Journal of Biogeography (*J. Biogeogr.*) (2006) **33**, 1461–1469

ORIGINAL ARTICLE

Long-distance seed dispersal, clone longevity and lack of phylogeographical structure in the European distributional range of the coastal *Calystegia soldanella* (L.) R. Br. (Convolvulaceae)

Rami Arafeh and Joachim W. Kadereit*

Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg-Universität Mainz, Mainz, Germany

ABSTRACT

Aim To explore the relative effects of Quaternary climatic history vs. species-specific biological properties (high seed dispersability, high seed longevity, clonal growth) on phylogeographical structure in European Mediterranean and Atlantic Ocean and Black Sea material of the coastal dune plant *Calystegia soldanella* (L.) R. Br.

Location Black Sea and European Mediterranean and Atlantic Ocean coasts.

Methods Variation in amplified fragment length polymorphism was analysed at two different sampling levels. First, an entire-range sample from the Black Sea to the North Sea, including single individuals from sites evenly spread along this entire coast was analysed. Second, in a population-level sample, seven populations from the European Mediterranean and Atlantic Ocean coasts were analysed.

Results Neither the entire-range nor the population-level sampling resulted in clear phylogeographical patterns. Instead, individuals from geographically distant areas were often genetically more similar to each other than individuals from the same area. Non-significant isolation-by-distance was found for both sampling approaches, and comparatively low levels of intrapopulational genetic variation were observed.

Main conclusions The lack of phylogeographical structure in *C. soldanella*, in comparison with the clear phylogeographical patterns observed in other coastal plant species analysed previously, is postulated to be the result of the specific biology of *C. soldanella*. The combination of high seed longevity, high dispersability of seeds by sea water and clonal growth and probable high clone age are likely to be responsible for the observed absence of phylogeographical structure. This implies that extreme biological properties such as those shown by *C. soldanella* can either erase or prevent the formation of historical patterns of genetic variation.

Keywords

AFLP, *Calystegia soldanella*, clonal growth, coastal distribution, long-distance dispersal, phylogeography, sea-water dispersal, seed longevity.

*Correspondence: Joachim Kadereit, Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg-Universität Mainz, D-55099 Mainz, Germany.
E-mail: kadereit@uni-mainz.de

INTRODUCTION

The investigation of the geographical distribution of intraspecific genetic variation and intraspecific phylogenetic or genetic lineages in a large number of plant and animal taxa from different parts of the world has resulted in the detection of often clear phylogeographical patterns (reviewed in, e.g. Soltis *et al.*, 1997; Comes & Kadereit, 1998, 2003; Taberlet *et al.*, 1998; Hewitt, 2000, 2004; Abbott & Brochmann, 2003; Brochmann *et al.*, 2003; Stehlik, 2003; Tribsch & Schönswetter, 2003; Lascoux *et al.*, 2004). These patterns were most commonly explained by climate-driven shifts in geographical distribution, particularly during the last glacial maximum (LGM) and the Holocene. When comparing the routes of post-glacial

doi:10.1111/j.1365-2699.2006.01512.x

(re)colonization of northern parts of Europe from southern European refugia of plant and animal species of often very different ecology, life form, reproductive biology and dispersal biology, a limited number of patterns shared by many different species can be identified (Comes & Kadereit, 1998; Taberlet *et al.*, 1998; Hewitt, 2000, 2004). Observation of such large-scale phylogeographical congruence is most plausibly interpreted to be a result of a shared biogeographical history (Avise, 2000). On a closer look, however, it is apparent that phylogeographical patterns are species specific, both spatially and temporally (Taberlet *et al.*, 1998; Comes & Kadereit, 2003; Kadereit & Comes, 2005). Few systematic attempts have been made to systematically identify the biological properties of species that are responsible for their species-specific phylogeographical patterns.

In a recent publication (Kadereit *et al.*, 2005), we reported the results of a comparative phylogeographical analysis of five coastal flowering plant species [*Cakile maritima* Scop., *Crithmum maritimum* L., *Eryngium maritimum* L., *Halimione portulacoides* (L.) Aellen and *Salsola kali* L.] from their entire European range. One important motivation for our investigation of coastal plants was the idea that the comparison of several species co-distributed over long stretches of coastline, and their dispersal only or almost only along the coast, would enable us to distinguish between shared historical and species-specific factors as determinants of phylogeographical structure. The five species investigated by Kadereit *et al.* (2005) were found to show high congruence in phylogeographical patterns. In most cases they were found to consist of Atlantic Ocean vs. Mediterranean Sea/Black Sea genetic clusters, and in most cases the Mediterranean Sea/Black Sea material was found to group into Black Sea/Aegean Sea, Adriatic Sea and West Mediterranean subclusters. Similarities and dissimilarities among the phylogeographical patterns detected in the different species were interpreted mainly in terms of species-specific LGM distribution limits and modern gene flow barriers in the form of sea currents (Kadereit *et al.*, 2005). Many of the phylogeographical patterns detected in this study have also been identified in several marine plant and animal species (Magoulas *et al.*, 1996; Borsa *et al.*, 1997a,b; Pannacciulli *et al.*, 1997; Röhner *et al.*, 1997; Garibaldi & Caddy, 1998; Naciri *et al.*, 1999; Pérez-Losada *et al.*, 1999; Bahri-Sfar *et al.*, 2000; Bianchi & Morri, 2000; Rios *et al.*, 2002; Bargelloni *et al.*, 2003; Nikula & Väinölä, 2003; Waters & Roy, 2003; Olsen *et al.*, 2004; Jolly *et al.*, 2005). We present here the results of an analysis of another coastal plant species, *Calystegia soldanella* (L.) R. Br. (sea bindweed), across its entire European distributional range. *Calystegia soldanella* is a herbaceous perennial creeper which grows in coastal sand dunes and occasionally on sandy beaches. It forms extensive rhizomes up to 1.5 m under the soil surface (Hegi, 1925), resulting in the formation of probably large but patchy clones. With this growth form, the species contributes significantly to the stabilization of sand dunes (Mun, 1984). Its showy flowers are self-incompatible (Ushimaru & Kikuzawa, 1999), and the large round seeds, released from a few-seeded capsule at maturity, have a very solid, water-impermeable seed coat and contain a large air-filled cavity. The seeds have been recorded to be able to float in sea water for up to 18 months and to retain 30% germination capacity in this period (Ridley, 1930). The genetic structure of *C. soldanella* in Korea has been investigated by Kim & Chung (1995) and Chung *et al.* (1995).

