *Mse*+CGA
	HEX	*Eco*+ATG - *Mse*+CGG
	6-FAM	*Eco*+AGA - *Mse*+CGG
S. kali	HEX	*Eco*+ATG - *Mse*+CGG
	6-FAM	*Eco*+ACT - *Mse*+CGA
E. maritimum	NED	*Eco*+ACG - *Mse*+CTG
	HEX	*Eco*+ATG - *Mse*+CCT
	6-FAM	*Eco*+ACT - *Mse*+CGA
H. portulacoides	NED	*Eco*+AGC - *Mse*+CGG
	HEX	*Eco*+ATG - *Mse*+CCT
	6-FAM	*Eco*+AGA - *Mse*+CTG
Cr. maritimum	NED	*Eco*+AGC - *Mse*+CGG
	HEX	*Eco*+ATG - *Mse*+CGG

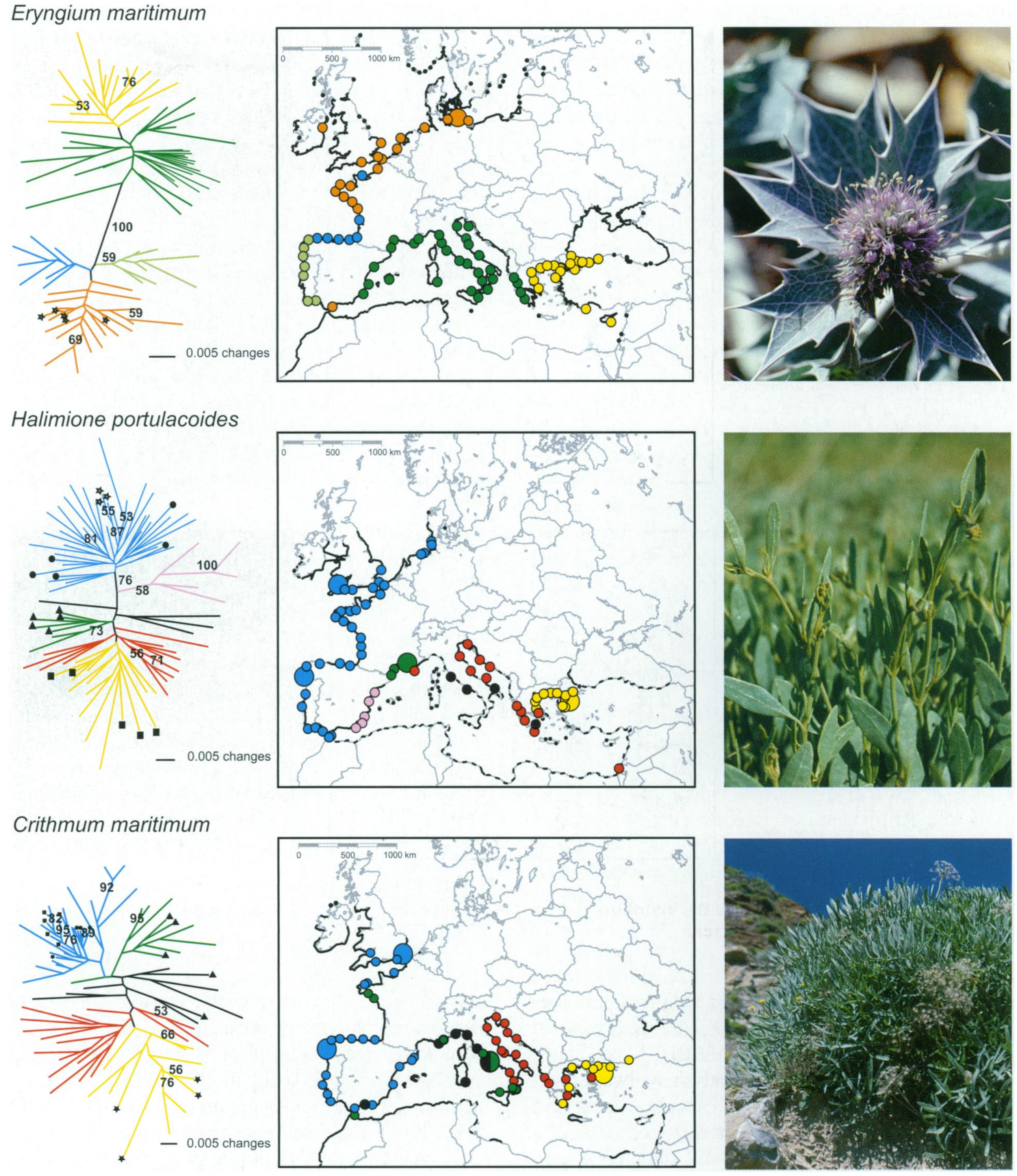
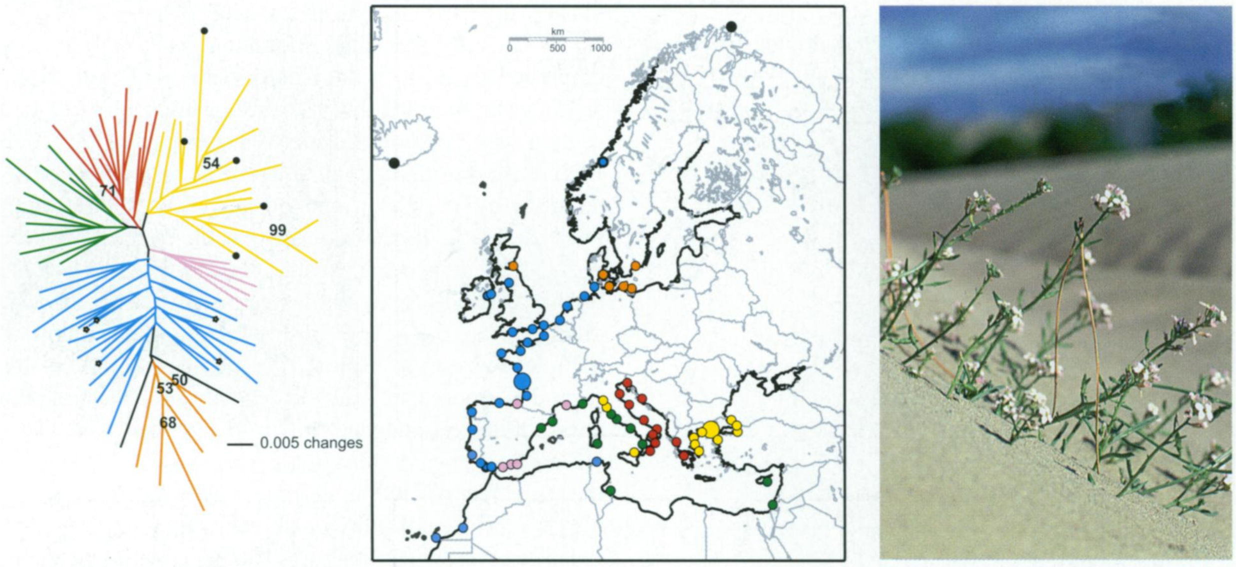


Fig. 1. Geographical distribution (lines and dots), sample localities (circles), Neighbor-Joining phenograms (with bootstrap percentages >50%) and geographical distribution of clusters in the five species investigated (continued on next page). Clusters and sampling localities of constituent individuals are marked in the same colour. Large circles indicate localities where populations (three to five individuals) were sampled. Individuals from these populations are marked by different symbols in the NJ phenograms. Areas where detailed knowledge about the distribution is not available are shown by dashed lines.

min at 60 °C. The primers used are listed in Table 1.

