Determinants of phylogeographic structure:
a comparative study of seven coastal flowering plant species across their European range

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ABSTRACT

We here synthesize our own mostly published work on the phylogeographic structure of seven flowering plant species (*Cakile maritima*, *Calystegia soldanella*, *Eryngium maritimum*, *Salsola kali*, *Halimione portulacoides*, *Triglochin maritima* and *Crithmum maritimum*) widely distributed along the European coasts. Three categories of factors were identified to determine phylogeographic structure and are illustrated with examples. These are 1) historical abiotic factors such as Quaternary climatic changes resulting in sea level changes which may explain, e.g., a phylogeographic break in the Dardanelles and Bosporus straits, 2) modern abiotic factors such as sea currents important for the dispersal of fruits or seeds of most of the species investigated which may explain, e.g., a phylogeographic break in the Strait of Gibraltar, and 3) factors related to the specific biology of the species which may explain, e.g., the absence of phylogeographic structure in *Calystegia soldanella* resulting from clonal growth and long-distance dispersal. These factors also act in all possible combinations. The combination of 1) and 2) may be responsible for differential magnitudes in different species of the Dardanelles and Bosporus genetic breaks, that of 2) and 3) for interspecific differences in the Gibraltar break, and 1) and 3) may explain the existence of phylogeographic breaks along the north coast of the west Mediterranean basin present in only some species, and also the deviant behaviour of *Triglochin maritima* which colonized northern European coasts from inland refugia.

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

INTRODUCTION

In recent years, the development of appropriate molecular methods has enabled the analysis of the geographical distribution of intraspecific genetic variation. The phylogeographic structure most commonly found is interpreted as the result of range shifts in response to Quaternary

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climatic oscillations (reviewed in, e.g., Soltis *et al.* 1997; Comes & Kadereit 1998, 2003; Taberlet *et al.* 1998; Hewitt 2000, 2004; Abbott & Brochmann 2003; Brochmann *et al.* 2003; Stehlik 2003; Tribsch & Schönswetter 2003; Lascoux *et al.* 2004). Intraspecific genetic structure, however, is also likely to be determined by species-specific biological attributes (Hamrick & Godt 1989; Nybom & Bartish 2000), and by modern barriers to gene flow. These three categories of factors influencing phylogeographic structure are not easy to separate.

In this context, we chose to investigate the geographical distribution of intraspecific variation in 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 current barriers to gene flow and of species-specific biology. 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, coastal species have an essentially linear distribution range. This limits the spatial options for migration and facilitates the reconstruction of distributional limits in Quaternary glacials.

