European phylogeography of the coastal plants *Cakile maritima* Scop. (Brassicaceae) and *Eryngium maritimum* L. (Apiaceae)

Dissertation
zur Erlangung des Grades

Doktor der Naturwissenschaften

Am Fachbereich Biologie
der Johannes Gutenberg-Universität
in Mainz

Erik Daniel Westberg
geboren in Uddevalla, Schweden
Mainz, Deutschland, Januar 2005

Tag der mündlichen Prüfung: 15 April 2005

CONTENTS

SUMMARY

Linear dispersal systems, such as coastal habitats, are well suited for phylogeographic studies because of their low spatial complexity compared to three dimensional habitats. Widely distributed coastal plant species additionally show azonal and often essentially continuous distributions. These properties, firstly, make it easier to reconstruct historical distributions of coastal plants and, secondly, make it more likely that present distributions contain both Quaternary refugia and recently colonized areas. Taken together this makes it easier to formulate phylogeographic hypotheses.

This work investigated the phylogeography of *Cakile maritima* and *Eryngium maritimum*, two species growing in sandy habitats along the north Atlantic Ocean and the Mediterranean Sea coasts on two different spatial scales using AFLP data. The genetic structure of these species was investigated by sampling single individuals along most of their distributions from Turkey to south Sweden. On a regional scale the population genetic structure of both species was also studied in detail in the Bosporus and Dardanelles straits, the Strait of Gibraltar and along a continuous stretch of dunes in western France. Additionally, populations of *C. maritima* were investigated in the Baltic Sea/Kattegat/North Sea area.

Over the complete sampling range the species show both differences and similarities in their genetic structure. In the Mediterranean Sea, both species contain Aegean Sea/Black Sea and west Mediterranean clusters. *Cakile maritima* additionally shows a clustering of Ionian Sea/Adriatic Sea collections. Further, both species show a subdivision of Atlantic Ocean/North Sea/Baltic Sea material from Mediterranean. Within the Atlantic Ocean group, *C. maritima* from the Baltic Sea and the most northern Atlantic localities form an additional cluster while no such substructure was found in *E. maritimum*.

In all three instances where population genetic investigations of both species were performed in the same area, the results showed almost complete congruency of spatial genetic patterns. In the Aegean/Black Sea/Marmara region a subdivision of populations into a Black Sea, a Sea of Marmara and an Aegean Sea group is shared by both species. In addition the Sea of Marmara populations are more close to the Aegean Sea populations than they are to the Black Sea populations in both cases.

Populations from the Atlantic side of the Strait of Gibraltar are differentiated from those on the Mediterranean side in both species, a pattern that confirms the results of the wide scale study. Along the dunes of West France no clear genetic structure could be detected in any of the species. Additionally, the results from the Baltic Sea/North Sea populations of *C. maritima* did not reveal any geographical genetic pattern.

It is postulated that the many congruencies between the species are mainly due to a predominantly sea water mediated seed dispersal in both species and their shared sandy habitat. The results are compared to hypothetical distributions for the last glacial maximum based on species specific temperature requirements. It is argued that in both species the geographical borders of the clusters in the Mediterranean area were not affected by quaternary temperature changes and that the Aegean/Black Sea/Marmara cluster, and possibly the Ionian Sea/Adriatic Sea cluster in *C. maritima*, is the result of sea currents that isolate these basins from the rest of the sampled areas.

The genetic gap in the Strait of Gibraltar between Atlantic Ocean and Mediterranean Sea populations in both species is also explained in terms of sea currents. The existence of three subgroups corresponding to the Aegean Sea, Black Sea and Sea of Marmara basins is suggested to have arisen due to geographical isolation during periods of global sea regressions in the glacials. The population genetic evidence was inconclusive regarding the Baltic Sea cluster of *C. Maritima* from the wide scale study.

The results of this study are very similar to those of an investigation of three other coastal plant species over a similar range. This suggests that the phylo-geographic patterns of widespread coastal plants may be more predictable than those of other terrestrial plants.

Zusammenfassung

Lineare Verbreitungssysteme wie Küstenstandorte sind für phylogeographische Untersuchungen aufgrund ihrer geringen räumlichen Komplexität im Vergleich zu dreidimensionalen Standorten gut geeignet. Weit verbreitete Arten von Küstenpflanzen zeigen zudem azonale und oft kontinuierliche Verbreitungen. Diese Eigenschaften machen es zum einen einfacher, historische Verbreitungen von Küstenpflanzen zu rekonstruieren und zum anderen auch wahrscheinlicher, dass rezente Verbreitungen sowohl quartäre als auch vor kurzer Zeit besiedelte Gebiete einschließen. Vor diesem Hintergrund wird die Formulierung phylogeographischer Hypothesen erleichtert.

In dieser Arbeit wurde die Phylogeographie von *Cakile maritima* und *Eryngium maritimum*, die beide an sandigen Standorten entlang des Nordatlantiks und des Mittelmeeres vorkommen, mittels AFLP Daten auf zwei verschiedenen räumlichen Maßstäben untersucht. Die genetische Struktur der Arten im großen Maßstab wurde ermittelt, indem einzelne Individuen entlang eines Großteils des europäischen Verbreitungsgebietes gesammelt wurden. Zusätzlich wurde die populationsgenetische Struktur beider Arten in den Meerengen des Bosporus, der Dardanellen, der Straße von Gibraltar sowie entlang eines zusammenhängenden Dünengebietes in Westfrankreich detailliert untersucht. Außerdem wurden Populationen *von C. maritima* in der Region Ostsee/Nordsee näher erkundet.

Die Arten zeigen in der europaweit untersuchten genetischen Struktur sowohl Ähnlichkeiten als auch Unterschiede. Im Mittelmeer findet man in beiden Arten Gruppierungen in den Regionen Ägäis/Schwarzes Meer sowie im westlichen Mittelmeer. *Cakile maritima* zeigt zusätzlich eine Gruppierung im Gebiet Ionisches Meer/Adria. Beide Arten zeigen eine Trennung der Proben der Atlantik/Nordsee/Ostsee Gruppierung von denen des Mittelmeeres. Am Atlantischen Ozean gruppieren Proben von *C. maritima* mit den am weitesten nördlich gelegenen Standorten, während bei *E. maritimum* keine solche Substrukturierung beobachtet werden konnte.

In allen drei Beispielen, in denen populationsgenetische Untersuchungen beider Arten im gleichen Gebiet durchgeführt wurden, zeigten die Ergebnisse eine nahezu vollständige Übereinstimmung der räumlich genetischen Muster. Im Gebiet Ägäis/Schwarzes Meer/Marmarameer tritt in beiden Arten eine Unterteilung der

Populationen in je eine Gruppe im Schwarzen Meer, Marmarameer und der Ägäis auf. Zudem sind die Populationen des Marmarameeres in beiden Fällen mehr von denen des Schwarzen Meer differenziert als von denen der Ägäis. Die Populationen der atlantischen Seite der Straße von Gibraltar sind in beiden Arten von denen der Mittelmeerseite abgegrenzt, ein Muster, das die Ergebnisse der europaweiten Untersuchung bestätigt. Entlang der westfranzösischen Küste konnte bei keiner der untersuchten Arten eine klare genetische Strukturierung festgestellt werden. Die Ergebnisse von *C. maritima* in der Region Ostsee/Nordsee zeigten ebenfalls kein geographisch gegliedertes genetisches Muster.

Es wird postuliert, dass die großen Übereinstimmungen zwischen den Arten vor allem auf eine vorwiegende Verbreitung der Samen durch das Meerwasser sowie das Vorkommen beider Arten auf sandigen Standorte Standort zurückzuführen sind. Diese Ergebnisse werden mit hypothetischen Verbreitungen während der letzten Eiszeit verglichen, die auf artspezifischer Temperaturanforderungen basieren. Wahrscheinlich war in beiden Arten die geographischen Grenzen der Gruppierungen im Mittelmeer nicht durch quartäre Temperaturänderungen beeinflusst worden sondern die Gruppierungen in der Region Ägäis/Schwarzes Meer/Marmarameer, und möglicherweise ebenso die der Region Ionisches Meer/Adria bei *C. maritima*, ein Ergebnis von Meeresströmungen sind, die diese Becken von den restlichen besammelten Gebieten abgrenzen.

Die genetische Kluft in der Straße von Gibraltar wird ebenfalls vornehmlich mit Meeresströmungen erklärt. Die Existenz der drei Untergruppen Ägäis, Schwarzes Meer und Marmarameer ist vermutlich aufgrund der geographischen Isolation in Perioden globaler Meeresspiegelsenkungen während der Eiszeiten entstanden. Die Beweise bezüglich der Gruppierungen in der Ostsee waren in der europaweiten Untersuchung nicht aussagekräftig.

Die europaweiten Ergebnisse dieser Untersuchung sind denen einer Studie zu drei anderen Küstenarten sehr ähnlich. Dies lässt vermuten, dass sich phylogeographische Muster weit verbreiteter Küstenpflanzen leichter vorhersagen lassen als die terrestrischer Pflanzen.

INTRODUCTION

A species is for practical reasons generally recognized on morphological grounds. Simplified, phenotypic variation is usually continuous within and discontinuous between species so that well defined and coherent units are formed. The evolutionary process does however not recognize species as units but acts on other organismal levels (Darwin, 1859). Evolution can narrowly be defined as changes in allele frequencies in a population from one generation to the next (Curtis & Barnes, 1989) and from the works of e.g. Turesson (1922, a; b), it has been established experimentally and theoretically that the unit of a species indeed is a simplification and that considerable variation exists in the genetic and phenotypic make up of a species.

In an island model of populations (i.e. migration is equally likely between all pairs of populations) without differential selection only random genetic structure should be seen. If migration is sufficiently low, populations can diverge from each other by genetic drift. It has been shown, however, that even very low migration rates are sufficient to prevent population differentiation in a system like this (Silvertown & Charlesworth, 1997). Perhaps in contrary to this, in natural populations this is often not the case over the distribution range of a species. Instead species often consist of geographically structured populations, that is, populations in one region are genetically different from those of another area (Ehrlich & Raven, 1969).

The question of what factors are responsible for intraspecific patterns of variation is central to population biology and biogeography. Reviews of genetic diversity measured by allozymes (Hamrick & Godt, 1989), dominant markers and microsatellites (Nybom & Bartish, 2000; Nybom, 2004) in natural plant populations have shown that the characteristics of a species influence genetic diversity. All reviews found that life form, breeding system, mode of seed dispersal and successional status had strong effects on population differentiation. Of these, breeding system and life form have the most marked effects where inbreeders and annuals showed markedly more differentiation than more long-lived or outcrossed species (Hamrick & Godt, 1989; Nybom & Bartish, 2000). The same factors also influence within population diversity (Nybom & Bartish, 2000). Additionally, the

geological history of species is considered to be of crucial importance in shaping genetic diversity. The question as to which factor is the cause of a given genetic pattern is, however, a difficult one since genetic structure can arise from several different processes. On the other hand, it is possible to obtain indirect information by comparing patterns of genetic variation with, e.g., known ecological characteristics and geological history. With this general approach researchers are trying to describe the history of species in space and time. This branch of historical biogeography has been coined phylogeography (Avise *et al.*, 1987).

Phylogeographic investigations are restricted mainly to species or species complexes, and the time scale is usually confined to approximately the last 2 million years (myrs), or the Quaternary, and is thus small in comparison to studies on generic or higher taxonomic level (Huang *et al.*, 2004). The Quaternary is characterized by dramatic climatic fluctuations, the last 700 thousand years (kyrs) thereof seeing extreme climate oscillations where shorter warm periods (interglacials) have been succeeded by longer periods of colder climates (glacials), lasting around 100 kyrs (Webb & Bartlein, 1992). The latest glacial ended about 12 kyrs ago and was followed by the warmer interglacial of today, the Holocene. The results of biome reconstructions based on pollen and macrofossil data show that southern Europe predominantly hosted steppe vegetation whereas more northern latitudes had tundra vegetation during the last glacial maximum (LGM) indicating not only colder but also drier climates than today (Elenga *et al.*, 2000; Tarasov *et al.*, 2000). The consequences of the Quaternary climate have clearly been dramatic for most organisms, causing range expansions/contractions and distribution shifts where temperate species retreated southwards to refugial areas during glacials and claimed the habitats we find them in today only after the cold period ended (Brown & Lomolino, 1998). Accordingly, the effects on genetic variation must have been profound. Provided with a well-defined climatic history, interest in phylogeographic research has been considerable over the last decade. More importantly, with the advent of easily applied molecular tools, researchers have been given the opportunity to describe the genetic variation of species. In zoology, interest in phylogeography has been further spurred by the high sequence variability and small effective population size of the mitochondrial genome of animals (Avise, 1998). In the context of coalescent theory, sequence variation is especially useful as it

contains a historical record of itself (Li, 1997). The variation in plant chloroplasts and mitochondria is lower, however, and the availability of variable genes in plants is more limited. The development of microsatellites and molecular fingerprinting techniques like amplified fragment length polymorphisms (AFLP) and random amplified polymorphic DNA (RAPD) has given plant researchers access to more molecular tools, too. While less suitable for phylogenetic reconstructions than DNA sequences, the high variability of these markers on the other hand enables researchers the detection of population level structure and dynamics.

Much new insight into the effects of ice ages on plant distribution has been added by phylogeographic studies. For European animals and plants several reviews covering temperate species have been made (Taberlet *et al.*, 1998; Comes & Kadereit, 1998; Hewitt, 1999; 2004; Lascoux *et al.*, 2004). In general it has been shown that species responded individually to Quaternary climate change. However, some general patterns can be recognized. As expected from pollen fossil data, molecular studies have confirmed that temperate species were in refugias in the south of Europe during the LGM. These were situated on the southern European peninsulas of Iberia, Italy and the Balkans but also further to the east in the Caucasus and Caspian Sea area (Comes & Kadereit, 1998; Hewitt, 1999; 2004; for challenges to this view see Stewart and Lister, 2001; Stewart, 2003). In general, post-glacial colonization could have taken place from any of these refugias for a species. Hewitt (1999) pointed to three broadly outlined colonization patterns exemplified by both plants and animals and since then added a fourth route exemplified by freshwater fish (Hewitt, 2004). Several variants to these phylogeographic patterns are noted, however, and in hindsight it therefore seems difficult to predict how species have responded to the Quaternary climate (Lascoux *et al.*, 2004). Comes and Kadereit (1998) suggested that likely factors responsible for the incongruence in pattern between plant species include species specific rates and directions of spread, differential rates of extinction in refugial areas and inability to colonize areas already inhabited by other species. These factors have been accentuated by the geography of Europe, some of whose features have likely played a large role. Particularly the mainly east-west oriented mountain ranges and the likewise oriented Mediterranean Sea will have acted as barriers to dispersal. This is emphasized by comparisons with phylogeographic studies from north-western North

America which show a higher degree of congruence (Soltis *et al.*, 1997; Brunsfeld *et al.*, 2001). Here, mountain ranges and hence obstacles to dispersal are north-west oriented. However, a comparison of a limited number of studies from Northern Rocky Mountain taxa shows more variation between patterns demonstrating that here, too, similar distributions have resulted from different histories (Brunsfeld *et al.*, 2001). Incongruent patterns are also found when comparing European alpine species (Hewitt, 2004).

Considering the above, the lack of a common pattern among plants and animals with very different ecological properties is not surprising and even more so when geography and the complex spatial distribution of suitable habitats are taken into account. A way around this could be to reduce the number of factors that can differ between species and their respective histories. In this context widely and continually distributed coastal species seem ideal. First, distribution, dispersal and colonization routes are likely to be along the coast which greatly reduces the spatial complexity of habitats. In such a linear dispersal system it should be easier to attribute found differences to the species history or ecological properties. Further, they are often azonally distributed showing large latitudal and longitudal ranges. It is therefore likely that the present distribution still contains both refugial and re-colonized areas. Moreover, the clear spatial limit to the species' more or less continuous habitat makes predictions about past distribution ranges easier. If present day distributions are assumed to be limited primarily by climate, then rather reliable historical distributions can be inferred directly from climate reconstructions by correlating distribution limits to climatic factors. In contrast, reconstructing distributions for organisms with more complex and three dimensional distributions may need computer modelling and fossil evidence.

This work investigates the biogeography of *Eryngium maritimum* L. (Apiaceae) and *Cakile maritima* Scop. (Brassicaceae) along the coasts of Europe. Both species are ecologically similar in much of their distribution range. They grow on sand, often together, and show similarities in dispersal and pollination biology. However, they differ in traits like life history and climatic limits.

The Sea Rocket, *Cakile maritima*, is an annual, prostrate to upright and more or less fleshy plant with lilac to white insect-pollinated flowers (Fig. 1). It grows predominantly on the shore lines or in the fore dunes but is also found in yellow dunes and dune slacks. The fruits are divided into two segments where the upper part is falling off when ripe. The seeds can be dispersed by water and germination is not affected by salt water. Its biology is well known due to researchers' interest in its recent introduction to North America and Australia where it is currently replacing *C. edentula* ecologically (Barbour & Rodman, 1970; Boyd & Barbour, 1993; Thrall *et al.*, 2000). *Cakile maritima* is diploid with a chromosome number of n=9 as reported from several areas across its native range (Rodman, 1974).

Several intraspecific classifications have been made for *C. maritima* (e.g. Pobedimova, 1963; 1964; Rodman, 1974; see also Elven and Gjelsås, 1981). The taxa recognized in these have biogeographic relevance since they often have corresponded to geographical regions. Accordingly, *C. maritima* subsp. *euxina* (Pobed) Nyárády was described from the Black Sea and/or the Aegean Sea region, *C. maritima* subsp. *baltica* (Rouy and Foucaud) P.W. Ball from the Baltic Sea, Kattegat and the remaining Swedish west coast, *C. maritima* subsp. *islandica* (Gand.) Elven from Iceland and in northern Norway, *C. maritima* subsp. *monosperma* (Lange) O. E. Schulz for the Atlantic coasts and *C. maritima* subsp. *aegyptica* (Willd.) Nyman from the Mediterranean Sea (see Rodman [1974] and Elven & Gjelsås [1981] for details). The northern distribution limitof *C. maritima*, including subsp. *islandica*, lies around the 71° N approximately corresponding to a mean temperature of 8°C for July. The corresponding value for the distribution limit of *C. maritima* subsp *maritima* is ca 12°C.

The Sea Holly, *Eryngium maritimum*, is a bluish-green, thorny, perennial plant with small blue flowers arranged in a head (Fig. 2). It is mostly found in sand dunes but also grows on other sandy beaches and shingle (Walmsley and Davy, 1997). Fruits can be dispersed by water or wind and they have a persistent, pointed calyx which according to Ridley (1930) serves as an anchor on sandy places. The spiny fruits have also been interpreted as an adaptation for epizoochory. This has not been confirmed and was doubted by Ridley. Its chromosome number, n=8, has been reported from several localities in the Atlantic and the Mediterranean (Pimenov *et al.*,

a

b

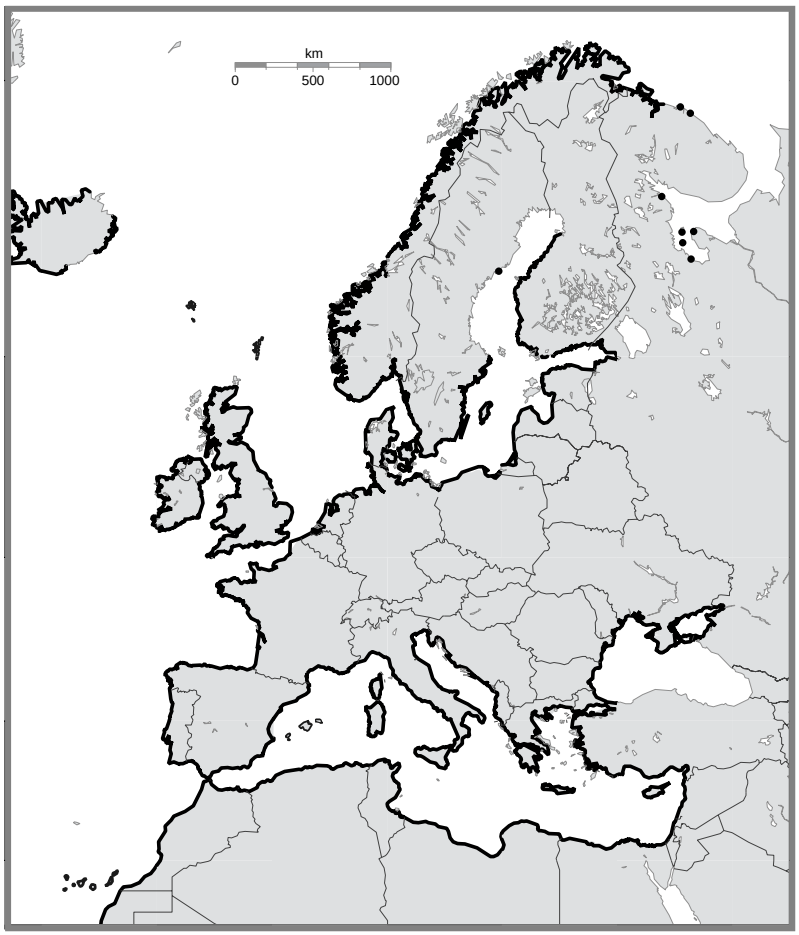


Figure 1. *Cakile maritima*, Sea Rocket. a) Close view and b) map of European and North African distribution, including subsp. *islandica* (after Meusel & Jäger, 1992).

a

b

Figure 2. *Eryngium maritimum*, Sea Holly. a) Habit and b) map of distribution (after Meusel & Jäger, 1992).

2002). No intraspecific classification has been made in *E. maritimum*. Its northern limit corresponds to 14°C mean July temperature at around 59°N.

We used AFLP and sequence variation to survey large scale geographic structuring of genetic diversity in these two species along the coasts of Europe. Here we focus mainly on two issues:

1. How has the climatic history shaped the distribution of genetic variation in *Cakile maritima* and *Eryngium maritimum*?
2. Can the genetic imprints of refugial areas be detected and where were they situated?
3. Can similarities and differences in geographical genetic patterns between the two species best be interpreted in light of ecological factors or the climatic history of the region?

To accomplish this we assess intraspecific genetic relationships using AFLPs among samples collected continuously along the coast from the Black Sea (Turkey) to south Sweden, including southern England. Theory predicts that refugial areas will have higher genetic diversity and larger genetic distances than recently colonized ones (Hewitt, 1996). For additional support, the present day isotherms correlating to the northern distribution limits of the species will be used to predict their distributions during the latest glacial maximum. If, e.g., areas situated north of the predicted LGM distribution show lower genetic diversity this can be taken as evidence for subsequent re-colonization of that area and *vice versa* (but see Coyer *et al.*, [2003] and Castric and Bernatchez, [2004] for examples where refugial areas show reduced genetic variation). The genetic patterns will also be compared between the two species in light of abiotic factors and differences and similarities in ecology.

The methodology used here analyses one individual per population and was designed to detect genetic patterns on a coarse scale, by improving on the sample scheme used by Clausing *et al.*, (2000). This essentially excludes population level inferences to be made. On the other hand their study showed that smaller scale processes, such as dispersal barriers, could not be ruled out and that they potentially could have a large influence even on a Europe wide scale.

For coastal plants, conditions should be favourable for effective dispersal (Bonn & Poschlod, 1998). However, several European sea straits correspond to distribution limits of proposed intraspecific taxa in *C. maritima* (Rodman, 1974) and also act as taxonomic borders in marine animals (e.g. Borsa *et al.*, 1997a). In marine species the influence of currents on dispersal, although never tested as far as we know, has been proposed as a reason for differentiation across straits.

To investigate what role small scale processes play in the study area we perform population genetic studies along three sea straits (Baltic Sea - North Sea, Strait of Gibraltar and the Bosporus - Dardanelles straits). The three straits differ in several properties. The Baltic Sea is a geologically young one, its connection to the North Sea opening only some 9 kyrs ago, before which it was a fresh water lake (Björck, 1995). Shortly after, the connection was closed again for approximately 2 kyrs (Yoldia Sea; Elven and Gjelsås, 1981). Consequently the Baltic Sea must have been colonized from the North Sea direction earliest around 9-7 kyrs ago. The Black Sea also lacked a connection to the Aegean Sea during the last glacial due to the drying out of the Dardanelles and the Bosporus strait (Aksu *et al.*, 2002a). Reconnection here occurred about 12 kyrs ago for the Marmara Sea and 9 kyrs ago for the Black Sea before which they both were brackish inland seas (Mudie *et al.*, 2004). Contrary, the Strait of Gibraltar has been open since the Messinean Crisis ca 5 myrs ago (Schmidt *et al.*, 2001). The primary focus of the population genetic studies in the three straits is to:

1. detect and assess the nature of recent barriers to gene flow,
2. estimate their influence on larger scale patterns, and
3. investigate the role of differential geological history on genetic diversity.

We study this by investigating genetic structure of populations sampled along the three sea straits and comparing them to a population system where no barriers to gene flow should be present. The coast from Bayonne to the Gironde in the Bay of Biscay, France, is a more or less continuous stretch of sand dunes and should provide a good approximation to unrestricted gene flow. These results are then viewed in light of all data. If a distinct barrier to gene flow is located within the investigated areas, it should be evident for both species given that their seeds likely

are dispersed primarily in the same way. Also its relative importance for the complete study system should be evident in the large scale phylogeographical patterns where a strong barrier to gene flow should result in clusters corresponding to geographic areas separated by that sea strait. Finally, because of the differential geological history of the three straits and since two ecologically similar species are used, we should be able to draw some conclusions about the relative importance of gene flow and history for the partitioning of genetic diversity for these study systems. For example, the Baltic Sea Strait and the Bosporus Strait opened at approximately the same time whereas the Baltic Sea is much younger than the Black Sea. Thus we would expect clear differences in genetic patterns between them if the longer history of the Black Sea is of significance.

MATERIAL AND METHODS

Collection

AFLP phylogeography. Collections were made throughout the summer and autumn of 2001. Parts of leaves were removed from plants and dried on silica gel beads in order to preserve the DNA. From each collection locality leaves from five plants of each species were taken 10 to 100 meters apart from each other. Collection localities were chosen approximately every 100 km where possible. For the AFLP analysis, one sample from each locality listed in tables 1 and 2 was included, except from locality no. 34 and 67 in *C. maritima* and no. 2 in *E. maritimum* where five individuals were analyzed. Efforts were made to collect plants only from natural habitats.

Table 1: Geographic origin of *Cakile maritima* samples. Localities are numbered according to geographical position starting from with the most North-western locality and following the coast to the most South-eastern Mediterranean one. One individual from each locality was included except where otherwise indicated. A negative longitude indicates degrees west of the Greenwich meridian.

No	Locality	Country	Eastern longitude	Northern latitude
1	Arnastapi	Iceland	-24.03	64.41
2	Berlevåg	Norway	29.06	70.51
3	Brekstad	Norway	9.42	63.41
4	Åhus	Sweden	14.20	56.01
5	Ahrenshoop	Germany	12.35	54.30
6	Peenemünde	Germany	13.46	54.09
7	Heiligenhafen	Germany	10.59	54.22
8	Hadarslev	Danmark	9.30	55.15
9	St. Andrews	Great Britain	-2.48	56.20
10	St. Peter Ording	Germany	8.37	54.18
11	Juist	Germany	7.00	53.41
12	Petten	Netherlands	4.39	52.46
13	Cadzand	Netherlands	3.25	51.22
14	Le Crotoy	France	1.37	50.14
15	Camber	Great Britain	0.49	50.55
16	East Head	Great Britain	-0.55	50.46
17	Exmouth	Great Britain	-3.23	50.35
18	Workington	Great Britain	-3.34	54.39
19	Greystones	Ireland	-6.04	53.08
20	Utah Beach	France	-1.10	49.25
21	St. Benoit des Ondes	France	-1.51	48.37
22	Champ du Tir	France	-3.17	47.49
23	Les Jars	France	-1.22	46.21
24	Grand Crohot Ocean (5 individuals)	France	-1.14	44.49
25	Rio Oka	Spain	-2.41	43.24
26	Playa la Espasa	Spain	-5.13	43.29
27	Playa Porcia	Spain	-6.53	43.34
28	A Illa de Arousa	Spain	-8.52	42.33
29	Torreira	Portugal	-8.42	40.40
30	Porto Covo	Portugal	-8.47	37.52
31	Playa el Hoyo	Spain	-7.17	37.12
32	Playa de Montillo	Spain	-6.24	36.46

Table 1, continued.

33	Agadir		Morocco	-9.66	30.45
34	Playa Jandia, Fuerteventura		Spain	-14.20	28.03
35	Cabo Pino		Spain	-4.44	36.29
36	Platja las Alberquillas		Spain	-3.50	36.45
37	Playa de Guardias Viejas		Spain	-2.49	36.42
38	Ruimar Platja		Spain	0.51	40.44
39	Punta de Tordera		Spain	2.46	41.39
40	St. Marie de la Mer		France	4.27	43.27
41	St. Aygulf		France	6.44	43.24
42	Raouel		Tunisia	10.18	36.54
43	Jerba		Tunisia	10.55	33.52
44	Torre di Lago Puccini		Italy	10.16	43.49
45	Olmaia		Italy	10.31	43.09
46	Boca di Albegna		Italy	11.11	42.30
47	Lago di Fogliano		Italy	12.55	41.23
48	Villasimius, Sardinia		Italy	9.33	39.05
49	Mondragone		Italy	13.53	41.07
50	Longobardi Marina		Italy	16.04	39.12
51	St. Pollina		Italy	14.10	38.01
52	Roccella		Italy	16.24	38.20
53	Punta Alicia		Italy	17.09	39.24
54	Marina di Pisticci		Italy	16.47	40.18
55	Egnazia		Italy	17.22	40.54
56	Marina di Lesina		Italy	15.21	41.54
57	Marcelli		Italy	13.38	43.29
58	Marina Romea		Italy	12.17	44.31
59	Valle Granato		Italy	13.33	45.44
60	Zadar		Croatia	15.15	44.06
61	Igoumenitsa		Greece	20.11	39.31
62	Lehena		Greece	21.15	37.59
63	Nei Kios		Greece	22.45	37.35
64	Anthidona		Greece	23.26	38.30
65	N. Poroi		Greece	22.40	39.59
66	Olympiada		Turkey	23.50	40.35
67	Makri	(5 individuals)	Greece	25.41	40.51
68	Odun Isk		Turkey	26.07	39.45
69	Esenköy		Turkey	28.57	40.37
70	Akpinar		Turkey	28.49	41.18
71	Igneada		Turkey	28.03	41.53
72	Voroklini		Cyprus	33.39	34.59
73	Herzliya		Israel	34.49	32.10

Table 2: Geographic origin of *Eryngium maritimum* samples. Localities are numbered according to geographical position, starting from the Baltic Sea and following the coast to the most eastern Mediterranean locations. One individual from each locality was included except where otherwise indicated. A negative longitude indicates degrees west of the Greenwich meridian.