AFLP products were separated on 6% polyacrylamide gels as a multiplex of three primer combinations labeled with different fluorescent dyes (6-FAM, NED

Cakile maritima



Salsola kali

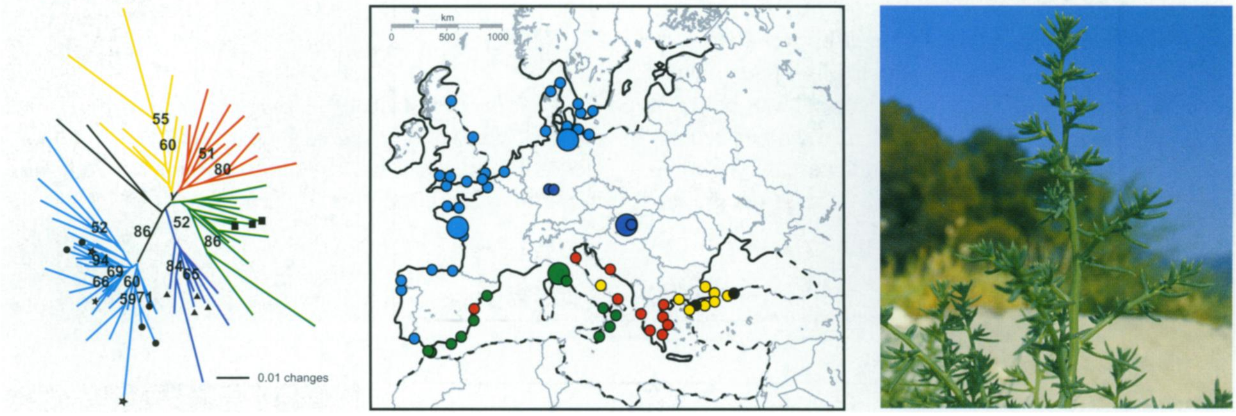


Fig. 1 (continued). For *Ca. maritima* the distribution shown is that of subsp. *maritima* Scop. Individuals of subsp. *islandica* (Gand.) Elven are coloured black.

and HEX; Applied Biosystems) and an internal size standard labelled with ROX (ROX 500, ABI). Gels were run for approximately 4 hours on an ABI 377 automated sequencer using the Genescan analysis software (v3.1, ABI). AFLP products were automatically scored with Genotyper (v2.1, ABI) as either absent (0) or present (1). Scoring was manually corrected and ambiguities were recorded as missing data.

Data analysis. — To detect geographical structure of genetic variation, pairwise Nei and Li (1979) genetic distances were calculated from the AFLP data and clustered with Neighbor Joining (NJ; Saitou & Nei, 1987) using PAUP* v4.0b10 (Swofford, 2002). The phenograms were inspected for clusters or genetically similar groups of clusters occupying a cohesive geographical area (Fig. 1). Branch support was evaluated by bootstrapping with 10,000 replicates. Pairwise genetic distances were also used to calculate the mean genetic distance within species. To quantify genetic differentiation among geographical groups, molecular variance was partitioned within and among groups by AMOVA (Excoffier & al., 1992) and tested for significance using Arlequin 2.0 (Schneider & al., 2000). Significance levels of the test statistics were evaluated with a non-parametric randomization test using 3000 permutations as implemented in Arlequin 2.0.

Correlation between genetic and geographical distances (Mantel's *r*), measured along the coast, was tested by a Mantel test (Mantel, 1967) with 9999 permutations using The R Package 4.0 (Casgrain & Legendre, 2000). This was done to investigate whether genetic diversity within the clusters identified in the NJ-analysis is struc-

Table 2. Comparison of genetic diversity (Shannon index, SI) between the Atlantic Ocean/North Sea/Baltic Sea and the Mediterranean Sea regions. Sample sizes (N) were adjusted (column 3) by excluding individuals from the larger-sized group at regular geographical intervals.

Species	N	SI	N	Adjusted SI
Cakile maritima				
Atlantic	34	0.334	34	0.334
Mediterranean	40	0.380	34	0.379
Salsola kali				
Atlantic	32	0.106	30	0.102
Mediterranean	32	0.148	30	0.148
Eryngium maritimum				
Atlantic	33	0.247	33	0.247
Mediterranean	48	0.317	33	0.296
Halimione portulacoides				
Atlantic	41	0.116	35	0.117
Mediterranean	44	0.125	35	0.125
Crithmum maritimum				
Atlantic/east Spain	30	0.212	27	0.118
Mediterranean	48	0.456	27	0.192

tured by an isolation-by-distance (IBD) process. Populations in areas with a long history would have had more time to reach migration-drift equilibrium than populations in areas re-colonized postglacially. Hence, lower values or absence of significant correlation between genetic and geographical distances in hypothesized re-colonized areas compared to refugial areas might be expected. Individuals from localities located geographically far distant from the bulk of their respective genetic cluster were excluded from this analysis.

Because of the single-individual sampling strategy employed for most of the material, no population diversity measures could be compared between postulated refugia and re-colonized areas. Instead, we pooled all individuals belonging to a region of interest and estimated the Shannon Index (Shannon & Weaver, 1949), calculated as $SI = -p_i \log2(p_i)$ where p_i is the frequency of the ith band. In all species except *Cr. maritimum*, the whole of the Atlantic Ocean/North Sea/Baltic Sea region was compared to the whole of the Mediterranean region. Sample sizes were adjusted by excluding individuals from the larger-sized group at regular intervals along the coast until sizes matched. In *Cr. maritimum* the Atlantic Ocean/North Sea/eastern Spain cluster was compared to the remainder of the Mediterranean material. From localities where several individuals were sampled, one individual was chosen at random to be included in the analysis.

RESULTS

Cakile maritima. — A total of 167 fragments could be consistently scored for 81 individuals. Of these, eleven fragments were monomorphic and 25 were only found in a single individual.

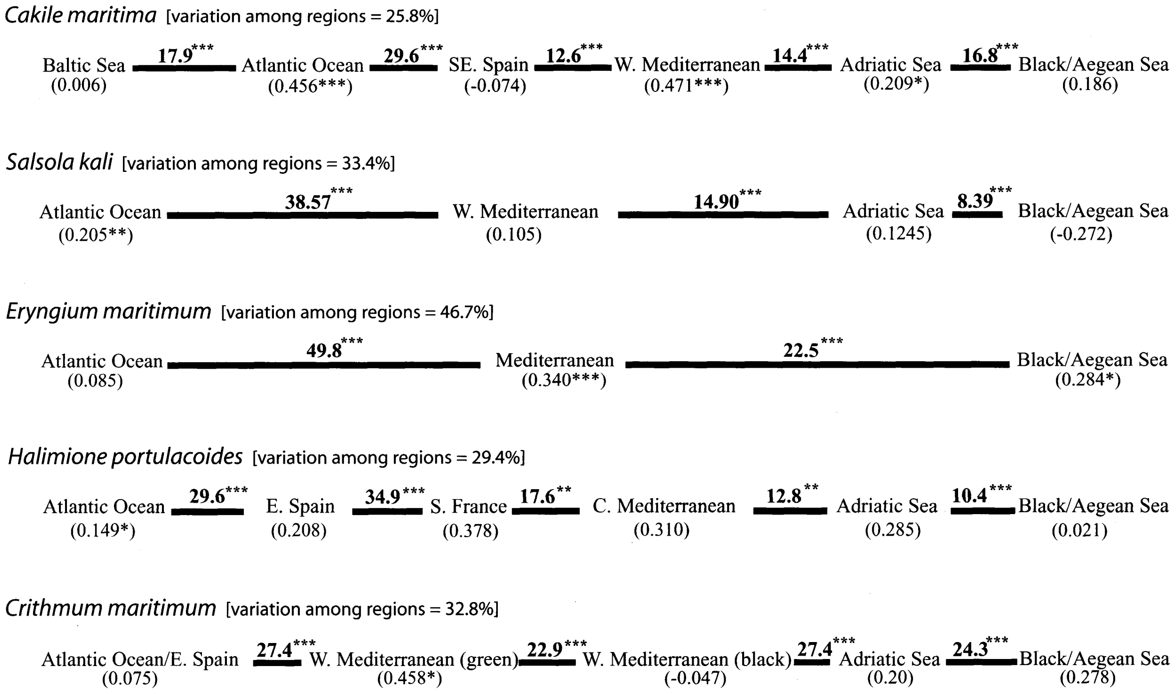


Fig. 2. Genetic differentiation among geographical regions and isolation-by-distance within geographical regions. The percentages of variation partitioned among regions (bold) are indicated between regions, and correlations between geographical and genetic distances (Mantel r; brackets) below regions (see Fig. 1). Significance is indicated by *0.05 > P > 0.01, **0.01 > P > 0.001, ***P <0.001.