The seven plant taxa investigated are Sea Rocket *Cakile maritima* Scop. (Brassicaceae), an annual often growing in the drift-line of sandy beaches or on shingle, Prickly Saltwort *Salsola kali* L. subsp. *kali* (Chenopodiaceae), another annual from essentially the same habitat as *Cakile maritima*, Sea Holly *Eryngium maritimum* L. (Apiaceae), a perennial from mostly stabilized sandy habitats such as dunes, Sea Bindweed *Calystegia* (= *Convolvulus*) *soldanella* (L.) R. et Sch. (Convolvulaceae), a
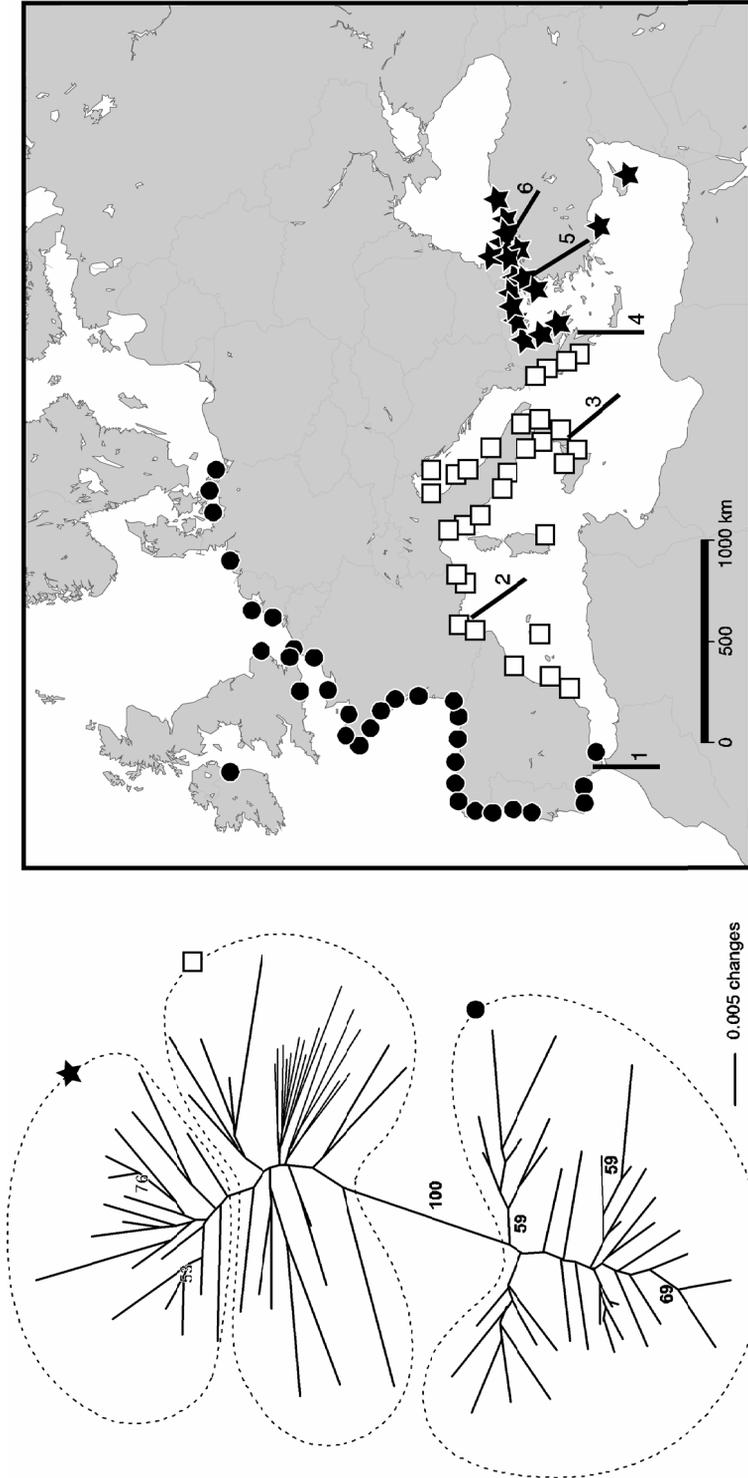
FIGURE 1. Geographical distribution of clusters obtained by Neighbor Joining analysis of Nei and Li's genetic distances between individuals. The analysis is exemplified with *E. maritimum*. The NJ phenogram was inspected for genetic clusters of individuals originating from a geographically cohesive area. Such clusters and the sampling localities of their constituent individuals are marked with the same symbol. The population genetic data of *E. maritimum* from sea straits have been analyzed separately and the results have been incorporated in the figure (see below). For *C. maritima, Cr. maritima, H. portulacoides* and *S. kali* the positions of intraspecific genetic gaps are indicated by thick lines (numbered from one to six). The location of these gaps are (1) the Strait of Gibraltar (*C. maritima, E. maritimum, H. portulacoides, S. kali*), (2) southwest France (*C. maritima, Cr. maritima, H. portulacoides*), (3) south Italy (*C. maritima, Cr. maritima, H. portulacoides, S. kali*), (4) south Greece (*C. maritima, Cr. maritima, E. maritimum, H. portulacoides, S. kali*), (5) the Dardanelles strait (*C. maritima*) and (6) the Bosporus strait (*C. maritima, E. maritimum*).

perennial creeper from dunes and sandy beaches, Rock Samphire *Crithmum maritimum* L. (Apiaceae), a perennial from rocky shores, and Sea Purslane *Halimione portulacoides* (L.) Aellen (Chenopodiaceae) and Sea Arrowgrass *Triglochin maritima* L. (Juncaginaceae), both perennials from salt marshes.