No	Locality		Country	Eastern longitude	Northern latitude
1	Peenemünde		Germany	13.46	54.09
2	Ahrenshoop	(5 individuals)	Germany	12.35	54.30
3	Heiligenhafen		Germany	10.59	54.22
4	Schillig		Germany	8.02	53.43
5	Petten		Netherlands	4.39	52.46
6	Kilcoole		Ireland	-6.04	55.08
7	East Head		Great Britain	-0.55	50.46
8	Wassenarseslag		Netherlands	4.23	52.09
9	Kessingland		Great Britain	1.43	52.23
10	Sandwich		Great Britain	1.23	51.17
11	Grand Fort Phillip		France	2.06	51.00
12	Le Crotoy		France	1.37	50.14
13	Gatteville		France	-1.18	49.41
14	Plage du Port Hue		France	-2.10	48.38
15	Penven		France	-3.35	48.46
16	Plage L'Aber		France	-4.26	48.14

Table 2, continued.

17	Champ du Tir	France	-3.17	47.49
18	Pen-Ar-Ran	France	-2.33	47.22
19	Les Jars	France	-1.22	46.21
20	Hourtin Plage	France	-1.10	45.13
21	Cap Breton	France	-1.27	43.37
22	Rio Oka	Spain	-2.41	43.24
23	Las Dunas de Liencres	Spain	-3.59	43.27
24	Playa la Espasa	Spain	-5.13	43.29
25	Playa Navia	Spain	-6.43	43.34
26	Praia grande de Mino	Spain	-8.12	43.22
27	A Illa de Arousa	Spain	-8.52	42.33
28	Cabeledo	Portugal	-8.50	41.41
29	Torreira	Portugal	-8.42	40.40
30	Praia de Vieira	Portugal	-8.59	39.52
31	Armacao de Pera	Portugal	-8.21	37.06
32	Playa el Hoyo	Spain	-7.17	37.12
33	Cabo Pino	Spain	-4.44	36.29
34	El Pinet	Spain	-0.37	38.09
35	Platja d'Oliva	Spain	-0.05	38.55
36	Palma Mallorca	Spain	2.39	39.35
37	Ruimar Platja	Spain	0.51	40.44
38	St. Cyprien Plage	France	3.02	42.40
39	La Tamarissiere	France	3.26	43.17
40	Berriaud Plage	France	6.12	43.06
41	St. Aygulf	France	6.44	43.24
42	Torre di Lago Puccini	Italy	10.16	43.49
43	Olmaia	Italy	10.31	43.09
44	Boca di Albegna	Italy	11.11	42.30
45	Villasimius, Sardinia	Italy	9.33	39.05
46	Lago di Fogliano	Italy	12.55	41.23
47	Mondragone	Italy	13.53	41.07
48	Villamare	Italy	15.31	40.05
49	Longobardi Marina	Italy	16.04	39.12
50	St. Pollina	Italy	14.10	38.01
51	Foce di Simeto	Italy	15.06	37.24
52	Roccella	Italy	16.24	38.20
53	Punta Alicia	Italy	17.09	39.24
54	Marina di Pisticci	Italy	16.47	40.18
55	Marina di Lesina	Italy	15.21	41.54
56	Porto d'Ascoli	Italy	13.54	42.55
57	Marcelli	Italy	13.38	43.29
58	Marina Romea	Italy	12.17	44.31
59	Medullin	Croatia	13.56	44.49
60	Igoumenitsa	Greece	20.11	39.31
61	Preveza	Greece	20.40	39.04
62	Lehena	Greece	21.15	37.59
63	Kyparissia	Greece	21.41	37.19
64	Anthidona	Greece	23.26	38.30
65	Nei Anchialos	Greece	22.49	39.15
66	Nei Poroi	Greece	22.40	39.59
67	Olymiada	Greece	23.50	40.35
68	Erasmio	Greece	24.50	40.52
69	Makri	Greece	25.41	40.51
70	Gallipoli	Turkey	26.50	40.36
71	Odun Isk.	Turkey	26.07	39.45
72	Musaköy	Turkey	26.31	40.14
73	Yeniciftlik	Turkey	27.51	40.59
74	Yeniköy	Turkey	28.24	40.23
75	Akpinar	Turkey	28.49	41.18
76	Sile	Turkey	29.35	41.11
77	Karasu	Turkey	30.40	41.08
78	Eregli	Turkey	31.26	41.19
79	Igneada	Turkey	28.03	41.53
80	Avsallar	Turkey	30.08	36.18
81	Voroklini	Cyprus	33.39	34.54

Population analyses. Population samples was collected from localities along the area of interest for both species and dried in silica gel beads. Collections were made in May and July 2001 as well as August 2002 and 2003. Only *C. maritima* was collected in the Baltic Sea/North Sea study system since *E. maritimum* is too rare to be collected consistently. Sample localities, and number of individuals included in the AFLP analysis is listed in table 3.

Table 3a: Geographic origin of population samples of ***Cakile maritima*** from the **Baltic Sea, Kattegat** and **North Sea** area, abbreviations, number of individuals included in the analyses and geographical coordinates**.** A negative longitude indicates degrees west of the Greenwich meridian.

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
DK1	Kalø	Denmark	10	10.25	54.17
DK2	Juelsmünde	Denmark	10	10.00	55.43
DK3	Tårups Strand	Denmark	10	10.47	55.14
DK4	Havnsø Strand	Denmark	9	11.21	55.46
DK5	Køge	Denmark	10	12.12	55.27
DK6	Hadarslev	Denmark	7	9.37	55.17
DK7	Lyngså	Denmark	9	10.31	57.15
DK8	Grønhøj	Denmark	10	9.40	57.19
SV1	Lernacken	Sweden	10	12.54	55.34
SV2	Trelleborg	Sweden	11	13.07	55.22

Table 3b: Geographical origin of population samples of ***Cakile maritima*** from **West France.**

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
WF9	Port d'Albret	France	9	-1.24	43.47
WF10	Hutchet	France	7	-1.23	43.54
WF11	Tuquelets du nord	France	6	-1.19	44.05
WF12	Mimizan plage	France	10	-1.18	44.14
WF13	Biscarrose plage	France	7	-1.15	44.27
WF14	Dune du Pilat	France	7	-1,13	44,35
WF17	Grand Crohot Ocean	France	9	-1.14	44.49
WF18	Lacanau ocean	France	10	-1.10	45.13
WF19	Hourtin plage	France	9	-1.12	45.00

Table 3c: : Geographical origin of population samples of ***Cakile maritima*** from the **Strait of Gibraltar.**

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
ATL1	Tarifa	Spain	10	-5.37	36.01
ATL2	Playa Bolonia	Spain	9	-5.47	36.05
ATL3	Rio Barbate	Spain	10	-5.55	36.11
ATL4	Playa de los Bateles	Spain	10	-6.06	36.17
ATL5	St. Petri	Spain	10	-6.13	36.23
MED1	Getares	Spain	9	-5.27	36.06
MED2	Playa de Atunara	Spain	9	-5.20	36.12
MED3	Playa Sabinillas	Spain	9	-5.13	36.22
MED4	Playa de Guadalmina	Spain	10	-4.59	36.29
MED5	Torre del Mar	Spain	10	-4.05	36.45

Table 3d: Geographical origin of population samples of ***Cakile maritima***,from the **Aegean Sea, Sea of Marmara** and the **Black Sea.**

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
BS1	Riva	Turkey	7	29.12	41.13
BS3	Sile	Turkey	8	29.35	41.11
MA11	Esenköy	Turkey	10	28.58	40.37
MA13	Bandirma kör	Turkey	7	27.53	40.23
MA14	Gönen	Turkey	10	27.37	40.19
MA15	Sevketiye	Turkey	11	26.52	40.24
MA17	Musaköy	Turkey	3	26.31	40.14
AE18	Kumkale	Turkey	10	26.11	40.01
AE19	Odun Isk	Turkey	8	26.07	39.45
AE22	Galipoli	Turkey	8	26.50	40.36
GR	Makri	Greece	5	25.41	40.51

Table 3e: Geographical origin of population samples of ***Eryngium maritimum*** from **West France.**

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
WF9	Port d'Albret	France	10	-1.24	43.47
WF10	Hutchet	France	10	-1.23	43.54
WF11	Tuquelets du Nord	France	10	-1.19	44.05
WF12	Mimizan plage	France	10	-1.18	44.14
WF13	Biscarrose plage	France	9	-1.15	44.27
WF16	La vigne	France	10	-1.15	44.41
WF17	Grand crohot ocean	France	10	-1.14	44.49
WF18	Lacanau ocean	France	10	-1.12	45.00
WF19	Hourtin plage	France	9	-1.10	45.13

Table 3f: Geographical origin of population samples of ***Eryngium maritimum*** from the **Strait of Gibraltar.**

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
ATL1	Tarifa	Spain	9	-5.37	36.01
ATL2	Playa Bolonia	Spain	10	-5.47	36.05
ATL3	Canos de Meca	Spain	9	-6.01	36.11
ATL4	St. Petri	Spain	10	-6.13	36.23
ATL5	Punta Condor	Spain	9	-6.23	36.37
MED1	Getares	Spain	9	-5.27	36.06
MED2	Playa de Atunara	Spain	10	-5.20	36.12
MED3	Playa Sabinillas	Spain	10	-5.13	36.22
MED4	Playa de Guadalmino	Spain	10	-4.59	36.29
MED5	Playa la Calahonda	Spain	10	-4.44	36.29

Table 3g: Geographical origin of population samples of ***Eryngium maritimum*** from the **Aegean Sea, Sea of Marmara** and the **Black Sea.**

Abbre-viation	Locality	Country	N	Eastern longitude	Northern latitude
BS2	Sahilköy	Turkey	10	29.23	41.12
BS3	Sile	Turkey	10	29.35	41.11
MA12	Yeniköy	Turkey	7	28.24	40.23
MA13	Bandirma kör	Turkey	4	27.53	40.23
MA14	Gönen	Turkey	7	27.37	40.19
MA17	Musaköy	Turkey	10	26.31	40.14
MA24	Yeniciftlik	Turkey	7	26.40	40.24
AE18	Kumkale	Turkey	9	26.11	40.01
AE19	Odun Isk	Turkey	9	26.07	39.45
AE22	Galipoli	Turkey	10	26.50	40.36
GR	Makri	Greece	5	25.41	40.51

DNA isolation

Approximately 100 mg of dried leaf material was ground with autoclaved sand (Roth). Total genomic DNA was extracted using the DNeasy^{TM} plant minikit (Qiagen) following the manufacturer's instructions and stored at -20°C. DNA concentration was measured spectrophotometrically with a GeneQuant RNA/DNA calculator (Pharmacia), or estimated visually by ethidiumbromide staining on agarose gels.

Fluorescent labelled AFLP

The AFLP method is a fingerprinting technique that can yield a large number of markers (Vos *et al*., 1995). The technique combines DNA restriction fragment length polymorphisms (RFLP) with the polymerase chain reaction and generates DNA fragments of different lengths. AFLPs were favoured over other fingerprinting methods such as RAPDs and microsatellites because it rapidly produces a large amount of reproducible markers without requiring species specific development. The reproducibility is achieved by combining cutting of DNA with restriction enzymes and ligating the yielded cuts with specifically synthesized dsDNA fragments, called adapters, which are used as priming sites for PCR amplification. From the population of produced fragments a subset is selected for amplification by using primers matching the adapter sequence but including three additional bases on the 3` end (selective bases). Because primer annealing can tolerate some mismatching on the third position from the 3` end, an intermediary selection step is included using primers with only one extra base. To visualize the amplified fragments, one of the selective primers is labelled with a fluorescent dye which can be detected by a scanner after excitation by a laser.

Two different restriction enzymes are used, one cutting frequently (*Mse*I: 5'-T^TAA-3') and one cutting more rarely (*Eco*RI: 5'-G^AATTC-3'). In addition, only the selective primer that fits to adapters of the rare cutting enzyme is labelled. This is done primarily to yield a suitable number of fragments of appropriate sizes for scoring. The result of this is that, more or less exclusively, fragments whose ends have different restriction sites are detected on the gel. This is because fragments with only frequent-cut ends are not labelled and those with only rare cut ends are usually larger than the size range that is chosen for scoring. The AFLP protocol often produces between 30 and 100 markers per primer combination.

Mutation of an AFLP fragment can happen in two different ways. Either a cutting site is mutated, in which case a dominantly inherited marker is yielded, or, alternatively, the length of the fragment is changed by an indel mutation, in which case inheritance is codominant. To determine which category a fragment belongs to, additional information from crossing experiments is needed. In the present study no such information was available and hence markers had to be treated exclusively as dominant. Other studies typically report 10-15% of AFLPs to be codominant (e.g. Becker *et al.*, 1995; Maheswaran *et al.*, 1997; Bai *et al.*, 1999).

Fluorescent labelled AFLP - Protocol

The AFLP protocol follows the modifications to the protocol of Vos *et al*. (1995) by Kropf *et al*. (2003). All reactions in the large scale studies were performed simultaneously for all samples of each species. Likewise, the population studies were done simultaneously in *E. maritimum* but were divided into two batches in *C. maritima* containing Aegean Sea/Black Sea and west France populations, and Strait of Gibraltar and Baltic Sea populations, respectively. All primers used are listed in table 4. Approximately 150 ng total genomic DNA were 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 10x PCR-buffer (GeneCraft), 0.25 µl 20mM dNTP, 1.25 µl 50mM MgCl2, 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 labelled *Eco*RI+3 primer, 0.15 µl 20mM dNTP, 0.75 µl 50mM $MgCl_2$, 1.5 µl BioTherm 10x 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 min at 60 °C.

Primer pairs were chosen after screening 12 and 16 primer combinations for variability in *E. maritimum* and *C. maritima*, respectively, using four samples in each species. For *C. maritima* two samples from the eastern Mediterranean region (Olympiada, Greece, and Sile, Turkey) and two from the North Sea (Thy and Grönhöj, Denmark) were used. Primers for *E. maritimum* were screened with two individuals from Turkey (Sahilköy, Yeniköy) and two from west France (Tuquelet du Nord, Cap Ferret). The same primers were used for both the AFLP phylogeography and the geneflow analyses. For a list of used primer combinations in the two species see table 4.

Table 4: Overview of the used primers and adapters.

Adapters	
EcoRI	5'-CTCGTAGACTGCGTACC-3' 3'-CATCTGACGCATGGTTAA-5'
MseI	5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'
Primers	**Sequence 5`- 3`**
Eco+A	GACTGCGTACCAATTCA
Mse+C	GATGAGTCCTGAGTAAC
E+ACG	GACTGCGTACCAATTCACG
E+ACT	GACTGCGTACCAATTCACT
E+AGA	GACTGCGTACCAATTCAGC
E+ATG	GACTGCGTACCAATTCATG
M+CCT	GATGAGTCCTGAGTAACCT
M+CGA	GATGAGTCCTGAGTAACGA
M+CGG	GATGAGTCCTGAGTAACGG
M+CTG	GATGAGTCCTGAGTAACTG
Combinations *C. maritima*	
NED	E+ACG - M+CGA
HEX	E+ATG - M+CGG
6-FAM	E+AGA - M+CGG
Combinations *E. maritimum*	
NED	E+ACG - M+CTG
HEX	E+ATG - M+CCT
6-FAM	E+ACT - M+CGA

AFLP products were separated on 6% polyacrylamide gels as a multiplex of three primer combinations labelled with different fluorescent dyes (6-FAM™, NED™ and HEX™; Applied Biosystems) and an internal size standard labelled with ROX™ (ROX 500TM, ABI). Gels were run for approximately 4 hours on an ABI 377™ automated sequencer using the Genescan analysis software (v3.1, ABI). AFLP products in the size range 75 - 500 base pairs 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

Phylogeographic structure. The primary instruments to detect genetic structure in the data were cluster analyses. Two different methods were used.

Neighbor Joining. First, genetic distances between individuals were calculated from the absence/presence matrix based on the AFLP patterns using the complementary value of the similarity coefficient of Nei and Li (1979) as implemented by PAUP* (Swofford, 2002). This similarity index was originally developed for RFLPs and can be seen as an estimation of the proportion of bands shared between two individuals because they had a common ancestor (Robinson & Harris, 1999). The resulting pairwise distance matrix was subjected to Neighbor Joining analysis (NJ; Saitou & Nei, 1987) using PAUP*. This clustering method gives an approximation of the tree with the smallest possible sum of branch lengths (minimum evolution tree; Li, 1997). The data sets were bootstrapped to produce statistical support for branches in the resulting phenogram.

The identification of clusters had to be done visually since bootstrapping in both species only gave support for one cluster and/or pairs of individuals. Because of this, distinct clusters were identified when they showed a clear geographical affinity.

Bayesian inference of population structure. Neighbor Joining dendrograms have several visual advantages in that relationships between individuals and clusters can be directly inspected in terms of genetic distances. However, as mentioned above, delimiting of clusters has to be done rather arbitrarily when branch support is lacking, and conclusions based on this may thus be erroneous. Because of this a different approach was also used. The program STRUCTURE (Pritchard *et al.,* 2000) implements a model-based clustering method using genotypic data. In this method a model is

assumed in which there are *K* populations (or clusters), each characterized by a set of allele frequencies at each locus. Using a Bayesian approach, population allele frequencies and assignment of individuals to these populations are inferred simultaneously. The number of populations, *K*, may be unknown and no prior information on the origin of individuals is used. The model further assumes Hardy-Weinberg equilibrium and linkage equilibrium within populations. To determine *K*, the program is run using different values of assumed number of populations in the model and the posterior probability for each *K* given the individual genotypes (Pr[*K* | *X*]) is calculated from the posterior probability of the data given *K* (Pr[*X* | *K*]). The result for the K-value that has the highest posterior probability is chosen as the best clustering solution.

In practice it may be difficult to use Pr(*K* | *X*) to choose the number of populations for complex data (Rosenberg *et al.*, 2002). In the case of data structured by an isolation-by-distance process, allele frequencies will vary gradually across the region. This will result in the study area being partitioned into smaller and smaller areas until all individuals are assigned according to their geographical proximities (Garnier *et al.*, 2004). In this case, an increment of *K* will increase the fit of the data to the model (Pr [*X* | *K*]) but each increment to a lesser and lesser extent. On the other hand, if there is a strong barrier to gene flow in the study area this should result in a clear structuring in the data, especially in a linear dispersal system such as the present, and thus a larger increase of Pr(*X* | *K*). Since we should be interested in finding the simplest model capturing the major structure in the data one should be able to find an appropriate value of *K* by looking at the increase in the fit of the data over the increase in *K*. However, because the goal of the present investigation is not to find the number of gene pools but instead to detect geographical structure, solutions of better fit that make biological sense will not be disregarded.

For the case of dominant markers like AFLP, the second allele is entered as missing data. According to the program manual this approach is valid under a model where individuals do not have mixed ancestry. This model, under the assumption of independent allele frequencies, was thus used in the large scale analyses.

The above approach was utilized for both phylogeography and population analyses. For the latter analyses the correlated allele frequencies setting was used. Basically, this setting assumes that allele frequencies are quite similar between

populations whereas the independent allele frequencies setting assumes that they are rather different (Pritchard *et al.*, 2000).

Population structure

Populations were clustered using UPGMA (Unweighted Pair-Group Method using arithmetic Averages; Sneath & Sokal, 1973) from the pairwise Nei's unbiased genetic distances (Nei, 1978) between populations using TFPGA V1.3 (Miller, 1997). The analysis was bootstrapped with 1000 permutations to obtain statistical branch support. Additionally, individuals were clustered into populations using STRUCTURE (see above).

Differentiation between populations was described using F-statistics Wright (1931; 1951) derived within the framework of the analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) as implemented in ARLEQUIN v2.0 (Schneider *et al.*, 2000). This method is an analogue to variance analysis of normally distributed data (ANOVA). Molecular variation is hierarchically distributed within populations and between populations, between clusters and/or between geographic regions. From the partitioning of the variance between the different hierarchical groups, analogues to F-statistics are computed. Differentiation between populations is here described with F_{st} or the proportion of the variance due to differences between populations, which in this case are the same. Significance of the F-statistics is tested by permutation of the individual genotypes over populations. In this case 3000 permutations were chosen. Groups of individuals, for the European scale analysis, and groups of populations, for hierarchical AMOVA for the population studies, was chosen based on the clustering results.

The significance test of an AMOVA uses the computed statistics (in the case of a non-hierarchical AMOVA they are F_{st} and the variance component due to differences among all populations) and tests them against a resampled null-distribution. Thus it does not give any information about which populations that differ from one another but only if there is significant difference between the populations in the analysis as a whole. Because of this the pairwise F_{st} values between populations were calculated and tested for significant difference from zero by a permutation test using ARLEQUIN v2.0.

Genetic diversity of populations was estimated from polymorphic markers with the Shannon Index ($SI=-\Sigma P_i \log_2$ where P_i is the frequency of the *i*th band; Shannon &

Weaver, 1949) and Nei's gene diversity, H_e, (Nei and Kumar, 2000) was estimated assuming Hardy-Weinberg equilibrium, using the program POPGENE (Yeh *et al.,* 1997). Differences in genetic diversity between groups of populations was tested for significance by resampling populations over groups following the approach of Goudet (2001) using the excel add-in POPTOOLS v2.5.2 (Hood, 2002). A marker was considered polymorphic if the marker frequency was within the interval 0.01-0.99 (99% level).

Gene flow and migration

Gene flow between populations was estimated using the indirect method of Wright (1931) based on F_{st}. This estimator is calculated as $N_em=(1-F_{st})/(4*F_{st})$, where N_e stands for effective population size and m is the migration rate. In those sea straits that showed a marked gene flow barrier, the rate of gene flow was estimated separately on each side of the barrier. Additionally the gene flow across the barrier was estimated by comparing only populations from different sides of the barrier. In cases where estimates of F_{st} were negative, these were adjusted to $F_{st}=0$, as suggested by Long (1986).

Another way of obtaining information about migration is to estimate from which gene pool an individual is drawn. Assignment tests compare the genotype of an individual to those of predefined populations and assigns it to the most probably one (or none). Comparing the assignment of individuals to the geographical origin of the sample gives an idea about whether dispersal occurs between populations or if they contain individuals from different areas. It also gives indirect information about dispersal distance. The above described Bayesian assignment method has drawback in that it may lack power, since it is primarily developed for co-dominant data, and does not allow an admixture model to be used when the data is dominant. Instead assignment was done with the reassignment or allocation procedure of AFLPOP v1.1 (Bernatchez & Duchesne, 1999) which computes the likelihood of an individual belonging to a population based on allele frequencies of the candidate populations. The reassignment procedure takes all individuals out of the populations, one at a time, and computes the likelihood of it belonging to each population whereas allocation assigns individuals of unknown origin (that is, individuals that has not been pre-assigned a population). Ten individuals per populations is a small number for population assignment studies (Campbell *et al.*, 2003) and consequently the analysis

suffered from a lack of power. Hence, populations clearly belonging to the same group in clustering analyses were pooled. When this was not possible the analysis was not performed. Closely linked loci or loci that show identical frequencies are redundant in information content and were excluded from the analysis, as were also monomorphic markers.

Test for correlation between geographic and genetic distances

For a species with limited dispersal ability compared to its spatial distribution, individuals or populations close to each other are expected to be genetically more similar than more distant ones (isolation by distance) due to migration-drift equilibrium. Refugial areas would have had more time to reach equilibrium than re-colonized ones. Hence, absence, or a marked reduction in IBD in a region compared to refugial areas, can be taken as evidence for that region being more recently colonized. To test the hypothesis that the data is structured by an isolation by distance process, a Mantel test (Mantel, 1967) between geographic and genetic distances was performed on clusters as well as the whole data using the software THE R PACKAGE v4.0 (Casgrain & Legendre, 2000). In this procedure the Normalized Mantel's correlation coefficient (Mantel's *r*) is computed between the pairwise geographical distances of sample localities and the pairwise genetic distances, measured as F_{st}/ (1- F_{st}) for populations as recommended by Rousset (1997), and Nei and Li's distances (Nei and Li, 1979) for single individuals, respectively. Negative F_{st}-values were adjusted to zero in this analysis, too. Significance of the obtained *r* is tested by permutation of one of the distance matrices to generate a randomized distribution of correlation values with which to compare the calculated *r*-value. The probability, *P*, that the obtained *r*-value is produced by chance is the proportion of values equal to or higher than r to the total number of random replicates, including *r* (following Hope, 1968).

The correlation should be strongest along the actual dispersal routes. To test the hypothesis that dispersal is mainly along the coast, two different testing strategies were used. First, correlations using coastal and Euclidean distances were compared for several areas with the expectation that Euclidean distances should exhibit lower correlations with genetic distances, than distances measured along the coast. Analogous, populations separated by large distances of water, e.g. on islands should produce lower correlations when included (Castric & Bernatchez, 2004).

ITS analysis

Ribosomal DNA (rDNA) codes for the RNA component of the ribosome. It is arranged in tandem arrays separated by the intergenic spacer and each unit codes for the large and small rDNA subunits 28S and 18S. Separated from, and between the two subunits lie the gene 5.8S as well as two spacers, ITS1 and ITS2. The ITS region is a widely used marker since the development of universal primers in the early nineties. Here the universal primers 18S and 28S (18S: CCT TMT CAT YTA GAG GAA GGA G and 28S: CCG CTT ATT KAT ATG CTT AAA; Muir & Schlötterer, 1999) were used to screen the complete ITS region comprising ITS1, 5.8S and ITS2 for sequence variation. All amplifications were performed in 25 µL volumes containing 1.0 µL of 50 mM $MgCl_2$, 2.5 µL 10x BioTherm PCR buffer (GeneCraft), 0.25 µL of 20 mM dNTP, 1.0 µL of each primer at 25 pmol/µL, 0.75 U BioTherm Taq polymerase (GenCraft), and 0.4 µL genomic DNA (ca 5–40 ng). Cycling conditions were as follows: 60 s at 94° was followed by 35 cycles of 18 s at 94°C, 30 s at 55°C and 60 s at 72°C and then by a post treatment of 78 s at 55°C and 8 min at 72°C.

Several other genes were also screened for variation for both species; however, after finding only little sequence variability and experiencing difficulties with amplifications in a large proportion of individuals, the efforts were halted. The genes screened and the sequences obtained can be found together with amplification protocols in appendix III.

PCR purification and sequencing

PCR products were purified with the QIAquick purification kit (QIAgen) according to the manufacturer's instructions. Purified PCR products were directly sequenced using BigDye 3.0/2.1 Terminator Cycle Sequencing Kit. The thermo cycling conditions were 30 cycles of 10 s at 96°C and 4 min at 55°C for version 3.0. For BigDye 2.1 an initial 60 s at 96°C was followed by 28 cycles of 6 s at 96°C, 12 s at 55°C and 4 min at 60°C after which a post treatment followed consisting of 6 s at 51.4°C and 4 min at 60°C. The same primers were used for sequencing as in the amplifications.

Sequencing reactions were analyzed on an ABI377 sequencer by GENterprise in Mainz, Germany. Sequences were aligned in Sequencher 3.0 (Gene Codes co.) with minor manual corrections.

RESULTS

Phylogeography

Cakile maritima - Biogeographic structure

A total of 164 fragments could be consistently scored for 81 individuals. Of these, eleven bands were monomorphic and 25 only found in a single individual. The remaining 138 fragments could differentiate all the 81 individuals from each other. A Neighbor Joining tree of the Nei and Li's distances shows several clusters corresponding to geographical regions; a north Atlantic, an Adriatic and an Aegean-Black Sea cluster as well as a split into Mediterranean and Atlantic material (Fig. 3). The same general patterns were found in the PCO-analysis of the data set (not shown). Separate NJ-analyses of the Atlantic and Mediterranean groups improve the geographical affinity of clusters: the three Italian samples found in the Aegean-Black Sea cluster groups with other material from west Italy and France and a sample from Trondheim clusters with other northern material (not shown).

The results of the model based Bayesian clustering shown in figure 4 confirm the result of the NJ analysis. The Pr[*X* | *K*] increased from K=1 to K=6. All clusters identified correspond to increasingly smaller geographical areas. First Atlantic and Mediterranean collections are divided. In K=3 individuals from the Aegean Sea and Black Sea are split followed by the northernmost Atlantic and Baltic Sea material. The remaining Mediterranean material is subdivided geographically into a West Mediterranean and an Ionian/Adriatic Sea cluster in the next model except that Jerba (Tunisia, no. 43), Oroklini (Cyprus, no. 72) and Herzliya (Israel, no. 73) are assigned to the west Mediterranean cluster. In K=6 a geographically heterogenous group is formed consisting of Raouel (Tunisia, no. 42) and most individuals close to the Strait of Gibraltar but also material from Northeast Spain.

Generally one of the identified clusters for a given K-value was split into two in the K+1 model. These results are consistent with those expected under isolation-by-distance. Nevertheless, no additional subdivision is found for higher values of K and large geographical areas remain undivided. As the clustering for K=5 corresponds closely to both the NJ analysis as well as being geographically plausible, whereas the last inferred cluster in K=6 contains several less strongly assigned individuals and seems difficult to interpret biologically, K=5 is chosen to be the best representation of

the data, although the information from K=6 will also be considered. This decision is also supported by the population genetic investigation from the Strait of Gibraltar (see below).

Differentiation

An AMOVA was calculated with the five groups inferred by the clustering method and the results are shown in table 5. The overall F_{st} is 0.242 among the clusters inferred by the K=5 model. Comparisons between adjacent areas generally give the lowest pairwise F_{st} values (Appendix I). The only exception is the Aegean Sea/Black Sea - west Mediterranean comparison showing lower differentiation than to their respective neighboring clusters. However, if the west Mediterranean cluster in fact should be extended to include the whole of the African and parts of the Asian coast, as is suggested by the clustering of the collections from Tunisia, Israel and Cyprus with the west Mediterranean samples (figures 3 and 4), this is an erroneous statement.