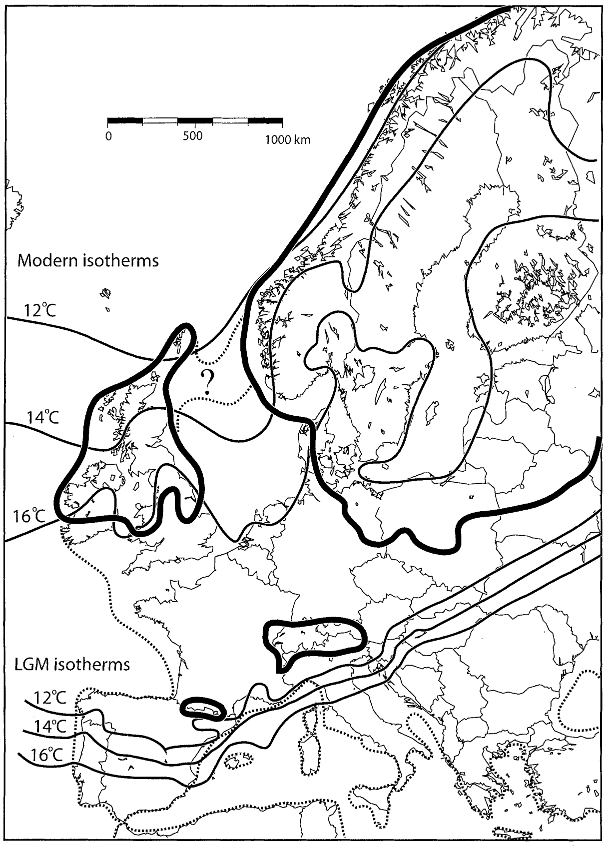


Fig. 3. Modern (northern Europe) and reconstructed (southern Europe) last glacial maximum (LGM) isotherms. The 12°C, 14°C and 16°C mean July (modern) or June/July/August isotherms (LGM) are shown. The position of the 14°C and 16°C LGM isotherms were interpolated from the position of the 12°C and 18°C isotherms. Modern isotherms were redrawn from Hann (1887), and LGM isotherms from van Andel (2002). The ice shield during the LGM is shown in bold, the coastline as a dotted line (Lang, 1994).

Several geographical clusters were identified in *Ca. maritima* (Fig. 1). The large Atlantic Ocean/North Sea/Baltic Sea cluster contained a distinct Baltic Sea subcluster also containing one Scottish individual. Mediterranean accessions were divided into a Black Sea/Aegean Sea, an Adriatic Sea and a western Mediterranean group which also includes a few eastern Mediterranean and northern African accessions. Finally, a southeastern Spanish cluster also including one individual from north Spain and south France each could be recognized. Geographical outliers are a Scottish individual in the otherwise Baltic Sea cluster, a Norwegian individual in an otherwise western European cluster, the northern Spain and the southern France individuals in the otherwise southeastern Spanish cluster, the eastern Mediterranean individuals in the otherwise western Mediterranean cluster.

Among-region variation accounted for 25.8% (P <0.001) of total variation (Fig. 2), and all regions were significantly different from each other in pairwise comparisons. The highest value of among-region variation, (29.6%; P <0.001) was found between the Atlantic Ocean cluster and the southeast Spanish cluster (Fig. 2). The *SI* was marginally lower in the Atlantic than in the Mediterranean area (Table 2). Significant correlation between genetic and geographical distances was found for the Atlantic (excl. Baltic Sea), western Mediterranean and Adriatic Sea groups (Fig. 2). Mean genetic distance among individuals was 0.0566 ± 0.0139.

Salsola kali. — Seventy-nine individuals from 57 localities were included in the AFLP analysis. Of a total of 233 fragments, four were monomorphic and 52 were autapomorphic. The NJ analysis revealed a clear Mediterranean-Atlantic subdivision (Fig. 1). In the Mediterranean, individuals from the Black/Aegean Seas and Adriatic/Ionian Seas fall into separate and neighbouring clusters. A third cluster contains all individuals from southern Italy to southern Spain (Strait of Gibraltar). The remaining accessions from west of Gibraltar to the Baltic Sea fall into a clearly separate cluster. Inland accessions from Hungary and Germany also form a separate cluster which is more similar to the Mediterranean than to the Atlantic material. Geographical outliers were one individual from eastern Italy falling into the Black Sea/Aegean Sea cluster, and one individual from eastern Spain grouping with Adriatic/south Greece accessions.

Among-region variation accounted for 33.4% (P <0.001) of the total variation. The strongest differentiation was found between the Atlantic area and the western Mediterranean cluster (Fig. 2). In the Atlantic area the *SI* was distinctly lower than in the Mediterranean area (Table 2). Significant IBD was found only in the Atlantic cluster (Fig. 2). Mean genetic distance among individuals was 0.0669 ± 0.023.

Eryngium maritimum. — In *E. maritimum* 170 fragments, of which 23 were monomorphic and 24 apomorphic, were included in the analysis of 85 individuals. The NJ analysis divided the material into three geographical groups (Fig. 1). These are an Atlantic, a Mediterranean and a Black Sea/Aegean Sea cluster. The material from the Atlantic Ocean was further divided into three geographical clusters. However, these could not be recovered when only Atlantic Ocean material was analyzed. Accordingly, these three subclusters were not included in further analyses.

Strong genetic differentiation was found between the three geographical groups in *E. maritimum* (Fig. 2). The total amount of variation among regions was 46.7% (P <0.001), and the strongest differentiation was between the Atlantic material and the western Mediterranean material. No IBD was found in the Atlantic cluster,

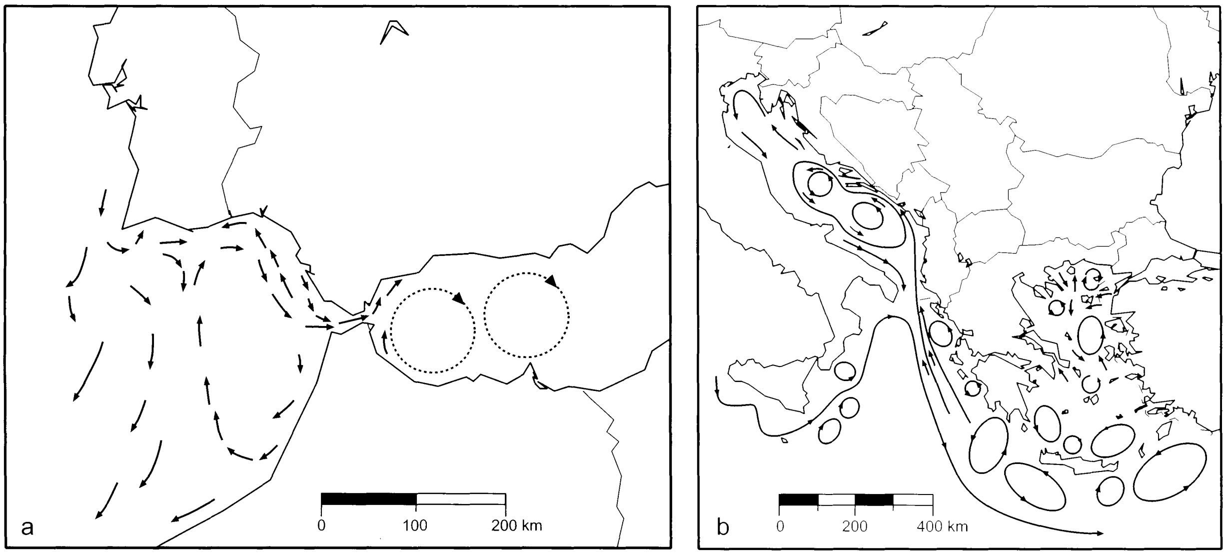


Fig. 4. Major surface currents in (a) the Iberian-Moroccan Bay and Alboran Sea and (b) the Ionean and Cretan Seas and the Adriatic and Aegean Seas. Redrawn from Ovchinnikov (1966), Rey (1983), Malanotte-Rizzoli & al. (1997), Millot (1999) and Lykousis & al. (2002).

whereas the two Mediterranean groups show significant correlations (Fig. 2). Genetic diversity in the Atlantic area was marginally lower than in the Mediterranean area (Table 2). Mean genetic distance among individuals was 0.0396 ± 0.015.

Halimione portulacoides. — The total number of scored fragments in 86 individuals of *H. portulacoides* was 295. Of these, 68 were monomorphic and 28 present in only one individual. The overall pattern revealed by the NJ analysis (Fig. 1) is a clear Mediterranean-Atlantic subdivision, with an intermediate group of eastern Spanish accessions. Most of the Aegean accessions fall into a separate cluster. Individuals from southern Greece and the Adriatic Sea fall into two genetically similar clusters (plus one neighbouring individual). Another cluster is formed by individuals from southern France, and the remaining individuals (except one) from western and southern Italy (plus one individual from south Greece) form another cluster. The large Atlantic Ocean/North Sea cluster extends from a few kilometers east of Gibraltar to Langeness in the North Sea. One individual from southern France and one from the eastern Mediterranean grouped with mostly Adriatic/southern Greece material.