Calystegia soldanella differs markedly from the five species investigated by Kadereit *et al.* (2005) in its substantial clonal growth and in having seeds with a very high potential for long-distance dispersal by sea water. These seeds could be responsible for the very wide distribution of the species. By looking at the geographical patterns of intraspecific amplified fragment length polymorphisms (AFLP) we investigate the question of whether these properties of *C. soldanella* have influenced the formation of phylogeographical pattern. Alternatively, irrespective of these properties, we will see whether *C. soldanella* has a phylogeographical structure similar to that found in the five coastal species investigated by Kadereit *et al.* (2005). We also performed a small-scale experiment on the buoyancy and viability of *C. soldanella* seeds.

MATERIALS AND METHODS

Plant material

In order to obtain a detailed picture of the spatial genetic structure of *C. soldanella*, our sampling employed two different strategies. First we sampled a single individual from each of various localities approximately 100–200 km apart along the species' entire European range, from the Black Sea (Akpinar) to southern Britain (Braunton Burrows). This sampling strategy (entire-range sampling) allows one to infer a broad-scale pattern of spatial genetic variation but essentially precludes any type of inference at the population level. In addition, four localities were represented by between four and seven individuals (see Fig. 1 & Table 1). In total, 72 individuals from 54 localities were included in this approach. In our second sampling approach (population-level sampling), 61 individuals representing 7 geographically widely separated populations were analysed (three populations from the English Channel area, one population from the Italian west coast, two populations from the Adriatic Sea and one population from Greece; see Table 1). This sampling strategy allowed the calculation of standard population genetic measures. To avoid sampling the same individual twice, samples were collected at distances of at least 30 m. The names of localities and geographical coordinates are listed in Table 1. All specimens for both approaches were collected in the field. Leaves were torn into small pieces and transferred into silica gel beads (Sigma-Aldrich Chemie Gmbh, Munich, Germany) for drying. All samples were kept at room temperature.

DNA extraction

Total genomic DNA was isolated from *c.* 100 mg of silica gel-dried leaf material. The DNA isolation and purification