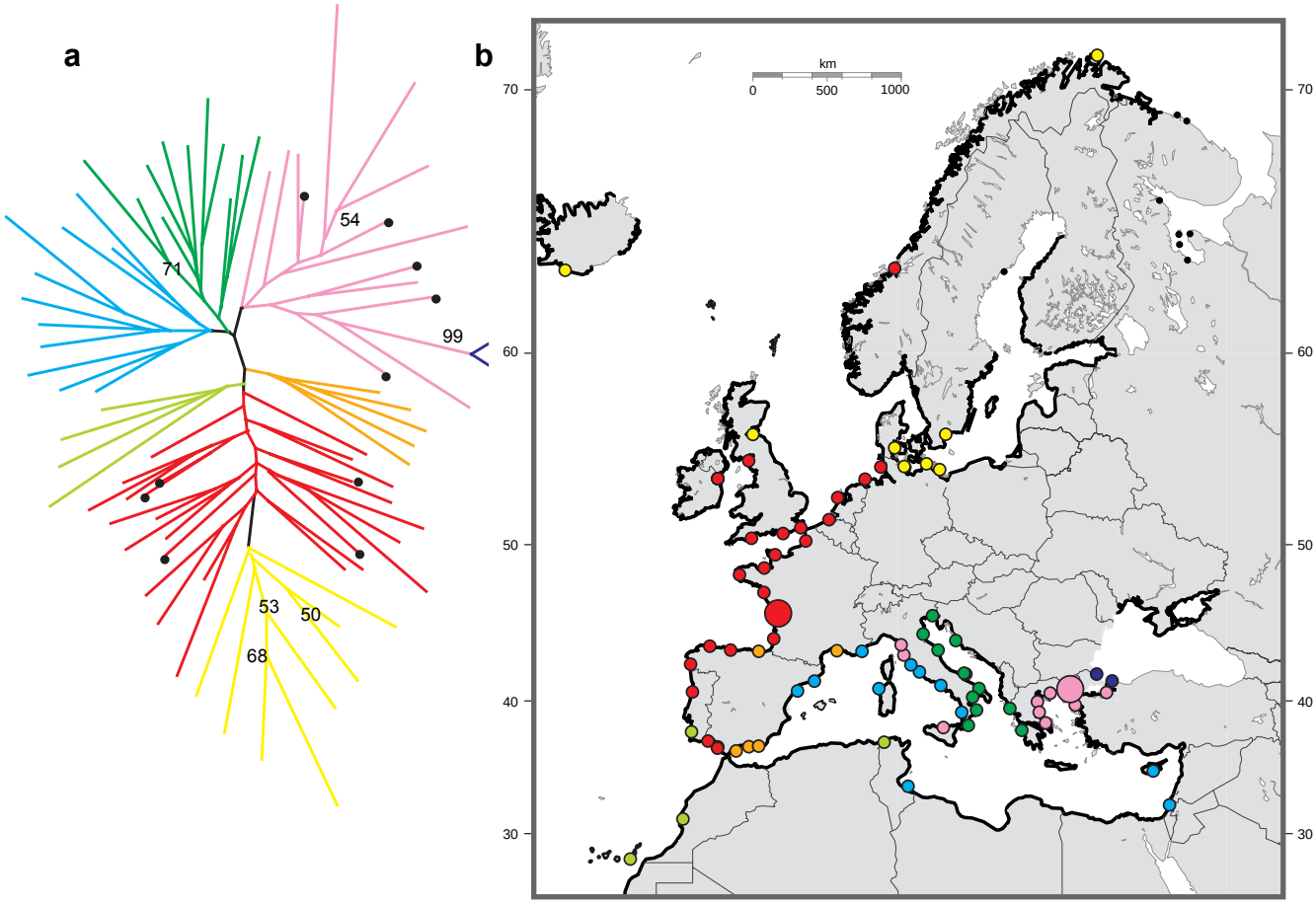


Figure 3. *Cakile maritima.* a) Neighbor Joining phenogram, and b) sampling localities and geographical distribution of the coloured NJ clusters. Large dots on the map indicate that five individuals were sampled from the locality. These individuals are marked in the NJ phenograms. Numbers indicate bootstrap support.

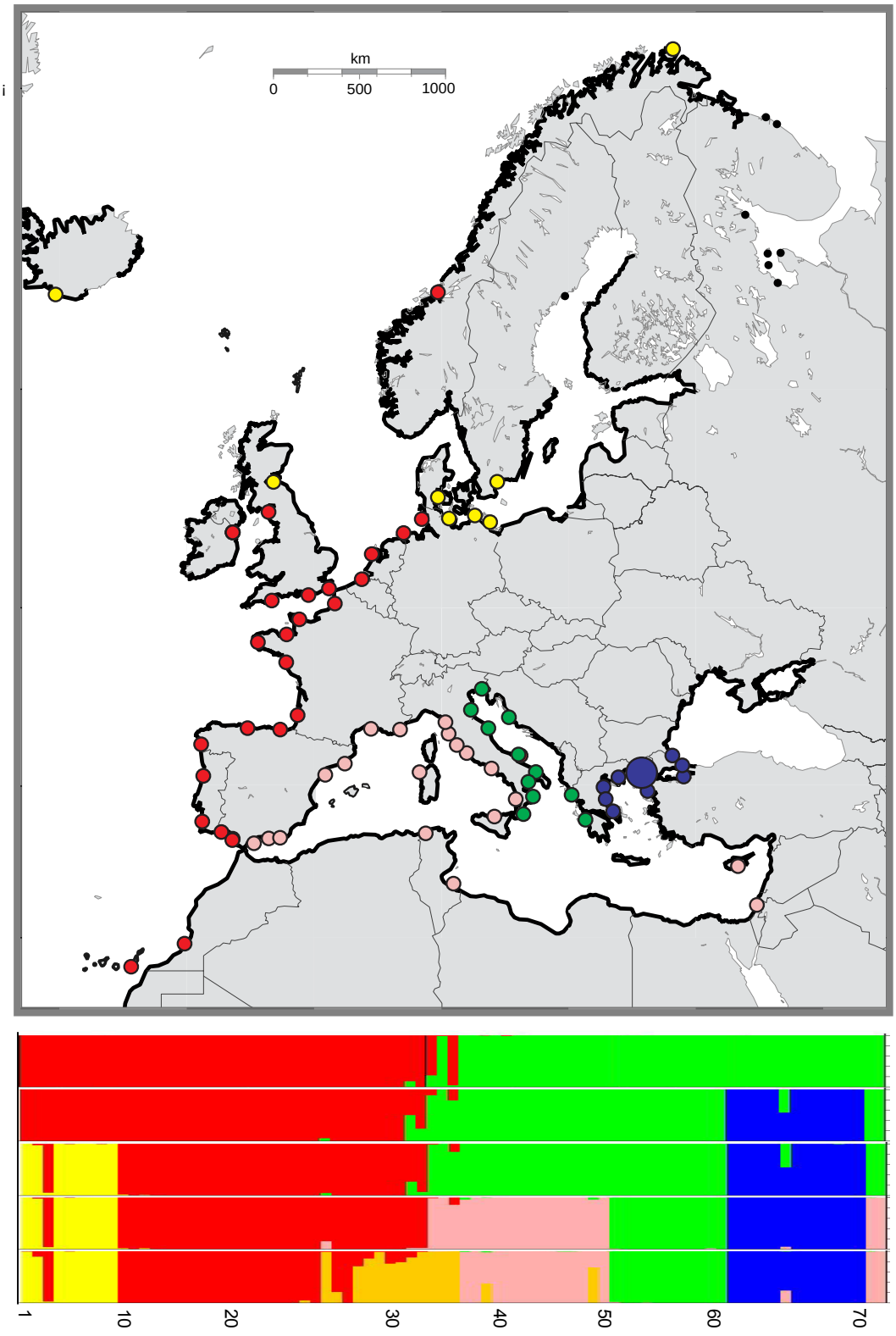


Figure 4. Result of the model based clustering (K=2-6), sampling localities and geographical distribution of clusters (K=5) of *C. maritima*. Each cluster is identified by its colour. Individuals and their assignment frequencies to the K coloured clusters are represented by vertical bars. Large dots on the map indicate that 5 individuals were sampled from this locality. These individuals are grouped together by thin dark lines in the bar plot. Sampling localities are ordered from north to south and numbers below bars refer to the localities in table 1. The figure is based on the runs with the highest posterior probability of the data given the implemented model. Probabilites are given in appendix IV.

Eryngium maritimum - Biogeographic structure

In *E. maritimum* 170 bands, of which 23 were monomorphic and 24 apomorphic, were included in the analysis of 85 individuals. The 123 informative bands could distinguish between all included individuals. The Neighbor Joining analysis of this dataset revealed a distinct division of the material into an Atlantic and a Mediterranean cluster (Fig. 5). The Atlantic cluster is comprised of three subclusters; a south Atlantic one containing collections south of Cadiz, Spain, a west Atlantic cluster which includes plants from the Bay of Biscay and one with the rest of the material from Bretagne and northwards but also including a sample from Cabo Pino east of Gibraltar. An additional NJ analysis performed on the Atlantic material recovered only some of this structure and the comparatively long branches seen between the three clusters in the analysis of the full dataset were lacking. The Mediterranean cluster is divided into an eastern and a western cluster with a division between them at Peloponnesus. The eastern cluster contains the Aegean and Black Sea material and two collections from Cyprus and south Turkey that branch off at the base.

Model based clustering gives a very similar result, finding the highest likelihood in K=5 (Fig. 6). Under the two population models STRUCTURE assigns all Atlantic individuals and Cabo Pino to one cluster. In the next model all individuals east of Peloponnesus are grouped. In the two subsequent models the Atlantic and the west Mediterranean clusters are subdivided, but individuals in the new groupings are drawn from throughout the geographical range of the respective original clusters.

The two major clusters were also analyzed separately with STRUCTURE. The subdivision of the Mediterranean material (K=5 in the analysis of the full data set) was not found in this analysis despite a large number of runs. Instead, K=2 was the best solution found, dividing the material into an east and a west Mediterranean cluster as in the K=3 model of the full dataset. The Atlantic material was divided into three groups with individuals of rather mixed geographical origin. The largest cluster contains much of the northern collections and a portion of the north French samples. However it also includes two south Iberian collections as well as excluding three samples from the British Isles. The other two clusters contain more southern material but also some French and British collections. In summary the data seem to contain at

least a weak north – south structure judging from the two clustering methods, but this should be regarded with caution.

Differentiation

The AMOVA was performed on the three groups identified by the clustering analyses. Over-all among population F_{st} was 0.509 ($P<0.001$). The pairwise F_{st}s reflected the pattern seen in the NJ analysis with a much higher proportion of among population variation found in the comparisons involving the Atlantic than between the Mediterranean and the Aegean/Black Sea clusters (Table 6).

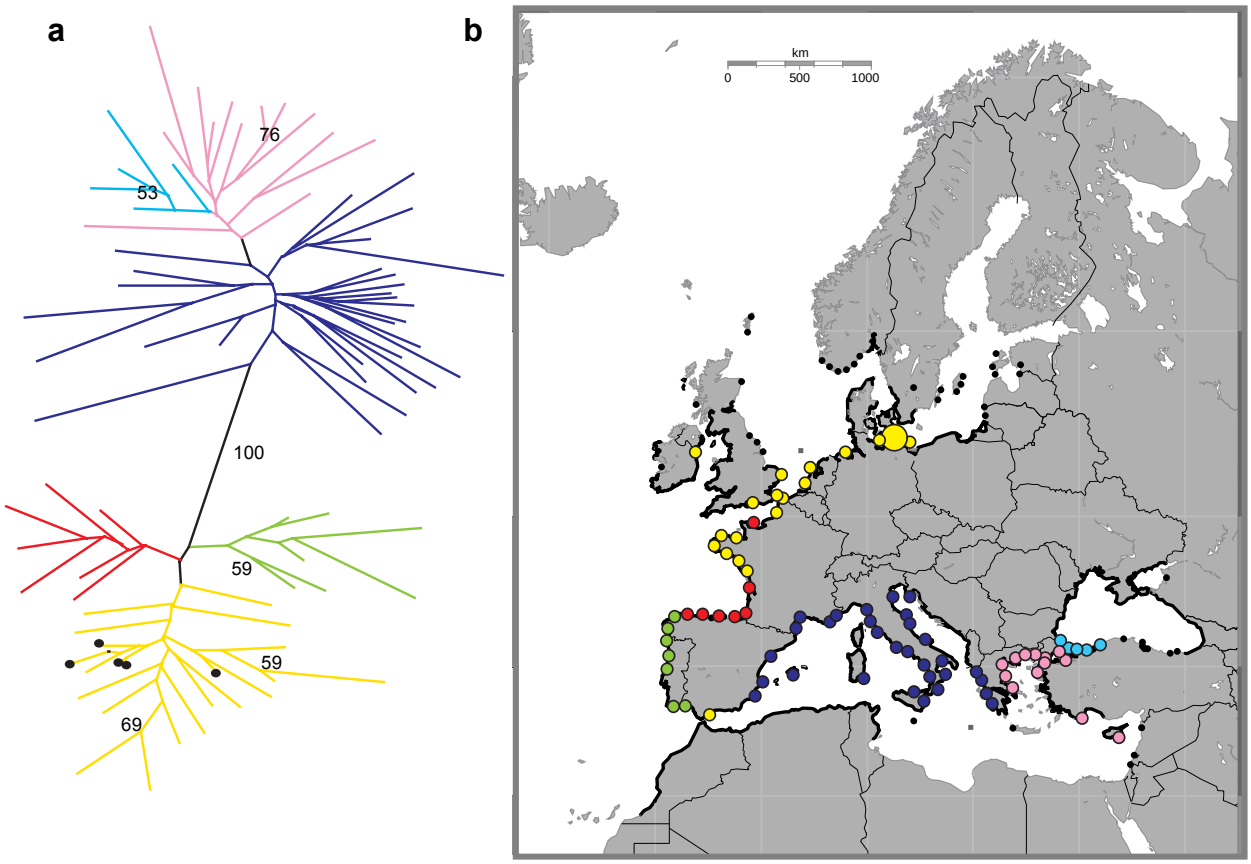


Figure 5. *Eryngium maritimum.* a) Neighbor Joining phenogram, and b) sampling localities and geographical distribution of the coloured NJ clusters. The large dot on the map indicates that five individuals were sampled from that locality. These individuals are marked in the NJ phenogram. Numbers in the tree indicate bootstrap support.

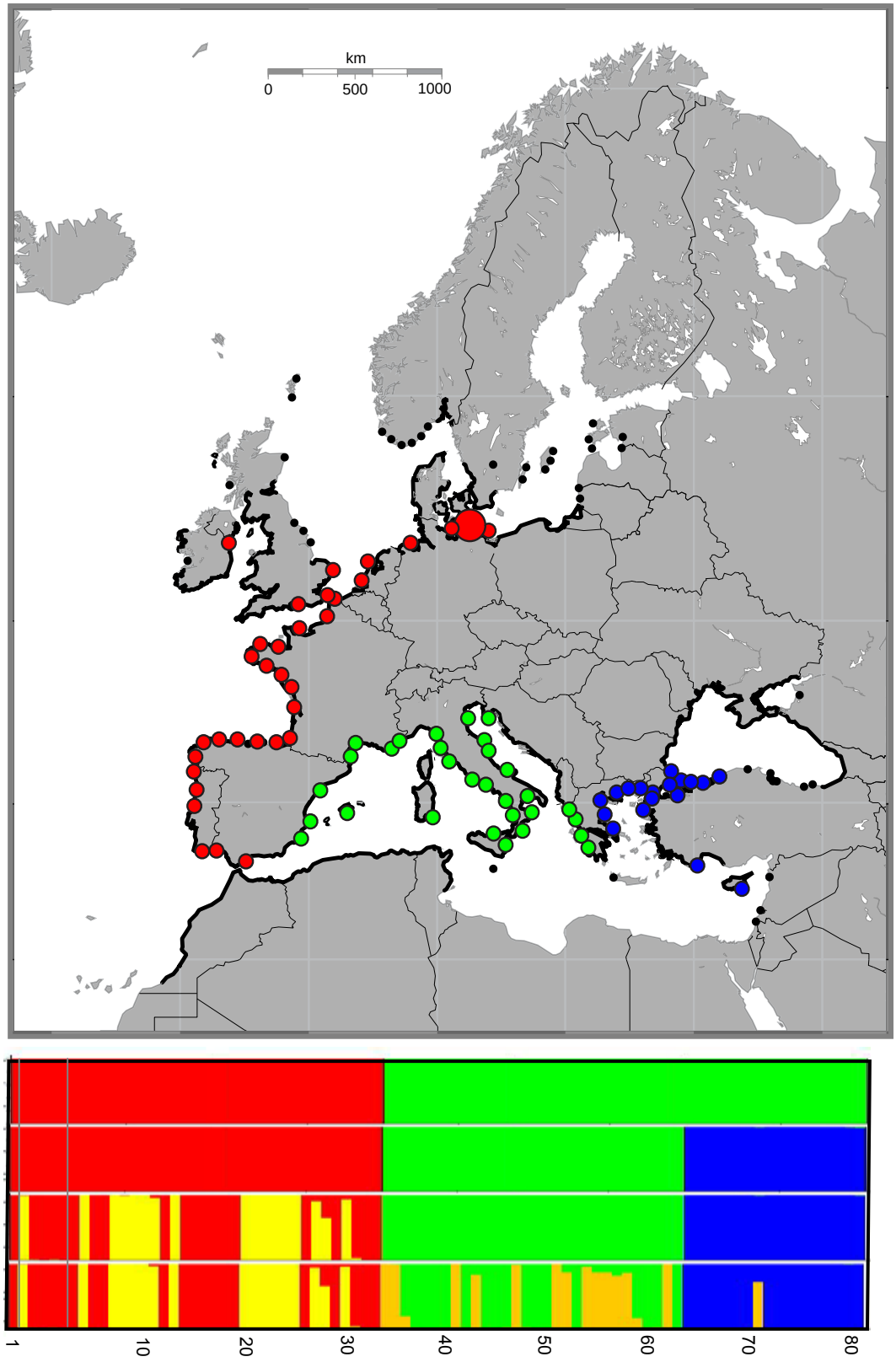


Figure 6. Result of the model based clustering (K=2-5), sampling localities and geographical distribution of clusters (K=3) of *E. maritimum*. Each cluster is identified by its colour. Individuals and their assignment frequencies to the K coloured clusters are represented by vertical bars. Large dots on the map indicate that 5 individuals were sampled from this locality. These individuals are grouped together by thin dark lines in the bar plot. Sampling localities are ordered from north to south and numbers below bars refer to the localities in table 2. The figure is based on the runs with the highest posterior probability of the data given the implemented model. Probabilities are given in appendix IV.

Table 5. Genetic differentiation in *Cakile maritima*. Results of a non-hierarchical AMOVA performed on the groups identified in the cluster analyses, and pairwise F_{st} values between adjacent regions.

Source of variance	Degrees of freedom	Sum of Squares	Variance components	Percentage of variation	P-value
Among regions	4	278.52	3.78610	24.24	0.000
Within regions	76	899.31	11.83302	75.76	–
Total	80	1177.83	15.61912		
F_{st} = 0.2424, P = 0.000					
Pairwise F_{st} between regions					
North Atlantic – South Atlantic				0.17113	0.000
South Atlantic – West Mediterranean				0.25279	0.000
West Mediterranean – Ionian/Adriatic				0.12712	0.000
Ionian/Adriatic – Aegean/Black Sea				0.19618	0.000

Table 6. Genetic differentiation in *Eryngium maritimum*. Results of a hierarchical AMOVA performed on the groups identified in the cluster analyses and pairwise F_{st} values between adjacent regions.

Source of variance	Degrees of freedom	Sum of Squares	Variance components	Percentage of variation	P-value
Among groups of regions	1	369.68	6.501	35.56	0.329
Among regions within groups	1	69.47	2.807	15.36	0.000
Within regions	82	735.72	8.972	49.08	0.000
Total	84	1174.87	18.280		
F_{st} = 0.50918, P = 0.000					
Pairwise F_{st} between regions					
Atlantic Ocean – West Mediterranean				0.49875	0.000
West Mediterranean – Aegean/Black Sea				0.22538	0.000

Isolation by distance

A mantel test performed on the whole range sampling shows a positive and significant correlation between pairwise Nei and Li's genetic distances and geographic distances for both species. In *C. maritimum* the Mantel's normalized correlation, *r*, was 0.59 (P<0.0001) when all individuals and localities were included. Significant correlations were also found when south Atlantic and west Mediterranean samples were analyzed separately (table 7).

At the largest scale there was a strong correlation in *E. maritimum* too (r=0.72, P<0.0001). A separate analysis of the Atlantic only showed isolation by distance when outliers were excluded whereas the west Mediterranean/Adriatic and

Aegean/Black Sea clusters showed significant positive correlations also when outliers were included (table 8).

Euclidean geographical distances generally produced somewhat higher correlations than coastal distances in *C. maritima*. In *E. maritimum* the two measures yielded comparable values.

Table 7. Correlations between geographical and Nei and Li's genetic distances. P-values for the Mantel test are given in italics.

C. maritima	Coastal distances	Coastal, no outliers	Euclidean distances	Euclidean, no outliers
Whole range	0.594 *0.0001*		0.422 *0.0001*	
North Atlantic	0.006 *0.436*		0.039 *0.376*	
South Atlantic	0.456 *<0.0001*	0.458 *<0.0001*	0.508 *<0.0001*	0.465 *<0.0001*
West Mediterranean	0.298 *0.006*	0.471 *<0.0001*	0.349 *0.002*	0.487 *<0.0001*
Adriatic/Ionean Sea	0.209 *0.080*		0.379 *0.003*	
Aegean/Black Sea	0.186 *0.144*		0.326 *0.034*	

Table 8. Correlations between geographical and Nei and Li's genetic distances. P-values for the Mantel test are given in italics.

E. maritimum	Coastal distances	Coastal, no outliers	Euclidean distances	Euclidean, no outliers
Whole range	0.720 *<0.0001*		0.531 *<0.0001*	
Atlantic	0,085 *0,112*	0,207 *0,008*	0,038 *0,294*	0,187 *0,018*
West Mediterranean/ Adriatic	0.316 *<0,0001*	0.340 *<0,0001*	0,356 *0,0009*	0,369 *0,002*
Aegean/Black Sea	0.284 *0.039*		0,293 *0,046*	
Adriatic only	0.452 *0.002*		0,560 *0,0003*	
West Mediterranean Basin	0,190 *0,086*	0,253 *0,061*	0,152 *0,100*	0,191 *0,076*

Regional scale studies

Genetic diversity

The number of markers scored for each region is given in table 9. In general, more monomorphic and fewer apomorphic markers were scored in *E. maritimum* than in *C. maritima*. The total number of scored markers was lower in the Atlantic regions than in the Strait of Gibraltar and the Aegean Sea/Black Sea areas. This is due to a larger number of monomorphic null alleles in the Atlantic regions that are not counted towards the total.

Table 9. Number of scored markers in different regions.

	C. maritima			***E. maritimum***		
Area	**Monomorphic**	**Apomorphic**	**Total**	**Monomorphic**	**Apomorphic**	**Total**
Baltic Sea – North Sea	16 (13%)	14 (12%)	123	-	-	-
West France	26 (21%)	13 (11%)	119	70 (74%)	4 (4%)	94
Strait of Gibraltar	21 (15%)	12 (9%)	137	48 (40%)	3 (3%)	119
Aegean Sea – Black Sea	19 (12%)	17 (11%)	158	56 (53%)	2 (2%)	106

The population diversity estimates, measured as the Shannon Index, SI, and gene diversity, H_e, in *C. maritima* and *E. maritimum*, were based on, respectively, 93 and 72 AFLP-markers that could be scored reliably in the entire material and were polymorphic at the 99% level. Markers that could be scored in some but not in other gels were excluded. These markers were excluded usually due to the fact that the base of large peaks covered the location of peaks of other markers, so that null alleles (missing bands) could not be scored comparably over all gels. Alternatively, in one gel (*C. maritima*, West France), data from fragments larger than ca 400 bp was not collected. As a result, only fragments shorter than 400 bp could be usad for this analysis.

The average population diversity measures within regions and the results of the significance tests are given in the tables 10 and 11. For both indices, the average

values over all populations were slightly higher in *C. maritima* (SI_{tot}=0.171 and H_{tot}=0.115) than in *E. maritimum* (SI_{tot}=0.139 and H_{tot}=0.093). Differences were also found between regions within species. The highest average regional diversities were found in the Gibraltar area followed by the Aegean/Black Sea/Marmara region for both species. In *E. maritimum,* west France had the lowest values and in *C. maritima* the Baltic Sea had slightly lower diversity than west France but the difference was not significant.

Table 10. Mean within populations diversities for different regions and tests for significant differences. Significance is indicated by asterisks: *0.05 > P > 0.01, **0.01 > P > 0.001, ***P <0.001

Region	SI	H_s	Balt	Wes	Gib	Atl	Med	Aeg
Baltic Sea	0.142	0.094	-	ns	***	**	***	**
West France	0.160	0.108	ns	-	**	*	***	ns
Gibraltar	0.213	0.142	***	***	-	-	-	*
Atlantic	0.192	0.129	**	*	-	-	*	**
Mediterranean	0.234	0.156	***	***	-	ns	-	**
Aegean/Black Sea	0.181	0.122	**	ns	*	**	**	-
					SI below diagonal			
All Mediterranean	0.190	0.128						
All Atlantic Ocean	0.159	0.107						
Test Med. vs. Atl.	**	*						
Average all pop.	0.171	0.115						

Table 11. Mean within populations diversities for different regions and tests for significant differences. Significance is indicated by asterisks: *0.05 > P > 0.01, **0.01 > P > 0.001, ***P <0.001

Region	SI	H_s	Wes	Gib	Atl	Med	Aeg
West France	0.109	0.073	-	*	*	**	*
Gibraltar	0.168	0.111	*	-	-		ns
Atlantic	0.093	0.058	ns	-	-	*	**
Mediterranean	0.249	0.163	***	-	**	-	***
Aegean/Black Sea	0.136	0.088	ns	ns	*	***	-
				SI below diagonal			
All Mediterranean	0.176	0.118					
All Atlantic Ocean	0.102	0.068					
test Med. vs. Atl.	***	***					
Average all pop.	0.139	0.093					

Differentiation between populations

The genetic differentiation among populations was highly significant for both species in all three sea straits with F_{st} values, calculated with AMOVA, ranging from 0.082 (P<0.001) for *C. maritimum* in the Baltic Sea/North Sea region to 0.31 (P<0.001) for *E. maritimum* in the Strait of Gibraltar (Table 12). In comparison the F_{st} values for the control region, presumably without restrictions to gene flow, was much lower, 0.015 (P=0.053) and 0.042 (P=0.008) for *C. maritimum* and *E. maritimum* respectively.

The test of pairwise population differentiation (F_{st}) reflects these results and gave different results within different areas (Appendix I). In the Strait of Gibraltar and the Aegean/Black Sea/Marmara area most population pairs were significantly different from each other (P<0.05 for the Strait of Gibraltar in 42 and 34 cases out of 45 compared to 2.25 expected by chance and for the Aegean/Black Sea/Marmara 49 and 43 cases out of 55 where 2.75 is expected by chance for *C. maritima* and *E. maritimum*, respectively). Fewer significantly different comparisons were found in the Baltic Sea/North Sea area (P<0.05 in 28 out of 45 cases) and in west France, six and eight comparisons out of 36 (1.8 expected by chance) were significant for *C. maritima* and *E. maritimum*, respectively. In the latter area the significant comparisons were found in cases involving mainly Port d'Albret (WF9) in both species (5 and 6 cases in *C. maritima* and *E. maritimum*, respectively).

Table 12. Genetic differentiation of populations. Analsysis of molecular variance (AMOVA) based on AFLP multilocus phenotypes of individuals sampled from populations of two species. The populations are divided into seven analyses according to species and geographical region.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	P-value
Eryngium maritimum					
West France					
Among populations	8	34.48	0.134 Va	4.23	0.0078
Within populations	77	233.37	3.031 Vb	95.77	
Total	85	267.85	3.165		
Fixation Index F_{st} : 0.0423	P=0.0078				
Strait of Gibraltar					
Among groups	1	95.31	1.840 Va	27.50	0.0107
Among populations within groups	8	56.38	0.256 Vb	3.82	<0.0001
Within populations	86	395.11	4.594 Vc	95.77	<0.0001
Total	95	546.80	6.689	68.68	
Fixation Index F_{st} : 0.3132	P<0.0001				

Table 12, continued.

Aegean Sea/Black Sea/Marmara						
Among groups			2	85.22	1.244 Va	23.40	<0.0001
Among populations within groups			8	53.04	0.380 Vb	7.16	<0.0001
Within populations			77	284.24	3.691 Vc	69.44	<0.0001
Total			87	422.49	5.315		
Fixation Index	F_{st} :	0.3047	P<0.0001				
Cakile maritima							
Baltic Sea/North Sea						
Among populations			9	133.93	0.714 Va	8.16	<0.0001
Within populations			86	690.87	8.033 Vb	91.84	
Total			95	824.80	8.748		
Fixation Index	F_{st} :	0.0816	P<0.0001				
West France						
Among populations			8	90.60	0.152 Va	1.49	0.0528
Within populations			65	655.14	10.079 Vb	98.51	
Total			73	745.73	10.231		
Fixation Index	F_{st} :	0.0149	P=0.0528				
Strait of Gibraltar						
Among groups			1	81.31	1.271 Va	8.97	<0.0001
Among populations within groups			8	173.51	1.046 Vb	7.38	<0.0001
Within populations			84	995.84	11.855 Vc	83.65	<0.0001
Total			93	1250.67	14.173		
Fixation Index	F_{st} :	0.1635	P<0.0001				
Aegean Sea/Black Sea/Marmara						
Among groups			2	206.591	3.169 Va	19.84	<0.0001
Among populations within groups			8	169.545	1.295 Vb	8.11	<0.0001
Within populations			73	840.007	11.507 Vc	72.05	<0.0001
Total			83	1216.143	15.971		
Fixation Index	F_{st} :	0.2795	P<0.0001				

Isolation by distance

Correlation between $F_{st}/(1-F_{st})$ and geographic distances was detected for both species in all the sea straits investigated (table 13). In the Strait of Gibraltar correlations were particularly high. However, when the Atlantic and Mediterranean subgroups identified in the cluster analyses were tested separately, this pattern disappeared and instead correlations were non-significant except for a negative correlation on the Atlantic side of the strait for *E. maritimum*. Likewise, when subgroups identified from the Aegean/Marmara/Black Sea region were tested separately, correlations were no longer significant. It appears that the correlation is due to comparisons across gene flow barriers (Husband & Barret, 1995).

In the Baltic – North Sea Strait, strong and significant correlation was found. However, this correlation was entirely due to the outlier Grönhöj. When removed, the correlation was much lower and not significant.

Table 13. Isolation by distance and mantel test.

	Mantel r	P
E. maritimum		
Aegean/Marmara/Black Sea	0.41	0.016
Aegean/Marmara	0.055	0.320
Marmara	0.060	0.410
E. maritimum		
West France	0.36	0.320
E. maritimum		
Gibraltar	0.64	0.002
Gibraltar Mediterranean	-0.56	0.007
Gibraltar Atlantic	0.31	0.226
C. maritima		
Baltic Sea	0.49	0.013
Baltic Sea - Grönhöj	0.13	0.210
C. maritima □		
Gibraltar	0.60	0.001
Gibraltar Mediterranean	0.29	0.220
Gibraltar Atlantic	0.15	0.360
C. maritima		
Aegean/Marmara/Black Sea	0.68	<0.0001
Aegean/Marmara	0.56	0.004
Marmara	0.38	0.205
Aegean	-0.50	0.210
C. maritima		
West France	0.47	0.011

Genetic structure

Aegean Sea/Black Sea/Sea of Marmara

Cakile maritima. 87 individuals from 11 populations went into the data set from the Aegean/Black Sea/Marmara area. A non-hierarchical AMOVA found 23% of the total AFLP variation among populations (P<0.001). The UPGMA based on Nei's genetic distances is shown in figure 7c. The most divergent populations are BS1 and BS3 from the Black sea which cluster together. The two other clusters are less divergent from each other but both reflect geographical relationships as well, one containing populations from the Sea of Marmara (MA11, MA13, MA14, MA15, MA17) and the other from the Aegean area (AE18, AE19, AE22, GR1). This grouping of populations is also supported by the model based Bayesian clustering of individuals where the two Black Sea populations, except one individual from BS3, were split off first and the Aegean Sea and Sea of Marmara populations were divided in the K=3 model (Fig. 7a). For K=4, the fourth cluster contained one individual from BS3 and seven individuals from MA1 with three further samples from other populations partially assigned. The highest likelihood was found for K=5, but individuals are not as strongly assigned as in other clusters and they are not geographically correlated. As it is not surprising that individuals group to their own population as in K=4, the results of the three populations model are considered the best representation of the data. A hierarchical AMOVA based on the three groups inferred by the Bayesian clustering showed that the three regions were significantly separated with 19.8% of

the variation between regions and 8.1% among populations within groups (Table 12). The estimated migration rates were lower between the regions than within regions (Table 14). An exception was, however, the two Black Sea populations with an estimated N_em of 0.68 which is unusually low for populations so close to each other. This is likely due to the population in BS1 which exhibit very low levels of variation (Appendix II). This locality was a small stretch of beach containing only few individuals and it's seems likely that bottlenecks could have depleted the population of variation. The assignment test allocates 79 out of 83 samples to a region with a likelihood difference of at least 1 (95.2%). Of these, 78 individuals are allocated to their own region of origin and one individual from BS1 to the Marmara populations. The allocation success on the population level was much lower on the other hand. In accordance with the lower pairwise F_{st} values when populations within groups were compared, this shows that the main differentiation lies in the two straits separating the Aegean Sea and the Black Sea from the Sea of Marmara, respectively. When using MA11 as a fourth candidate region, the mis-aligned BS1 individual was allocated to this population.