Among-region variation was 29.39% ($P < 0.001$) of total variation. The highest value of among-region variation was found between the eastern Spanish and the southern France material (Fig. 2). The Atlantic region contained marginally lower genetic diversity than the Mediterranean area (Table 2). Significant IBD was found only in the Atlantic cluster. Mean genetic distance among individuals was 0.051 ± 0.0138.

Crithmum maritimum. — The total number of AFLP fragments scored in 78 individuals was 114, of which eight were monomorphic and 17 were autapomorphic. Accessions from the Black and Aegean Seas fall into a separate cluster that is neighbour to a cluster of all Adriatic Sea accessions (Fig. 1). Individuals from eastern Spain to the English Channel fall into another cluster. The remaining accessions in the western Mediterranean form one cluster, which is closer to the Atlantic/eastern Spain material, and several small groups that are closer to the eastern Mediterranean material. One Aegean individual grouped with mostly Adriatic Sea accessions.

The proportion of variation among regions was 32.8% ($P < 0.001$). Equally strong differentiation was found between Atlantic/eastern Spain and one of the western Mediterranean groups, and between the other of the western Mediterranean groups and the Adriatic Sea material (Fig. 2). The *SI* was distinctly smaller in the Atlantic/eastern Spain area than in the Mediterranean area (Table 2). Significant IBD was observed only in one of the two western Mediterranean groups (Fig. 2). Mean genetic distance among individuals was 0.0609 ± 0.0211.

DISCUSSION

All five species investigated show pronounced geographical structure in their genetic variation (Fig. 1). Bootstrap support of the major NJ clusters, however, was generally low. Irrespective of this, we here assume that the clusters identified in general validly represent patterns of genetic similarity. Mean and genetic distances within the material investigated are roughly comparable among species. An exception to this is *E. maritimum* with a distinctly smaller mean value. From this we conclude that, assuming a rough correlation of genetic diversification and absolute time, the history of our five species in the area investigated is of roughly the same absolute age. This is an important pre-requisite for their comparison. In terms of geographical patterns observed, comparison among the five species reveals a large amount of interspecific congruency, but also some differences. The following discussion attempts to explain these similarities and dissimilarities in terms of a combination of historical and extant abiotic and biotic factors.

Black Sea /Aegean Sea. — In all five species a distinct Black Sea/Aegean Sea cluster could be recovered. In the west, this cluster includes the east coast of Greece in *Ca. maritima*, *E. maritimum* and *Cr. maritimum*, while particularly in *S. kali* and to some extent in *H. portulacoides* the east coast of Greece is inhabited by individuals from the neighboring western cluster or clusters. Our material, as far as our only very small sample from the Black Sea permits to draw meaningful conclusions, does not allow to distinguish a separate Black Sea cluster. Considering that the Aegean Sea and the Black Sea were not connected during Quaternary glacials due to the fall of sea level, it seems possible that this repeated isolation was counterbalanced by high levels of gene flow in interglacials and the Holocene. Today the Black Sea and the Aegean Sea are connected by surface currents from the former into the latter (Zodiatis & al., 1996). However, population-level sampling of *Ca. maritima* and *E. maritimum* in the Black Sea, Marmara Sea and Aegean Sea indicates low levels of gene flow (Westberg & Kadereit, unpubl.). In consequence, our failure to recognize a distinct Black Sea cluster in the species examined probably results from insufficient sampling.

A primary subdivision between the Aegean Sea (and marine waters to the east) and the remainder of the Mediterranean basin and the Atlantic region has been detected in a cockle (Nikula & Väinölä, 2003), a distinct Aegean lineage was reported for a flounder (Borsa & al., 1997a), and in *Zostera marina* L. a distinct genetic lineage in the Black Sea in comparison to the Mediterranean Sea was found by Olsen & al. (2004). Among terrestrial plants, the Aegean/Black Sea area has been recognized as a separate biogeographic region by Adamovic (1909), Tahktajan (1986) and Meusel & Jäger (1992).

Adriatic Sea. — Except for *E. maritimum*, which shows no clear genetic differentiation across the entire Mediterranean basin west of the Aegean Sea, all species contain a more or less distinct Adriatic Sea cluster or a group of genetically very similar clusters containing individuals from the west coast of the Balkan Peninsula and the east coast of Italy towards its southeastern or partly southwestern tip. In *Ca. maritima* and *S. kali*, this cluster is positioned somewhat between the Black Sea/Aegean Sea cluster and the remainder of the material from the Mediterranean basin. In *H. portulacoides* and *Cr. maritimum* the Adriatic material is more similar to the Black Sea/Aegean Sea cluster.

An Adriatic lineage has also been reported for a flounder by Borsa & al. (1997a). For terrestrial plants, the Adriatic region was recognized as a separate biogeographic area by Engler (1904), Adamovic (1909), Tahktajan (1986) and Meusel & Jäger (1992).

Western Mediterranean basin. — The division of the Mediterranean basin into an eastern and a western part meeting near Sicily has been recognized for both marine and terrestrial organisms. For example, the comparison of 16 marine animal species revealed a more or less clear distinction between populations from the eastern and western Mediterranean basins (Borsa & al., 1997b), and essentially the same conclusion for marine organisms was reached by Bianchi & Morri (2000) and Garibaldi & Caddy (1998). For terrestrial plants, these two major regions were recognized by Markgraf (1934) and Rikli (1946), and with somewhat different boundaries also by Zohary (1973). Other authors, however, divided the Mediterranean area into a western, central and eastern region for terrestrial plants (Meusel & Jäger, 1992).

Except for *E. maritimum*, all species sampled by us show a separation into eastern and western Mediterranean accessions. Among the species investigated, conspicuous differences are recognizable within the western Mediterranean basin (southwestern Italy/Sicily to Gibraltar). Material from this area falls into one uniform cluster in *S. kali*, and into several genetically similar clusters in *E. maritimum*. In contrast to this, a subdivision can be recognized in *Ca. maritima* and *H. portulacoides*. In *Ca. maritima*, individuals from southeastern Spain fall into a cluster which is more similar to the Atlantic material of this species, and material from western Italy and northeastern Spain forms a cluster which is more similar to the Adriatic Sea cluster of this species. Both clusters intermingle in southern France. Essentially the same pattern is found in *H. portulacoides*: Material from the entire Mediterranean coast of Spain falls into one cluster, which is intermediate between the Atlantic cluster and the remaining Mediterranean material. On the other hand, individuals from southern France and the Italian west coast (including one sample from southeastern Italy) are more similar to the Adriatic/Aegean Seas clusters of this species. In *Cr. maritimum*, most material from the east coast of Spain groups with the Atlantic material, and plants from southern France and western Italy group somewhat between this Atlantic/eastern Spain cluster and the Adriatic/Aegean/Black Seas clusters.

Interestingly, the subdivision of the north coast of the western Mediterranean basin to some extant reflects the recognition of a separate biogeographical region for terrestrial plants, the Liguro-Tyrrhenian Province including southern France and western Italy, by, e.g., Tahktajan (1986).

Atlantic Ocean/North Sea/Baltic Sea. — Except for *Cr. maritimum*, in which eastern Spanish material can not be separated from the Atlantic material, all species share a distinct Atlantic Ocean/North Sea/Baltic Sea cluster that reaches from Gibraltar along the entire coasts of these seas. Whereas this cluster is not further differentiated in *S. kali* and *H. portulacoides*, and the Atlantic Ocean/English Channel material of *Cr. maritimum* also shows no further substructure; such substructure is recognizable in *E. maritimum* and *Ca. maritima*. Three subclusters from the Iberian west coast, the Iberian north coast and the remaining coasts northwards are recognizable in *E. maritimum* and show decreasing similarity to the Mediterranean material. However, when Atlantic Ocean/North Sea/Baltic Sea material of *E. maritimum* is analyzed alone, these three subclusters are not recognizable. In *Ca. maritima*, the Baltic Sea material represents a distinct subcluster (also containing one individual from Scotland). Irrespective of this, the existence of a Baltic Sea subcluster might provide genetic support for the recognition of a distinct subspecies of *Ca. maritima* in the Baltic Sea, i.e., subsp. *baltica* (Rouy & Fouc.) P.W. Ball. A separation of North Sea and Baltic Sea material has also been reported for *Zostera marina* (Olsen & al., 2004) and a polychaetous worm (Röhner & al., 1997).