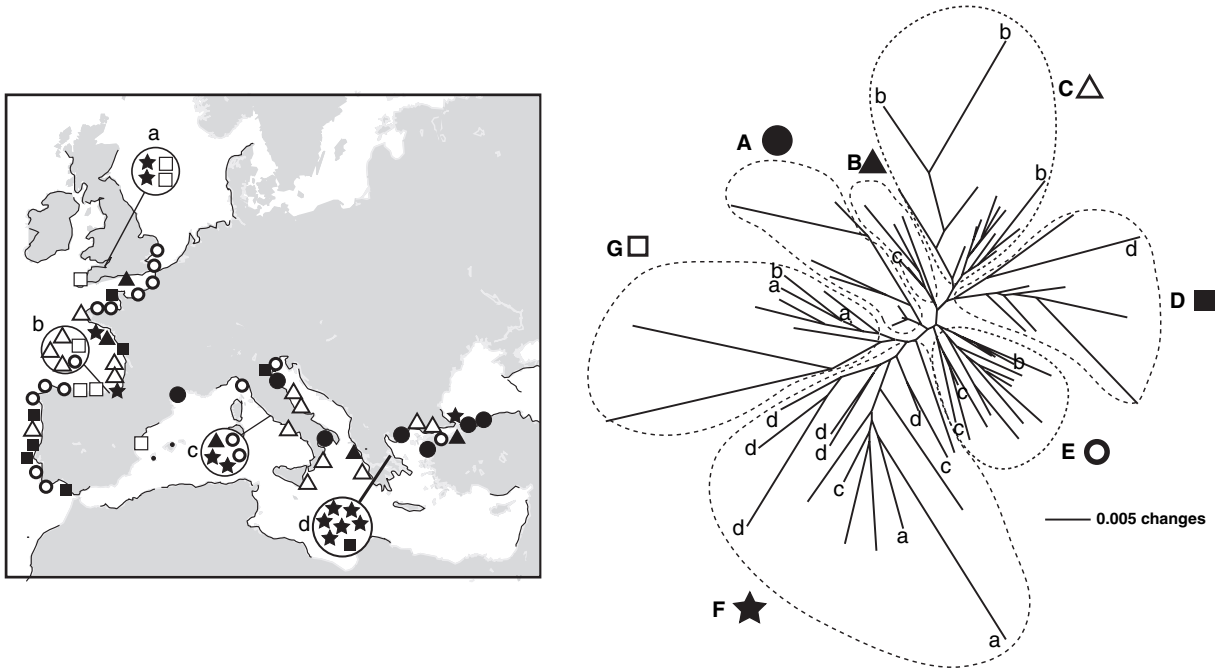

Figure 1 Left: Geographical distribution (bold coast line; Meusel *et al.*, 1965) of *C. soldanella* and sample localities for the entire-range sample. Different symbols indicate the different NJ clusters (A–G, see NJ phenogram) identified. Large circles indicate localities where more than one individual was sampled in the entire-range sample. Right: NJ phenogram of all accessions of the entire-range sample. A–G denote the seven clusters identified. a–d denote individuals from the four populations sampled in the entire-range sample and shown in the map.

procedure was carried out using the DNeasy™ Plant Minikit (Qiagen, Hilden, Germany) following the manufacturer's instructions with slight modifications: 600 µL of buffer AP1 and 195 µL of buffer AP2 were used. Quantification of DNA was carried out spectrophotometrically with a GeneQuant RNA/DNA calculator (Pharmacia, Uppsala, Sweden) or estimated visually in ethidium bromide stained agarose gels (1.4%). Crude DNA was diluted to 30 ng μL^{-1} with molecular-grade H2O (Roth, Karlsruhe, Germany) and stored at −20 °C.

AFLP fingerprinting

The AFLP protocol of Vos *et al.* (1995) and the ingredients and modifications described in Kropf *et al.* (2002) and Kadereit *et al.* (2005) were followed in this study. For the entire-range sampling the primer combinations used for selective amplification were *Eco*RI + AGA/*Mse*I + GTG and *Eco*RI + AAG/*Mse*I + GGG. In the population-level sampling the primer combinations used were *Eco*RI + AGA/*Mse*I + GTG, *Eco*RI + ATG/*Mse*I + GGG and *Eco*RI + ACG/*Mse*I + GCT.

Data analysis

The resulting fragments were scored 1 when present and 0 when absent. After generating a binary data matrix, pairwise genetic distances (*D*) for all accessions were calculated using the complementary value of Nei & Li's (1979) similarity coefficient implemented in PAUP version 4.010 for Macintosh PPC (Swofford, 2002): $D = 1 - SC = 1 - [2n_{xy}/(n_x + n_y)]$ where SC is the similarity coefficient, n_{xy} is the number of identical fragments shared between two accessions and n_x and n_y are the total number of fragments in accessions *x* and *y*, respectively.

Estimates of genetic distance (*D*) between accessions were subjected to a neighbour-joining (NJ) analysis (Saitou & Nei, 1987) using PAUP in order to detect geographical patterns of genetic variation. Genetic structure [partitioning of genetic variation within and among groups (AMOVA)] was calculated using ARLEQUIN v.2.0 (Excoffier *et al.*, 1992; Schneider *et al.*, 1997). The levels of significance of the variance components were evaluated with a nonparametric randomization test using 3000 permutations as implemented in ARLEQUIN. For the entire-range sampling, the groups included in the AMOVA were the clusters identified in the NJ analysis. In the population-level approach, two different AMOVAS were conducted: (1) a non-hierarchical AMOVA with all seven populations included and (2) a hierarchical AMOVA placing six populations (the small population from Evia was excluded from this analysis) into three geographical groups (three populations from the English Channel area, one from west Italy, two from the Adriatic Sea). For both the population-level and entire-range sampling approaches, isolation by distance (IBD) was tested using Mantel's test (Mantel, 1967) with 999 random permutations using NTSYS-PC software (version 2.10z; Rohlf, 2002). This procedure tests whether genetic distances among accessions are correlated with their geographical distances. Based on

Table 1 Origin of plant material. Localities in bold indicate populations used in the population-level analysis and number of individuals sampled (in parentheses). Localities represented by more than one individual in the entire-range analysis are indicated by the number of individuals sampled underlined