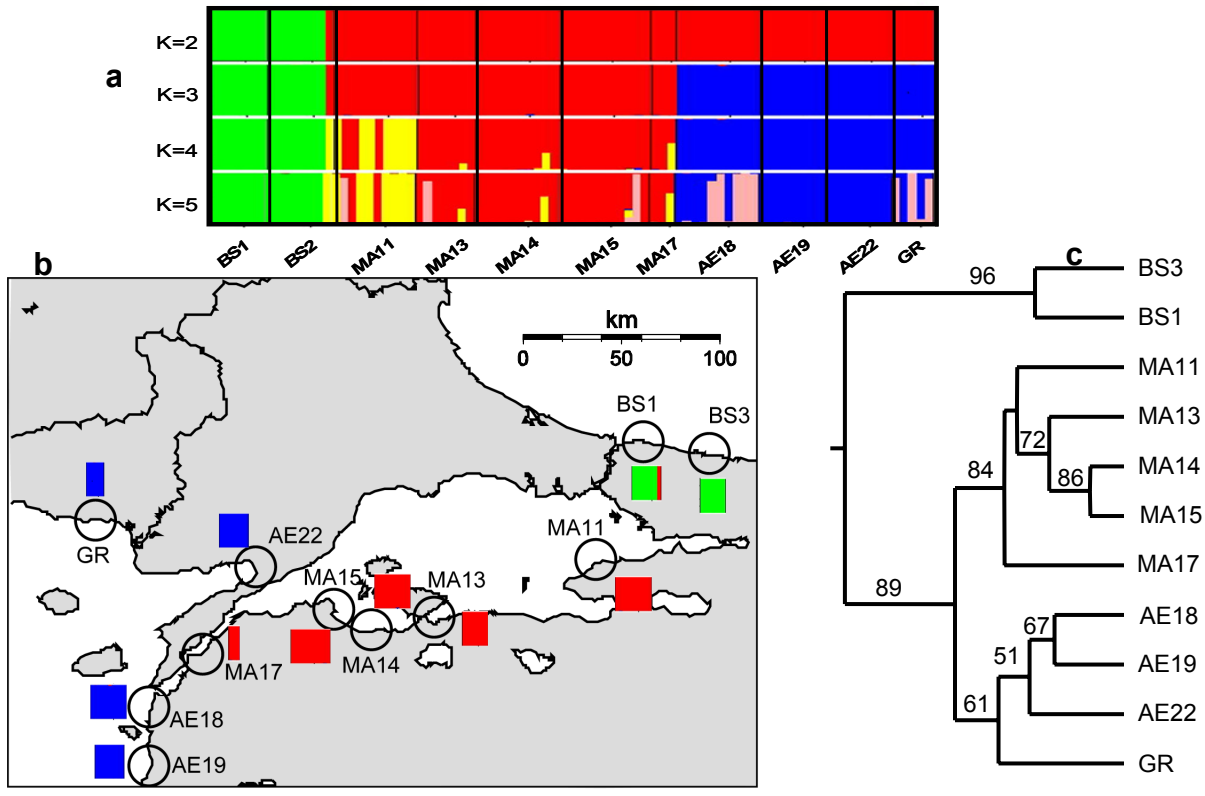


Figure 7. a). Result of the model based clustering (K=2-5) of *C. maritima* from the Aegean Sea/Sea of Marmara/Black Sea. Each cluster is identified by its colour. Individuals and their assignment frequencies to the K coloured clusters are represented by vertical bars. Populations are separated by dark lines and labelled below the figure. The population abbreviations refer to the sampling localities listed in table 3. The figure is based on the runs with the highest posterior probability of the data, given the implemented model (appendix IV). b) Geographical distribution and assignment of individuals to the clusters in K=3. c) UPGMA phenogram based on Nei's genetic distance (1978) between populations. Numbers on branches are bootstrap support.

Eryngium maritimum. In *E. maritimum,* 11 populations consisting of 88 samples were analyzed. Genetic distances were lower than for *C. maritima* but the resulting dendrogram shows a similar pattern (Fig. 8c). Again two Black Sea populations form a cluster separated from the remainder and the populations from the Sea of Marmara form another cluster. Of the Aegean populations GR, AE22 and AE18 form a cluster that doesn't contain AE19 although it has its lowest genetic distance to AE18. MA13 was not included in this analysis as only four individuals from this population were included.

The model based clustering reinforces this pattern partitioning the samples roughly into three groups (Fig. 8a). First the two Black Sea populations are split off from the rest and in the subsequent model most individuals from populations AE18 and AE19 are split from MA12, MA15, MA16 and MA24. The individuals from the three remaining populations were assigned to these two latter groups. The K=4 model gave a slightly higher likelihood but most of the individuals in the fourth group are only weakly assigned.

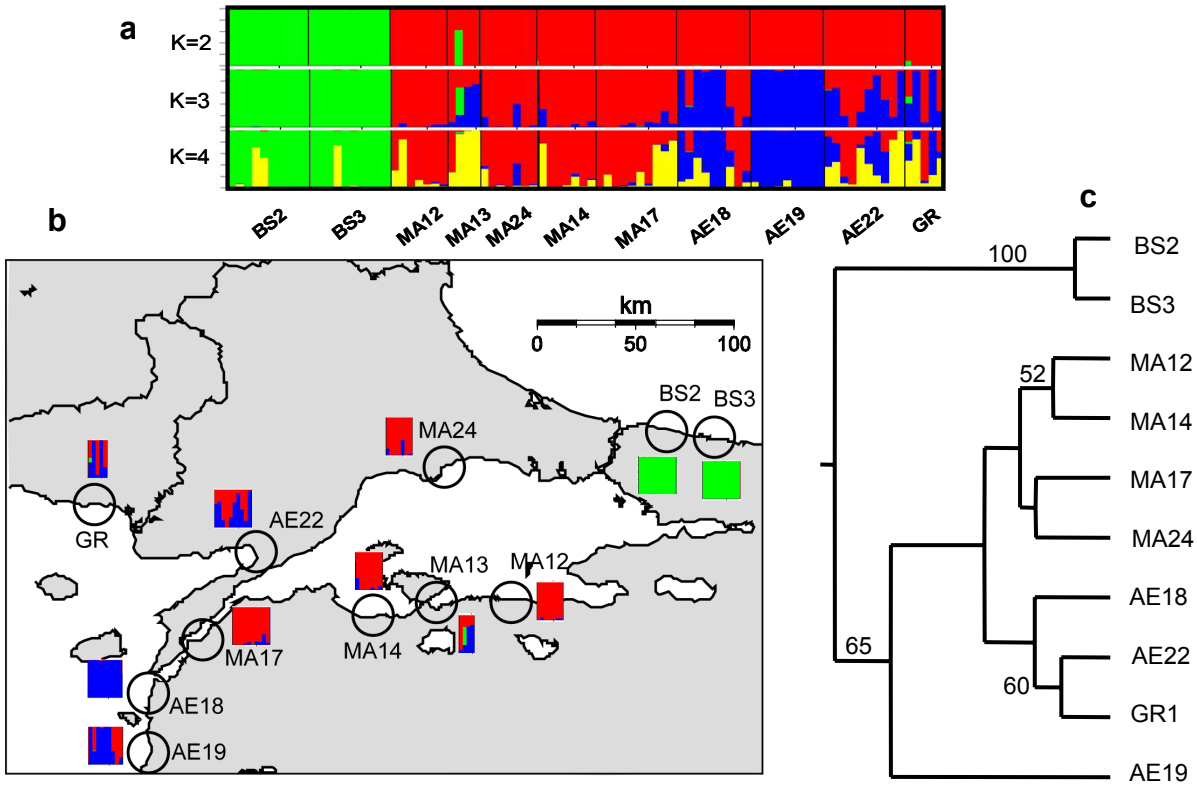


Figure 8. a) Result of the model based clustering (K=2-4) of *E. maritimum* from the Aegean Sea/Sea of Marmara/Black Sea. For details see figure 7. b) Geographical distribution and assignment of individuals of the populations for K=3. c) UPGMA phenogram based on Nei's genetic distance between populations. MA13 was excluded from the analysis because of low sample size. Numbers on branches indicate bootstrap support.

Table 14. Effective numbers of migrants between populations within regions and populations of different regions, estimated as $N_em=(1-Fst)/(4*Fst)$. The values are means of all compared populations.

Regions	Effective number of migrants, N_em
Cakile maritima	
Baltic Sea	
Mean between all populations	2,96
West France	
Mean between all populations	11.52
Gibraltar	
Mean between all populations	1.74
Mean between Mediterranean populations	3.93
Mean between Atlantic populations	2.23
Mean between Atlantic and Mediterranean regions	1.29
Aegean Sea, Black Sea	
Mean between all populations	0.83
Between Black Sea populations	0.69
Mean between Marmara populations	3.11
Mean between Aegean populations	2.42
Mean between Aegean Sea and Marmara regions	1.00
Mean between Marmara and Black Sea regions	0.44
Mean between Aegean Sea and Black Sea regions	0.32
Eryngium maritimum	
West France	
Mean value between all populations	5.29
Gibraltar	
Mean between all populations	1.03
Mean between Mediterranean populations	3.54
Mean between Atlantic populations	4.31
Mean between Atlantic and Mediterranean regions	0.57
Aegean Sea, Black Sea	
Mean between all populations	0.86
Between Black Sea populations	infinit (no differentiation)
Mean between Marmara populations	2.13
Mean between Aegean Sea populations	2.01
Mean between Marmara and Aegean Sea populations	1.37
Mean between Marmara and Black Sea regions	0.40
Mean between Marmara and Aegean Sea regions	1.05
Mean between Black Sea and Aegean Sea regions	0.40

A hierarchical AMOVA using the three regions as groups shows strong differentiation, partitioning 23.4% of the variation among groups and 7.2% between populations within groups (Table 12). Gene flow estimates between the Black Sea and the other regions were about one fifth of those within regions as well as substantially lower than between Aegean and Marmara populations (Table 14).

Strait of Gibraltar

Cakile maritima. Five populations from each side of the Strait of Gibraltar were sampled and a total of 94 individuals were scored. There is a clear division between the five Atlantic and Mediterranean populations in the UPGMA dendrogram based on Nei's genetic distances (Fig. 9c). The model based clustering also finds the Mediterranean-Atlantic subdivision, assigning all but three individuals in accordance with geography. These two clusters are further subdivided in K=3 and K=4, the latter for which the highest probability was found (Fig. 9a). In K=4, most individuals from ATL3, ATL4 and ATL5 form a geographical cluster. An AMOVA partitions 9.0% of the variation among Atlantic and Mediterranean groups and 7.4% among populations within groups (Table 12). The average effective migration rate, N_em, was 1.29 across the subdivision as compared to 2.23 and 3.93 within the Atlantic and Mediterranean regions, respectively (Table 14). Using the reassignment procedure of AFLPOP the geographical origin of individuals was tested. Allocation success was high; 86 out of 91 individuals (94.5%) could be assigned to a region with a log-likelihood difference of at least 1. Of these, 84 individuals were assigned to the region where it was sampled, one from ATL1 was assigned to the Mediterranean region and one sample from MED3 was assigned to the Atlantic region.

Eryngium maritimum. 96 individuals from 10 populations were analyzed from the Strait of Gibraltar. The AFLP data from the Strait of Gibraltar showed a similar pattern for *E. maritimum* as in *C. maritima*. As seen in figure 10c, Atlantic and Mediterranean populations form separate clusters except for MED1 that occupies an intermediate position close to the Atlantic ones. The model based clustering methods also separated the Atlantic populations from the Mediterranean ones in K=2 (Fig. 10a). In the subsequent model the Atlantic subcluster was subdivided with individuals drawn from all populations. In K=4 and K=5, the Mediterranean was divided into three

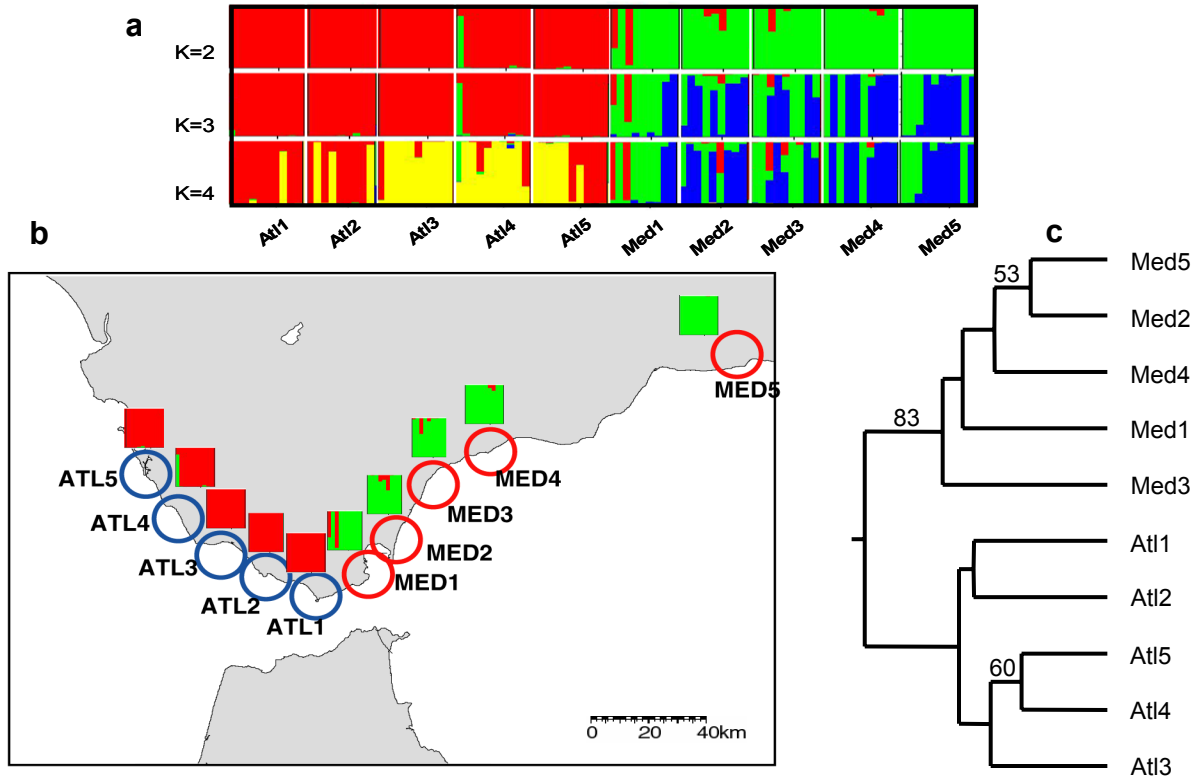


Figure 9. Result of the model based clustering (K=2-4) of *C. maritima* from the Strait of Gibraltar. For details see figure 7. b) Geographical distribution and assignment of individuals to the clusters in K=2. c) UPGMA phenogram based on Nei's genetic distance between populations. Numbers indicate bootstrap support.

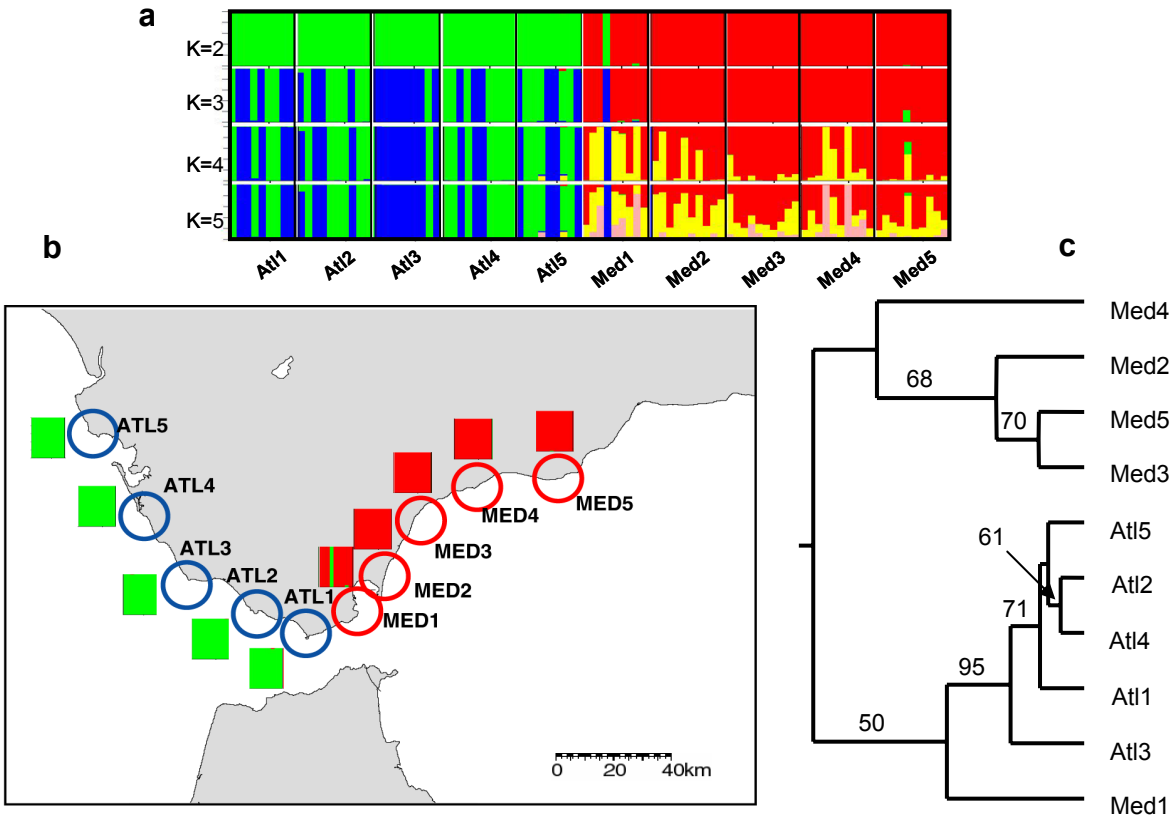


Figure 10. a) Result of the model based clustering (K=2-5) of *E. maritimum* from the Strait of Gibraltar. For details see figure 7. b) Geographical distribution of the clusters and assignment of individuals to the clusters in K=2. c) UPGMA phenogram based on Nei's genetic distance between populations. Numbers indicate bootstrap support

clusters but no individuals were strongly assigned. An AMOVA shows a very strong differentiation between the two regions partitioning 27.5% of the variation between the Atlantic and the Mediterranean and only 3.82% among populations within regions (Table 12). The effective migration rate was 0.57 across the subdivision compared to 4.31 and 3.54 for the within region estimates (Table 14). The assignment test allocates 97 of 98 individuals (99.0%) to one of the two regions. Only one of these is assigned to a different region from where it was collected; an individual being collected in MED1 was assigned with high likelihood to the Atlantic populations.

The average Shannon Index was 0.093 for Atlantic populations compared to 0.249 for Mediterranean populations and was significantly different ($P<0.01$). Likewise, the average gene diversity was significantly lower on the Atlantic side than on the Mediterranean one (0.058 vs. 0.163 $P<0.05$; Table 11).

Baltic Sea – North Sea

Cakile maritima. The analyses of *C. maritima* from the Baltic Sea/North Sea area included 96 individuals distributed over ten populations. No clear pattern could be seen in the UPGMA analysis of Nei's genetic distances although the geographically most distant population Gönhöj also is the most genetically divergent one (Fig. 11c). In the rest of the tree there is no pattern visible. The model based clustering also does not show any geographic structuring of the data. Here the inferred clusters more or less contain individuals from all populations for K=2 and 3 and the solutions for a higher assumed number of populations do not improve the pattern (Fig. 11a). Two subspecies have been recognized in the area (Elven & Gjelsås, 1981) and it is possible that individuals representing two lineages have been sampled. Because of this both clusters found in K=2 were analyzed separately but no additional geographic structure was found (not shown). A non-hierarchical AMOVA showed a significant differentiation between populations with an over all F_{st} of 0.082 (Table 12). The average N_em was 2,96 over all populations (Table 14).

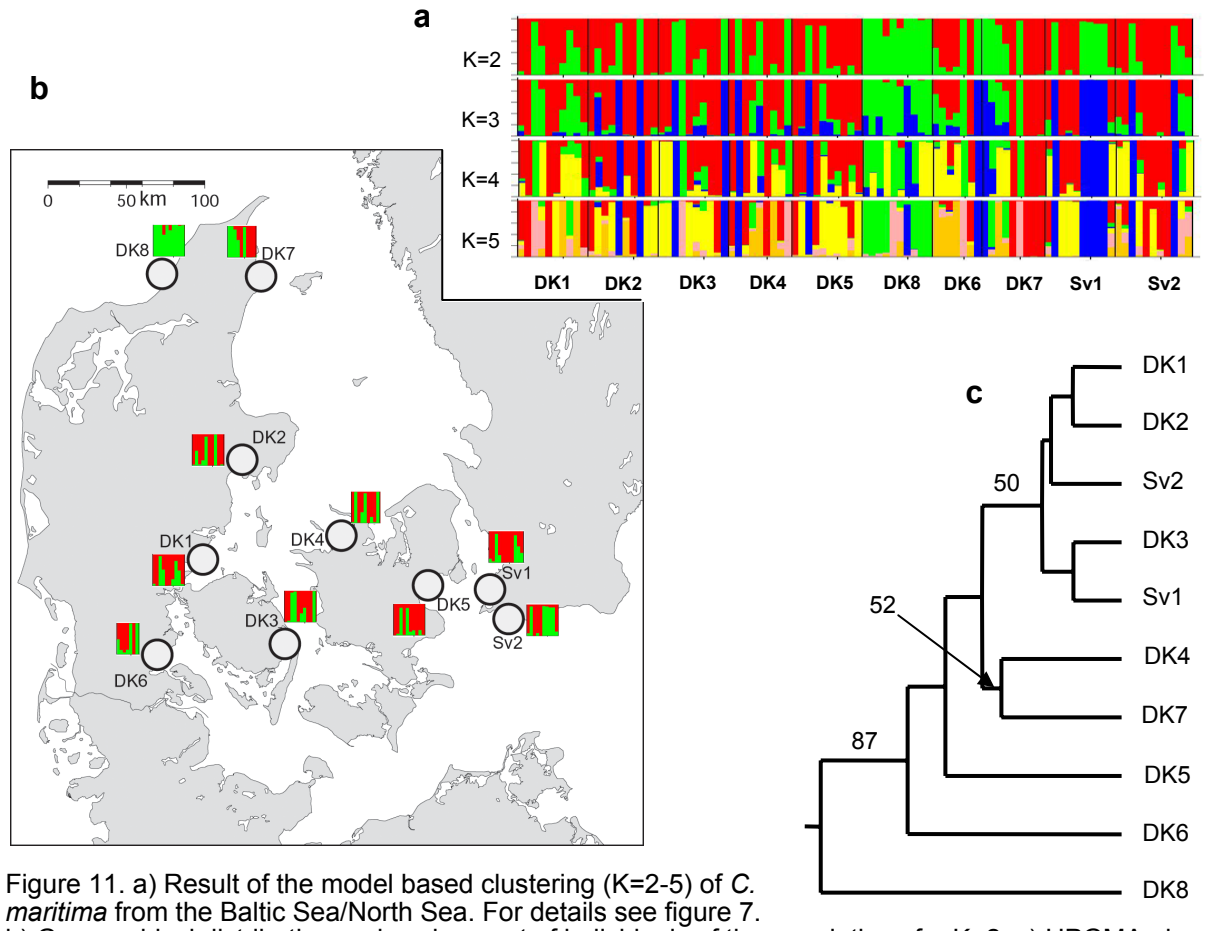


Figure 11. a) Result of the model based clustering (K=2-5) of *C. maritima* from the Baltic Sea/North Sea. For details see figure 7. b) Geographical distribution and assignment of individuals of the populations for K=2. c) UPGMA phenogram based on Nei's genetic distances between populations. Numbers indicate bootstrap support.

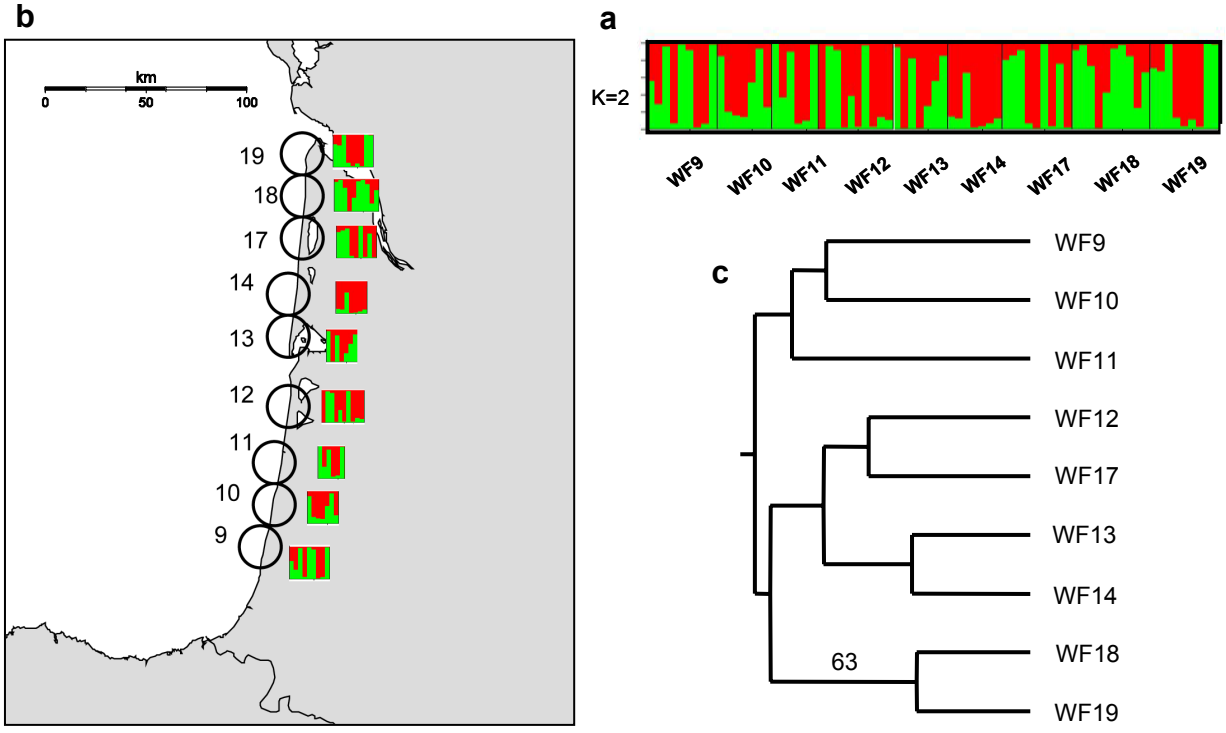


Figure 12. a) Result of the model based clustering (K=2) of *C. maritima* from west France. For details see figure 7. b) Geographical distribution and assignment of individuals of the populations for K=2. c) UPGMA phenogram based on Nei's genetic distances between populations. Bootstrap support is indicated by numbers.

West France

Cakile maritima. Very little differentiation was found in the control area from west France as evident from AMOVA performed on 74 individuals from 9 populations; only 1.5 % of the variation was partitioned among populations and this was not significantly different from zero (p= 0.053; Table 12). Populations cluster roughly according to geographical proximity in the UPGMA although no clear division is found in the dendrogram and bootstrap support is low (Fig. 12c). In the model based clustering method, K=2 gave only a slightly higher likelihood than K=1 and many individuals are weakly assigned as well as being drawn from all populations (Fig. 12a). Therefore the K=1 model is thought to represent the data better. The average migration rate, N_em, was 11.5 (Table 14).

Eryngium maritimum. Slightly more differentiation between populations was found in *E. maritimum* than in *C. maritima*. This amount, too, was the lowest found for any region in this species. A non-hierarchical AMOVA performed on 88 individuals from 9 populations gave an F_{st} of 0.042 (P<0.01; Table 12). Based on genetic distances the southernmost population, WF9, is the most divergent and the UPGMA places this population isolated from the others (Fig. 13c). The remaining populations form two clusters but without a discernable geographic pattern and bootstrap support is essentially lacking. The model-based clustering subdivides only three individuals in K=2 (Fig. 13a) and subsequent models with higher likelihood are non-sensical. The amount of polymorphic bands was also very low; only 20 bands out of 94 were polymorphic over the region. The average migration rate, N_em, was 5.29 (Table 14).

ITS data

Eryngium maritimum. Screening of 11 individuals revealed some variation between sequences. The variation was, however, not sufficient to produce a meaningful or resolved tree. A list of sequences are given in appendix III for reference.

Cakile maritima. Screening for variation showed only very few mutations. Comparison with eleven available ITS sequences (Kate Vickers, unpublished data) showed no new information.