Congruence and incongruence in the Mediterranean basin. — The high level of congruence in phylogeographic pattern among species in the eastern Mediterranean basin, and the finding of two species groups (*Ca. maritima*, *Cr. maritimum*, *H. portulacoides* vs. *E. maritimum*, *S. kali*) in terms of phylogeographic pattern in the western Mediterranean basin probably can be explained by climatic conditions during the last glacial period. When considering the geographical position during the last glacial maximum (van Andel, 2002; Fig. 3) of those June/July/August temperature isotherms now found at the northern distributional limits of the species investigated (*Ca. maritima* subsp. *maritima*, 12°C, *S. kali* subsp. *kali* and *E. maritimum*, 14°C, *H. portulacoides* and *Cr. maritimum*, 16°C), it is obvious that these were pushed much further south in the western than in the eastern Mediterranean area. Assuming that these summer isotherms are either directly, as postulated for *H. portulacoides* by Chapman (1950), or indirectly responsible for the northern distributional limit of the studied species, all five of them, including those with the highest temperature requirements, should have been able to inhabit the eastern Mediterranean coast throughout the last glacial maximum and probably throughout all other glacials of the Quaternary. They thus are likely to have shared the same history, i.e., their distribution range in the eastern Mediterranean area probably was not affected by Quaternary temperature oscillations.

The subdivision of the eastern Mediterranean region into the Black Sea/Aegean Sea and Adriatic Sea clusters or groups of similar clusters in all species but *E. maritimum*, i.e., the subdivision of an essentially continuous distribution range, most likely results from sea currents as the probable primary dispersal agent of all species. As obvious from Fig. 4, the Aegean Sea is bordered by complex circular currents at its southern edges. The Adriatic Sea contains a large circular current system, and no major currents appear to connect them with each other or the Adriatic Sea with the western Mediterranean basin (Ovchinnikov, 1966; Lykousis & al., 2002). These currents apparently are a sufficient dispersal barrier for genetic divergence to evolve. No explanation can be offered for the exceptional behaviour of *E. maritimum*, in which the Adriatic material could not be separated from material from more western parts of the Mediterranean region.

In the western Mediterranean area, the shift of isotherms in combination with changes of coastline (Fig. 3) probably did not allow *H. portulacoides* and *Cr. maritimum* as the two most cold-sensitive species to survive the last glacial maximum along the north coast of this area. This hypothesis, based on the assumption of summer temperature (or summer temperature correlated) range limitations and the position of relevant isotherms during the last glacial maximum, is also supported by vegetation reconstructions. Vegetation along the north coast of the western Mediterranean basin has been claimed to have been boreal forest (Tichy & al., 2001), which today is located to the north of the distribution ranges of *H. portulacoides* and *Cr. maritimum*. Accordingly, these two species may have survived the last glacial maximum in refugia in the southwest and southeast of the western Mediterranean area. This might be recognizable in the genetic subdivision of the western Mediterranean material of both species, with material from the east of the western Mediterranean basin being more similar to the eastern Mediterranean material in both species, and material from the western west Mediterranean being either intermediate between the Atlantic and remaining Mediterranean material (*H. portulacoides*) or being part of the Atlantic cluster (*Cr. maritimum*). In the absence of dense population-level sampling, this hypothesis regarding the location of refugial and recolonized areas in the western Mediterranean can not be investigated further. In contrast to *H. portula-*

coides and *Cr. maritimum*, particularly *Ca. maritima* but also *S. kali* and *E. maritimum* should have had a continuous history of settlement along the north coast of the western Mediterranean. This is recognizable in the existence of uniform western Mediterranean clusters in *S. kali* and *E. maritimum*. We can offer no explanation for the exceptional behaviour of *Ca. maritima*, which shows essentially the same subdivision as *H. portulacoides*. This may indicate that other factors than temperature limited the distribution of this species in glacial periods.

The Gibraltar gap. — For all species except *Cr. maritimum* a distinct genetic gap was found at the Strait of Gibraltar. This gap is most pronounced in *E. maritimum* and *S. kali*, and less distinct in *H. portulacoides* and *Ca. maritima*. Except for *H. portulacoides*, it is the clearest separation in terms of among-region differentiation (Fig. 2). The highest among-region differentiation in *Cr. maritimum* instead is found between the Atlantic Ocean/eastern Spain material vs. some of the western Mediterranean material, and between other western Mediterranean material and Adriatic Sea accessions, and in *H. portulacoides* between the eastern Spanish and the southern France material (Fig. 2). For *E. maritimum* and *Ca. maritima* this confirms the findings by Clausing & al. (2000). Population-level sampling east and west of Gibraltar of *E. maritimum* and *Ca. maritima* confirms the existence of a strong barrier to gene flow in this area (Westberg & Kadereit, unpubl.) for both these species.

Based on their summer temperature requirements (or correlated factors), all four species potentially could have occupied a continuous distribution range along the Atlantic and Mediterranean coasts of southern Iberia during the last glacial maximum. This makes the existence of this gap difficult to understand. However, a very distinct gap between conspecific Atlantic Ocean and Mediterranean Sea populations has also been identified in many (Borsa & al., 1997a, b; Pérez-Losada, & al., 1999; Rios & al., 2002; Bargelloni & al., 2003; Nikula & Väinölä, 2003; Waters & Roy, 2003; Olsen & al., 2004) but not in all (Magoulas & al., 1996; Bargelloni & al., 2003) marine organisms. Where discussed in detail (Bargelloni & al., 2003), either the glacial fall of sea level, reducing the width and depth of the Strait of Gibraltar (Nilsson, 1982), or the presence of strong anticyclonic gyres in the Alboran Sea east of Gibraltar, resulting in the so-called Almeria-Oran front (Fig. 4), were responsible for this gap in marine species, both believed to have acted and still act as efficient dispersal barriers. In those species not showing this gap, either means of dispersal able to transgress this barrier or only recent colonization of the Mediterranean area from the Atlantic area have been advocated. The latter seems to be the case in *Cr. maritimum*, where the eastern Spanish material very clearly is part of the larger Atlantic cluster. A biogeographical barrier for terrestrial plants in the area of Gibraltar has been identified by Takhtajan (1986) and Meusel & Jäger (1992).

Interestingly, different depths of divergence between Atlantic and Mediterranean material could be identified among the five species investigated here. This is very similar to what Bargelloni & al. (2003) reported for a comparison of five species of fish in this area. Thus, in *S. kali*, ITS sequence data (Arafeh & Kadereit, unpubl.) indicate the existence of Atlantic and Mediterranean clades, which must have separated far earlier than the last glacial maximum but probably within the Quaternary. Equally, the existence of a distinct ITS clade of *H. portulacoides* along the Mediterranean coast of Spain (Arafeh & Kadereit, unpubl.) indicates an age of this split older than the last glacial maximum in this species. No such split was found in ITS sequences of *Ca. maritima* and *E. maritimum* (Westberg & Kadereit, unpubl.). For *S. kali* and *H. portulacoides* these findings may indicate that the origin of the Gibraltar gap dates back further than in *Ca. maritima* and *E. maritimum*. The amount of ITS sequence divergence in *S. kali* and *H. portulacoides* may indicate that hybrid incompatibility is the present cause of absence of gene flow in these two species. In the absence of experimental crossing data this, however, is entirely speculative.

Recolonization of the Atlantic Ocean/North Sea/Baltic Sea coasts. — If indeed the 12°C (*Ca. maritima*), 14°C (*S. kali* and *E. maritimum*) and 16°C (*H. portulacoides* and *Cr. maritimum*) June-July-August isotherms or closely correlated factors should have limited the northern distribution of the species investigated during the last glacial maximum, as supported by the reconstruction of last glacial maximum vegetation in the Mediterranean area (see above), they must have been restricted to the southern two- to one-third of the Iberian Atlantic coast in that period. In consequence, their present-day distribution along much of the Atlantic Ocean/North Sea/Baltic Sea coasts originated only in the Holocene. To some extent this hypothesis is supported by our data. When comparing genetic variation in the Atlantic and Mediterranean areas in terms of Shannon Index, genetic diversity is smaller, although partly only marginally, in the Atlantic than in the Mediterranean area (Table 2). Only in *E. maritimum* IBD is insignificant only in the Atlantic region. Otherwise IBD provides no clear signal for recency of colonization (Fig. 2).