No.	Origin	Locality name	Co-ordinates		Collector(s)
			N	E/W	
1	Turkey	Akpinar	41°18′	28°49′	R. Arafeh & E. Westberg
2		Sahilköy	41°12′	29°23′	R. Arafeh & E. Westberg
3		Yeniköy Plage	40°23′	28°24′	R. Arafeh & E. Westberg
4		Gonen	40°19′	27°37′	R. Arafeh & E. Westberg
5		Ezine	39°45′	26°07′	R. Arafeh & E. Westberg
6	Greece	Erasmio Beach	40°52′	24°50′	R. Arafeh & E. Westberg
7		Igoumenitsa	39°31′	20°11′	R. Arafeh & E. Westberg
8		Lehena	37°59′	21°15′	R. Arafeh & E. Westberg
9		Olimbiada	40°35′	23°50′	R. Arafeh & E. Westberg
10		**Evia–Oreio (4/7)**	38°57′	23°06′	R. Arafeh & E. Westberg
11	Italy	Foce di Simeto	37°24′	15°06′	R. Arafeh & E. Westberg
12		**Laguna di Vinicia-Cavallino (10)**	45°28′	12°34′	J. W. Kadereit & G. Kadereit
13		**Porto Garibaldi (8)**	44°40′	12°14′	J. W. Kadereit & G. Kadereit
14		Macchia di Migliorino	43°49′	10°16′	R. Arafeh & E. Westberg
15		Marina di Lesina	41°54′	15°21′	R. Arafeh & E. Westberg
16		Marina di Pisticci	40°18′	16°47′	R. Arafeh & E. Westberg
17		Marina Romea	44°31′	12°17′	R. Arafeh & E. Westberg
18		Mondragone	41°07′	13°53′	R. Arafeh & E. Westberg
19		Parco Nazionale del Circe (5)	41°23′	12°55′	R. Arafeh & E. Westberg
20		Porto Civitianova	43°18′	13°44′	R. Arafeh & E. Westberg
21		**Marina deTorre del Lago Puccini (18)**	43°49′	10°15′	R. Arafeh & E. westberg
22		San Ferdinando	38°3′	15°54′	R. Arafeh & E. Westberg
23	France	Bourgneuf	47°02′	−2°02′	R. Arafeh & E. Westberg
24		Labenne Ocean	43°37′	−1°27′	R. Arafeh & E. Westberg
25		Las Castellas	43°21′	3°33′	R. Arafeh & E. Westberg
26		**Le Crotoy (5)**	50°14′	1°37′	R. Arafeh & E. Westberg
27		**Boulogne-Hardelot Plage (12)**	50°38′	1°34′	R. Arafeh
28		Les Jars	46°21′	−1°22′	R. Arafeh & E. Westberg
29		Les Sables d'Or	48°39′	−2°25′	R. Arafeh & E. Westberg
30		N. Parc de la Joie	48°4′	−3°39′	R. Arafeh & E. Westberg
31		Piscarrose Plage	44°27′	−1°15′	R. Arafeh & E. Westberg
32		Plaga N. Hourtin Ocean	45°13′	1°10′	R. Arafeh & E. Westberg
33		Ars en Re	49°23′	−1°00′	R. Arafeh & E. Westberg
34		Plage l'Aber	48°14′	−4°26′	R. Arafeh & E. Westberg
35		Port Louis	47°41′	−3°17′	R. Arafeh & E. Westberg
36		Agon Countin Ville	49°03′	−1°36′	R. Arafeh & E. Westberg
37		Utah beach museum	49°25′	−1°10′	R. Arafeh & E. Westberg
38	Spain	Ailla d' Arrousa	42°32′	−8°51′	R. Arafeh & E. Westberg
39		Lieneres	43°26′	−3°58′	R. Arafeh & E. Westberg
40		Menio	43°21′	−8°12′	R. Arafeh & E. Westberg
41		Cadiz	36°10′	−6°00′	R. Arafeh & E. Westberg
42		Platja de Olivia	38°55′	−0°04′	R. Arafeh & E. Westberg
43		Playa la Espasa	43°29′	−5°13′	R. Arafeh & E. Westberg
44		Playa de Navia	43°34′	−6°43′	R. Arafeh & E. Westberg
45		Rio oka (Playa de Layda; 5)	43°24′	−2°41′	R. Arafeh & E. Westberg
46	Portugal	Armacao de Pera	37°06′	−8°21′	R. Arafeh & E. Westberg
47		Foz do Arelho	39°26′	−9°13′	R. Arafeh & E. Westberg
48		Cabeledo	41°41′	−8°50′	R. Arafeh & E. Westberg
49		Torreira	40°45′	−8°42′	R. Arafeh & E. Westberg
50		Santa Andrea	38°07′	−8°48′	R. Arafeh & E. Westberg
51		Praia de Vieira	39°53′	−8°59′	R. Arafeh & E. Westberg
52	England	Studland	50°39′	−1°57′	R. Arafeh & E. Westberg
53		**Sandwich (4/7)**	51°16′	1°20′	R. Arafeh & E. Westberg
54		Widmouth	51°13′	−4°04′	R. Arafeh & E. Westberg
55		Braunton Burrows	51°04′	−4°12′	R. Arafeh & E. Westberg

the assumption that dispersal occurs only along the coast, geographical distances between localities were measured along the coastline.

To obtain a measure of intrapopulation genetic diversity, Nei's (1973) gene diversity was calculated using POPGENE version 3.2 assuming dominant-diploid markers under Hardy–Weinberg equilibrium. Only the four populations including eight or more individuals (see Table 1) were used for this.

Seed buoyancy and viability

Fifty seeds of *C. soldanella* were placed in a closed bottle containing 250 mL of sea water on 19 September 2003. The number of seeds that sank was recorded over time. On 19 September 2004 the viability of the seeds was tested. For this purpose, 10 scarified seeds were sown on sand and kept moist with tap water.