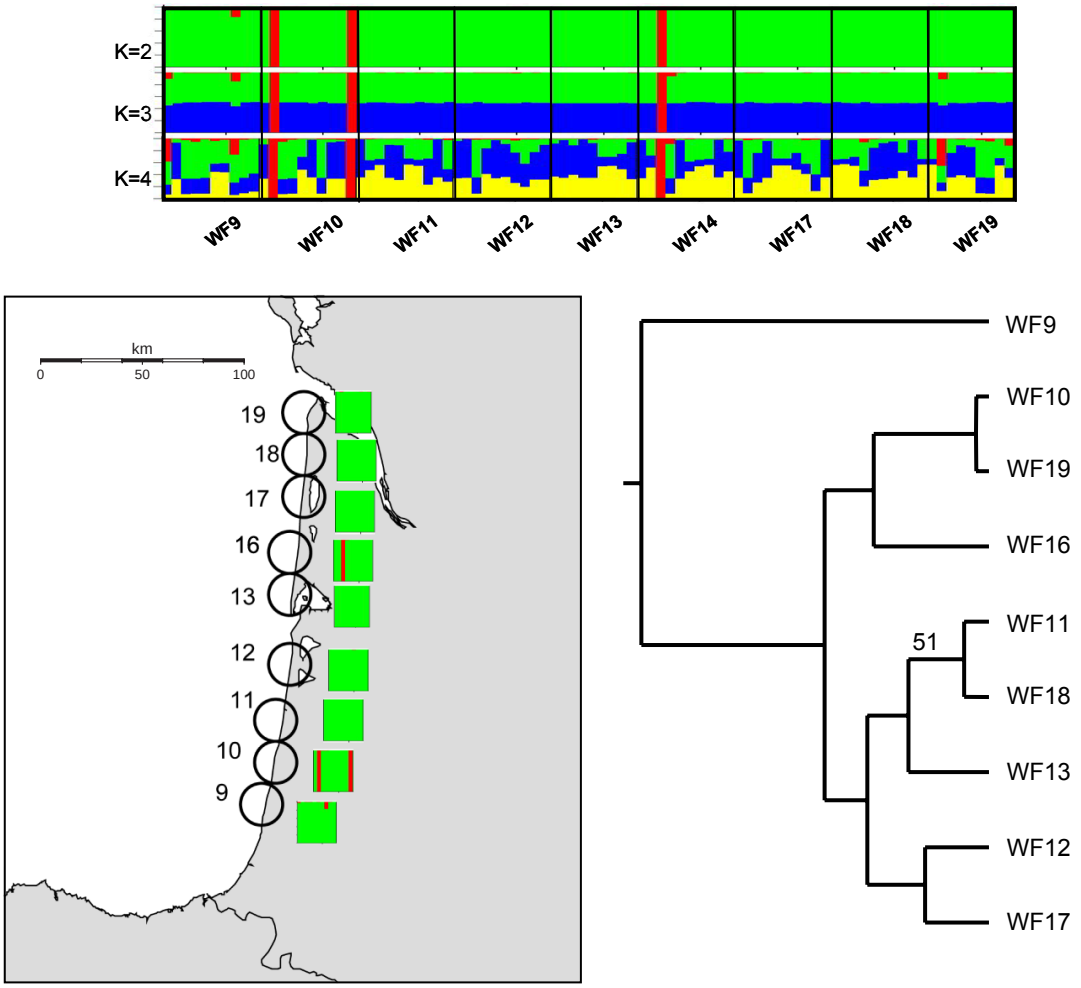


Figure 13. a) Result of the model based clustering (K=2-4) of *E. maritimum* from the Strait of Gibraltar. For details see figure 7. b) Geographical distribution and assignment of individuals of the populations for K=2. c) UPGMA phenogram based on Nei's genetic distances between populations. Numbers indicate bootstrap support.

DISCUSSION

Genetic structure

Both species show pronounced genetic structuring on a European scale (Figs. 3-6). The genetic variation is partitioned into cohesive geographical groups with almost no individuals originating as geographical outliers relative to the remainder of their cluster. The geographical clusters more or less correspond to marine basins and will hereafter be referred to accordingly.

Cakile maritima is divided into five, or possibly six, genetic groups by STRUCTURE at the largest scale, a result that is also visible in the NJ-phenogram (Figs. 3 & 4). Starting from the north, these groups are: a north Atlantic group containing all individuals from the Baltic, the subsp. *islandica* material and one individual from Scotland; the rest of the Atlantic including one individual from Trondheim (Norway, no. 3); all west Mediterranean collections and individuals from Tunisia, Israel and Cyprus; Adriatic Sea and Ionian Sea individuals; Aegean Sea, Marmara and Black Sea material. The sixth group is rather heterogeneous, both in terms of individual assignment frequencies and, to some extent, geographical origins of the samples assigned. Geographical outliers to this group are Raouel (Tunisia) and Rio Oka (Spain). Individuals that are not unambiguously assigned to this group alternatively cluster according to their geographical region so that even within this group an Atlantic Ocean and a Mediterranean Sea grouping can be recognized.

Eryngium maritimum is divided into three groups without geographical outliers (Figs. 5 & 6). All material from the Atlantic Ocean/North Sea/Baltic Sea forms a distinct cluster, also including a sample from Cabo Pino (Spain), east of the Strait of Gibraltar. Collections from the west Mediterranean Sea/Adriatic Sea/Ionian Sea form another group which is closest to a cluster composed by Aegean Sea/Black Sea material and the collections from Avsallar (Turkey, no. 80) and Oroklini (Cyprus, no. 81).

Corresponding to the results of the large scale sampling, a strong genetic division was found in Spain in the Strait of Gibraltar at the population scale in both species with reduced levels of gene flow compared to populations on the same side of the divide (Figs. 9 & 10; Table 14). This shows that there is a strong barrier to gene flow in this area over a small geographical distance, evidently somewhere between Tarifa and Algeciras. Apparently this barrier is strong enough to have a

profound effect on genetic variation on a Europe wide scale in *E. maritimum* and, albeit less markedly, it is also evident in the large scale pattern in *C. maritima*. In comparison the Dardanelles/Bosporus straits from the Aegean/Black Sea/Marmara region show similar levels of gene flow as across the Strait of Gibraltar (Table 14). No clear effect of this is seen on the large scale pattern in either of the species in the Bayesian clustering. However, the Black Sea collections are grouped in the NJ analyses although the sample is small (Figs. 3 & 5). The five Black Sea individuals that were included in *E. maritimum* form a separate subcluster within the Aegean Sea/Black Sea cluster. Individuals from the Sea of Marmara intermix with the rest of the Aegean Sea material. In *C. maritima* only two individuals are included from the Black Sea and one from the Sea of Marmara. Although the sample size is not large enough to permit recognition as a separate cluster, the Black Sea individuals do cluster together with a long branch connecting them to the sample from the Sea of Marmara in the NJ analysis (Fig. 3). Although caution about conclusions is warranted, this pattern, in fact, precisely matches the results from the population studies (Figs. 7 & 8). In the Bayesian analysis, *C. maritima* collections from the three basins formed separate clusters whereas the Aegean Sea and Sea of Marmara populations intermix in *E. maritimum*.

The fact that all borders of the clusters in *E. maritimum* and all, except the border between the northern and southern clusters in the Atlantic region, in *C. maritima* correspond to marine basins and that substantial reduction of gene flow in sea straits between basins was found where investigated, intuitively suggests that these basins are somehow responsible for these patterns. In both terrestrial and marine taxa, biogeographical regions corresponding more or less closely to the present geographical pattern have been recognized. In plants, for instance, Meusel and Jäger (1992) recognize as such the Aegean Sea/Black Sea region and further divide the Mediterranean in a west, a central and an eastern zone. Also the Strait of Gibraltar was associated with a phytogeographical barrier by the same authors. The usual explanation for the occurrence of biogeographical areas are climate-related (Gjaerevoll, 1992) and in the Mediterranean particularly precipitation has been emphasized. Compared to each other, the similarities between biogeographical zones and the intraspecific patterns in *C. maritima* and *E. maritimum* are striking. Intraspecific studies of genetic diversity in plants along the Mediterranean coasts are

lacking or are not designed to detect genetic structure on Mediterranean scales (but see below).

In marine organisms the Mediterranean is its own biogeographical region with the Strait of Gibraltar as the meeting point for the Mediterranean and the (Atlantic) Lusitanian and Mauretanian regions (Carballo *et al.*, 1997; Castelló & Carballo, 2001). The Mediterranean Sea has been further divided into around ten areas in addition to the delimitation of the Sea of Marmara and the Black Sea as separate regions (Garibaldi & Caddy, 1998; Bianchi & Morri, 2000). These divisions encompass all the geographical groups found in this study. In contrast to terrestrial plants, many intraspecific studies of plants and animals have been made in marine taxa. The genetic structure found in many of these studies show similarities to the patterns in *C. maritima* and *E. maritimum*. More often than not do populations from the Atlantic and Mediterranean show distinct differentiation between them. This differentiation has been shown in fishes (Borsa *et al.*, 1997a; Bargelloni *et al.*, 2003), bivalves (Nikula & Väinölä, 2003; Rios *et al.*, 2002), a cuttlefish (Pérez-Losada *et al*., 1999), a chaetognath (Peijnenburg *et al.*, 2004) a sea-star (Waters & Roy, 2003), and a sea grass (Olsen *et al.*, 2004). A comparison of sixteen marine animals showed the same pattern for a majority of species (Borsa *et al.*, 1997b). In a mussel (Quesada *et al.*, 1995) and fish (Naciri et al., 1999), a break was found slightly more to the east, between Almeria and Cullera. As noted above, it is not always found, however, as has been noted in fish (Magoulas *et al.* 1996; Bargelloni *et al.* 2003). An east/west Mediterranean divide, as seen in *C. maritima* is also found in several studies. Borsa *et al.* (1997b) noted that a proportion of the species included in their comparison exhibit this pattern, e.g. in the flounder (Borsa *et al.* 1997 a, b). The planktonic chaetognath *Sagitta setosa* exhibits a similar pattern as in *C. maritima* with samples from Tunisia clustering with the west Mediterranean and Adriatic and Black Sea as separate clusters (Peijnenburg *et al.*, 2004). Other studies than the one above have also found separate Aegean Sea/Black Sea lineages (Magoulas *et al.*, 1996; Borsa *et al.*, 1997a; Nikula & Väinölä, 2003; Olsen *et al.*, 2004). In the instances where reasons for the found patterns have been discussed, both historical (e.g. quaternary climate) and recent factors (e.g. sea currents) have been postulated as explanations (e.g. Procaccini *et al.*, 2001; Magoulas *et al.*, 2002).

The congruency between patterns in much of the range in *E. maritimum* and *C. maritima* is mirrored in three other plant species (*Salsola kali*, *Halimione*

portulacoides, *Crithmum maritimum*; R. Arafeh & J. W. Kadereit, pers. com.). These were studied within the same project along essentially the same geographical range. All three species basically show a primary subdivision into Atlantic Ocean and Mediterranean Sea material as well as exhibiting further clusters corresponding to the Aegean Sea/Black Sea, Adriatic Sea/Ionian Sea and the west Mediterranean. *Crithmum maritimum* is an exception from this general pattern in that the Atlantic clusters extends into the Mediterranean and includes eastern Spain. Since all these species are more or less azonally and co-distributed, it seems at hand that the same processes or historical events are responsible for the shared pattern between the species.

Hypothetical distributions of both species at the LGM can be extrapolated from figure 14. These are based on the isotherms now found at the northern limit of their present day distributions. Judging from this, none of the borders of the proposed clusters in the Mediterranean should have been uninhabitable during the LGM for any of the two species investigated here. Rather, at least the eastern Mediterranean should have provided suitable climatic conditions throughout the last glacial. This

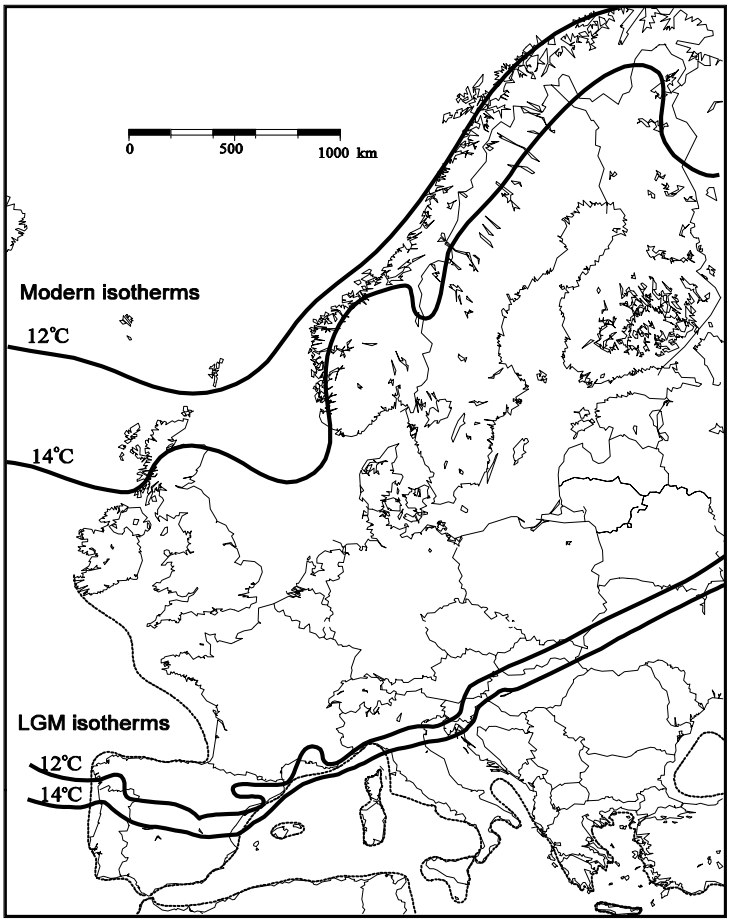


Figure 14. Modern (northern Europe) and reconstructed (southern Europe) isotherms for the last glacial maximum. The 12°C and 14°C mean July isotherms (modern) and June/ July/August (LGM) are shown. The position of the 14°C LGM isotherm was interpolated from the position of the 12°C and 18°C isotherms. Modern isotherms were redrawn from Hann (1892), and LGM isotherms from van Andel (2002). The LGM coast line is shown as a dotted line (Lang 1994). The modern 12°C and 14°C isotherms correspond to the recent distribution limits of *C. maritima* subsp. *maritima* and *E. maritimum*. The locations of the 12°C and 14°C LGM isotherms are taken as the hypothetical northern distribution limit of the species during the LGM.

reconstruction of distributions therefore does not provide evidence for a direct climatic impact on the Mediterranean clustering patterns.

If the clusters found do not represent historical isolation, and consequently the areas where the clusters meet are not zones of secondary contact, then, instead, barriers to gene flow in these areas could explain the congruency in pattern between species. Consequently this would suggest recent factors accounting for the apparent isolation.

Lacking detailed knowledge about pollen (over land) and seed movement (by water or land) in these species, it is in principle not possible to decide if the explanations for the apparent isolations are to be sought in terms of sea currents or on land, or if a combination of both would provide a better answer. However, assuming that the congruency is due to the same factors in all five species, there are arguments suggesting that seed dispersal by water is of overriding importance.

A comparison of the results of the Mantel correlations for different testing strategies suggests that significant gene flow takes place over water in the large scale investigation. In *C. maritima* correlations for the sub-areas identified in the Europe wide cluster analyses were in most cases higher and had better P-values when Euclidean distances were used compared to coastal distances (Table 7). In the south Atlantic and west Mediterranean the higher correlation was due to the inclusion of outliers, that is, mainly British and far removed collections in the case of the south Atlantic, and Sardinian and Tunisian as well as distant collections in the west Mediterranean cluster. These outliers are often separated from the rest by water (e.g. no. 48, Sardinia; Fig 4). The difference in geographical distances, depending on the choice of measure, is illustrated in figure 15, using the Tyrrhenian Sea as an example. The fact that correlations are better when Euclidean distances are used as opposed to when the distance to outliers is measured along the point with the shortest distance from the mainland, suggests that dispersal across water is the reason for this. Also, when the outliers are removed from the analysis the differences in correlations between the two distance measures likewise disappear (Table 7).

The correlations in *E. maritimum* are instead almost the same for coastal distances compared to Euclidean distances in the Atlantic, Aegean and west Mediterranean regions (Table 8). When outliers are removed from the analysis, correlations increase for both geographic distances. The differences between the two species could be due to lower dispersal distance of *E. maritimum* seeds, which is

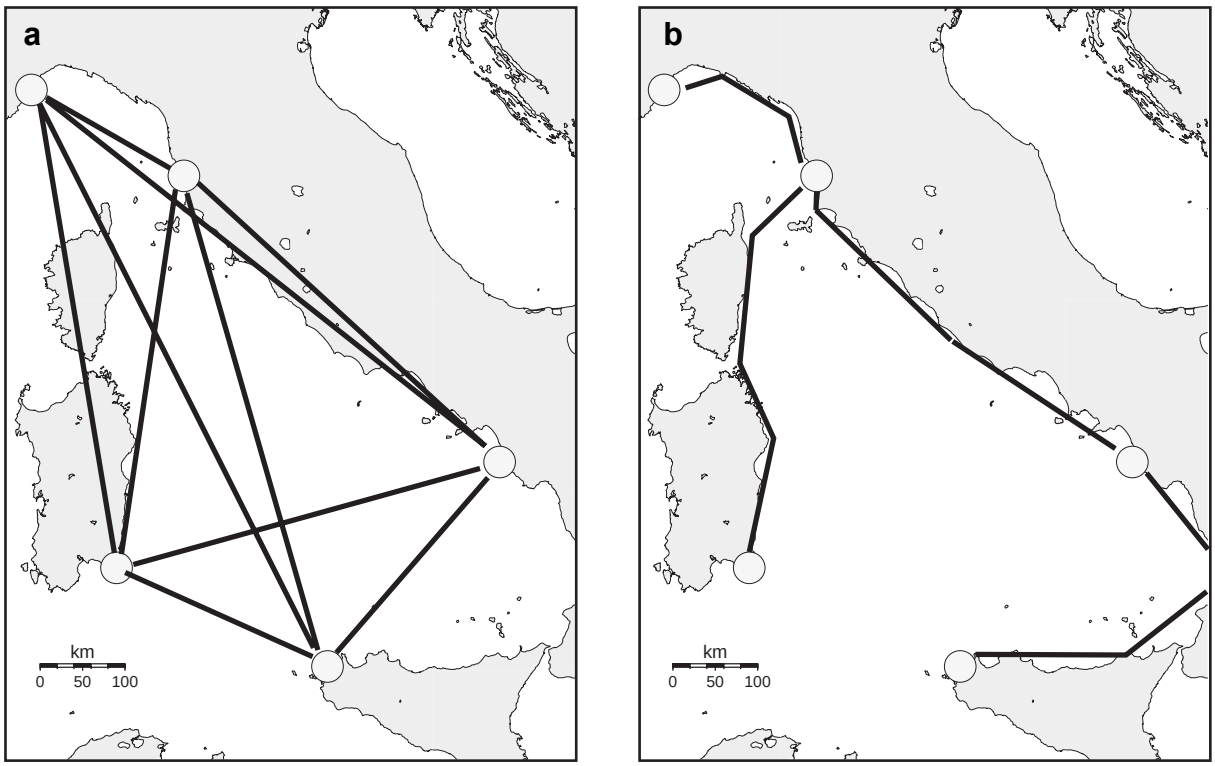


Figure 15. Geographical distances between sampling localities in the Tyrrhenian Sea. a) Euclidean distances are measured “as the bird flies” and allows for dispersal over large bodies of water. b) Coastal distances assume that seeds cross water only where land masses are close to each other. Otherwise distances are measured along the coast.

also suggested by their generally shorter floating times (Ridley, 1930; Rodman, 1974; Maune & Payne, 1989).

At the smaller scales of the population studies there was little evidence of isolation-by-distance despite significant F_{st} values (Table 12). This suggests high dispersability in both species over the smaller distances separating populations in these studies. However, in several cases the regions had to be divided into smaller subsets to avoid producing artefactual isolation-by-distance. Thus the lack of isolation-by-distance could simply be due to small sample size in the Mantel tests.

Another fact favouring the seed dispersal hypothesis is that the seeds of all five plant species mentioned above show adaptations to dispersal by sea water whereas pollen moves differently in the five species: whereas *C. maritima*, *E. maritimum* and *Cr. maritimum* are visited by insects, *S. kali* and *H. portulacoides* are likely to be wind-pollinated (pers. obs; R. Arafeh, pers. com.). Since essentially the same patterns were found in all species one would expect that the similarities in seed dispersal are the common factor between the species.

If barriers to dispersal by seeds produced the clusters found in the Mediterranean Sea, then one likely explanation is that sea currents act as dispersal barriers to plants dispersed by sea water. In figure 16 the surface circulation of the western and central East Mediterranean is depicted. The Aegean Sea is surrounded by more or less permanent gyres. These apparently prevent drifters deployed in the Mediterranean Sea from entering the Aegean Sea (Fig. 17) and consequently would have the same effect on seeds dispersed by water. The currents are thus probably enough to produce the observed genetic divergence of the Aegean Sea material. It is also possible that the split of the Algerian current at the Strait of Sicily acts in a similar manner to prevent dispersal between the Ionian and the Thyrrenian Seas (Fig. 16). A dispersal barrier in the Strait of Sicily would not be supported by the pattern in *E. maritimum*, however and an explanation for its incongruent pattern can not be offered.

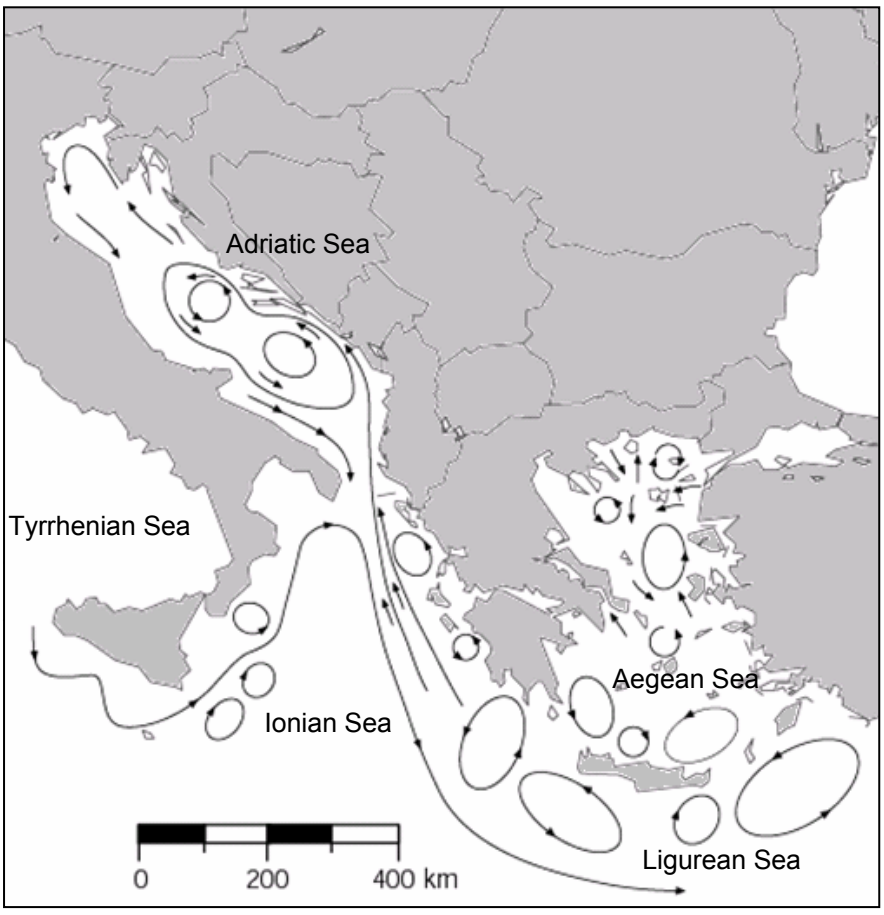


Figure 16. Major features of the surface circulation in the Adriatic, Ionian, Aegean and Ligurean seas.
Redrawn from Ovchinnikov (1966), Malanotte-Rizzoli *et al*. (1997), Millot (1999) and Lykousis *et al*. (2002).

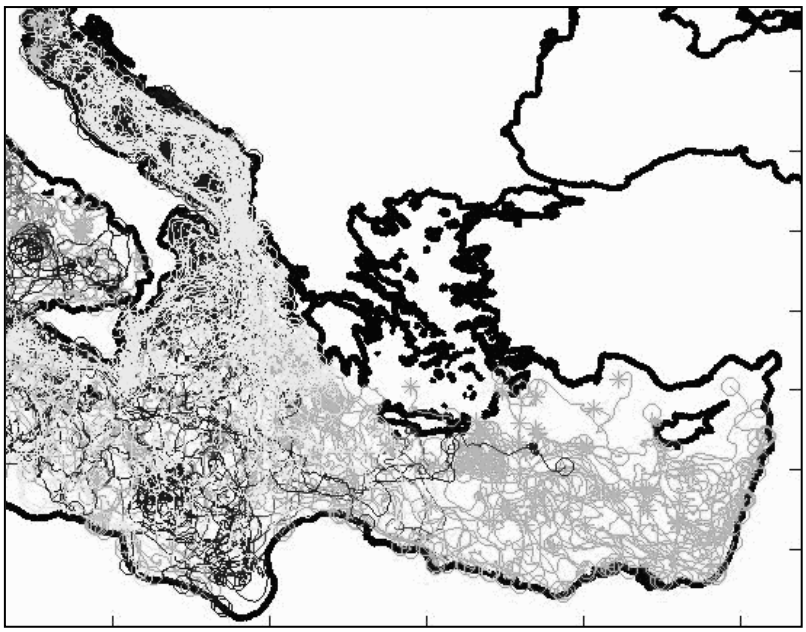


Figure 17. Trajectories of drifting buoys in the East Mediterranean Sea shown as a “spaghetti” plot. The drifting path of each buoy is represented by a line. Both surface and near surface drifters are shown. Picture modified from the Mediterranean Surface Drifter Database (1986-1999) website (http://poseidon.ogs.trieste.it/drifter/database_med/).

Strait of Gibraltar and the Atlantic – Mediterranean subdivision

The reconstructions of hypothetical distribution indicate that *C. maritima* subsp. *maritima* had its northern distribution limit in northern Portugal and that *C*. *m*. subsp. *islandica* could have survived up to south Bretagne (France). The limit of *E. maritimum* would have been close to that of *C. maritima* subsp. *maritima*, approximately around mid Portugal according to LGM isotherms. This implies that Northwest Africa, the area around the Strait of Gibraltar and the whole of the Mediterranean would have been continuously inhabited, perhaps with the exception of part of the northern West Mediterranean in *E. maritimum*. Based solely on distribution limits, here, too, recent factors should be sought in order to explain the Atlantic - Mediterranean subdivision in the AFLP data. Indeed, the population analysis in the Strait of Gibraltar found evidence for a barrier to gene flow between MED1 and ATL1 in both species (see below). Since two of the three above mentioned, additionally investigated species (*S. kali* and *H. portulacoides*) also show an Atlantic - Mediterranean subdivision, this would again seem to support the influence of sea currents on seed dispersal as the cause of the genetic differentiation.

The surface currents in the Strait of Gibraltar are influenced by the tidal differences between the Gulf of Cadiz on the west side 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. The net effect is,

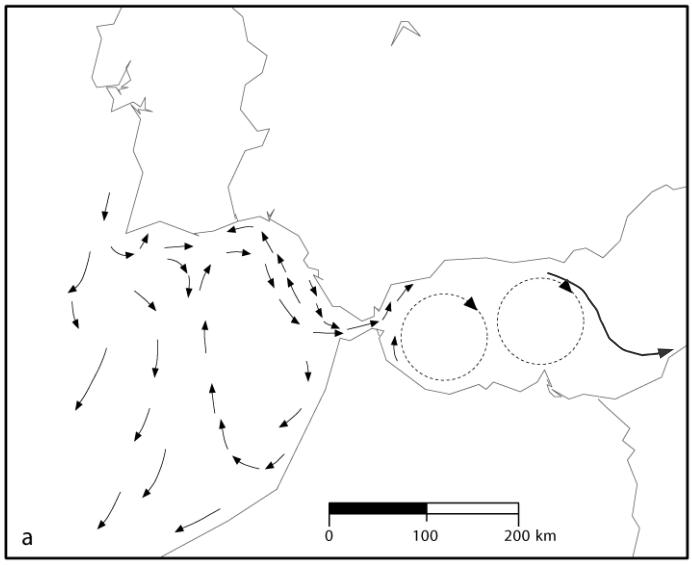


Figure 18. Major surface currents in the Iberian-Moroccan Bay and Alboran Sea. Redrawn from Rey (1983).

however, a continuous inflow of Atlantic surface water (Fig. 18). No major current patterns, other than that the current changes direction with the tides, seem to isolate the Atlantic from the Mediterranean in the Strait of Gibraltar.

Judging from this it is questionable if currents in the strait alone can be the primary cause of the strong differentiation seen in *E. maritimum*. The perennial habit of *E. maritimum* further would be expected to promote less differentiation (Hamrick & Godt, 1991; Nybom & Bartitsh, 2000; Nybom, 2004) but in fact the opposite is seen when compared to the annual *C. maritima*. On the other hand *C. maritima* should be more readily dispersed, judging from the time seeds can remain afloat, which could connect populations by gene flow and thus account for the lower differentiation seen in *C. maritima* in both the population and the large scale study. In the literature, seeds of *C. maritima* have been reported to float longer than seeds of *E. maritimum*. Maun and Payne (1989) reported 50% of seeds still floating after 100 days in tap water, and between seven to ten days floating time in sea water were given in Ridley (1930) and Rodman (1974) for *C. maritima*. Seeds of *E. maritimum* on the other hand can float between two and four days in sea water according to Ridley (1930). In own floating experiments (not shown) conducted in flasks filled with sea water, 100% of *C. maritima* seeds had sunken after ten (N=60) and three days (N=26), respectively. In *E. maritimum*, 37 seeds out of 40 had sunken after six days but one seed stayed afloat for 21 days. The large differences in these results indicate that floating times are likely to vary considerably within populations and individuals.

A competing explanation to dispersability, given by Clausing *et al.* (2000) emphasized the qualitative difference between *C. maritima* and *E. maritimum* in the genetic gap between the Atlantic Ocean and the Mediterranean Sea, a pattern that could be confirmed in this study. The strong differentiation seen in *E. maritimum* was attributed to its recent, less northerly distribution which could have caused the species to retreat further south than the Strait of Gibraltar during the LGM, thus leaving a distribution gap between the Atlantic and the Mediterranean. Conversely, the more northerly distributed *C. maritima* could have maintained a continuous distribution thus preventing differentiation. This hypothesis is not compatible with LGM isotherms and would require that *E. maritimum* is limited by some other factor, not closely correlated to temperature. However, the within population genetic diversities give some support to this scenario. It is generally assumed that re-colonised areas contain lower genetic diversity than refugia, mainly due to repeated founder effects during migration (Hewitt, 1996). Although admixture effects could increase gene diversity when immigrating populations meet (Comps *et al.*, 2001), intuitively this should be less common in a coastal habitat based on spatial considerations. Thus we would expect diversity to be highest in populations south of the postulated LGM distribution limit if these were indeed refugial areas. For *C. maritima* the mean H_s and SI are significantly higher in the Atlantic Strait of Gibraltar populations than in more northern populations (Table 11) which is in accordance with this prediction. However, when only strictly Atlantic populations are considered for *E. maritimum* there is no corresponding decrease of mean H_s and SI in west France compared to the Atlantic part of the Gibraltar strait, but rather a slight increase (Table 12). This is more in accordance with the hypothesis of Clausing *et al*. (2000) that *E. maritimum* had to re-colonise the whole of the European Atlantic coast. At the same time, however, this raises another question, namely why diversities at the Mediterranean side of the strait are, in fact, by far the highest of all regions (Table 12). If the Atlantic side was not inhabitable for *E. maritimum*, then it seems highly unlikely that in contrast the Mediterranean side was, since only a small geographical distance separates the two areas. If correct, then the high diversities on the Mediterranean side would have to be accounted for by some other process. This could include admixture of divergent lineages on the Mediterranean side, or a change to a higher degree of inbreeding in the Atlantic. While theoretically conceivable, it remains highly speculative and single populations in the Aegean Sea/Marmara/Black Sea region

also exhibit diversities comparable to both Atlantic and Mediterranean populations from the Strait of Gibraltar.

Allthough likely, it is not entirely clear whether this genetic break is equivalent to the one seen in the European scale analysis. The differences in diversities in *E. maritimum* around the Strait of Gibraltar are marked and a strong differentiation was found between the sides of the strait. However, the AFLP data (Figs. 5 & 6) implies that material just east of the Strait of Gibraltar may be most closely related to Atlantic material as sample no. 33 (Cabo Pino) clearly belongs to the Atlantic Ocean cluster. Also in *C. maritima* a tendency for Alboran Sea collections to cluster with Atlantic collections (samples 35, 36 and 37) can be seen (Figs. 3 & 4) which is paralleled in *H. portulacoides* (R. Arafeh, pers. com.).