We here summarize our published studies on these seven species (Kadereit *et al.* 2005; Arafeh & Kadereit 2006; Lambracht *et al.* 2007) and unpublished work on population level analyses of *Cakile maritima* and *Eryngium maritimum* across sea straits between the North and Baltic Seas, the Atlantic Ocean and the Mediterranean Sea, and the Aegean, Marmara and Black Seas (Westberg & Kadereit in prep.). Based on previous conclusions, we here offer a new synthesis of our results.

MATERIAL AND METHODS

PLANT MATERIAL

Leaf material of the seven species was collected every 100–200 km along the coast from the Turkish coast of the Black Sea to the southeast coast of Sweden on the Baltic Sea and dried in silica gel to preserve DNA. A few collections from outside this range were also included as well as inland material of *S. kali* and *T. maritima*. The latter species is rare in the Mediterranean area and is therefore represented from only three localities in that region. A few localities were represented by up to seven individuals in each species (Kadereit *et al.* 2005), and seven additional populations were sampled in *C. soldanella* from the same general range (Arafeh & Kadereit 2006). In *C. maritima* and *E. maritimum* population samples were taken on a more regional scale around the Bosporus and Dardanelles straits, the Strait of Gibraltar and along the coast of west France, as well as in the Baltic Sea and Kattegat in *C. maritima*. Usually, ten individuals from ten populations were sampled in each region.

AFLP PROCEDURE AND DATA ANALYSIS

Details of DNA isolation and the AFLP protocol (amplified fragment length polymorphism; Vos *et al*. 1995) can be found in Kadereit *et al.* (2005), Arafeh & Kadereit (2006) and Lambracht *et al.* (2007). To detect geographical structure of genetic variation, cluster analyses were performed on the AFLP data. In most cases individuals were clustered with Neighbour Joining (NJ; Saitou & Nei 1987) based on Nei and Li's genetic distances (Nei & Li 1979). In general bootstrap support for clusters was low. Therefore, the resulting phenograms were visually inspected for clusters or groups of genetically similar clusters corresponding to cohesive geographical areas. Such clusters or groups of clusters contained all or nearly all individuals from a continuous area (see Fig. 1 for more details). For the population samples of *C. maritima* and *E. maritimum* individuals were clustered with a Bayesian method implemented in BAPS 3.2 (Corander *et al.* 2003; 2006). With this method clusters are recognized without using information on the geographical origin of samples. For more details about methods see Kadereit *et al.* (2005), Arafeh & Kadereit (2006), Lambracht *et al.* (2007) and Westberg & Kadereit (in prep.).

RESULTS

CAKILE MARITIMA, CRITHMUM MARITIMUM, ERYNGIUM MARITIMUM, HALIMIONE PORTULACOIDES AND SALSOLA KALI

Little geographic substructure was found along the Atlantic and northern European coasts. Only in *C. maritima* a genetic cluster dividing Baltic Sea material (including Skagerrak and Kattegat) from the rest was detected.

Atlantic – Mediterranean subdivision. In four (*C. maritima*, *E. maritimum*, *H. portulacoides* and *S. kali*) of the five species sampled from southern Iberia a genetic gap was found between Atlantic and Mediterranean material. This gap coincided with the Strait of Gibraltar in *C. maritima*, *H. portulacoides* and *S. kali*, and included one sample slightly east of the strait in *E. maritimum* (Fig. 1). The differentiation between these two clusters was especially marked in *E. maritimum* and *S. kali*, whereas in *C. maritima* and *H. portulacoides* the genetic gap was of smaller magnitude. In *Cr. maritimum* no genetic gap was found between the Atlantic and Mediterranean material, and instead the Atlantic cluster traverses the Strait of Gibraltar and reaches the Mediterranean coast of northeast Spain.

The population level analysis of *C. maritima* and *E. maritimum* from this area also showed a distinct gap between Atlantic and Mediterranean samples, in both species occurring between Tarifa and Algeciras. As in the large scale analysis, the magnitude of differentiation between the two regions was higher in *E. maritimum* than in *C. maritima*.