RESULTS

Entire-range sampling

In the 72 individuals included, a total of 98 unambiguous AFLP characters (fragments) from 70–416 base pairs (bp) in size could be scored. Of these, 68 were polymorphic, 13 monomorphic and 17 were unique to individuals. No identical samples were found among the accessions studied. The mean genetic distance (D) among individuals was 0.0309 ± 0.011 SD. The genetically most similar accessions ($D = 0.00224$) were samples from Marina Romea and Marina di Pisticci (both from the Adriatic Sea with a coastal distance of *c.* 600 km between sites), followed by samples from Marina di Pisticci, Adriatic Sea and Las Castellas, southern France ($D = 0.0023$, coastal distance between sites *c.* 2000 km). The highest genetic distance was found between samples from Foz Arelho, western Portugal and Braunton Burrows, southern England ($D = 0.0798$; coastal distance between sites *c.* 3100 km).

The NJ tree based on genetic distances between all pairwise combinations of the 98 AFLP phenotypes is shown in Fig. 1. Seven clusters (A–G) could be recognized. These clusters do not reflect any broad-scale geographical pattern. Instead, each cluster contains individuals from at least two geographically neighbouring areas (Fig. 1) which were recognized as distinct phylogeographical regions by Kadereit *et al.* (2005).

The total amount of variation among clusters was 25.6% ($P < 0.001$), and the strongest differentiation (35.3%; $P < 0.01$) was between clusters C (including samples from the Aegean Sea, Adriatic Sea, west Mediterranean Sea and Atlantic Ocean) and G (containing samples from the Atlantic Ocean coasts). Variation among groups ranged between 11.9% and 35.3% (Table 2). The correlation between geographical and genetic distances (Mantel test) resulted in a non-significant IBD ($r_M = 0.06394$; $P = 0.0530$) across the sampling range.

Table 2 Percentage of among-group (A–G in NJ tree) variation as obtained by AMOVA analysis

Groups	A	C	D	B	G	F	E
A	0.0						
C	34.3	0.0					
D	27.9	19.3	0.0				
B	27.5	19.7	21.0	0.0			
G	26.6	35.3	34.9	32.3	0.0		
F	25.1	30.5	33.4	28.5	11.9	0.0	
E	19.2	23.6	26.4	20.0	16.9	20.3	0.0

Population-level sampling

Sixty-one individuals from seven populations were included in the analysis. Within the range of 70–455 bp, 154 characters were scored, of which 106 were polymorphic, 37 monomorphic and 11 unique to individuals. The mean genetic distance (D) among individuals was 0.0294 ± 0.0102 SD. No identical genotypes were found. The most similar genotypes were two samples from Porto-Garibaldi, Adriatic Sea with $D = 0.00533$, and the highest genetic distance among genotypes was found between one sample from Punto Torre del Lago del Puccini, western Italy and one sample from Cavallino, Adriatic Sea ($D = 0.0825$). The overall pattern observed in the NJ phenogram (Fig. 2) does not reflect a clear spatial structure of

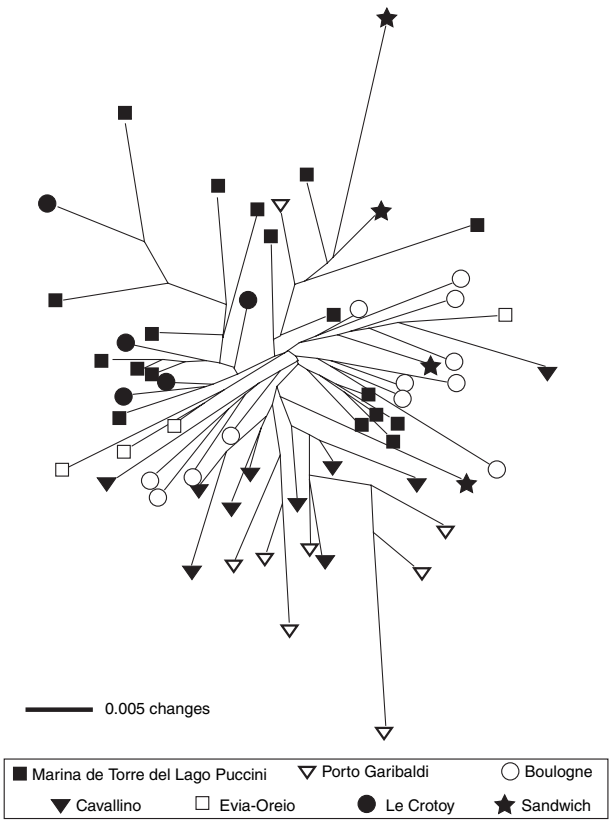

Figure 2 Unrooted NJ phenogram of samples from seven populations of *C. soldanella*, illustrating individual clustering.

genetic variation, especially with respect to the accessions from western Italy and the English Channel. Fifteen of 18 individuals from the Adriatic Sea fall into one cluster, while the remaining samples from western Italy and the English Channel are scattered in the phenogram without a clear geographical pattern.

The genetic variation partitioned among the seven populations was 18.02% of the total. Only 14.2% of the total genetic variation was partitioned among regions when six populations were divided into three geographical groups. Within-population and within-region variation were 81.98% and 85.00%, respectively.