Parallels can be seen in studies of marine organisms, some of which have found a genetic break between north Atlantic and Mediterranean populations east of Gibraltar to coincide with the so called Almeria-Oran front, a hydrographic boundary between Atlantic and Mediterranean surface water (Quesada *et al.*, 1995). When the Atlantic surface water comes into the Alboran Sea, it flows anticyclonically in the western part while further east another less stable gyre may be present off the coast of Almeria. Where Atlantic water encounters Mediterranean water, the Almeria-Oran Jet that flows from Spain to Algeria is induced (Fig. 18). At the African coast the jet is split into one branch turning west and another forming the Algerian current that continues east along the African coast. In none of the three studies where the role of this hydrographic barrier has been discussed, did the authors believe that it could be the sole cause of the Atlantic - Mediterranean divergence. Quesada *et al.*, (1995), Panacciulli *et al.*, (1997) and Naciri *et al.*, (1999) all discussed physical isolation due to Pleistocene and/or Pleiocene climate. Naciri *et al.*, (1999) further discussed a possible role for selection in maintaining this genetic break.

If only dispersal by present day currents is considered one would expect dispersal of Atlantic material into the Alboran Sea and further to the North African coasts instead of along the Spanish coast. More data from these regions could have provided a good test to the hypothesis that gene flow by seed dispersal predominates and also would have provided more insights into the nature of the genetic break in both species, but unfortunately no collections were available from eastern Morocco or Algeria. Only one individual of *C. maritima* from west Mediterranean Tunisia was available (no. 42). Although this individual clusters more closely to

Atlantic material, as would be predicted by sea currents (Figs. 3 and 4), no conclusions can be drawn solely based on this fact.

Re-colonisation of the northeast Atlantic coast

The hypothetical reconstructions of the species distribution ranges have shown that much of the Atlantic European coast would have been colonised earliest after the LGM (Fig. 14). A genetic trace of this re-colonisation is the progressively lower genetic diversity of more northerly populations in *C. maritima*. For example the mean gene diversity, H_s, of populations from the Atlantic side of the Strait of Gibraltar was 0.129, compared to 0.108 and 0.094 for West France and Baltic Sea/North Sea, respectively. Another prediction for recently colonised areas compared to refugia is a weaker isolation-by-distance pattern since less time has been available for populations to reach migration-drift equilibrium (Castric & Bernatchez, 2004). In *C. maritima* correlations are only marginally higher for the west Mediterranean compared to the south Atlantic cluster when outliers are removed ($r=0.471$, $P<0.001$ and $r=0.458$, $P<0.001$, respectively). In *E. maritimum* the correlations are higher in the continuously inhabited West Mediterranean/Adriatic Sea cluster ($r=0.340$, $p<0.0001$) than in the re-colonised Atlantic cluster ($r=0.207$, $p=0.006$). In this case, the higher value in the west Mediterranean/Adriatic Sea is likely a sign of a longer history in that region than in the Atlantic, where apparently a genetic trace of its post-glacial re-colonisation is still present. Since geographical substructure is known to produce higher correlation between genetic and geographic distances (Husband & Barrett, 1995) it is interesting to note that a weaker isolation-by-distance pattern is found in the Atlantic cluster even though a weak North/South genetic substructure was detected along the Atlantic coast (Fig. 5).

These results indicate that the high rate of seed dispersal in combination with an annual habit has allowed *C. maritima* to reach migration-drift equilibrium in much of its Atlantic range, which explains the similarity between correlation values in the refugial and re-colonised clusters. In *E. maritimum* the perennial habit would have slowed this process. The higher correlation in *C. maritima* than *E. maritimum* can also be attributed to another, related, factor. The sampling range of the compared Atlantic clusters (without outliers) is essentially the same for both species but relative to their distribution ranges it is much closer to the northern limit of *E. maritimum* than that of *C. maritima*. That should mean that the investigated area has been inhabited

longer by *C. maritima* than *E. maritimum*. Both these factors indicate that the number of generations available to reach equilibrium has been less for *E. maritimum* which explains why it exhibits a weaker isolation-by-distance pattern than *C. maritima*.

Additional evidence suggesting that *C. maritima*, too, shows traces of a recent colonisation, is the observation that the populations from west France exhibit isolation-by-distance whereas Baltic Sea/North Sea populations do not. A possible explanation is again that more time has been available for migration-drift equilibrium to arise in west France. On the other hand the more complex spatial structure of the Baltic Sea/North Sea area could also have an influence as equilibrium patterns should be more obvious in a linear system, such as west France, than when dispersal occurs in a two dimensional area (Kimura & Weiss, 1964).

Gene flow in Sea straits and impact of geological history

The population studies from the Strait of Gibraltar and the Aegean/Black Sea/Marmara region show that these sea straits constitute a barrier to gene flow in coastal species. This is seen clearly in the geographical genetic structure and in the differentiation between populations (Figs. 8-11, Table AMOVA). In all cases when a significant genetic break was found it corresponded to the strait itself. Estimation of N_em, across gene flow barriers, based on pairwise F_{st} values, show similar values for both species in the Aegean/Black Sea/Marmara region, about 0.4 and 1 effective migrants across the Bosporus and Dardanelles straits, respectively, but more gene flow across the Strait of Gibraltar in *C. maritima* (ca. 1.3) than in *E. maritimum* (ca. 0.6). Theory predicts that an effective migration rate <1 causes divergence to increase over time (Dunphy et al., 2004). These estimations, each being close to 1, would thus indicate relatively low effective dispersal rates. Indirect estimates of migration based on F_{st} should be regarded with extreme caution, however, since this method makes several assumptions that are most likely to be violated (Whitlock & McCauley, 1998).

The allocation test suggests that the gene flow barrier is of a physical nature. The high re-allocation success (94.5 - 99.0%) confirms that the data set could effectively identify the origin of individuals in all three analyses. Out of the 262 samples (96.3%) that could be assigned to a region with a log-likelihood difference of at least 1, four cases were detected where an individual was assigned to a different region than where it was sampled (i.e. mis-assigned). These were two *C. maritima*

individuals from the Strait of Gibraltar and one from the Aegean/Black Sea/Marmara region as well as an *E. maritimum* individual from the Strait of Gibraltar. However, a simulation of artificial genotypes, based on the allele frequencies of the different regions (a parametric bootstrap procedure implemented in AFLPOP) showed that the two *C. maritima* individuals from the Strait of Gibraltar that were mis-assigned are not likely to belong to the assigned population (P=0.005 and P=0.04 that the individuals belong to their respective assigned populations). Instead it is more likely that the origin for these two individuals is not among the candidate populations. Thus only two out of the four individuals remain confidently assigned to a different population than its sample origin.

That correctly "mis-assigned" individuals measure dispersal, has been shown in a skink meta-population system where assignment tests yielded similar estimates of the proportion of dispersers as mark-and-recapture experiments (Berry *et al.*, 2004). The two remaining individuals thus probably represent migrants. This means that only one individual in *E. maritimum* (in MED1), and one in *C. maritima* (BS2) could be identified as dispersers. These results thus confirm the conclusions of the indirect method that dispersal across the gene flow barrier is rare. In both cases were dispersal could be inferred, the mis-assigned individual was sampled in the population closest to the region of their origin, and in the case of the Black Sea individual, also assigned to the closest population on the other side of the barrier (AEG11). If immigration into a region is (only) restricted due to habitat disadvantages (e.g. abiotic factors or competition) migration would be equally likely to take place into any of the populations of that region. Since these migrants were only found at the shortest possible dispersal distance in both cases this indicates that dispersal is primarily physically limited across the gene flow barriers (e.g. by distance or currents).

In contrast to the Strait of Gibraltar and the Aegean/Black Sea/Marmara area there is no evidence for clear population structure or barriers to gene flow in the Baltic Sea/North Sea. This is perhaps surprising given that *C. maritima* subsp. *baltica* is described from this area. Several other studies have compared populations from the Baltic Sea, Kattegat and the North Sea. The results regarding differentiation of Baltic Sea and North Sea samples have been varying. Some of the studies have found distinct groups or a sharp genetic cline between the North Sea and the Baltic Sea (Gabrielsen *et al.*, 2002; Coyer *et al.*,2003; Nikula & Väinölä, 2003; Nielsen *et al.*, 2004; Olsen *et al.*, 2004) but not all (e.g. van Oppen *et al.*, 1995; Röhner *et al.*, 1997).

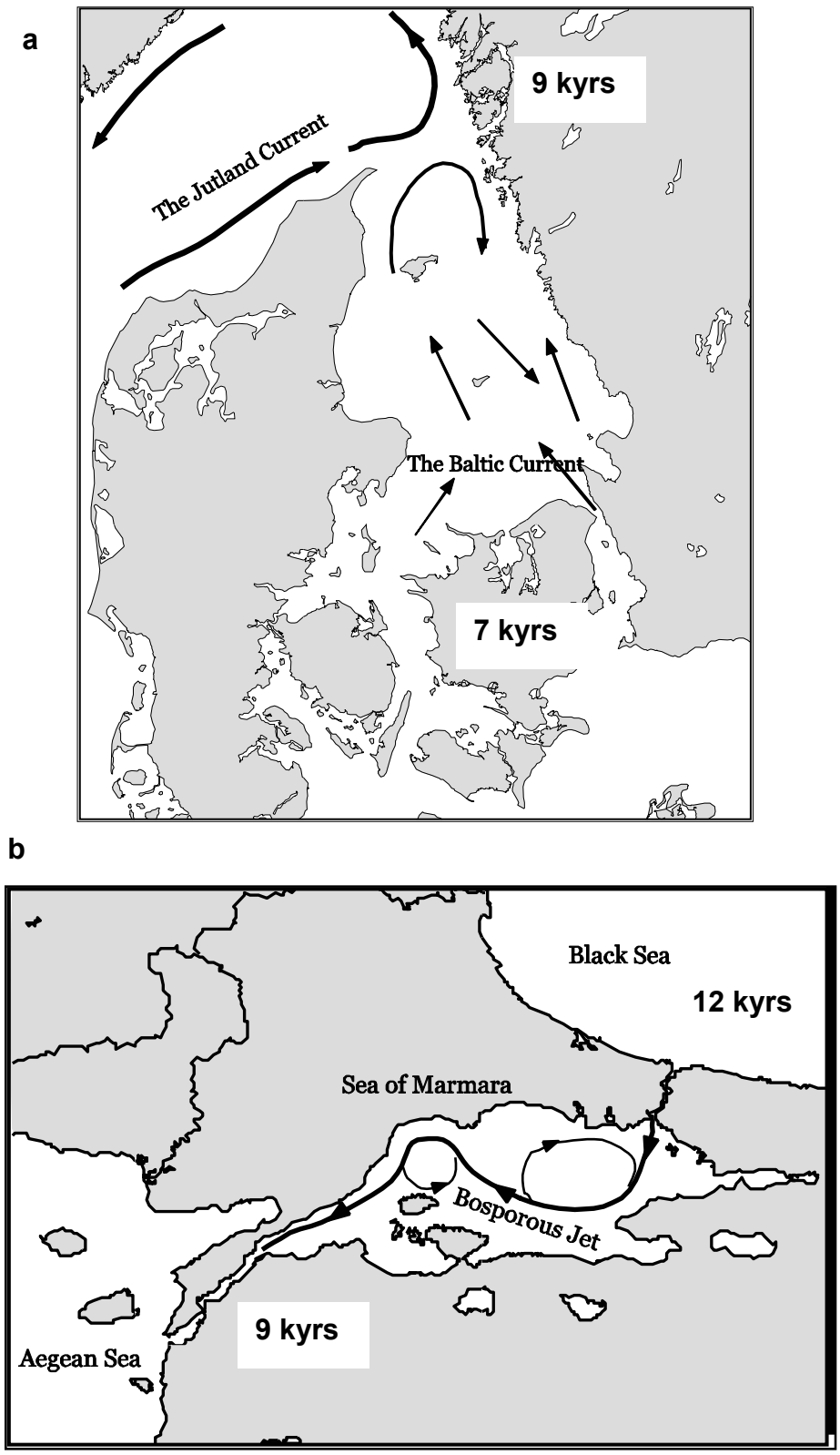


Figure 19. a) Major sea surface circulation in the Baltic Sea/Kattegat/North Sea region. The ages given denotes the time of connection of the Baltic Sea to the global ocean. Redrawn after Matthews *et al.* (1999). b) Major surface currents in the Sea of Marmara. The ages given represents the opening of the two straits to the Aegean Sea and the Black Sea, respectively. Redrawn after Aksu *et al*. (2002b).

In the study by van Oppen *et al.* (1995) both RAPDs and allozyme variation failed to detect any geographical genetic pattern in a species of red algae despite demonstrated ecotypic differentiation between North Sea and Baltic Sea populations.

It is interesting to compare the Baltic Sea/North Sea to the Aegean/Black Sea/Marmara region as the two study areas share hydrological and geological features (Fig. 19). The Baltic Sea arose as a freshwater lake after the ice retreated after the last glacial. The connection of the Baltic Sea to the North Sea took place trough the Great Belt and Öresund straits around 7 kyrs ago. A more northern connection was established ca. 9 kyrs ago but was soon after closed (Björck 1985). These connections are of comparable age to the Dardanelles (12 kyrs) and Bosporus straits (10 kyrs) in the Aegean/Black Sea/Marmara region (Aksu *et al.*, 2002a). Additionally the Baltic Sea, the Black Sea and the Sea of Marmara are all brackish to low salinity basins with more or less constant outflow trough their respective straits (Aksu *et al.,* 2002b; Matthews *et al.,* 1999; Fig 19). However, a direct comparison of the genetic patterns in *C. maritima* from the Baltic Sea/North Sea and the Aegean/Black Sea/Marmara region show little congruence. The Aegean/Black Sea/Marmara region has a distinct genetic structure whereas the Baltic Sea/North Sea show none of the kind. These differences are best explained by differences in the geological history of the brackish basins. The major geological difference of the Baltic Sea in comparison to the other sea straits is its low age. The Black Sea on the other hand became its outline in the late tertiary when it formed as a vestigal of the paratethys and the Aegean/Black Sea/Marmara region system is thus of a much greater age. During the Quaternary glacials the sea levels of the Black Sea and the Aegean were repeatedly lower than the sills of the Dardanelles and the Bosporus which lead to the two basins being isolated from the Mediterranean during such periods (Aksu *et al*., 2002b). During periods of isolation the basins were likely brackish (Mudie *et al.*, 2004) and thus could have supported halophyte vegetation on their shores. The subdivision of the *C. maritima* populations into Black Sea, Sea of Marmara and Aegean Sea groups can be explained by isolation of Black Sea, Sea of Marmara populations and Aegean Sea populations from each other during glacials. In contrast the history of the Baltic Sea/North Sea has not promoted population differentiation to the same extent, probably because of a lack of a perhaps necessary phase of isolation in this region. From this one has to conclude that *C. maritima* subsp. *baltica* has not arisen as a consequence of isolation in the Baltic Sea as have

been hypothesized (Elven & Gjelsås, 1981). Instead it may be that the characters distinguishing it from subsp. *maritima* (less fleshy and more deeply lobed leaves, smaller fruits constricted at the point of articulation; Rodman 1974) are adaptive responses to the lower salinity of Kattegat and the Baltic Sea and that no genetic differentiation has taken place. However, this appears to be in conflict with the result of the Europe wide study where Baltic material forms a cluster together with more northerly material. This could indicate that the area of contact between the two subspecies is situated outside the area sampled in the population study, somewhere else along the Danish coast. Judging by current patterns a candidate area could be the northern tip of Denmark where current directions could contribute to reduced migration between the North Sea and the Kattegat coasts (Fig. 19). The only population sampled from the Danish North Sea coast indeed clusters outside the remaining populations with high bootstrap support (87%; Fig. 11). However, until further populations are studied no firm conclusions can be drawn.

Paralleled by the findings on the European wide scale, both species show highly congruent patterns in the population studies, too. Whenever a major geographical structure was found among populations it was reflected in both species. This indicates that the two species have reacted similarly to historical and contemporary factors also on the much smaller scale of the population studies and further suggests that the mode of gene flow is similar in both species. Consequently, the similarity of patterns in the Aegean/Black Sea/Marmara region between *E. maritimum* and *C. maritima* suggests that, if the genetic gap in *C. maritima* is due to disconnection of the Black Sea and Sea of Marmara, then *E. maritimum*, too, has had a history of isolation of Black Sea populations. If so, reconnection of the Sea Marmara and the Aegean Sea populations happened about 12 kyrs ago when the global sea level reached the sill of the Dardanelles strait and spilled over into the Sea of Marmara. Subsequently the Black Sea was connected when the Black Sea reached the Bosporus sill. The order of these events can also explain why Marmara populations cluster more closely to Aegean populations than to Black Sea populations in the UPGMAs of both species (Figs. 7c & 8c).

The partitioning of molecular variance between groups of populations in the Aegean/Black Sea/Marmara region is also of similar magnitude in both species (table AMOVA). The longer generation time should cause lower differentiation in *E. maritimum* but this is not observed. That *E. maritimum* shows higher differentiation

than *C. maritima* could be the result of higher gene flow after the reconnection in the latter species but caution is warranted since it seems difficult to distinguish between the role of dispersal and historical differentiation. The differentiation may be transient if gene flow is sufficiently high but it may also be that Black Sea and Sea of Marmara populations are in migration-drift equilibrium or are even continuing to diverge due to low gene flow and no hypothesis can be favoured over the other. In addition, the longer generation time in *E. maritimum* not only causes differentiation to take more time but also the time for postgalcial re-homogenization, subsequent to the isolation, to be longer.

A comparison of sea straits can also be done between the Strait of Gibraltar and the Aegean/Black Sea/Marmara region. The Mediterranean Sea and the Atlantic Ocean have been connected continuously since about 5.3 myrs ago (Krijgisman *et al.*, 1999), much longer than the Aegean and Black Seas. In both the Strait of Gibraltar and Aegean/Black Sea/Marmara region both species show congruent patterns: where there is genetic differentiation, it is present in both species. The proportion of variation between clusters of population differs in the Strait of Gibraltar between the species, however. Given that the two areas have been continuously connected by coast line for a long time and likely have been inhabited by *C. maritima* during glacials, the populations should be in more or less equilibrium in this species. If the same can be said for *E. maritimum* this would indicate a higher effective gene flow in *C. maritima* since differentiation by historical isolation can be ruled out. That is, the differentiation of the Atlantic from the Mediterranean populations of both species would primarily be due to low dispersal.

The populations from the coast of west France are sampled from a large area of almost continuous sand dunes. This region was thus assumed to provide a good approximation of unrestricted gene flow. This region indeed exhibited the lowest F_{st}-values of all regions in both species which confirms this assumption. *Cakile maritima* is less differentiated than *E. maritimum* which is expected if dispersal is more effective in *C. maritima*. Isolation-by-distance is detected only in *C. maritima.* This is in accordance with an earlier re-colonisation and shorter generation time in *C. maritima*.

The proportion of variation between populations that reside within groups (i.e. when the proportion of variation residing between regions separated by genetic breaks is subtracted) is a product of species characteristics as well as habitat

structure. Spatially, the sandy habitats of the Strait of Gibraltar and West France are both more or less continuously distributed within groups and the stretches of coast that has been sampled are of approximately similar lengths (Figs. 9, 10, 12 & 13). One would thus expect these two areas to have similar values but that is only the case for *E. maritimum*. In *E. maritimum*, similar values are found in West France (4.23%) and Strait of Gibraltar (3.82%) whereas the Aegean Sea, Black Sea and Marmara regions has a lager proportion of variation between populations (7.16%). In *C. maritima*, on the other hand, the amount of variation is similar in the Baltic Sea/North Sea (8.16%), Strait of Gibraltar (7.38%) and Aegean/Black Sea/Marmara region (8.11%). The West France populations are here the exception with 1.49% (non significant, P=0.053) of the variation between populations. This is contrary to what one would expect. Further it is observed that *Eryngium maritimum* generally have lower proportions of variation among populations within groups than *C. maritima*, even though we have several indications that gene flow is higher in the latter species. However this can be accounted for by the higher generation time in *E. maritimum* since perennial plants generally show less population differentiation than annuals (Hamrick & Godt, 1989; Nybom & Bartitsh, 2000). One may be tempted to conclude that the differences between the species, and the similarities in differentiation within species between several of the areas, reflects properties of these plants. However, the scales of these areas differ and the two species show different patterns and so caution is needed in the interpretations of these results.

Conclusions

A comparison of the investigated species revealed considerable congruency in their phylogeographic patterns. These patterns are similar to those found in three other widespread species from partly different coastal habitats. Thus phylogeographic patterns of widespread coastal plants appear to be more congruent than those of other terrestrial plant taxa. An important prerequisite for this correspondence is the low spatial complexity of the coastal habitat. This has greatly reduced the number of possible migratory routes the plants could have taken in response to quaternary climate change.

The patterns of phylogeographic structure in this study can be explained by a small number of factors. Much of the similar structure found is best accounted for by

a predominance of seed dispersal by sea water. This has allowed for a marked influence by sea currents on the gene flow between regions as seen in the Strait of Gibraltar and the genetic gap between Aegean Sea and Ionian Sea collections. A role for sea currents is also possible in the Baltic Sea/North Sea area and may further contribute to upholding the regional differentiation in the Aegean/Black Sea/Marmara region.

Another important factor is the largely shared climatic history, which could be inferred from climate reconstructions. The distribution of Mediterranean populations of both species was most likely left largely unaffected by the colder glacial climate. This prevented divergent patterns to evolve due to differential historical distributions of the species. In connection with the re-colonisation of the European Atlantic coast, on the other hand, climate apparently caused differences to arise. Although re-colonisation took place simultaneous the time available has allowed *C. maritima*, but not *E. maritimum*, to reach migration-drift equilibrium in the Atlantic due to the effects of the different life-spans of the species on isolation-by-distance patterns.

The European scale patterns could be confirmed by the population genetic studies in the sea straits. This applies especially to the Strait of Gibraltar but also to some extent in the Aegean Sea/Black Sea Marmara region. Thus, in conclusion, the combined use of wide scale and regional scale studies has allowed more detailed insights both into phylogeographic patterns and as well as the processes shaping genetic diversity in these two species.

LITERATURE

Aksu, A.E., Hiscott, R.N., Kaminski, M.A., Mudie, P.J., Gillespie, H., Abrajano, T., Yasar, D. 2002a. Last glacial-Holocene paleoceanography of the Black Sea and Marmara Sea: stable isotopic foraminiferal and coccolith. *Marine Geology* 190: 119-149

Aksu, A.E., Hiscott, R.N., Mudie, P.J., Rochon, A., Kaminski, M.A., Abrajano, T., Yasar, D. 2002b. Persistent Holocene Outflow from the Black Sea to the Eastern Mediterranean Contradicts Noah's Flood Hypothesis. *GSA Today* 12: 4-10.

van Andel, T. H. 2002. The climate and landscape of the middle part of the Weichselian glaciation in Europe: the stage 3 project. *Quat. Res*. 57: 2-8.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C. 1987. Intraspecifc phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18: 489-522.

Avise, J.C. 1998. The history and purview of phylogeography: a personal reflection. *Molec. Ecol.* 7: 371-379.

Bai, G., Tefera, H., Ayele, M., Nguyen, H.T. 1999. A genetic linkage map of tef [*Eragrostis tef* (Zucc.) Trotter] based on amplified fragment length polymorphism. *Theor. Appl. Genet.* 99: 599-604.

Barbour, M.G., Rodman, J.E. 1970. Saga of the westcoast sea rockets: *Cakile edentula* ssp. *californica* and *C. maritima*. *Rhodora* 70: 370-386.

Bargelloni, L., Alarcon, J. A., Alvarez, M. C., Penzo, E., Magoulas, A., Reis, C., Patarnello, T. 2003. Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. *J. Evol. Biol.* 16: 1149-1158.

Becker, J., Vos, P., Kuiper, M., Salamini, F., Heun, M. 1995. Combined mapping of AFLP and RFLP markers in barley. *Mol. Gen. Genet.* 249: 65–73.

Berry, O., Tocher, M.D., Sarre, S.D. 2004. Can assignment tests measure dispersal? *Molec. Ecol.* 13: 551-561

Bianchi, N., Morri, C. 2000. Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Mar. Pollut. Bull.* 40: 367-376.

Björck, S. 1995. A review of the history of the Baltic Sea, 13.0-8.0 ka BP. *Quat. Int.* 27: 19-40.

Bonn, S., Poschlod, P. 1998. Ausbreitungsbiologie der Pflanzen Mitteleuropas. Wiesbaden

Borsa, P., Blanquer, A., Berrebi, P. 1997a. Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Mar. Biol.* 129: 233-246.

Borsa, P., Naciri, M., Bahiri, L., Chikhi, L., Garcia de Leon, F.J., Kotoulas, G., Bonhomme, F. 1997b. Zoogéographie infa-spécifique de la mer Méditerranée. Analyse des données génétiques populationelles sur seize espèces atlanto-méditerranéennes (Poissons et Invertébrés). *Vie Milieu.* 47: 295-305.

Boyd, R.S., Barbour, M.G. 1993. Replacement of *Cakile edentula* by *C. maritima* in the strand habitat of California. *Am. Mid. Nat.t* 130: 209-228.

Brown, J.H., Lomolino, M.V. 1998. Biogeography, 2nd edn. Sinauer, Sunderland, MA.

Brunsfeld, S.J., Sullivan, J., Soltis, D.E., Soltis, P.S. 2001. Comparative phylogeography of Northwestern North America: a synthesis. In: Silvertown, J., Antonovics, J. (eds) Integrating Ecological and Evolutionary Processes in a SpatialContext.. BlackwellScience, Oxford.

Campbell, D., Duchesne, P., Bernatchez, L. 2003. AFLP utility for population assignment studies: analytical investigation and empirical comparison with microsatellites. *Molec. Ecol.* 12: 1979-1992.

Castelló, J., Carballo, J.L. 2001. Isopod fauna, excluding Epicaridea, from the Strait of Gibraltar and nearby areas (Southern Iberian Peninsula). *Sci. Mar.* 65: 221-241.

Castric, V. Bernatchez, L. 2004. The rise and fall of Isolation by Distance in the Anadromous Brook Charr (*Salvelinus fontinalis* Mitchill). Genetics 163: 983-996

Carballo, J.L., Naranjo, S., García-Gómez, J.C. 1997. Where does the Mediterranean Sea begin? Zoogeographical affinities of the littoral sponges of the Straits of Gibraltar. *J. Biogeogr.* 24: 223-232.

Clausing, G., Vickers, K., Kadereit, J.W. 2000. Historical biogeography in a linear system: genetic variation of Sea Rocket (*Cakile maritima*) and Sea Holly (*Eryngium maritimum*) along european costs. *Molec. Ecol.* 9: 1823-1833.

Comes, H.P., Kadereit, J.W. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends Plant. Sci.* 3: 432-438

Comps, B., Gömöry, D., Letouzey, J., Thiébaut, B., Petit, R.J. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the european beech. *Genetics* 157:389-397.

Coyer, J.A., Peters, A.F., Stam, W.T., Olsen, J.L. 2003. Post-ice age recolonization and differentiation of *Fucus serratus* L. (Fucaceae: Phaeophyta) populations in Northern Europe. *Molec. Ecol.* 12: 1817-1829.

Curtis, H. Barnes, N.S. 1989. Biology. 5th edition Worth Publishers New York.

Darwin, C.M. 1859. On the origin of species by means of natural selection. John Murray, London.

Duchesne, P., Bernatchez, L. 2002. AFLPOP: a computer program for simulated and real population allocation based on AFLP data. Molecular Ecology Notes 2: 380-383.

Dunphy, B.K., Hamrick, J.L., Schwagerl, J. 2004. A comparison of direct and indirect measures of gene flow in the bat-pollinated tree *Hymenaea courbaril* in the dry forest life zone of southwestern Puerto Rico. *Int. J. Plant Sci.* 165: 427-436

Erhlich, P.R., Raven, P.H. 1969. Differentiations in populations. *Science*, 165: 1228-1231.

Elenga, H., Peyron, O., Bonnefille, R., Jolly, D., Cheddadi, R., Guiot, J., Andrieu, V., Bottema, S., Buchet, G., de Beaulieu, J.L., Hamilton, A.C., Maley, J., Marchant, R., Perez-Obiol, R., Reille, M., Riollet, G., Scott, L., Straka, H., Taylor, D., Van Campo, E., Vincens, A., Laarif, F., and Jonson, H. 2000. Pollen-based biome reconstruction for southern Europe and Africa 18,000 yr BP. *J. Biogeogr.* 27: 621-634.

Elven, R., Gjelsås, T. 1981. Strandreddik (*Cakile mill.*) i Norge. *Blyttia* 39:87-106.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 86: 991-1000.

Gabrielsen, T.M., Brochmann, C., Rueness, J. 2002. The baltic sea as a model system for studying postglacial colonization and ecological differentiation, exemplified by the red alga *Ceramium tetnuicorne*. *Molec. Ecol.* 11:1083-2095.

Garibaldi, L., Caddy, J.F. 1998. Biogeographic characterization of Mediterranean and Black Seas faunal provinces using GIS procedures. *Ocean Coast. Manag.* 139: 211-227.

Garnier, S., Alibert, P., Audiot, P., Prieur, B., Rasplus, J.Y. 2004. Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. *Molec. Ecol.* 13: 1883-1897.

Gjaerevoll, O. 1992. Plantegeografi. Tapir forlag. Trondheim.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hamrick, J.L., Godt. M.J. 1989. Allozyme diversity in plant species. pp 43-63 In: Brown, A.H.D., Clegg, M.T., Kahler, A. L., Weir, B.S. (eds) Plant Population Genetics, Breeding and Germplasm Resources. Sinauer, Sunderland, Mass.

Hann, J. 1892. Atlas der Meteorologie. In: Berghaus, H. C. W. (ed.), Berghaus' Physikalischer Atlas. Ed. 3. Justus Perthes, Gotha.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* 58: 247-276.

Hewitt, G.M. 1999. Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.* 68: 87-112.

Hewitt, G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philos. Trans.*, Ser. B 359: 183-195.

Hirner, A.A., Seitz, H.U. 2000. Isoforms of chalcone synthase in *Daucus carota* L. and their differential expression in organs from the European wild carrot and in untraviolet-A-irradiated cell cultures. *Planta* 210: 993-998.

Hood, G. 2002. PopTools, v2.5.2. CSIRO, Canberra.

Hope, A.C.A. 1968. A simplified Monte Carlo significance test procedure. *J. R. Statist. Soc. Ser. B* 30: 582–598.

Huang, S.F., Hwang, S.Y., Wang, J.C., Lin, T.P. 2004 Phylogeography of *Trochodendron aralioides* (Trochodendraceae) in Taiwan and its adjacent areas. *J. Biogeogr.* 31: 1251-1259

Husband, B.C., Barrett, S.C.H. 1995. Estimates of gene flow in *Eichhornia paniculata* (Pontederiaceae): Effects of range substructure. *Heredity* 75: 549-560

Kimura, M., Weiss, G.H. 1964. The stepping-stone model of population structure and the decrease of genetic correlation with distance. *Genetics* 49: 561-576.

Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J., Wilson, D.S. 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature* 400: 652-655.

Kropf, M., Kadereit, J.W., Comes, H.P. 2003. Differential cycles of range contraction and expansion in European high mountain plants during the Late Quaternary: insights from *Pritzelago alpina* (L.) O. Kuntze (Brassicaceae). *Molec. Ecol.* 12: 931-949.

Lange, G. 1994. Quartäre Vegetationsgeschichte Europas. G. Fischer, Jena.

Lascoux, M., Palmé, A.E., Cheddadi, R., Latta, R.G. 2004. Impact of ice ages on the genetic structure of trees and shrubs. *Philos. Trans.*, Ser. B 359: 197-207.

Casgrain, P., Legendre, P. 2000. The R package for multivariate and spatial analysis, v.4.0. (development release 2). University of Montréal, Québec.

Li, W.H. 1997. Molecular Evolution. Sinauer Associates, Sunderland, MA.

Long, J. C . 1986 . The allelic correlation structure of Gainj- and Kalam-speaking people. I . The estimation and interpretation of Wright's *F*-statistics . *Genetics* 112: 629-647.

Lykousis, V., Chronis, G., Tselepidis, A., Price, N.B., Theocharis, A., Sikou-Frangou, I., Van Wambeke, F., Danovaro, R., Stavrakakis, S., Duineveld, G., Georgopoulos, D., Ignatiades, L., Souvermezoglou, A., Voutsinou-Taliadouri, F. 2002. Major outputs of the recent multidisciplinary biogeochemical researches undertaken in the Aegean Sea. *J. Mar. Sys.* 33-34: 313-334.

Maheswaran, M., Subudhi, P.K., Nandi, S., Xu, J.C., Parco, Yang, D.C., Huang, N. 1997. Polymorphism, distribution, and segregation of AFLP markers in a double haploid rice population. *Theor. Appl. Genet.* 94: 39-45.

Malanotte-Rizzoli, P., Manca, B. B., Ribera d'Alcala, M., Theocharis, A., Bergamasco, A., Bregant, D., Budillon, G., Civitarese, G., Georgopoulos, D., Michelato, A., Sansone, E., Scarazzato, P., Souvermezoglou, E. 1997. A synthesis of the Ionian Sea hydrography, circulation and water mass pathways during POEM-Phase I. *Prog. Oceanogr.* 39: 153-204.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27: 209-220.

Magoulas, A., Tsimenides, N., Zouros, E. 1996. Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the European anchovy (*Engraulis encrasicolus*). *Molec. Biol. Evol.* 13: 178-190.

Matthews, J.B.L., Buchholz, F., Saborowski, R., Tarling, G.A., Dallot, S.. Labat, J.P. 1999. On the physical oceanography of the Kattegat and Clyde Sea area, 1996–98, as background to ecophysiological studies on the planktonic crustacean, *Meganyctiphanes norvegica* (Euphausiacea). *Helgol. Mar. Res.* 53: 70–84.

Maun, M.A., Payne, A.M. 1989. Fruit ans seed polymorphism and its relation to seedling growth in the genus *Cakile*. *Can. J. Bot.* 67: 2743-2750.

Meusel, H., Jäger, E.J. 1992. Vergleichende Chorologie der zentraleuropäischen Flora. G. Fischer, Stuttgart & New York.

Miller, M.P. 1997. Tools for Population Genetic Analysis. Version 1.3. Department of Biological Sciences, Northern Arizona University, Flagstaff.

Millot, C. 1999. Circulation in the Western Mediterranean sea. *J. Mar. Syst.* 20: 423-442.

Mudie, P.J., Rochon, A., Aksu, A.E., Gillespie, H. 2004. Late glacial, Holocene and modern dinoflagellate cyst assemblages in the Aegean-Marmara-Black Sea corridor: statistical analysis and re-interpretation of the early Holocene Noah's Flood hypothesis. Rev. Palaeobot. Palyno. 128: 143-167.

Naciri, M., Lemaire, C., Borsa, P., Bonhomme. F. 1999. Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). *J. Heredity* 90: 591-596.

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

Nei, M., Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.* 76: 5269-5273.

Nei, M., Kumar, S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Nielsen, E.E., Nielsen, P.H., Meldrup, D., Hansen, M.M. 2004. Genetic population structure of turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. *Molec. Ecol*. 13: 585-595.

Nikula, R., Väinölä, R. 2003. Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Mar. Biol.* 143: 339-350.

Nybom, H., Bartish, I.V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives Plant Ecol. Evol. Syst.* 3: 93-114.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molec. Ecol*. 13: 1143-1155.

Olsen, J.L., Stam, W.T., Coyer, J.A., Reusch, T.B.H., Billingham, M., Boström, C., Calvert, E., Christie, H., Granger, S., La Lumière, R., Milchakova, N., Oudot-Le Secq, M.P., Procaccini, G., Sanjabi, B., Serrão, E., Veldsink, J., Widdicombe, S., Wyllie-Echeverria, S. 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molec. Ecol.* 13: 1923-1941.

van Oppen, M.J.H., Olsen, J.L., Stam, W.T. 1995. Genetic variation within and among North Atlantic and Baltic populations of the benthic alga *Phycodrys rubens* (Rhodophyta). *Eur. J. Phycol.* 30: 251-260.

Ovchinnikov, I. M. 1966. Circulation in the surface and intermediate layers of the Mediterranean. *Oceanology* 6: 48-59.

Pannacciulli, F.G., Bishop D.D., Hawkins, S.J. 1997. Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. *Mar. Biol.* 128: 73-82.

Peijnenburg, K.T.C.A., Breeuwer, J.A.J., Pierrot-Bults, A.C., Menken, S.B.J. 2004. Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. *Evolution* 58: 1472-1487.

Perez-Losada, M., Guerra, A., Sanjuan, A. 1999. Allozyme differentiation in the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) from the NE Atlantic and Mediterranean. *Heredity* 83: 280-289.

Pimenov, M.G., Vasileva, M.G., Leonov, M.V., Daushkevich, J.V. 2002. Karyotaxonomical analysis in the Umbelliferae. Science Publishers Inc. Enfield (NH), USA.

Pobedimova, E.G. 1963. A review of the genus *Cakile* Mill. *Bot. Zhurn.* 48: 1762-1775.

Pobedimova, E.G. 1964. Genus *Cakile* Mill. (pars specialis). *Nov. Sist. Vysshikh Rast.* 1: 90-128.

Pritchard, J. K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Procaccini, G., Orsini, L., Ruggiero, M.V., Scardi, M. 2001. Patterns of genetic diversity in *Posidonia oceanica*, an endemic Mediterranean seagrass. *Molec. Ecol.* 10: 1413-1422.

Quesada, H., Beynon, C.M., Skibinski, D.O.F. 1995. A mitochondrial DNA discontinuity in the mussel *Mytilus galloprovincialis* Lmk: pleistocene vicariance biogeography and secondary integration. *Mol. Biol. Evol.* 12: 521-524.

Rey, J.C. 1983. El paso del atún rojo, *Thunnus thynnus* (Linnaeus, 1758), a través del Estrecho de Gibraltar y su relación. Esquemas de migración. *Bol. Inst. Esp. Oceanog.* 1: 85-94.

Ridley, H.N. 1930. The dispersal of plants throughout the world. Reeve, Ashford, Kent.

Rios, C., Sanz, S., Saavedra, C., Peña, J.B. 2002. Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria-Oran front. *J. Exp. Mar. Biol. Ecol.* 267: 223-244.

Robinson, J.P., Harris, S.A. 1999. Amplified fragment length polymorphism and microsatellites: a phylogenetic perspective. In: Which DNA Marker for Which Purpose? Final Compendium of the Research Project Development, Optimization and Validation of Molecular Tools for Assessment of Biodiversity in Forest Trees http://webdoc.sub.gwdg.de/ebook/y/1999/whichmarker/index.htm

Rodman, J.E. 1974. Systematics and evolution of the genus *Cakile* (Cruciferae). *Contr. Gray Herb.* 205: 3-146.

Rosenberg, N.A., Pritchard, J.K., Weber, J.L., Cann, H.M., Kidd, K.K., Zhivotovsky, L.A., Feldman, M.W. 2002. Genetic structure of human populations. *Science* 298: 2381-2385

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145: 1219-1228.

Rozen, S., Skaletsky, H.J. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S., Misener, S. (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386

Röhner, M.R., Bastrop, R., Jürss, K. 1997. Genetic differentiation in *Hediste diversicolor* (Polychaetea: Nereididae) for the North Sea and the Baltic Sea. *Mar. Biol.* 130: 171-180.

Saitou, N., Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* 4: 406-425.

Schmidt, I., Glaubrecht, M., Golani, D. 2001. Biogeographie und Biodiversität. In: Hofrichter, R. (ed) *Das Mittelmeer: Fauna, Flora, Ökologie. Allgemeiner Teil.* Spektrum Akadedmischer Verlag. Heidelberg, Berlin.

Schneider, S., Roessli, D., Excoffier, L. 2000. Arlequin: A software for population genetic data. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shannon, C.E., Weaver, W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana.

Silvertown, J., Charlesworth, D. 2001. Introduction to Plant Population Biology. 4th Edition. Blackwell Science. Oxford, UK.

Sneath, P.H.A., Sokal, R.R. 1973. Numerical Taxonomy. Freeman, San Francisco, CA.

Soltis, D.E., Gitzendanner, M.A., Strenge, D.D., Soltis, P.S. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Pl. Syst. Evol.* 206: 353-373.

Stewart, J.R., and Lister, A.M. 2001. Cryptic northern refugia and the origins of the modern biota. *Trends Ecol. Evol.* 16: 608-613.

Stewart J.R. 2003. Comment on "Buffered Tree Population Changes in a Quaternary Refugium: Evolutionary Implications". *Science* 299: 825.

Strand, A.E., Leebens-Mack, J., Milligan, B.G. 1997. Nuclear DNA-based markers for plant evolutionary biology. *Molec. Ecol*. 6: 113-118.

Swofford, D.L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, MA.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molec. Ecol.* 7: 453-464.

Tarasov, P.E., Volkova, V.S., Webb III, T., Guiot, J., Andreev, A.A., Bezusko, L.G., Bezusko, T.V., Bykova, G.V., Dorofeyuk, N.I., Kvavadze, E.V., Osipova, I.M., Panova, N.K., Sevastyanov, D.V. 2000. Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from northern Eurasia. *J. Biogeogr*. 27: 609-620.

Thrall, P.H., Young, A.G., Burdon, J.J. 2000. An analysis of mating structure in populations of the annual sea rocket, *Cakile maritima* (Brassicaceae). *Austr. J. Bot.* 48: 731-738.

Turesson, G. 1922a. The species and variety as ecological units. *Hereditas* 3: 100-113.

Turesson, G. 1922b. The genotypical response of the plant species to the habitat. *Hereditas* 3: 211-350.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407-4414.

Walmsley, C.A., and Davy, A.J. 1997. Germination characteristics of shingle beach species, effects of seed ageing and their implicarions for vegetation restoration. *J. Appl. Ecol.* 34: 131-142.

Waters, J.M., Roy, M.S. 2003. Global phylogeography of the fissiparous sea-star *Coscinasterias*. *Mar. Biol.* 142: 185-191.

Webb, T., Bartlein, P.J. 1992. Global Changes During the Last 3 Million Years: Climatic Controls and Biotic Responses. *Annu. Rev. Ecol. Syst.* 23: 141-173.

Whitlock, M.C. McCauley, D.E. 1998. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity* 82: 117-125.

Wright, S. 1931. Evolution in mendelian populations. *Genetics* 16: 97-159.

Wright, S. 1951. The genetical structure of populations. *Ann. Eugen*. 15: 323-354.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X. 1997. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

APPENDIX I

Pairwise F_{st} values and significance tests between populations (Tables A1-A7) and clusters (A8).

Population name	BS1	BS3	MA11	MA13	MA14	MA15	AE18	AE19	AE22	GR	MA17
BS1	0 *										
BS3	0.26684 *0.00195*	0 *									
MA11	0.43986 *0.00000*	0.28674 *0.00000*	0 *								
MA13	0.37892 *0.0014*	0.26838 *0.00098*	0.14392 *0.00391*	0 *							
MA14	0.43811 *0.0000*	0.31701 *0.00098*	0.1054 *0.00000*	0.09164 *0.00000*	0 *						
MA15	0.36193 *0.0000*	0.25495 *0.00000*	0.09771 *0.00293*	0.04751 *0.07031*	0.0279 *0.07715*	0 *					
AE18	0.44041 *0.0010*	0.33519 *0.00000*	0.16387 *0.00000*	0.13719 *0.00000*	0.12979 *0.00000*	0.1512 *0.00000*	0 *				
AE19	0.50654 *0.00195*	0.42999 *0.00098*	0.27407 *0.00000*	0.25866 *0.00098*	0.21785 *0.00000*	0.27048 *0.00000*	0.08804 *0.00098*	0 *			
AE22	0.5091 *0.00195*	0.4163 *0.00098*	0.25863 *0.00098*	0.22797 *0.00000*	0.20908 *0.00000*	0.25504 *0.00000*	0.09114 *0.00000*	0.10377 *0.00098*	0 *		
GR	0.47753 *0.00879*	0.41756 *0.00000*	0.21901 *0.00098*	0.23643 *0.00098*	0.18137 *0.00000*	0.23285 *0.00000*	0.08225 *0.00000*	0.06409 *0.02930*	0.13221 *0.00098*	0 *	
MA17	0.50109 *0.02051*	0.35919 *0.01855*	0.08949 *0.06250*	0.10477 *0.01758*	0.02385 *0.27930*	0.01112 *0.36035*	0.10681 *0.02539*	0.18227 *0.00391*	0.1774 *0.00488*	0.12013 *0.05957*	0 *

Table A1 : *Cakile maritima,* Aegean Sea/Black Sea. Population pairwise F_{st} and P-values (italics).

	BS5	BS4	MA12	MA24	MA14	MA17	AE18	AE19	MA13	AE22	GR
BS3	0 *										
BS2	-0.05868 *0.97656*	0 *									
MA12	0.36983 *0.00000*	0.36499 *0.00000*	0 *								
MA24	0.42143 *0.00000*	0.4094 *0.00000*	0.04422 *0.14453*	0 *							
MA14	0.40543 *0.00000*	0.40089 *0.00000*	0.05065 *0.10547*	0.02 *0.32715*	0 *						
MA17	0.34855 *0.00000*	0.35018 *0.00000*	0.05055 *0.14355*	-0.00686 *0.51270*	0.02146 *0.27930*	0 *					
AE18	0.34103 *0.00000*	0.32541 *0.00000*	0.17996 *0.00000*	0.1504 *0.00000*	0.15299 *0.00293*	0.12713 *0.00586*	0 *				
AE19	0.55695 *0.00000*	0.53876 *0.00000*	0.44831 *0.00000*	0.36413 *0.00000*	0.40756 *0.00000*	0.32256 *0.00000*	0.1367 *0.00000*	0 *			
MA13	0.35269 *0.00098*	0.34634 *0.00098*	0.22222 *0.00098*	0.26694 *0.00098*	0.17855 *0.02441*	0.19474 *0.01074*	0.22792 *0.00000*	0.42393 *0.00098*	0 *		
AE22	0.30393 *0.00000*	0.31226 *0.00000*	0.12087 *0.00098*	0.05961 *0.06641*	0.05416 *0.06738*	0.02282 *0.22461*	0.09834 *0.00000*	0.23579 *0.00000*	0.09873 *0.04395*	0 *	
GR	0.35839 *0.00000*	0.35339 *0.00000*	0.17965 *0.00391*	0.09065 *0.04297*	0.14116 *0.02344*	0.09985 *0.03418*	0.02399 *0.28418*	0.15796 *0.00098*	0.16823 *0.02148*	0.01087 *0.36523*	0 *

Table A2 : ***Eryngium maritimum,*** **Aegean Sea/Black Sea.** Population pairwise F_{st} and P-values (italics)..

	DK1	DK2	DK3	DK4	DK5	SV2	SV1	DK7	DK6	DK8
DK1	0 *									
DK2	0.01439 *0.24805*	0 *								
DK3	0.04136 *0.04785*	0.01094 *0.27734*	0 *							
DK4	0.05948 *0.01270*	0.05067 *0.03223*	0.0874 *0.00000*	0 *						
DK5	0.09234 *0.00293*	0.02988 *0.13281*	0.02931 *0.08984*	0.11034 *0.00195*	0 *					
SV2	0.04151 *0.04688*	0.03733 *0.07715*	0.06253 *0.00391*	0.03761 *0.07227*	0.03552 *0.07813*	0 *				
SV1	0.07102 *0.02148*	0.02982 *0.12500*	-0.02787 *0.86426*	0.07955 *0.00684*	0.02983 *0.13086*	0.03391 *0.12305*	0 *			
DK7	0.03078 *0.11816*	0.06 *0.01953*	0.0688 *0.00879*	0.00908 *0.34082*	0.11406 *0.00293*	0.03338 *0.09766*	0.0799 *0.01270*	0 *		
DK6	0.07877 *0.00684*	0.12027 *0.00000*	0.11515 *0.00098*	0.01326 *0.32520*	0.16199 *0.00098*	0.0473 *0.06836*	0.14113 *0.00098*	0.04195 *0.10840*	0 *	
DK8	0.16888 *0.00000*	0.13586 *0.00000*	0.13921 *0.00000*	0.15394 *0.00000*	0.21321 *0.00000*	0.16218 *0.00000*	0.13866 *0.00000*	0.11303 *0.00000*	0.1796 *0.00000*	0 *

Table A3 : *Cakile maritima,* Baltic Sea/North Sea. Population pairwise F_{st} and P-values (italics).

	MED5	MED4	MED3	MED2	MED1	ATL1	ATL2	ATL3	ATL4	ATL5
MED5	0 *									
MED4	0.07144 *0.01660*	0 *								
MED3	0.06933 *0.01172*	0.09694 *0.00195*	0 *							
MED2	0.04601 *0.01660*	0.0681 *0.00781*	0.02504 *0.15039*	0 *						
MED1	0.06462 *0.00879*	0.07588 *0.00488*	0.05683 *0.01367*	0.02423 *0.13770*	0 *					
ATL1	0.23038 *0.00000*	0.2049 *0.00000*	0.18185 *0.00000*	0.15477 *0.00000*	0.1468 *0.00000*	0 *				
ATL2	0.23492 *0.00000*	0.20335 *0.00000*	0.14124 *0.00098*	0.11034 *0.00000*	0.12139 *0.00098*	0.11832 *0.00000*	0 *			
ATL3	0.25991 *0.00000*	0.1865 *0.00000*	0.18422 *0.00000*	0.15689 *0.00000*	0.15298 *0.00000*	0.15747 *0.00000*	0.09265 *0.00293*	0 *		
ATL4	0.16724 *0.00000*	0.14507 *0.00000*	0.11422 *0.00000*	0.06371 *0.01563*	0.11031 *0.00000*	0.12665 *0.00000*	0.10422 *0.00293*	0.08695 *0.00098*	0 *	
ATL5	0.21929 *0.00000*	0.18201 *0.00000*	0.14332 *0.00000*	0.11035 *0.00098*	0.12378 *0.00000*	0.13332 *0.00000*	0.05532 *0.01367*	0.09465 *0.00195*	0.03898 *0.06641*	0 *

Table A4 : *Cakile maritima,* Strait of Gibraltar. Population pairwise F_{st} and P-values (italics).

	MED5	ATL5	ATL3	ATL4	ATL3	MED2	MED1	MED3	MED4	ATL1
MED5	0 *									
ATL5	0.29037 *0.00000*	0 *								
ATL3	0.33245 *0.00000*	-0.04056 *0.85156*	0 *							
ATL4	0.33646 *0.00000*	-0.01754 *0.59180*	-0.03604 *0.85156*	0 *						
ATL3	0.34445 *0.00000*	0.08687 *0.05176*	0.15516 *0.00684*	0.19796 *0.00391*	0 *					
MED2	0.06722 *0.00684*	0.30788 *0.00000*	0.34387 *0.00000*	0.34307 *0.00000*	0.39092 *0.00000*	0 *				
MED1	0.082 *0.00000*	0.15899 *0.00000*	0.18597 *0.00000*	0.1712 *0.00000*	0.26868 *0.00000*	0.07334 *0.01465*	0 *			
MED3	0.04939 *0.03125*	0.33928 *0.00000*	0.3653 *0.00098*	0.37086 *0.00000*	0.41617 *0.00000*	0.02927 *0.12402*	0.09927 *0.00293*	0 *		
MED4	0.06436 *0.01074*	0.27799 *0.00000*	0.32234 *0.00000*	0.31684 *0.00000*	0.36077 *0.00000*	0.0062 *0.36328*	0.07725 *0.01367*	-0.00525 *0.53125*	0 *	
ATL1	0.27852 *0.00000*	-0.01405 *0.53906*	0.07398 *0.05762*	0.05582 *0.11035*	0.08901 *0.05078*	0.29825 *0.00000*	0.16298 *0.00098*	0.33301 *0.00000*	0.26614 *0.00000*	0 *

Table A5 : *Eryngium maritimum,* Strait of Gibraltar. Population pairwise F_{st} and P-values (italics).

	WF 9	WF 10	WF 11	WF 12	WF 13	WF 14	WF 17	WF 18	WF 19
WF9	0 *								
WF10	0.03025 *0.16016*	0 *							
WF11	0.00957 *0.34082*	-0.00622 *0.50488*	0 *						
WF12	0.05757 *0.01563*	-0.00028 *0.47266*	0.00082 *0.45215*	0 *					
WF13	0.04086 *0.07715*	-0.03892 *0.85156*	0.03011 *0.17090*	-0.00664 *0.55762*	0 *				
WF14	0.06922 *0.01172*	-0.01137 *0.59766*	0.03071 *0.11816*	-0.02519 *0.81836*	-0.05248 *0.94824*	0 *			
WF17	0.08038 *0.00000*	-0.00022 *0.48242*	0.03688 *0.10449*	-0.01981 *0.81445*	-0.01564 *0.70117*	-0.0183 *0.71875*	0 *		
WF18	0.05553 *0.00586*	-0.00154 *0.48535*	-0.01488 *0.74707*	-0.0171 *0.80566*	0.03336 *0.11328*	0.00443 *0.42188*	-0.00445 *0.56348*	0 *	
WF19	0.07585 *0.00977*	0.02189 *0.22559*	0.02788 *0.14551*	0.0023 *0.45996*	0.07076 *0.00977*	0.03193 *0.10547*	0.012 *0.27246*	-0.01454 *0.75488*	0 *

Table A6 : *Cakile maritima,* West France. Population pairwise F_{st} and P-values (italics).

	WF9	WF 10	WF 11	WF 12	WF 13	WF 16	WF 17	WF 18	WF 19
WF9	0 *								
WF10	0,03682 *0.19336*	0 *							
WF11	0,08657 *0.03516*	0,01894 *0.27246*	0 *						
WF12	0,14603 *0.00000*	0,03267 *0.19922*	0,03413 *0.17090*	0 *					
WF13	0,1586 *0.00195*	0,03539 *0.20215*	-0,0394 *0.81445*	0,00854 *0.37207*	0 *				
WF16	0,10443 *0.00977*	0,03574 *0.19043*	-0,01707 *0.60840*	0,05362 *0.06250*	-0,05426 *0.93652*	0 *			
WF17	0,11616 *0.00684*	0,0134 *0.32129*	0,00916 *0.37305*	0,04788 *0.11719*	-0,0446 *0.83301*	-0,02358 *0.71094*	0 *		
WF18	0,17661 *0.00000*	0,09314 *0.01758*	-0,01218 *0.59180*	0,04915 *0.11523*	-0,02636 *0.69922*	-0,02861 *0.77051*	0,00019 *0.43750*	0 *	
WF19	0,05361 *0.09668*	-0,01245 *0.59570*	0,02969 *0.20605*	0,11934 *0.00879*	0,04615 *0.10156*	0,01984 *0.27441*	0,04739 *0.12988*	0,05087 *0.09668*	0 *

Table A7 : *Eryngium maritimum,* West France. Population pairwise F_{st} and P-values (italics).

Table A8. Pairwise F_{st} between the clusters identified on the Europe wide scale.

REGION	F_{ST} VALUE	REGION
	Cakile maritima	
IONIAN/ADRIATIC SEA	0.12712	WEST MEDITERRANEAN
SOUTH ATLANTIC	0.17113	NORTH ATLANTIC
WEST MEDITERRANEAN	0.19618	AEGEAN SEA/BLACK SEA
SOUTH ATLANTIC	0.20194	WEST MEDITERRANEAN
IONIAN/ADRIATIC SEA	0.25279	SOUTH ATLANTIC
IONIAN/ADRIATIC SEA	0.25306	AEGEAN SEA/BLACK SEA
WEST MEDITERRANEAN	0.28951	NORTH ATLANTIC
SOUTH ATLANTIC	0.31421	AEGEAN SEA/BLACK SEA
IONIAN/ADRIATIC SEA	0.33325	NORTH ATLANTIC
AEGEAN SEA/BLACK SEA	0.37005	NORTH ATLANTIC
	Eryngium maritimum	
WEST MEDITERRANEAN	0.49875	ATLANTIC OCEAN
AEGEAN SEA/BLACK SEA	0.54094	ATLANTIC OCEAN
WEST MEDITERRANEAN	0.22538	AEGEAN SEA/BLACK SEA

APPENDIX II. Genetic diversity of individual populations.

Cakile maritima - Baltic Sea/North Sea

ID	N	% polymorphic loci	H_e	Si
DK1	10	26.88 %	0,0911	0,1361
DK2	10	27.96 %	0,0903	0,1365
DK3	10	31.18 %	0,1073	0,1621
DK4	9	24.73 %	0,0853	0,1281
DK5	10	25.81 %	0,0864	0,1300
DK6	7	20.43 %	0,0539	0,0862
DK7	9	32.26 %	0,1097	0,1646
DK8	10	34.41 %	0,1236	0,1838
SV1	10	29.03 %	0,0955	0,1438
SV2	11	31.18 %	0,0985	0,1490

Cakile maritima - Strait of Gibraltar

ID	N	% polymorphic loci	H_e	Si
MED5	10	47.31 %	0,1647	0,2460
MED4	10	41.94 %	0,1343	0,2042
MED3	9	47.31 %	0,1597	0,2404
MED2	9	45.16 %	0,1553	0,2326
MED1	9	50.54 %	0,1639	0,2467
ATL1	10	37.63 %	0,1224	0,1852
ATL2	9	29.03 %	0,0981	0,1476
ATL3	10	35.48 %	0,1328	0,1965
ATL4	10	39.78 %	0,1613	0,2341
ATL5	10	39.78 %	0,1311	0,1975

Cakile maritima - West France

ID	N	% polymorphic loci	H_e	Si
WF9	9	27.96 %	0,0887	0,1359
WF10	7	27.96 %	0,1094	0,1600
WF11	6	27.96 %	0,1036	0,1531
WF12	10	27.96 %	0,1078	0,1578
WF13	7	26.88 %	0,1078	0,1578
WF14	7	26.88 %	0,1057	0,1544
WF17	9	36.56 %	0,1308	0,1946
WF18	10	33.33 %	0,1118	0,1675
WF19	9	31.18 %	0,1031	0,1550

Cakile maritima – Aegean Sea/Sea of Marmara/Black Sea

ID	N	% polymorphic loci	H_e	Si
BS1	7	10.75 %	0,0369	0,0554
BS3	8	26.88 %	0,0895	0,1342
MA11	11	33.33 %	0,1282	0,1886
MA13	7	26.88 %	0,0991	0,1465
MA14	10	32.26 %	0,1183	0,1749
MA15	11	34.41 %	0,1248	0,1846
MA17	3	20.43 %	0.0835	0.1216
AE18	10	43.01 %	0,1455	0,2184
AE19	8	38.71 %	0,1355	0,2031
AE22	8	37.63 %	0,1382	0,2054
GR	5	30.11 %	0,1205	0,1760

Eryngium maritimum - Strait of Gibraltar

ID	N	% polymorphic loci	H_e	Si
MED5	10	53.52 %	0,1803	0,2717
MED4	10	50.70 %	0,1731	0,2604
MED3	10	56.34 %	0,1821	0,2757
MED2	10	50.70 %	0,1556	0,2379
MED1	9	43.66 %	0,1260	0,1970
ATL1	9	19.72 %	0,0624	0,0956
ATL2	10	18.31 %	0,0603	0,0866
ATL3	9	16.90 %	0,0464	0,0729
ATL4	10	19.72 %	0,0570	0,0915
ATL5	9	19.72 %	0,0630	0,0947

Eryngium maritimum - West France

ID	N	% polymorphic loci	H_e	Si
WF9	10	19.72 %	0,0577	0,0893
WF10	10	28.17 %	0,0960	0,1440
WF11	10	21.13 %	0,0714	0,1071
WF12	10	18.31 %	0,0654	0,0978
WF13	9	16.90 %	0,0534	0,0815
WF16	10	23.94 %	0,0874	0,1296
WF17	10	16.90 %	0,0664	0,0976
WF18	10	19.72 %	0,0750	0,1110
WF19	9	21.13 %	0,0867	0,1255

Eryngium maritimum – Aegean Sea/Sea of Marmara/Black Sea

ID	N	% polymorphic loci	H_e	Si
BS2	10	23.94 %	0,0951	0,1380
BS3	10	19.72 %	0,0640	0,0966
MA13	7	21.13 %	0.0821	0.1204
MA12	7	21.13 %	0,0783	0,1147
MA14	4	16.90 %	0,0708	0,1022
MA17	10	29.58 %	0,1129	0,1647
MA24	7	19.72 %	0,0720	0,1065
AE18	9	30.99 %	0,1145	0,1687
AE19	9	28.17 %	0,0949	0,1420
AE22	10	33.80 %	0,1306	0,1928
GR	5	25.35 %	0.0974	0.1428

APPENDIX III

APETALA1

The intron between exons seven and eight in a putative *APETALA1* homologue was screened for variation in *C. maritima*. Primers were designed from sequences published in Genebank by identifying regions conserved in taxa related to *C. maritima*. Potential locations for primers were chosen with Primer3 (Rozen & Skaletsky, 2000) and further analyzed with NetPrimer (PREMIER Biosoft International). The forward primer AP17f (TCA GCC ATC TCC TTT TCT CAA) was located in exon no. 7 and the reverse primer AP18r (CGT TCC TCC TCA TTN CCA TT) in exon no. 8. Two loci were amplified by these primers in *C. maritima*. The amplicons were separated on agarose and purified with QiaQuick (Qiagen) according to the manufacturers instructions and the shoreter fragment was sequenced with the forward and reverse primers. Amplifications were carried out under the same PCR conditions as for ITS.