The Mediterranean. In three species the clustering showed a subdivision of material along the south coast of France (Fig. 1). Samples of *H. portulacoides* from east Spain appear as a distinct cluster and material from France and western Italy forms several closely related clusters. A similar pattern is seen in *Cr. maritimum* where the Atlantic cluster reaches northeast Spain. In *C. maritima* two western Mediterranean clusters overlap in northeast Spain and southern France. In *E. maritimum* and *S. kali* the samples from the west Mediterranean basin all fall into the same geographical cluster or the same group of clusters.

In all species except *E. maritimum* there is a subdivision of eastern and western Mediterranean samples with Sicily as the approximate geographical border. Italian samples of *S. kali* from the Ionian Sea fall into the western Mediterranean cluster but otherwise samples from the Adriatic and Ionian Seas fall into their own cluster in *C. maritima*, *Cr. maritimum*, *H. portulacoides* and *S. kali*. With the exception of *C. maritima* this cluster additionally includes some samples from the western Aegean Sea. East of the Ionian Sea the Aegean and Black Sea samples form additional geographical clusters in all five species. Although no further subdivision of these clusters was found in the large-scale analysis, the population level analysis from the straits between the Black Sea, Marmara Sea and Aegean Sea revealed clear genetic gaps at the Bosporus strait in *C. maritima* and *E. maritimum* as well as at the Dardanelles strait in *C. maritima*. The amount of genetic differentiation in the Bosporus was higher than in the Dardanelles in *C. maritima*, but in both cases lower than in *E. maritimum* from the Bosporus.

TRIGLOCHIN MARITIMA

In the NJ (Fig. 2) and Bayesian analyses two groups were inferred. One Atlantic group contained samples from the Portuguese, Spanish and French coasts and the British west coast, and another group consisted of material from the North Sea, Baltic Sea and Mediterranean coasts as well as Norwegian and Central European inland material.

CALYSTEGIA SOLDANELLA

The results from *C. soldanella* contrasted with those from the other species in that no geographical clusters could be identified in the NJ analysis (Fig. 3). Any genetic cluster recognized in the large scale or population level analyses consisted of geographically widely scattered individuals.

DISCUSSION

Our comparative analysis of phylogeographic patterns in seven flowering plant species of partly different biology, and the consideration of historical and modern abiotic factors allow us to formulate hypotheses about the possible causes of the patterns observed.

DARDANELLES AND BOSPORUS: EVIDENCE FOR THE PERSISTENCE OF HISTORICAL GENE FLOW BARRIERS

In the population level analysis of *C. maritima* and *E. maritimum*, a distinct genetic gap was found in the Bosporus, and also in the Dardanelles in *C. maritima*. This gap was not detected either in these two species or in the other species investigated in the large scale analysis of Kadereit *et al.* (2005), probably as a result of limited sampling of only individual genotypes in this area. Following Kadereit *et al.* (2005), both species could have grown along the coasts of this area during the last glacial maximum (LGM). During the Quaternary glacials the sea levels of the Black Sea, the Sea of Marmara and the Aegean Sea were repeatedly lower than the sills of the Dardanelles and the Bosporus (Aksu *et al.* 2002). This resulted in the isolation of the three seas from each other during such periods. As a consequence, populations in these areas would have been isolated from each other. On the other hand, currents in both the Bosporus and the Dardanelles are unidirectional outflows from the Black Sea to the Marmara Sea and from here to the Aegean Sea. These currents should result in high westward gene flow. The combination of geological history and modern sea currents in conclusion suggest that the genetic differentiation across the Bosporus (and the Dardanelles in *C. maritima*) is the result of historical isolation during the LGM.