A single Mantel test involving all populations yielded a statistically non-significant correlation between genetic and geographical distance ($r_M = 0.1083$; $P = 0.3301$).

The highest gene diversity was observed in the population from western Italy (0.1301 ± 0.1714), followed by the Adriatic Sea populations of Cavallino (0.1292 ± 0.1735) and Porto Garibaldi (0.1162 ± 0.1740), and lowest gene diversity was found in the Bolougne population (0.1034 ± 0.1603).

Seed buoyancy and viability

Up to 8 December 2005, 4 of the 40 seeds left after the germination experiment had sunk and the remaining 36 were still afloat. Of the 10 seeds tested for viability, 9 had germinated within 2 weeks.

DISCUSSION

In contrast to the findings of Kadereit *et al.* (2005), little geographical pattern is recognizable in the European range of *C. soldanella*. Kadereit *et al.* (2005) suggested that some aspects of the phylogeographic patterns found for different species can plausibly be explained by their northern distributional limits during the LGM, as deduced from their present northern distributional limits and the southward displacement of isotherms in the LGM. Of the five species compared by Kadereit *et al.* (2005), *E. maritimum* and *S. kali* are most similar to *C. soldanella* by sharing a correlation of their present northern distributional limits with the 14 °C July isotherm. Clear geographical groups could be identified in both *E. maritimum* and *S. kali*. Both species showed a separation into Atlantic/North Sea/Baltic Sea vs. Mediterranean/Aegean Sea/Black Sea material: in both species the Aegean Sea/Black Sea material was separated from the remainder of the Mediterranean material, and in *S. kali* an additional cluster of Adriatic Sea/southern Greece material could be identified. No such pattern could be found in *C. soldanella* across the entire range investigated. Where more than one individual from a given locality was sampled in our entire-range analysis, they never grouped into one major genetic cluster (Fig. 1) as was found for the populations sampled by Kadereit *et al.* (2005). In our population-level analysis, individuals from different local populations are also strongly intermingled in the NJ phenogram. The major exception to this is the Adriatic Sea material where 15 of 18 sampled individuals fell into one cluster. Our findings are similar to the results of Kim & Chung (1995) for *C. soldanella* in Korea, where little geographical structure could be detected, at least along the west coast of the Korean peninsula and islands off its southern coast. Similar results have been described for other strongly clonal coastal plant species by Franks *et al.* (2004) and Jonsson & Prentice (2000).

The virtual absence of phylogeographical structure in *C. soldanella* requires explanation. One obvious explanation would be that the species only arrived in the study area in post-glacial times and consequently the distribution range was not affected by the Quaternary climatic changes. Apart from the important fact that fossil pollen from interglacial (Ipswichian) and post-glacial (Flandrian) deposits in England has been attributed to *C. soldanella* (Godwin, 1975), the level of intraspecific variation found in this species lies within the range, although at its lower limit, of that found in those species investigated by Kadereit *et al.* (2005). Thus, the mean genetic distance among individuals across the entire geographical range is 0.0309 ± 0.011 in *C. soldanella* as compared with 0.0566 ± 0.0139 in *C. maritima*, 0.0609 ± 0.0211 in *C. maritimum*, 0.0396 ± 0.015 in *E. maritimum*, 0.051 ± 0.0138 in *H. portulacoides* and 0.0669 ± 0.023 in *S. kali*. If these values are regarded at least as a rough proxy of evolutionary age within a given area, and can be compared across species, then *C. soldanella* is about as old as *E. maritimum* in the area investigated. The latter species shows a clear phylogeographical pattern in the study area. Equally, partitioning of genetic variation among clusters in *C. soldanella* (25.64% among-cluster variation) is similar to partitioning of genetic variation among phylogeographical groups in the species studied by Kadereit *et al.* (2005) (*C. maritima* 25.8%, *C. maritimum* 32.8%, *E. maritimum* 46.7%, *H. portulacoides* 29.39%, *S. kali* 33.4%). Thus, differentiation among lineages is similar in all these species, but genetic lineages in *C. soldanella* do not constitute geographically defined groups. In consequence, it is not likely that *C. soldanella* is a recent arrival in the European flora. This supports the assumption (Hegi, 1925) that *C. soldanella* is a species of Mediterranean origin.

We postulate here that a combination of high frequency of long-distance dispersal and great clone longevity is responsible for the absence of phylogeographical pattern observed in this species. There is strong evidence for the potential of long-distance dispersal in *C. soldanella*. The seeds of this species have been recorded to be able to float in sea water for up to 18 months and to retain 30% germination capacity in this period (Ridley, 1930). In our own small-scale experiment we recorded 90% seed germination after 1 year of immersion in sea water, and 90% buoyancy after almost 27 months (September 2003 to December 2005). These values for seed buoyancy and viability are much higher than those reported for the other coastal species investigated by Kadereit *et al.* (2005). Although sea current systems in the Aegean/Black Sea, the Adriatic Sea and the Strait of Gibraltar area have been postulated to be strong barriers to gene flow for most of the

species investigated by Kadereit *et al.* (2005), small-scale investigations of gene flow in the Gibraltar and Black Sea/Aegean Sea areas have shown that these barriers are not insurmountable (Westberg, 2005). The chance of seed dispersal across these barriers certainly increases with time of buoyancy of seeds. As regards the longevity of clones in *C. soldanella*, we do not have comprehensive direct evidence for their size and growth rate that would allow us to calculate the ages of the clones.