The existence of a Baltic Sea cluster (including one geographical outlier from Scotland) in *Ca. maritima* is somewhat unexpected in view of the probably very recent colonization of northern latitudes and in view of the very young age—c. 8000 yrs (Björck, 1995)—of the Baltic Sea. This finding may indicate that phylogeographic pattern can arise within a few thousand years.

Why such pattern has arisen in the recent past in *Ca. maritima*, but not in *S. kali*, *H. portulacoides* and *Cr. maritimum*, remains to be explained.

Scale of dispersal. — To some extent our data permit conclusions to be drawn regarding the range of long-distance dispersal in the species studied. While most individuals sampled fall into geographically well-defined clusters, exceptions exist in all species except *E. maritimum* and have been listed above (Fig. 1 and Results). Assuming that these exceptionally placed individuals do not represent artifacts resulting from either confusion of samples or problems of AFLP data collection or analysis, they indicate that occasional dispersal over large distances, i.e., out of the cluster areas found here, is possible. On the other hand, when several individuals from single localities were analyzed, they fell into one cluster only in case of a French site of *H. portulacoides* (Fig. 1). In all other cases individuals from single populations did not form one cluster but (except for samples from Parco Nationale del Cicre/western Italy of *Cr. maritimum*; Fig. 1) grouped within their major phylogeographic group (Fig. 1). It thus seems justified to conclude that most dispersal takes place within the major phylogeographic areas identified (Black Sea/Aegean Sea - Adriatic Sea - western Mediterranean area - Atlantic Ocean/North Sea/Baltic Sea), but rather rarely between these areas. At a smaller scale, however, it has been shown that differentiation among populations can be found in *Posidonia oceanica* in the Tyrrhenian Sea (Procaccini & al., 2001).

CONCLUSIONS

Summarizing the above discussion, we believe that the patterns of phylogeographic structure at our scale of analysis are the result of the interplay of two pairs of biotic and abiotic factors.

First, fairly minor differences in species-specific temperature requirements or properties closely correlated with temperature requirements, in combination with temperature oscillations during the Quaternary explain differences found among species in the western Mediterranean basin. Here, the more cold-sensitive species (*H. portulacoides*, *Cr. maritimum*), but not the more cold-tolerant species (*S. kali*, *E. maritimum*), had to retreat from northern coasts. Their re-colonization of these areas is recognizable in the subdivision of the western Mediterranean cluster in the former two species. An exception to this is *Ca. maritima*.

Second, seed or fruit dispersal mainly by sea water in combination with present-day sea currents explain most major phylogeographic gaps identified in the essentially continuous distribution areas of all five species. This applies particularly to the Gibraltar gap, but also to the existence of distinct Black Sea/Aegean Sea and Adriatic Sea clusters.

When comparing the phylogeographic patterns found with biogeographic boundaries identified for either terrestrial plants or marine animals (and plants), great similarity is found with both. Considering that fruits or seeds of all species investigated except *E. maritimum* and *S. kali* show adaptations to dispersal by sea water, that all can float in sea water and remain viable there, and that the distribution ranges of all species are essentially continuous and are not interrupted in those regions where genetic discontinuities were identified, we conclude that dispersal of seeds and fruits by sea-water is of overriding importance in shaping the genetic structure of geographical ranges.

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Appendix. List of sampling localities with latitude and longitude of plants used in the AFLP analyses. When more than one plant from one locality was analyzed, the number of specimens is given in brackets after the name of the locality. Collectors: R. M. Arafeh - RMA; E. Westberg - EW; J. W. Kadereit - JWK; G. Kadereit - GK; B. von Hagen - BvH; P. Vargas - PV; M. Höhn - MH; K. Vickers - KV; M. Kropf - MK; R. Milne - RM; N. Schmalz - NS; O. Fragman - OF; E. Lambracht - EL; W. Licht - WL; A. Debez - AD; Unknown - NN, Botanical Garden Hamburg (Germany) - BGH; Botanical Garden Dublin (Ireland) - BGD; Botanical Garden Berlin-Dahlem (Germany) - BGB; Botanical Garden Cagliari (Italy) - BGC; Botanical Garden Chemnitz (Germany) - BGK. DNA vouchers have been deposited at MJG.

Cakile maritima Scop. – **Croatia**: Zadar, 44.06, 15.15, EW; **Cyprus**: Voroklini, 34.59, 33.39, BGH; **Denmark**: Hadarslev, 55.15, 9.30, JWK & GK; **England**: St. Andrews, 56.20, -2.47, RM; Exmouth, 50.35, -3.23, RMA & EW; Workington, 54.39, -3.34, KV; Camber, 50.55, 0.49, RMA & EW; East Head, 50.46, -0.55, RMA & EW; **France**: Grand Crohot Ocean (5), 44.49, -1.14, RMA & EW; St. Marie de la Mer, 43.27, 4.27, RMA & EW; Champ du Tir, 47.49, -3.17, RMA & EW; Le Crotoy, 50.14, 1.37, RMA & EW; Les Jars, 46.21, -1.22, RMA & EW; St. Aygulf, 43.24, 6.44, RMA & EW; Utah Beach, 49.25, -1.10, RMA & EW; St. Benoit des Ondes, 48.37, -1.51, RMA & EW; **Germany**: St. Peter Ording, 54.18, 8.37, JWK; Heiligenhafen, 54.22, 10.59, JWK; Ahrenshoop, 54.30, 12.35, JWK & GK; Peenemünde, 54.09, 13.46, JWK & GK; Juist, 53.41, 7.00, JWK & GK; **Greece**: Makri (5), 40.51, 25.41, RMA & EW; Igoumenitsa, 39.31, 20.11, RMA & EW; Lehena, 37.59, 21.15, RMA & EW; Nei Kios, 37.35, 22.45, RMA & EW; Nei Poroi, 39.59, 22.40, RMA & EW; Anthidona, 38.30, 23.26, RMA & EW; Olympiada, 40.35, 23.50, RMA & EW; **Iceland**: Arnastapi, 64.41, -24.03, PV; **Ireland**: Greystones, 53.08, -6.04, BGD; **Israel**: Herzliya, 32.10, 34.49, OF; **Italy**: Boca di Albegna, 42.30, 11.11, RMA & EW; Egnazia, 40.54, 17.22, RMA & EW; Marina Romea, 44.31, 12.17, RMA & EW; Lago di Fogliano, 41.23, 12.55, RMA & EW; Roccella, 38.20, 16.24, RMA & EW; Olmaia, 43.09, 10.31, RMA & EW; Marina di Lesina, 41.54, 15.21, RMA & EW; Punta Alicia, 39.24, 17.09, RMA & EW; Longobardi Marina, 39.12, 16.04, RMA & EW; Villasimius, 39.05, 9.33, BGC; St. Pollina, 38.01, 14.10, RMA & EW; Valle Cavanato, 45.44, 13.33, EL; Torre di Lago Puccini, 43.49, 10.16, RMA & EW; Marcelli, 43.29, 13.38, RMA & EW; Mondragone, 41.07, 13.53, RMA & EW; Marina di Pisticci, 40.18, 16.47, RMA & EW; **Morocco**: Agadir, 30.45, -9.66, RMA & EW; **Netherlands**: Petten, 52.46, 4.39, RMA & EW; Cadzand, 51.22, 3.25, RMA & EW; **Norway**: Berlevåg, 70.51, 29.06, NN; Brekstad, 63.41, 9.42, EL; **Portugal**: Porto Covo, 37.52, -8.47, RMA & EW; Torreira, 40.40, -8.42, RMA & EW; **Spain**: A Illa de Arousa, 42.33, -8.52, RMA & EW; Playa el Hoyo, 37.12, -7.17, RMA & EW; Ruimar Platja, 40.44, 0.51, RMA & EW; Playa la Espasa, 43.29, -5.13, RMA & EW; Punta de Tordera, 41.39, 2.46, RMA & EW; Platja las Alberquillas, 36.45, -3.50, RMA & EW; Playa Porcia, 43.34, -6.53, RMA & EW; Rio Oka, 43.24, -2.41, RMA & EW; Playa de Montillo, 36.46, -6.24, RMA & EW; Playa de Guardias Viejas, 36.42, -2.49, RMA & EW; Cabo Pino, 36.29, -4.44, RMA & EW; Playa Jandia, 28.03, -14.20, EW; **Sweden**: Åhus, 56.01, 14.20, EW; **Tunisia**: Raouel, 36.54, 10.18, AD; Jerba, 33.52, 10.55, AD; **Turkey**: Igneada, 41.53, 28.03, RMA & EW; Esenköy, 40.37, 28.57, RMA & EW; Akpinar, 41.18, 28.49, RMA & EW; Odun Isk, 39.45, 26.07, RMA & EW.