STRAIT OF GIBRALTAR: SEA CURRENTS AS A BARRIER TO GENE FLOW

For all species growing in the Gibraltar area except *Cr. maritimum* a more or less distinct genetic gap was found. This gap is most pronounced in *E. maritimum* and *S. kali*, and less distinct in *H. portulacoides* and *C. maritima* in the large scale analysis (Kadereit
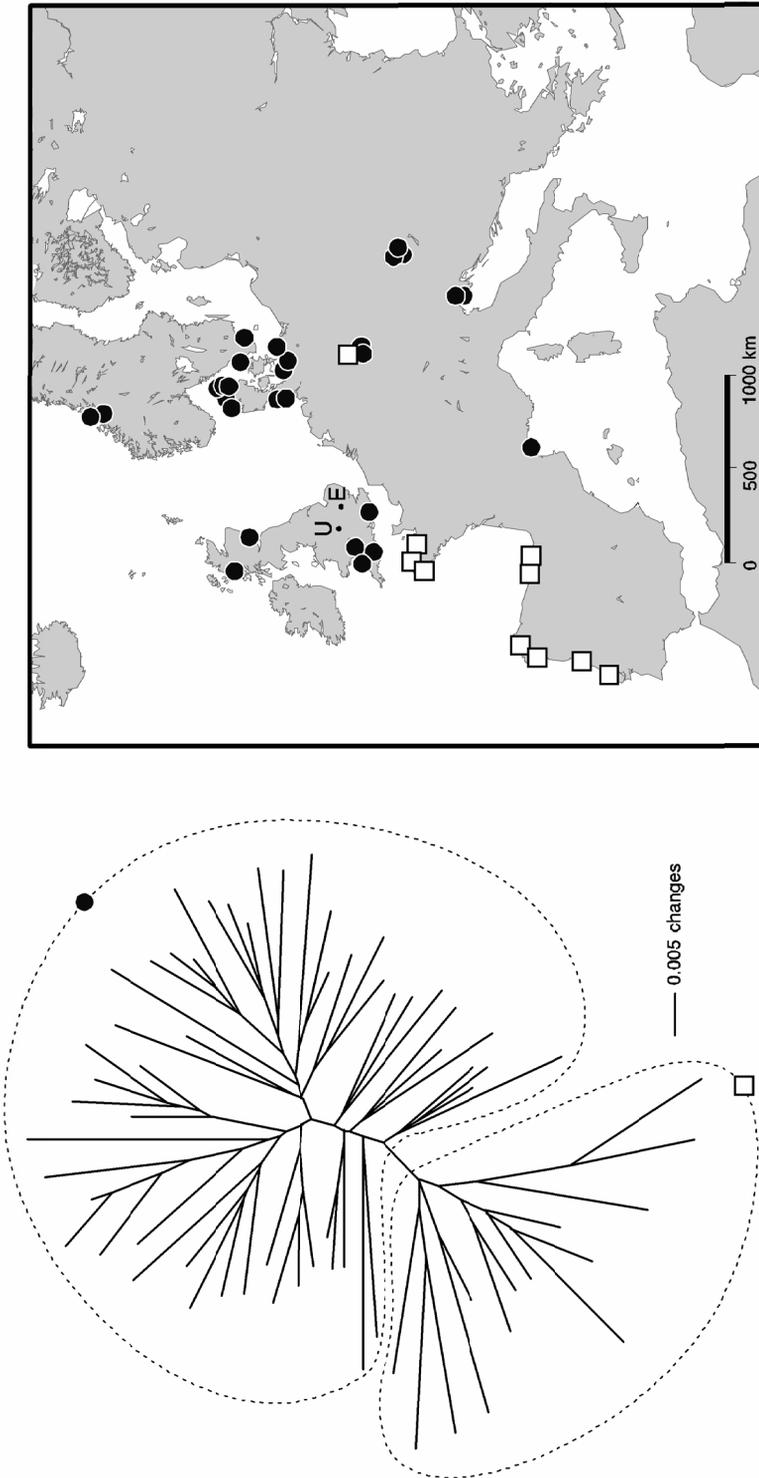
FIGURE 2. Neighbor Joining analysis and geographical distribution of clusters in *T. maritima*. Squares represent individuals belonging to an Atlantic group and circles samples assigned to a North Sea/Baltic Sea group that also contains Mediterranean and Norwegian coastal and Central European inland material. The letters E and U indicate sites of fossil *T. maritima*.
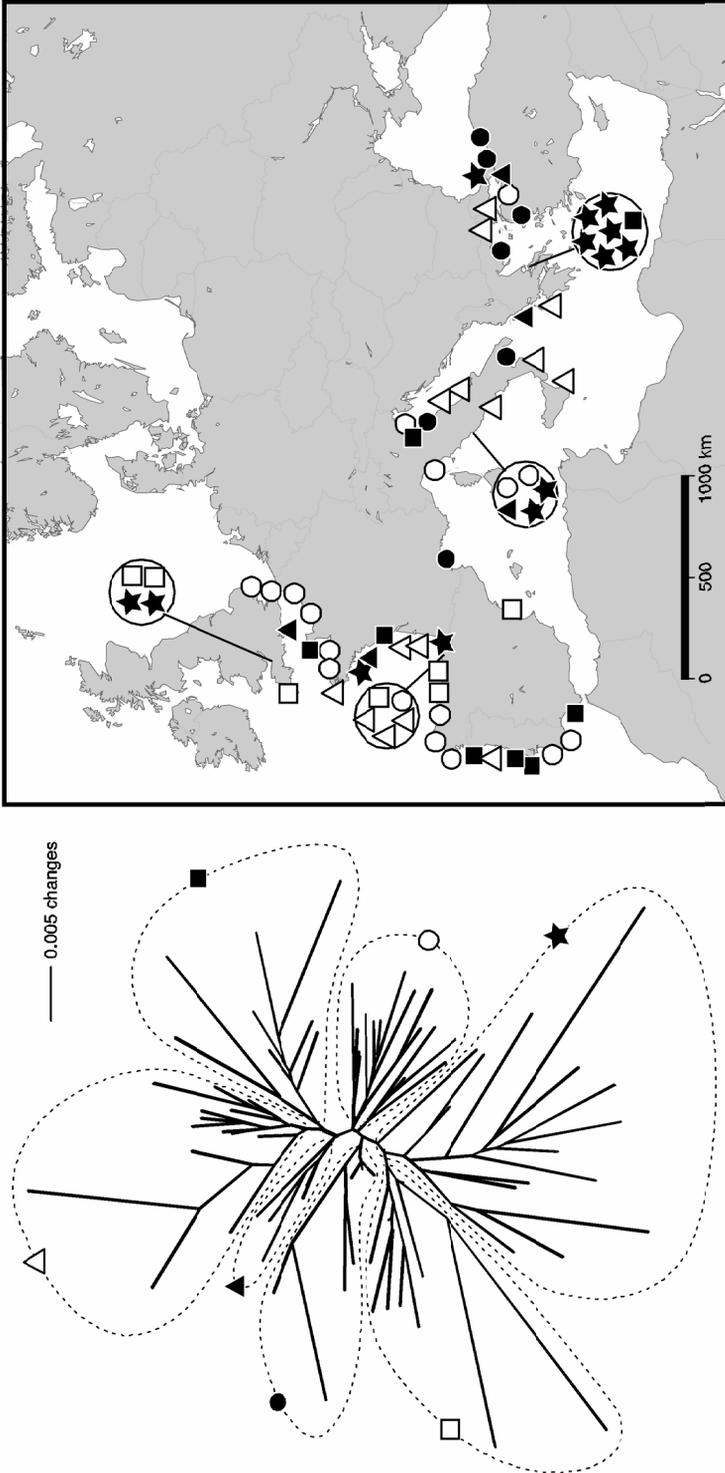
FIGURE 3. Neighbour Joining analysis and geographical distribution of clusters in *C. soldanella*. Individuals do not group according to geographical origin.