The growth form of *C. soldanella*, with rhizomes buried to up to 1.5 m, is suitable for the formation of clones (Richards, 1986). Our data imply that as a rule clones of *C. soldanella* are no larger than 30 m in spatial expansion, and that populations of *C. soldanella* consist of more than one clone. This is implied by our population-level sampling strategy, where leaf material was sampled at distances of at least 30 m, by our molecular results, where no identical genotypes were detected and where populations were shown to contain genotypes falling into different clusters, and from the observation of good seed set in local populations which can be achieved only by out-breeding in this self-incompatible species. The presence of several clones in local populations of clonal species has been found in several studies (Ellstrand & Roose, 1987; Widén *et al.*, 1994; Jonsson & Prentice, 2000; Franks *et al.*, 2004). More importantly, the levels of intrapopulational genetic variation found in the four large populations studied by us is low in comparison with other out-breeding species, as also reported by Kim & Chung (1995) for *C. soldanella* in Korea. For AFLP variation, the average gene diversity is 0.12 for *C. soldanella*, compared with a mean of 0.214 ± 0.177 as reported by Nybom & Bartish (2000) for random amplified polymorphic DNA (RAPD) studies of several species. When the populations investigated also contain individuals falling into different NJ clusters, implying comparatively high levels of genetic divergence of at least some genotypes, the finding of comparatively low levels of genetic variation in local populations implies that these most commonly consist of a mixture of mainly closely related clones and few immigrant genets.

High longevity of clones may imply that even if the establishment of seeds dispersed over long distances is rare, and individuals arising from seeds dispersed over long distances are outnumbered in a local population, the likelihood of detection of such individuals is increased because they probably persist for long periods of time. We postulate here that this combination of long-distance dispersal and great clone age is responsible for the absence of a phylogeographical pattern in *C. soldanella*. This implies that extreme biological properties, such as shown by *C. soldanella*, can either erase or prevent the formation of historical patterns of genetic variation.

REFERENCES

Abbott, R.J. & Brochmann, C. (2003) History and evolution of the arctic flora: in the footsteps of Eric Hultén. *Molecular Ecology*, **12,** 299–313.

Avise, J.C. (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.

Bahri-Sfar, L., Lemaire, C., Ben Hassine, O. & Bonhomme, F. (2000) Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **267,** 929–935.

Bargelloni, L., Alarcon, J.A., Alvarez, M.C., Penzo, E., Magoulas, A., Ries, C. & Patarnello, T. (2003) Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. *Journal of Evolutionary Biology*, **16,** 1149–1158.

Bianchi, C.N. & Morri, C. (2000) Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Marine Pollution Bulletin*, **40,** 367–376.

Borsa, P., Blanquer, A. & Berrebi, P. (1997a) Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Marine Biology*, **129,** 233–246.

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

Brochmann, C., Gabrielsen, T.M., Nordal, I., Landvik, J.Y. & Elven, R. (2003) Glacial survival or tabula rasa? The history of North Atlantic biota revisited. *Taxon*, **52,** 417–450.

Chung, M.G., Kim, S.T., Chung, H.G. & Chung, M.S. (1995) Allozyme diversity in Korean populations of *Calystegia soldanella* and *C. japonica* (Convolvulaceae): implications for conservation. *Journal of Plant Biology*, **38,** 173–180.

Comes, H.P. & Kadereit, J.W. (1998) The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science*, **11,** 432–438.

Comes, H.P. & Kadereit, J.W. (2003) Spatial and temporal patterns in the evolution of the flora of the European Alpine System. *Taxon*, **52,** 451–462.

Ellstrand, N.C. & Roose, M.L. (1987) Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, **74,** 123–131.

Excoffier, L., Smouse, P. & Quattro, J. (1992) Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131,** 479–491.

Franks, S.J., Richrads, C.L., Gonzales, E., Cousins, J.E. & Hamrick, J.L. (2004) Multi-scale genetic analysis of *Uniola paniculata* (Poaceae): a coastal species with a linear, fragmented distribution. *American Journal of Botany*, **91,** 1345–1351.

Garibaldi, L. & Caddy, J.F. (1998) Biogeographic characterization of Mediterranean and Black Seas faunal provinces using GIS procedures. *Ocean Coast Management*, **139,** 211–227.

Godwin, H. (1975) *The history of the British flora*, 2nd edn. Cambridge University Press, Cambridge.

Hegi, G. (1925) *Illustrierte Flora von Mitteleuropa*, Vol. 3. Paul Parey Verlag, Berlin, pp. 2083–2085 (reprinted 1966).

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405,** 907–913.

Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B*, **359,** 183–195.

Jolly, M.T., Jollivet, D., Gentil, F., Thiébaut, E. & Viard, F. (2005) Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the north coast of France. *Heredity*, **94,** 23–32.