Crithmum maritimum L. – **Croatia**: Senj, 45.00, 14.54, EW; Zadar, 44.06, 15.15, EW; Split, 43.30, 16.27, EW; **England**: Sandwich (4), 51.16, 1.23, RMA & EW; Sandy Bay, 51.22, -2.56, RMA & EW; Little Hampton, 50.47, -0.34, RMA & EW; Lullworth Cove, 50.37, -2.14, RMA & EW; **France**: Beg Legeur, 48.44, -3.32, RMA & EW; Corniche dell Armorique, 48.40, -3.39, RMA & EW; Champ du Tir, 47.49, -3.17, RMA & EW; Pen-Ar-Ran, 47.22, -2.33, RMA & EW; Le Collet, 47.02, -2.02, RMA & EW; St. Aygulf, 43.24, 6.44, RMA & EW; Gines, 43.02, 6.06, RMA & EW; **Greece**: Makri, 40.51, 25.41, RMA & EW; Olympiada, 40.35, 23.50, RMA & EW; Igoumenitsa, 39.31, 20.11, RMA & EW; Agria, 39.21, 22.59, RMA & EW; Preveza, 39.04, 20.40, RMA & EW; Anthidona, 38.30, 23.26, RMA & EW; Palea Epidavros, 37.38, 23.10, RMA & EW; Githio, 36.46, 22.34, RMA & EW; **Italy**: Parco Nazionale del Circe (5), 41.22, 12.57, RMA & EW; Trieste, 54.36, 13.47, RMA & EW; Laguna di Venezia, 45.28, 12.34, JWK; Moneglia, 44.14, 9.29, RMA & EW; Bergeggio, 44.14, 8.27, RMA & EW; Porto Civitianova, 43.18, 13.44, RMA & EW; Boca di Albegna, 42.30, 11.11, RMA & EW; Marina di Savito, 42.18, 12.51, RMA & EW; Marina di Montenero, 42.04, 14.48, RMA & EW; Pyros, 42.02, 11.57, RMA & EW; Gargano, 41.47, 16.12, WL; Egnazia, 40.54, 17.22, RMA & EW; Villamare, 40.05, 15.31, RMA & EW; Longobardi Marina, 39.12, 16.04, RMA & EW; Villasimius, 39.05, 9.33, BGC; San Ferdinando, 38.30, 15.45, RMA & EW; Roccella, 38.20, 16.24, RMA & EW; St. Pollina, 38.01, 14.10, RMA & EW; **Portugal**: Praia de Vieira, 39.52, -8.59, RMA & EW; Largo de Praja da Arguda, 38.51, -9.28, RMA & EW; Porto Covo, 37.52, -8.47, RMA & EW; Armacao de Pera, 37.06, -8.21, RMA & EW; **Spain**: Rinlo (5), 43.33, -7.05, RMA & EW; Playa la Espasa, 43.29, -5.13, RMA & EW; Las Dunas de Liencres, 43.27, -3.59, RMA & EW; Paredes, 43.24, -8.12, RMA & EW; Playa de Laida, 43.24, -2.39, RMA & EW; A Illa de Arousa, 42.33, -8.52, RMA & EW; Punta de Tordera, 41.39, 2.46, RMA & EW; Sant Pol del Mar, 41.36, 2.37, RMA & EW; Ruimar Platja, 40.44, 0.51, RMA & EW; Platja de l'almadraba, 38.51, 0.01, RMA & EW; Platja las Alberquillas, 36.45, -3.50, RMA & EW; Plaja de San Miguel, 36.43, -2.52, RMA & EW; Playa de la Barrosa, 36.21, -6.12, RMA & EW; Cueta, 35.54, -5.17, RMA & EW; **Turkey**: Sevketiye (4), 40.24, 26.40, RMA & EW; Eregli, 41.19, 31.26, RMA & EW; Gaziköy, 40.43, 27.18, RMA & EW; Esenköy, 40.37, 28.57, RMA & EW; Bandirma Bay, 40.23, 27.23, RMA & EW; Kücükkuyu, 39.31, 26.26, RMA & EW.

Eryngium maritimum L. – **Croatia**: Medullin, 44.49, 13.56, MK; **Cyprus**: Voroklini, 34.59, 33.39, BGH; **England**: East Head, 50.46, -0.55, RMA & EW; Sandwich, 51.16, 1.23, RMA & EW; Kessingland, 52.23, 1.43, BvH; **France**: Champ du Tir, 47.49, -3.17, RMA & EW; Le Crotoy, 50.14, 1.37, RMA & EW; Les Jars, 46.21, -1.22, RMA & EW; St. Aygulf, 43.24, 6.44, RMA & EW; Grand Fort Phillip, 51.00, 2.06, RMA & EW; Plage L'Aber, 48.14, -4.26, RMA & EW; Agde, 43.17, 3.26, RMA & EW; Berriaud Plage, 43.06, 6.12, RMA & EW; Pen-Ar-Ran, 47.22, -2.33, RMA & EW; Gatteville, 49.41, -1.18, RMA & EW; Penven, 48.46, -3.35, RMA & EW; Plage du Port Hue, 48.38, -2.10, RMA & EW; Hourtin Plage, 45.13, -1.10, RMA & EW; Cap Breton, 43.37, -1.27, RMA & EW; St. Cyprien Plage, 42.40, 3.02, RMA & EW; **Germany**: Ahrenshoop (5), 54.30, 12.35, JWK & GK; Heiligenhafen, 54.22, 10.59, JWK; Peenemünde, 54.09, 13.46, JWK & GK; Schillig, 53.43, 8.02, JWK & GK; **Greece**: Makri, 40.51, 25.41, RMA & EW; Igoumenitsa, 39.31, 20.11, RMA & EW; Lehena, 37.59, 21.15, RMA & EW; Nei Poroi, 39.59, 22.40, RMA & EW; Anthidona, 38.30, 23.26, RMA & EW; Olympiada, 40.35, 23.50, RMA & EW; Erasmio, 40.52, 24.50, RMA & EW; Nei Anchialos, 39.15, 22.49, RMA & EW; Preveza, 39.04, 20.40, RMA & EW; Kyparissia, 37.19, 21.41, RMA & EW; **Ireland**: Kilcoole, 53.06, -6.04, BGD; **Italy**: Boca di Albegna, 42.30, 11.11, RMA & EW; Marina Romea, 44.31, 12.17, RMA & EW; Lago di Fogliano, 41.23, 12.55, RMA & EW; Roccella, 38.20, 16.24, RMA & EW; Olmaia, 43.09, 10.31, RMA & EW; Marina di Lesina, 41.54, 15.21, RMA & EW; Punta Alicia, 39.24, 17.09, RMA & EW; Longobardi Marina, 39.12, 16.04, RMA & EW; Villasimius, 39.05, 9.33, BGC; St. Pollina, 38.01, 14.10, RMA & EW; Torre di Lago Puccini, 43.49, 10.16, RMA & EW; Marcelli, 43.29, 13.38, RMA & EW; Mondragone, 41.07, 13.53, RMA & EW; Marina di Pisticci, 40.18, 16.47, RMA & EW;

Appendix (continued).

Playa de la Barrosa, 36.21, -6.12, RMA & EW; Playa de Navia, 43.33, -6.43, RMA & EW; Praia Grande de Mino, 43.22, -8.12, RMA & EW; El Pinet, 38.09, -0.37, RMA & EW; Mar Menor, 37.43, -0.44, RMA & EW; Puerto del Rey, 37.12, -1.48, RMA & EW; La Algarda, 36.49, -6.20, RMA & EW; Algeciras River, 36.10, -5.27, RMA & EW; **Turkey**: Gallipoli (5), 40.36, 26.50, RMA & EW; Bandirma Bay, 40.23, 27.23, RMA & EW; Kücükkuyu, 39.31, 26.26, RMA & EW; Yeniciftlik, 40.59, 27.51, RMA & EW; Cardak, 40.23, 26.24, RMA & EW; Gömec, 39.24, 26.46, RMA & EW.