et al. 2005), and distinct and of geographically identical position in the population level analysis of *C. maritima* and *E. maritimum*. Except for *H. portulacoides*, it is the clearest separation in terms of among region genetic differentiation (Kadereit *et al.* 2005). The Strait of Gibraltar has been open for the last c. 5my (Krijgisman *et al.* 1999), and the coasts of the area were likely to have been inhabitable for all species concerned during the LGM (Kadereit *et al.* 2005). This implies that only a modern barrier to gene flow across the Gibraltar strait can be responsible for the genetic gap found. This gene flow barrier could be in the form of sea currents. The surface currents in the Strait of Gibraltar are influenced by the tidal differences between the Gulf of Cadiz to the west and the Alboran Sea on the east side of the strait (Rey 1983). During high tide surface water is flowing east into the Mediterranean and at low tide the currents are reversed. Possibly the gene flow barrier arises because the changes in sea current direction make it difficult for seeds to pass the strait. This explanation implies that dispersal of the coastal flowering plant species investigated here is largely by sea currents. Similar explanations have been proposed to explain the existence of a genetic gap in the Gibraltar area in several (but not all) marine organisms investigated (Magoulas *et al.* 1996; Borsa *et al.* 1997a, b; Pérez-Losada, *et al.* 1999; Rios *et al.* 2002; Bargelloni *et al.* 2003; Nikula & Väinölä 2003; Waters & Roy 2003; Olsen *et al.* 2004). The exceptional behaviour of *Cr. maritimum*, where the eastern Spanish material is very clearly part of the larger Atlantic cluster (Kadereit *et al.* 2005) is perhaps best explained by a recent colonization of the Mediterranean coast of Spain from the Atlantic coast. This does not explain, however, how *Cr. maritimum* could surmount the Gibraltar barrier postulated here to act in the other species investigated.