Jonsson, B.O. & Prentice, C.H. (2000) Allozyme diversity and geographic variation in the widespread coastal sedge, *Carex arenaria*. *Diversity and Distributions*, **6,** 65–80.

Kadereit, J.W. & Comes, H.P. (2005) The temporal course of alpine plant diversification in the Quaternary. *Plant species systematics. New perspectives on pattern and process. Regnum Vegetabile*, Vol. 143 (ed. by F.T. Bakker, L.W. Chatrou, B. Gravendeel and P. Pelser), pp. 117–130. Koeltz, Künigstein, Germany.

Kadereit, J.W., Arafeh, R., Somogyi, G. & Westberg, E. (2005) Terrestrial growth and marine dispersal? Comparative phylogeography of five coastal plant species at a European scale. *Taxon*, **54,** 861–876.

Kim, S.T. & Chung, M.G. (1995) Genetic and clonal diversity in Korean populations of *Calystegia soldanella* (Convolvulaceae). *Israel Journal of Plant Sciences*, **43,** 213–226.

Kropf, M., Kadereit, J.W. & Comes, H.P. (2002) Late Quaternary distributional stasis in the sub-Mediterranean mountain plant *Anthyllis montana* L. (Fabacae) inferred from ITS sequences and amplified fragment length polymorphism markers. *Molecular Ecology*, **11,** 447–463.

Lascoux, M., Palmé, A.E., Cheddadi, R. & Latta, R.G. (2004) Impact of ice ages on the genetic structure of trees and shrubs. *Philosophical Transactions of the Royal Society of London Series B*, **359,** 197–207.

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*). *Molecular Biology and Evolution*, **13,** 178–190.

Mantel, N. (1967) The detection of disease clustering and generalized regression approach. *Cancer Research*, **27,** 209–220.

Meusel, H., Jäger, E. & Weinert, E. (1965) *Vergleichende Chronologie der Zentraleuropäischen Flora. Karten*. VEB Gustav Fischer Verlag, Jena.

Mun, H.T. (1984) On the plant succession of sand bars at the estuary of the Nagdong River. PhD Dissertation, Seoul National University, Seoul.

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

Nei, M. (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America*, **70,** 3321–3323.

Nei, M. & Li, W. (1979) Mathematical model for studying genetic variance in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **76,** 5269–5273.

Nikula, R. & Väinölä, R. (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology*, **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 in Plant Ecology, Evolution and Systematics*, **3,** 93–114.

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. *Molecular Ecology*, **13,** 1923–1941.

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

Pérez-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.

Richards, A.J. (1986) *Plant breeding systems*. George Allen & Unwin, London.

Ridley, H. (1930) *The dispersal of plants throughout the world*. Reeve, Ashford.

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. *Journal of Experimental Marine Biology and Ecology*, **267,** 223–244.

Rohlf, F.J. (2002) *NTSYS-PC: numerical taxonomy and multivariate analysis system*, Ver. 2.10z. Exeter Software, Setauket, NY.

Röhner, M., Bastrop, R. & Jürss, K. (1997) Genetic differentiation in *Hediste iversicolor* Polychaeta: Nereididae) for the North Sea and Baltic Sea. *Marine Biology*, **130,** 171–180.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4,** 406–425.

Schneider, S., Kueffer, J.M., Roessli, D. & Excoffier, D. (1997) *ARLEQUIN: a software for population genetic data analysis*, Ver. 2.0. Genetics and Biometry Laboratory, University of Geneva, Geneva.

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. *Plant Systematics and Evolution*, **206,** 353–373.

Stehlik, I. (2003) Resistance or emigration? Response of alpine plants to the ice ages. *Taxon*, **52,** 499–510.

Swofford, D.L. (2002) *PAUP: phylogenetic analysis using parsimony*, Ver. 4.0b10. Sinauer Associates, Sunderland, MA.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G. & Cosson, J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7,** 353–364.

Tribsch, A. & Schönswetter, P. (2003) Patterns of endemism and comparative phylogeography confirm palaeo-environmental evidence for Pleistocene refugia in the Eastern Alps. *Taxon*, **52,** 477–497.

Ushimaru, A. & Kikuzawa, K. (1999) Variation in breeding system, floral rewards, and reproductive success in clonal *Calystegia* species (Convolvulaceae). *American Journal of Botany*, **86,** 436–446.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Pelman, J., Kuiper, M. & Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23,** 4407–4414.

Waters, J.M. & Roy, M.S. (2003) Global phylogeography of the fissiparous sea-star genus *Coscinasterias*. *Marine Biology*, **142,** 185–191.

Westberg, E. (2005) European phylogeography of the coastal plants *Cakile maritima* Scop. (Brassicaceae) and *Eryngium maritimum* L. (Apiaceae). PhD Dissertation, Mainz University, Mainz.

Widén, B., Cronberg, N. & Widén, M. (1994) Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants, a literature survey. *Folia Geobotanica & Phytotaxonomica*, **29,** 245–263.

BIOSKETCHES

Rami Arafeh did his PhD on the molecular phylogeography of four European coastal plant species and the influence of historical and modern factors on phylogeographical patterns.

Joachim W. Kadereit has broad interests in the systematics, phylogeny, evolution and biogeography of Eurasian flowering plants.

Editor: Peter Linder