Halimione portulacoides (L.) Aellen – **Croatia**: Split, 43.30, 16.27, EW; Zadar, 44.06, 15.15, EW; **England**: Braunton Burrows (3), 51.04, -4.12, RMA & EW; Sandwich, 51.16, 1.23, RMA & EW; Little Hampton, 50.47, -0.34, RMA & EW; Lullworth Cove, 50.37, -2.14, RMA & EW; Walpersweck, 52.18, 1.39, BvH; River Otter's mouth, 50.37, -3.18, RMA & EW; **France**: St. Marie de la Mer (5), 43.27, 4.27, RMA & EW; Champ du Tir, 47.49, -3.17, RMA & EW; Grand Fort Phillip, 51.00, 2.06, RMA & EW; Le Dourduff, 48.37, -3.50, RMA & EW; Mont St. Michel, 48.37, -1.30, RMA & EW; Le Greves, 48.30, -2.41, RMA & EW; Plage L'Aber, 48.14, -4.26, RMA & EW; Saille , 47.18, -2.26, RMA & EW; Charron, 46.18, -1.08, RMA & EW; Neyran, 45.30, -1.05, RMA & EW; Avdenge, 44.41, -1.00, RMA & EW; Agde, 43.17, 3.26, RMA & EW; Berriaud Plage, 43.06, 6.12, RMA & EW; Canet, 42.40, 3.02, RMA & EW; **Germany**: Norderney (2), 53.43, 7.15, JWK & GK; St. Peter Ording, 54.18, 8.37, JWK; Langeness, 54.38, 8.35, JWK & GK; Harlesiel, 53.42, 7.48, JWK & GK; **Greece**: Igoumenitsa, 39.31, 20.11, RMA & EW; Erasmio, 40.52, 24.50, RMA & EW; Potamos, 40.23, 22.55, RMA & EW; Nei Anchialos, 39.15, 22.49, RMA & EW; Lehena, 37.59, 21.15, RMA & EW; Nei Kios, 37.35, 22.45, RMA & EW; Makri, 40.51, 25.41, RMA & EW; Preveza, 39.04, 20.40, RMA & EW; Githio, 36.46, 22.34, RMA & EW; Strimonas, 40.47, 23.51, RMA & EW; Nei Poroi, 39.59, 22.40, RMA & EW; Evia (Oreoi), 38.57, 23.05, Not known; **Israel**: Yerekon River, 34.54, 32.08, OF; **Italy**: Boca di Albegna, 42.30, 11.11, RMA & EW; Egnazia, 40.54, 17.22, RMA & EW; Marina Romea, 44.31, 12.17, RMA & EW; Porto Civitianova, 43.18, 13.44, RMA & EW; Riva Longa, 45.45, 13.31, RMA & EW; Gargano, 41.57, 16.01, WL; Lago di Fogliano, 41.23, 12.55, RMA & EW; **Portugal**: Armacao de Pera, 37.06, -8.21, RMA & EW; Bestiada, 40.46, -8.41, RMA & EW; Foz de Arelho, 39.27, -9.13, RMA & EW; **Spain**: A Illa de Arousa (5), 42.33, -8.52, RMA & EW; Playa de Laida, 43.24, -2.39, RMA & EW; Playa el Serrada, 40.00, -0.01, RMA & EW; Playa el Hoyo, 37.12, -7.17, RMA & EW; Las Dunas de Liencres, 43.27, -3.59, RMA & EW; Ruimar Platja, 40.44, 0.51, RMA & EW; Playa de la Barrosa, 36.21, -6.12, RMA & EW; Playa de Navia, 43.33, -6.43, RMA & EW; Praia Grande de Mino, 43.22, -8.12, RMA & EW; El Pinet, 38.09, -0.37, RMA & EW; Mar Menor, 37.43, -0.44, RMA & EW; Puerto del Rey, 37.12, -1.48, RMA & EW; La Algarda, 36.49, -6.20, RMA & EW; Algeciras River, 36.10, -5.27, RMA & EW; **Turkey**: Gallipoli (5), 40.36, 26.50, RMA & EW; Bandirma Bay, 40.23, 27.23, RMA & EW; Kücükkuyu, 39.31, 26.26, RMA & EW; Yeniciftlik, 40.59, 27.51, RMA & EW; Cardak, 40.23, 26.24, RMA & EW; Gömec, 39.24, 26.46, RMA & EW.

Salsola kali L. – **Croatia**: Split, 43.30, 16.27, EW; **Denmark**: Jerup strand, 57.31, 10.25, JWK & GK; Løgstør, 56.58, 9.15, JWK & GK; **England**: Sandwich, 51.16, 1.23, RMA & EW; Sandy Bay, 51.22, -2.56, RMA & EW; St. Andrews, 56.20, -2.47, RM; Mappelton, 53.52, -0.08, BvH; Braunton Burrows, 51.04, -4.12, RMA & EW; Romney Sand, 50.58, 0.58, RMA & EW; Wittering, 50.46, -0.55, RMA & EW; Exmouth, 50.35, -3.23, RMA & EW; **France**: Bourgneut en Rete (4), 47.02, -2.02, RMA & EW; Le Crotoy, 50.14, 1.37, RMA & EW; St. Efflam, 48.44, -3.16, RMA & EW; St. Benoit des Ondes, 48.37, -1.51, RMA & EW; Les Jars, 46.21, -1.22, RMA & EW; **Germany**: Boinsdorf (5), 54.01, 11.32, JWK & GK; Fehrman, 54.30, 11.13, JWK & GK; Prerow, 54.26, 12.34, JWK & GK; Heiligenhafen, 54.22, 10.59, JWK; St. Peter Ording, 54.18, 8.37, JWK; Zinnowitz, 54.04, 13.54, JWK & GK; Budenheim, 50.01, 8.10, NS; Gonsenheim, 50.00, 8.11, NS; **Greece**: Igoumenitsa, 39.31, 20.11, RMA & EW; Anthidona, 38.30, 23.26, RMA & EW; Erasmio, 40.52, 24.50, RMA & EW; Potamos, 40.23, 22.55, RMA & EW; Nei Anchialos, 39.15, 22.49, RMA & EW; Lehena, 37.59, 21.15, RMA & EW; Nei Kios, 37.35, 22.45, RMA & EW; **Hungary**: Tahito (4), 47.54, 19.06, MH; Soroksar (2), 47.24, 19.07, MH; **Italy**: Boca di Albegna (3), 42.30, 11.11, RMA & EW; Egnazia, 40.54, 17.22, RMA & EW; Villamare, 40.05, 15.31, RMA & EW; Roccella, 38.20, 16.24, RMA & EW; Marina Romea, 44.31, 12.17, RMA & EW; Olmaia, 43.09, 10.31, RMA & EW; Marina di Lesina, 41.54, 15.21, RMA & EW; St. Salvador, 41.11, 1.32, RMA & EW; Punta Alicia, 39.24, 17.09, RMA & EW; Foce di Simeto, 37.24, 15.06, RMA & EW; **Netherlands**: Hoofdplaat, 51.22, 3.39, RMA & EW; **Portugal**: Cabeledo, 41.41, -8.50, RMA & EW; **Spain**: Playa la Espasa, 43.29, -5.13, RMA & EW; Playa de Laida, 43.24, -2.39, RMA & EW; A Illa de Arousa, 42.33, -8.52, RMA & EW; Plaja de San Miguel, 36.43, -2.52, RMA & EW; Cueta, 35.54, -5.17, RMA & EW; Playa el Serrada, 40.00, -0.01, RMA & EW; Platja d'Oliva, 38.55, -0.05, RMA & EW; Playa la Cabana, 37.23, -1.37, RMA & EW; Playa el Hoyo, 37.12, -7.17, RMA & EW; Playa de los Lances, 36.01, -5.37, RMA & EW; **Sweden**: Viken, 56.09, 12.34, EW; Nyehusen, 55.50, 14.13, EW; Lernacken, 55.33, 12.53, EW; **Turkey**: Sevketye, 40.24, 26.40, RMA & EW; Eregli, 41.19, 31.26, RMA & EW; Bandirma Bay, 40.23, 27.23, RMA & EW; Igneada, 41.53, 28.03, RMA & EW; Sahilköy, 41.12, 29.23, RMA & EW; Karasu, 41.08, 30.40, RMA & EW; Yaluva, 40.39, 29.12, RMA & EW; Kumkale, 40.01, 26.11, RMA & EW.