CALYSTEGIA SOLDANELLA: CLONAL GROWTH AND LONG-DISTANCE DISPERSAL PREVENT THE FORMATION OF PHYLOGEOGRAPHIC PATTERN

Among the species investigated, *C. soldanella* is exceptional in showing essentially no phylogeographic structure. This was found not only in the large scale analysis, but also in a population level study where individuals from geographically widely separated populations did not group according to population (Arafeh & Kadereit 2006). A postglacial arrival of *C. soldanella* in the study area as explanation for

the absence of phylogeographic pattern was ruled out by Arafeh & Kadereit (2006) on the basis of interglacial fossil evidence for the species in the British Isles (Godwin 1975) and on the basis of the observation that the magnitude of intraspecific genetic differentiation in *C. soldanella* is as high as in the other species investigated. Instead, Arafeh & Kadereit (2006) argued that the exceptional behaviour of *C. soldanella* is best explained by its biology. The species shows substantial clonal growth, and seeds have been reported to be able to float in sea water for at least 27 months (Arafeh & Kadereit 2006) and to have 90% viability after 1 year of floating (Arafeh & Kadereit 2006) and 30% viability after 18 months of floating (Ridley 1930). The exceptional floating ability results from a very robust seed coat in combination with an air cavity in the seeds. Arafeh & Kadereit (2006) argued that the absence of phylogeographic structure is the combined result of a high frequency of long-distance dispersal resulting from long floating time and long viability of the seeds, and clone longevity, where the age of clones, however, is unknown. High longevity of clones implies that even if establishment of seeds after long-distance dispersal is rare, the chance of their detection is increased by their long persistence. If this interpretation of *C. soldanella* is correct, it would illustrate that the formation of phylogeographic pattern through historical or modern abiotic factors can be prevented by a species' biology.

THE DARDANELLES AND BOSPORUS GENETIC BREAKS: THE INTERACTION OF HISTORICAL AND MODERN ABIOTIC FACTORS

As discussed above, distinct genetic gaps are found in *C. maritima* across the Dardanelles and across the Bosporus. In view of ongoing gene flow in this area, these gaps were interpreted as signatures of the closure of these two straits during Quaternary glacials. Interestingly, differentiation among regions in *C. maritima* is stronger across the Bosporus than across the Dardanelles. This, in our opinion, reflects the interaction of modern and historical abiotic factors. Whereas the Dardanelles land barrier was broken c. 12 kyr ago (Aksu *et al.* 2002), the reconnection of the Marmara Sea to the Black Sea happened c. 9·5 kyrs ago (Bahr *et al.* 2006; Mudie *et al.* 2004). Accordingly, erosion of the signal left by the glacial closure of the two straits started some

2·5 kyrs earlier in the Dardanelles than in the Bosporus, and this is recognizable in the weaker differentiation across the Dardanelles than across the Bosporus. The comparison of these two areas offers the unique opportunity to calculate rates of gene flow through seed dispersal and to predict when the historical signal recognizable will be completely eroded.