Consensus sequence of the complete intron of a putative *APETALA1* homologue from *Cakile maritima*. 353 bp:

```
  1 TTACT AATTT AAGTA TACAT ATTTA GCCCA TGTGT TATAC AATCT CACTT
 51 AGGTG TTATA ATTCC AGTGA TGATA GTTAA ATGTG TAAGT TGTGA GTTAT
101 AATTA CACCC TACTA AAACT ATTCA CGTAT TAACG TATGT TAATG AAATG
151 GTCGT CAAAC ACACT TGTAT TTTAT TGTAA GGGTT ATGAA AAGCC TAACA
201 AAGGG ATCAG CTCCT TTTTT TTTTT AACAT TCAAC TGATC ACTTT GATAC
251 TTTCA ATATA TGGAC TTTTA CCAAT TTTAA GACTA AACAT ATGAT TTTTA
301 ATGCA CGTAT GTCGT TAAGG TGAAT TATAT TCATG TACAT ATGAA TGGAT
351 GCC
```

Chalcone Synthase

An attempt to screen the intron of chalcone synthase for variation was made in both species. In *C. maritima* short DNA fragments corresponding to an intron length of about 100 bp were amplified which is in agreement with other taxa within the Brassicaceae and was therefore not further considered. In *E. maritimum* the universal primers of Strand *et al.* (1997) did not yield clear amplification products and thus more taxon specific primers were designed as above. The forward primer CHS2dcF (CAA ACT CAA ATT CAA GCG AAT G) corresponds to the same primer position as the forward primer CHSX1F in Strand *et al.*, (1997). The reverse primer CHSpetR (GAA GTG GTG CAG AAA A TG AGG) was designed so as to only amplify the CHS2 isoform of Hirner and Seitz (2000) since gene bank searches suggested that there might a duplication of CHS in Apiaceae. Amplifications were performed using the thermo cycling protocol of Strand *et al.*, (1997) but with an annealing temperature of 55°C. Amplifications were carried out in 25 µl total volume containing 2.5 µl 10x BioTherm PCR

buffer (GeneCraft), 0.75 µL of 50 mM $MgCl_2$, 0.25 µL of 20 mM dNTP, 0.5 µL of each primer at 10 pmol/µL, 0.75 U BioTherm Taq polymerase (GenCraft), and ca 100 ng genomic DNA.

Partial intron of putative Chalcone Synthase from *Eryngium maritimum*. 295 bp:

```
  1 CGTCT TTACT CTTCT TTTCT  GCTCA ATATT ATCAT ATCAT  ATGTA TGTCT
 51 GTGTT CTATA TATTT CTCTC  GGATG TTGCA CTCAT TAATC  TGCTA TAAAG
101 CAGTA CACTC CAATT TTAAA  ACAAC AAAAT TTAAA GATCT  GACAT ATGCC
151 TCGAG CTCAG TTTTC TGTCG  CAAGT AAGCC TTTCT GTTGT  AGTGT ATGTT
201 CAAAC ACAAC TTCTA GTCCG  AAGAA TGAGA ATGTT TAAAC  TCTCC GTTTG
251 GNTTT CTACT TTGGA TAACA  TAACT TANTT ATCAC AACTA  TGTCT
```

Internal Transcribed Spacer

ITS from eleven individuals yielded seven different sequences. An alignment is given below with mutations marked in the consensus sequence by a star and ambiguities with (+). Geographical origin is given.

Summary view of alignment of ITS from *Eryngium maritimum*

```
> Loc no.35       #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc MED5        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc no.57       #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc no.41       #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc MED4        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc MED2        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc ATL2        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc ATL3        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc no.3        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc no.30       #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
> Loc no.8        #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC
                              ........................................
                  #1          GCGGTCGAGG CACACACCGT TGGTGGCGCC AGAGGGTCAC


> Loc no.35       #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc MED5        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc no.57       #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc no.41       #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc MED4        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc MED2        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc ATL2        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc ATL3        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc no.3        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc no.30       #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
> Loc no.8        #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
                              ........................................
                  #41         AGGAGCTCCC ACCGACGACG AGGCGACGAC GCGGCACTGA
```

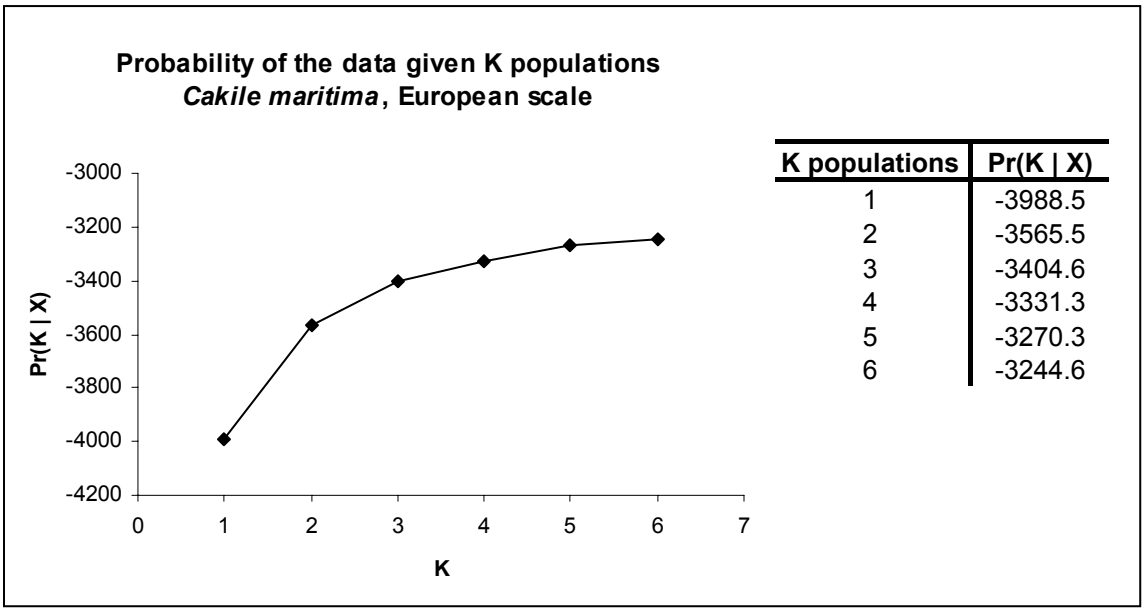
```
> Loc no.35       #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc MED5        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc no.57       #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc no.41       #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc MED4        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc MED2        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc ATL2        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc ATL3        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc no.3        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc no.30       #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
> Loc no.8        #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT
                               ..........................................
                  #81          GAGACGGCCT TTTTACAACC ACCACATGCC GCGACATCCT


> Loc no.35       #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc MED5        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc no.57       #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc no.41       #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc MED4        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc MED2        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc ATL2        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc ATL3        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
> Loc no.3        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCTGCAAT
> Loc no.30       #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCTGCAAT
> Loc no.8        #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCTGCAAT
                               ..........................................
                  #121         GTCGCACGGG GACTCGCTTT TGGGCCAACC GCGCAGCAAT
                                                                    *

> Loc no.35       #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc MED5        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc no.57       #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc no.41       #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc MED4        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc MED2        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc ATL2        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc ATL3        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc no.3        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc no.30       #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
> Loc no.8        #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC
                               ..........................................
                  #161         GCACGGGAGG CCATTATCCG CCCCTCAGAC CGCGCATCCC


> Loc no.35       #201         TCGAGAGAGT GCATGRTTTG GGGGGCGACG CGATGCGTGA
> Loc MED5        #201         TCGAGAGAGT GCATGATTTG GGGGGCGACG CGATGCGTGA
> Loc no.57       #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc no.41       #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc MED4        #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc MED2        #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc ATL2        #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc ATL3        #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc no.3        #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc no.30       #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
> Loc no.8        #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
                               ..........................................
                  #201         TCGAGAGAGT GCATGGTTTG GGGGGCGACG CGATGCGTGA
                                           *
```

```
> Loc no.35       #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc MED5        #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc no.57       #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc no.41       #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc MED4        #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc MED2        #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc ATL2        #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc ATL3        #241        CGCCCAGGSA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc no.3        #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc no.30       #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
> Loc no.8        #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
                              ..........................................
                  #241        CGCCCAGGCA GACGTGCCCT CCGCCTAATG GCTTCGGGCG
                                     +

> Loc no.35       #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc MED5        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc no.57       #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc no.41       #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc MED4        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc MED2        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc ATL2        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc ATL3        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc no.3        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc no.30       #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
> Loc no.8        #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC
                              ..........................................
                  #281        CAACTTGCGT TCAAAGACTC GATGGTTCAC GGGATTCTGC

> Loc no.35       #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc MED5        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc no.57       #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc no.41       #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc MED4        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc MED2        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc ATL2        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc ATL3        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc no.3        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc no.30       #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
> Loc no.8        #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG
                              ..........................................
                  #321        AATTCACACC AAGTATCGCA TTTCGCTACG TTCTTCATCG

> Loc no.35       #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc MED5        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc no.57       #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc no.41       #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc MED4        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc MED2        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc ATL2        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc ATL3        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc no.3        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc no.30       #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
> Loc no.8        #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
                              ..........................................
                  #361        ATGCGAGAGC CGAGATATCC GTTGCCGAGA GTCGTTTGTG
```

```
> Loc no.35       #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc MED5        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc no.57       #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc no.41       #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc MED4        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc MED2        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc ATL2        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc ATL3        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc no.3        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc no.30       #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
> Loc no.8        #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC
                              ..........................................
                  #401        TTTCTGAAAG ACGCCGGCGC CGCCCGCGAA CGGGGGCGAC


> Loc no.35       #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc MED5        #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc no.57       #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc no.41       #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc MED4        #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc MED2        #441        AACGTGCAGT TCGTTTCTGT TTCCTTGGCG CATTCCGCGC
> Loc ATL2        #441        AACGTGCAGT TCGTTTCTGT TTCCTTGGCG CATTCCGCGC
> Loc ATL3        #441        AACGTGCAGT TCGTTTCTGT TTCCTTGGCG CATTCCGCGC
> Loc no.3        #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc no.30       #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
> Loc no.8        #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
                              ..........................................
                  #441        AACGTGCAGT TCGTTTCTAT TTCCTTGGCG CATTCCGCGC
                                         *

> Loc no.35       #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc MED5        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc no.57       #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc no.41       #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc MED4        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGACCG AGGACGCCCT
> Loc MED2        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc ATL2        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc ATL3        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc no.3        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc no.30       #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
> Loc no.8        #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
                              ..........................................
                  #481        CGGGGTGAGT TTGTGGCCGG CGAGCGCCCG AGGACGCCCT
                                                    *

> Loc no.35       #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc MED5        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc no.57       #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc no.41       #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc MED4        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc MED2        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc ATL2        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc ATL3        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc no.3        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc no.30       #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
> Loc no.8        #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
                              ..........................................
                  #521        CCGCCAGCGG GGTCACGGAC ACGGGGAGCT CGCGCACCCC
```

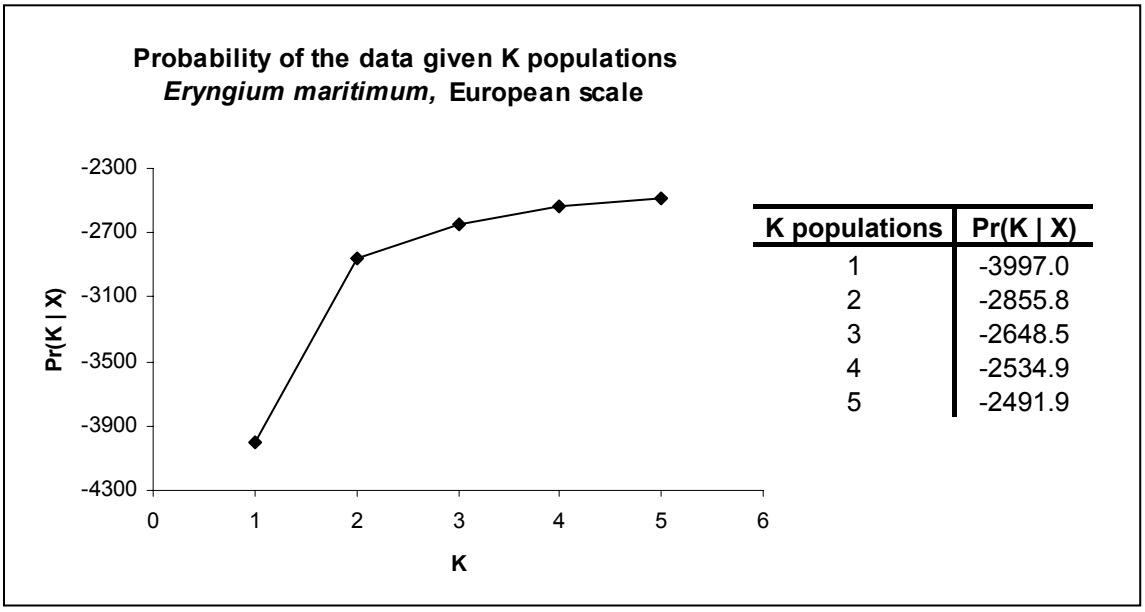
```
> Loc no.35       #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc MED5        #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc no.57       #561        GGGACCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc no.41       #561        GGGACCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc MED4        #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc MED2        #561        GGGGCCGATC CCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc ATL2        #561        GGGGCCGATC CCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc ATL3        #561        GGGGCCGATC CCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc no.3        #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc no.30       #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
> Loc no.8        #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
                              ........................................
                  #561        GGGGCCGATC GCCCGATTTT TTAACGTGTT CGCGGGTCGT
                                 *       *

> Loc no.35       #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc MED5        #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc no.57       #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc no.41       #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc MED4        #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc MED2        #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc ATL2        #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc ATL3        #601        TCTGCTTTGC AAGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc no.3        #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc no.30       #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
> Loc no.8        #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
                              ........................................
                  #601        TCTGCTTTGC AGGCATCGAC AATGATCCTT CCGCAGGTTC
                                          *

> Loc no.35       #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc MED5        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc no.57       #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc no.41       #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc MED4        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc MED2        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc ATL2        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc ATL3        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc no.3        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc no.30       #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
> Loc no.8        #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
                              ...................................
                  #641        ACCTACGGAA ACCTTGTTAC GACTTCTCCT TCCTCTA
```

APPENDIX IV. Posterior probabilites (Pr[K | X]) of the results from the Bayesian clustering method (STRUCTURE) .



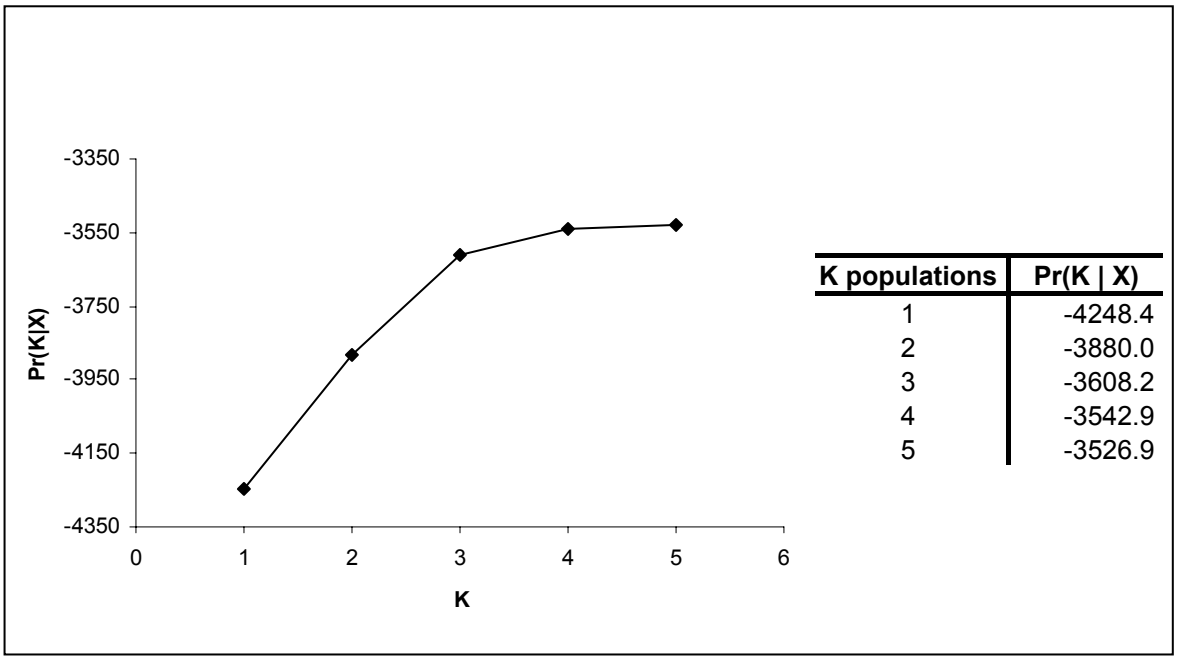
K populations	Pr(K | X)
1	-3988.5
2	-3565.5
3	-3404.6
4	-3331.3
5	-3270.3
6	-3244.6

Figure 1. Probability of the data for *K* populations for *Cakile maritima* in the European scale analysis.



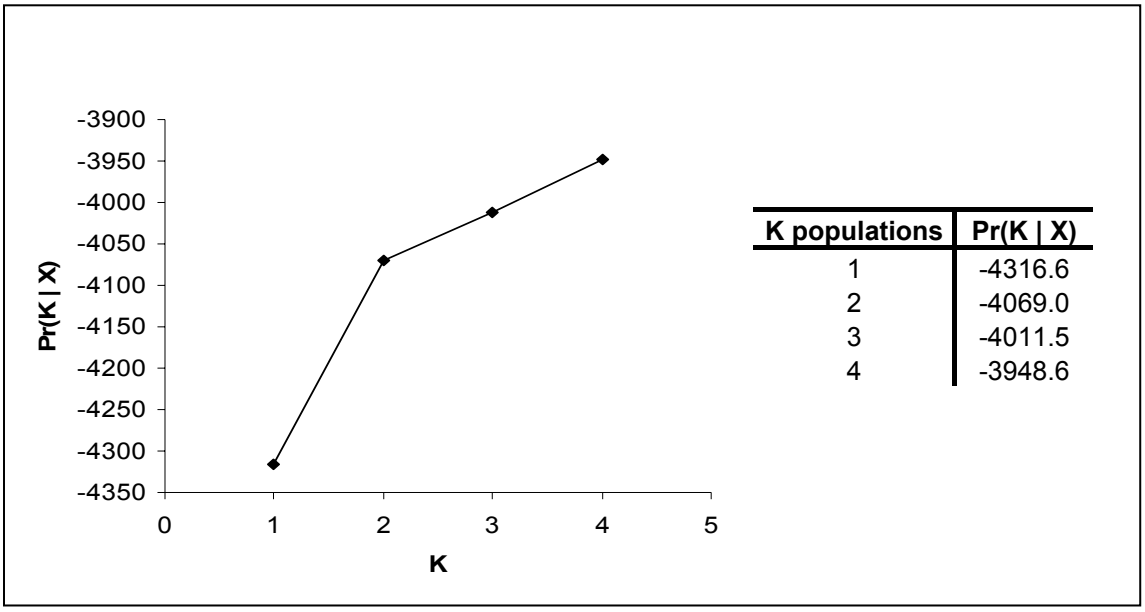
K populations	Pr(K | X)
1	-3997.0
2	-2855.8
3	-2648.5
4	-2534.9
5	-2491.9

Figure 2. Probability of the data for *K* populations for *Eryngium maritimum* in the European scale analysis.



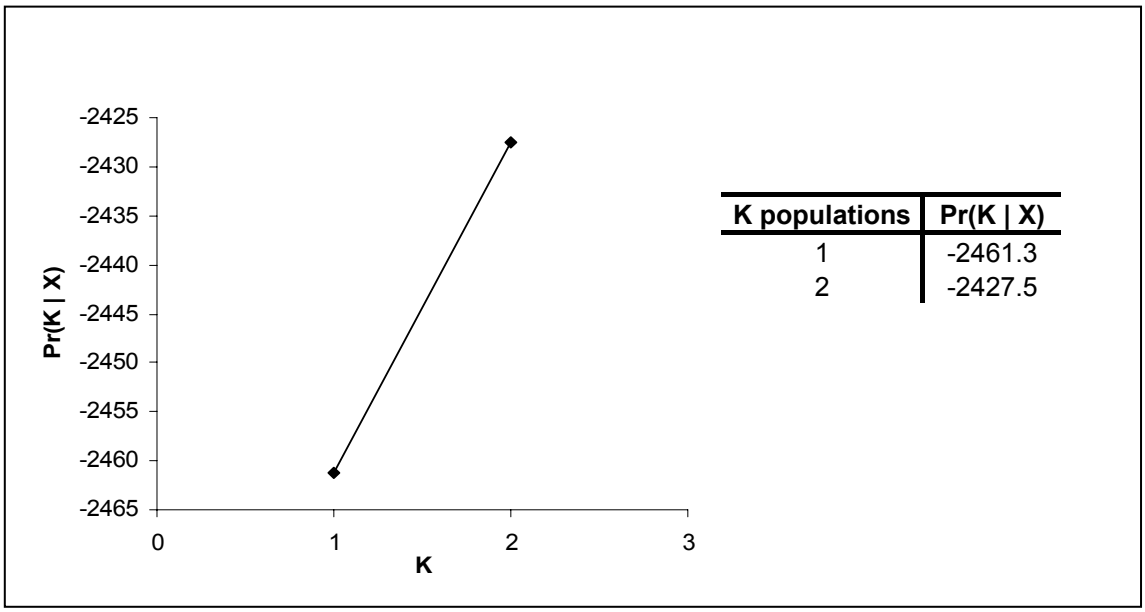
K populations	Pr(K | X)
1	-4248.4
2	-3880.0
3	-3608.2
4	-3542.9
5	-3526.9

Figure 3. Probability of the data for *K* populations for *Cakile maritima* in the Aegean Sea/Sea of Marmara/Black Sea region.



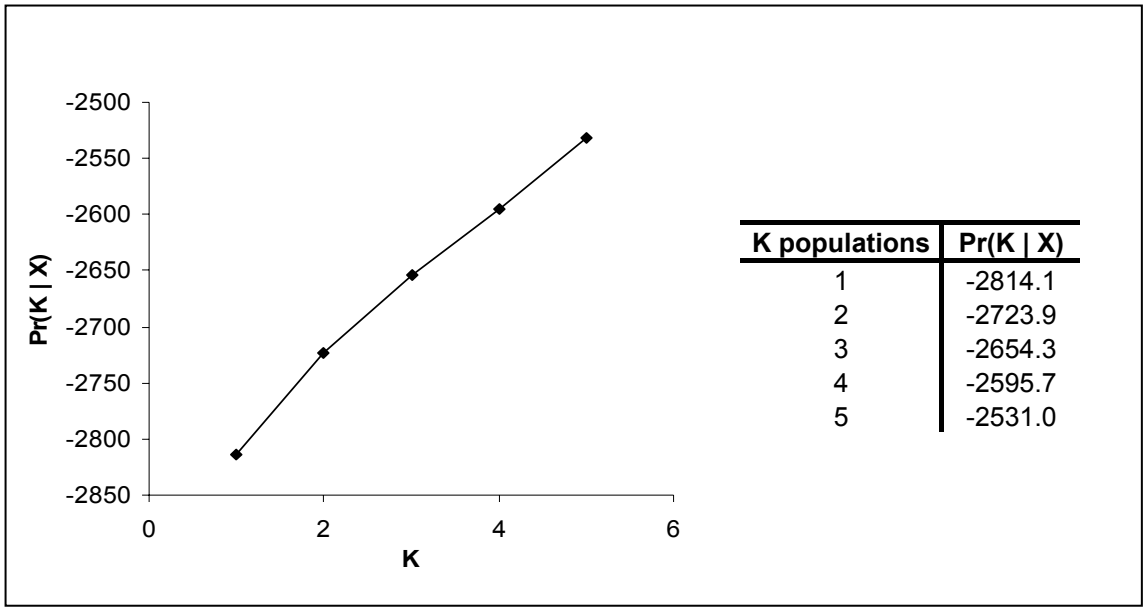
K populations	Pr(K | X)
1	-4316.6
2	-4069.0
3	-4011.5
4	-3948.6

Figure 4. Probability of the data for *K* populations for *Cakile maritima* in the Strait of Gibraltar region.



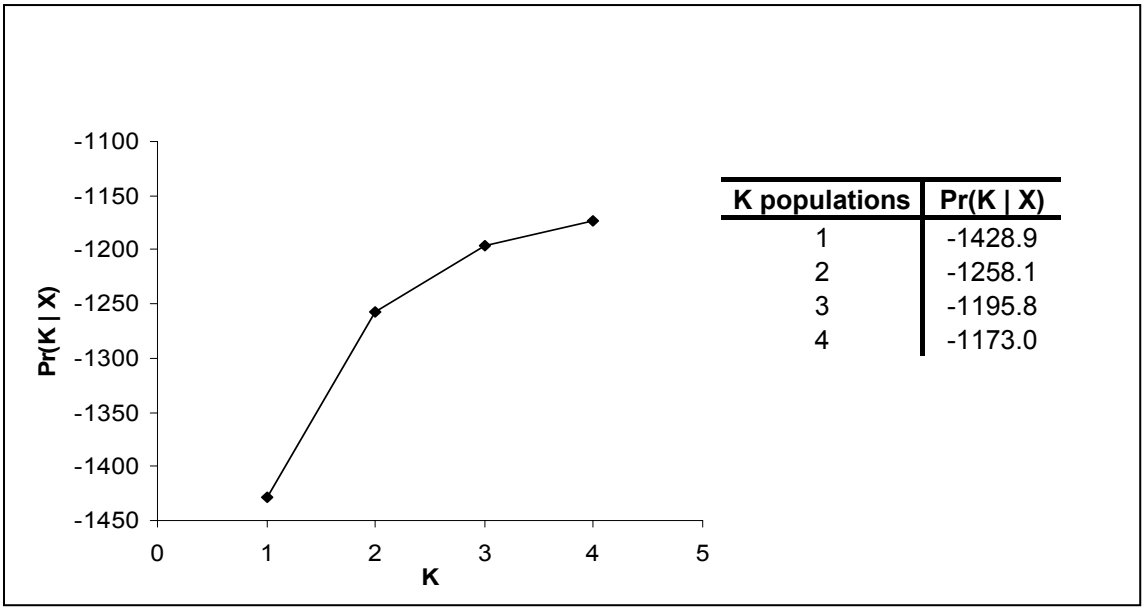
K populations	Pr(K | X)
1	-2461.3
2	-2427.5

Figure 5. Probability of the data for *K* populations for *Cakile maritima* in the West France region.



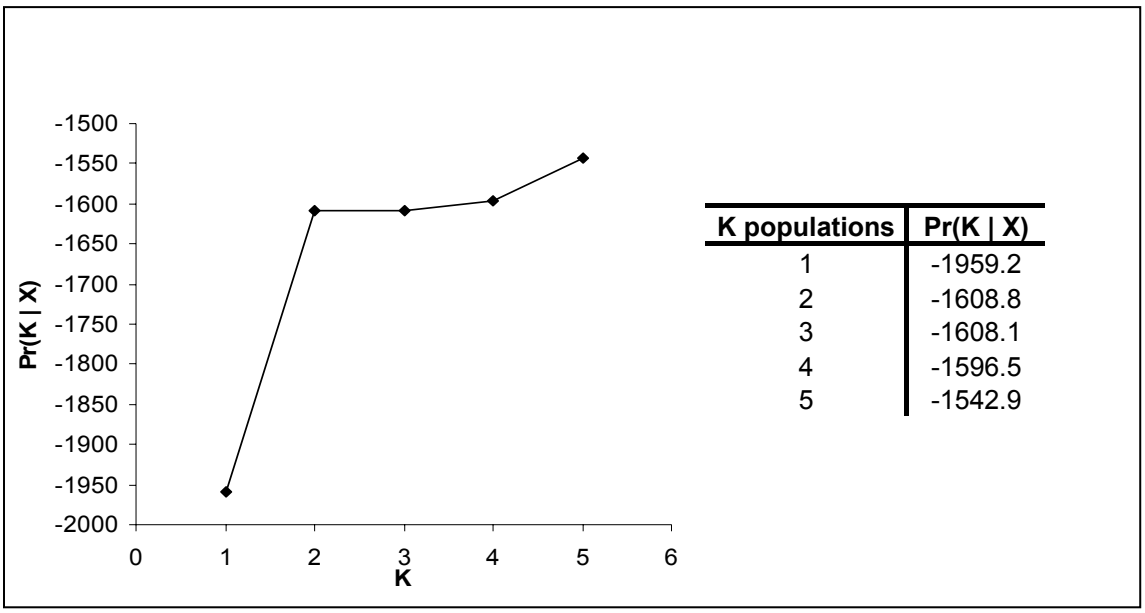
K populations	Pr(K | X)
1	-2814.1
2	-2723.9
3	-2654.3
4	-2595.7
5	-2531.0

Figure 6. Probability of the data for *K* populations for *Cakile maritima* in the Baltic Sea/North Sea region.



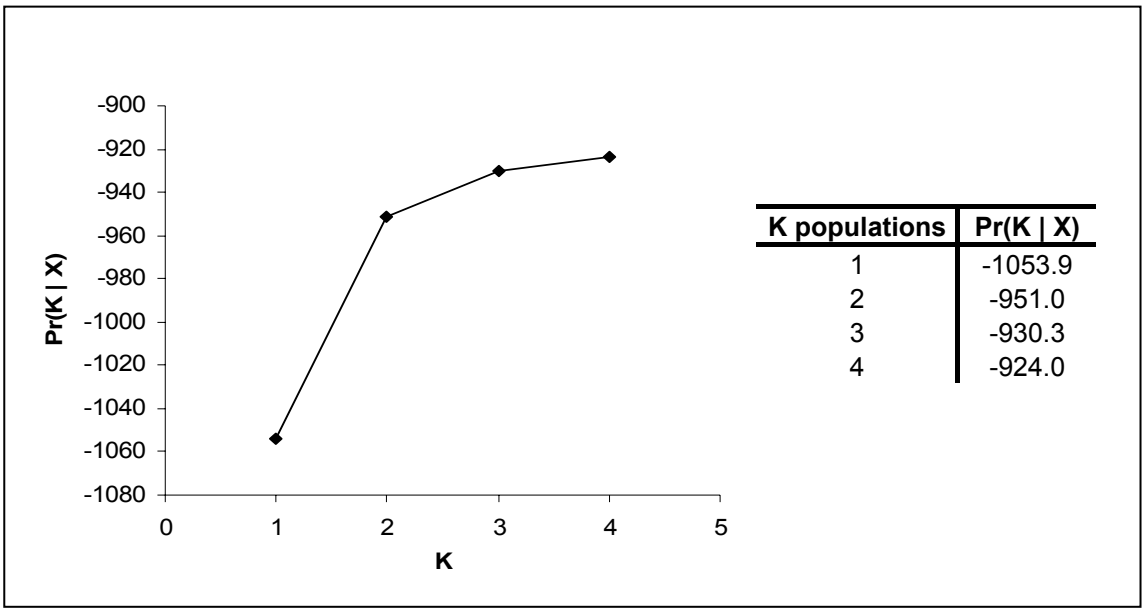
K populations	Pr(K | X)
1	-1428.9
2	-1258.1
3	-1195.8
4	-1173.0

Figure 7. Probability of the data for *K* populations for *Eryngium maritimum* in the Aegean Sea/Sea of Marmara/Black Sea region.



K populations	Pr(K | X)
1	-1959.2
2	-1608.8
3	-1608.1
4	-1596.5
5	-1542.9

Figure 8. Probability of the data for *K* populations for *Eryngium maritimum* in the Strait of Gibraltar region.



K populations	Pr(K | X)
1	-1053.9
2	-951.0
3	-930.3
4	-924.0

Figure 9. Probability of the data for *K* populations for *Eryngium maritimum* in the West France region.