INTERSPECIFIC DIFFERENCES IN GIBRALTAR: THE MAGNITUDE OF MODERN BARRIERS TO GENE FLOW DEPENDS ON SPECIES BIOLOGY

The population-level comparison of *C. maritima* and *E. maritimum* across the Strait of Gibraltar revealed the existence of a distinct phylogeographic gap at exactly the same location. Considering the history of the Strait of Gibraltar, which has been continuously open for the last c. 5 my, and the failure to identify a terrestrial barrier to gene flow in the area, modern sea currents were hypothesized to explain the Gibraltar gap found. When comparing the two species investigated, the gap in *C. maritima* is noticeably smaller than in *E. maritimum*. This difference can be attributed to the differential dispersability of the two species, where dispersability in terms of floating time in sea water and viability of seeds is higher in *C. maritima* than in *E. maritimum*. This finding illustrates that sea currents in the Gibraltar area are not an unsurmountable barrier to gene flow, and, more importantly in the present context, that the strength of modern gene flow barriers is relative and depends on species-specific dispersability.

HISTORICAL FACTORS LEAVE SPECIES-SPECIFIC SIGNATURES

Among the species investigated, differences in phylogeographic structure exist in the west Mediterranean basin (southwest Italy/Sicily to Gibraltar; Fig. 1). Material from this area falls into one genetic cluster in *S. kali*, and into several genetically similar clusters in *E. maritimum*. In *C. maritima*, *H. portulacoides* and *Cr. maritimum* material essentially separates into two groups, of which a western group (e.g., southeast Spain in *C. maritima*) is more similar to Atlantic material, and an eastern group (e.g., northeast Spain and west Italy in *C. maritima*) is more similar to Adriatic/east Mediterranean material. The difference between these two species groups (*C. maritima*, *Cr. maritimum*, *H. portulacoides* vs. *E. maritimum*, *S. kali*) probably can be explained with species-specific responses to Quaternary climatic changes (Kadereit *et al.* 2005). When

considering the geographical position of June/July/August temperature isotherms now found at the northern distributional limits of the species investigated (*C. maritima* subsp. *maritima*: 12°C, *S. kali* subsp. *kali* and *E. maritimum*: 14°C, *H. portulacoides* and *Cr. maritimum*: 16°C) during the last glacial maximum (van Andel 2002), and when 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 species investigated, it is likely that *E. maritimum* and *S. kali* could have persisted along the entire north coast of the west Mediterranean basin during the LGM, whereas *Cr. maritimum* and *H. portulacoides* had to reatreat south. Retreat along the coasts would have meant retreat to the southwest and southeast. The phylogeographic subdivision of the latter two species along the north coast of the west Mediterranean basin would then be the result of re-colonization from these two directions. No explanation can be offered for *C. maritima* which, considering its temperature requirements, might have persisted along the entire north coast but still shows a subdivision similar to that of *Cr. maritimum* and *H. portulacoides*. The among-species phylogeographic incongruencies in the west Mediterranean basin thus illustrate that responses to historical climatic changes are species-specific and leave a distinct phylogeographic signal which may eventually disappear through ongoing gene flow.

Among the species investigated by us, the most striking example for a species-specific response is provided by *Triglochin maritima*. Today, *T. maritima* can grow much further north than the remaining species, and its northern distributional limit is correlated with the 8°C July isotherm. This, the identification of an Atlantic cluster and a cluster containing all material sampled from the North Sea area, the Baltic Sea area, Central European inland localities, the northern Adriatic Sea coast and the Mediterranean coast of southwest France, and the existence of macrofossils of *T. maritima* near Earith and Upton Warren in southern England (Fig. 3) dated to approximately 40 000 years ago (Bell 1969), led Lambracht *et al.* (2007) to postulate that modern populations of this species from the North and Baltic Sea areas originated from inland populations rather than from Atlantic Ocean populations as in the other species investigated.

CONCLUSIONS

The above hypotheses on the possible causes of phylogeographic structure in the species investigated certainly can be taken to even higher levels of complexity. It should be noted, however, that all hypotheses formulated are the result of a comparative approach and are based on rather incomplete knowledge of past and present abiotic factors considered relevant for the distribution of species. Because the plant species compared differ in a large number of characters beyond those discussed above, and the effect of historical and modern abiotic factors on these species may well have been quite different from what we assume, our interpretations should be considered with the necessary caution.

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