Genetic diversity in peripheral and subcentral populations of *Corrigiola litoralis* L. (Illecebraceae)

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Genetic diversity and differentiation were studied in *Corrigiola litoralis* L., an annual plant species growing on seasonally flooded river banks. Plant species that are restricted to river systems may consist of highly isolated populations. For this species, pronounced genetic differentiation among rivers was expected. Plants were sampled from the river Loire (France) representing subcentral populations and the rivers Rhine, Weser and Elbe (Germany) representing peripheral ones. Allozyme electrophoresis revealed 17 putative loci in 11 enzyme systems. At the species level, percentage polymorphic loci, mean number of alleles, observed heterozygosity and expected heterozygosity were $P = 29\%$, $A = 1.5 \pm 0.2$, $H_o = 0.007 \pm 0.005$ and $H_e = 0.065 \pm 0.035$, respectively. Peripheral populations were smaller in number and showed decreased levels of genetic diversity relative to central populations. *Corrigiola litoralis* was highly inbreeding as indicated by a mean F_{IS} of 0.755. Genetic differentiation among populations was high with a mean F_{ST}-value of 0.585. Hierarchical *F*-statistics revealed that genetic variability was partitioned at 57% among sites, 52% among countries and 11% among sites within countries. Genetic distances between French and German populations were 0.08, indicative of considerable differentiation at the intraspecific level. The overall low level of allozyme diversity is attributed to the breeding system and to habitat conditions homogenized by regular flooding. The decrease in diversity from subcentral to peripheral populations is considered to be a result of drift and founder effects during postglacial recolonization. Peripheral populations were characterized by a single fixed allele at locus IDH, thus representing an evolutionarily significant unit.

Keywords: allozymes, genetic diversity, inbreeding, isolation, river system.

Introduction

The spatial structure of genetic variability has long been studied, both on local and large geographical scales (Brussard, 1984; Lesica & Allendorf, 1995; Linhart & Grant, 1996). The spatial structure of allozyme diversity in plant species is influenced by a variety of species-specific traits like life form, seed dispersal or geographical range, but most importantly by the breeding system (Hamrick & Godt, 1990, 1996). In contrast to outbreeding species, selfers or species with mixed mating systems show a substantial proportion of allozyme diversity partitioned among populations. The general patterns of allozyme diversity as influenced by species traits, however, may be affected by site-specific constraints. Whether a given population is located centrally or peripherally in the species range may exert an influence on its genetic diversity. At the periphery of the species range populations are often smaller and more isolated and selection could be more intense than in the centre of the species range, possibly leading to genetic differentiation. Thus, peripheral populations of selfing species with short generation time have been identified as potentially valuable for conservation of genetic diversity (Lesica & Allendorf, 1995).

Because species ranges are dynamic, historical range changes may also have influenced current genetic structure. In central Europe, postglacial recolonization may have played an important role in shaping the genetic structure of plant populations (Hewitt, 1996). A decrease in genetic diversity of central European populations relative to glacial refugia has been found in several European tree species (e.g. Lagercrantz & Ryman, 1990;

Konnert & Bergmann, 1995; Demesure *et al.*, 1996; Dumolin-Lapègue *et al.*, 1997). In general, wind-pollinated outbreeding tree species show high levels of allozyme diversity and low levels of spatial differentiation (Hamrick & Godt, 1990). Thus, for species with lower levels of gene flow like selfers, comparatively large effects of historical range expansions on genetic diversity and differentiation can be expected.

The level of isolation of local populations will influence the genetic structure of a species, predominantly in species restricted to small and spatially isolated habitats. Rivers, flood plains and river margins are habitats with strong spatial structure. River systems exert two major influences: strong isolation between watersheds and potentially high connectivity along the river caused by running water, both of which will affect gene flow and genetic differentiation of organisms restricted to these habitats. The varying degree of isolation among and within watersheds has been shown for several animal species to lead to strong genetic differentiation and functional isolation-by-distance, assuming gene flow along the drainage system (e.g. Arter, 1990; Hänfling & Brandl, 1998). In aquatic plants, however, very low levels of genetic diversity and differentiation are often found and the homogeneity of aquatic habitats is stated as a possible explanation (Barrett *et al.*, 1993). There are only a few studies that have addressed genetic differentiation of plants growing on river margins. It has been shown that gene flow occurring mainly within rivers leads to considerable differentiation among rivers (Ritland, 1989; Akimoto *et al.*, 1998; Liao & Hsiao, 1998). In this paper genetic differentiation of *Corrigiola litoralis* L., a plant species mainly restricted to river margins (Coker, 1962; Vogel, 1997) is addressed.

Corrigiola litoralis (Illecebraceae, Caryophyllales) is an annual herb primarily growing on sandy or gravelly alluvial river bank soils at the flood-limit of rivers that are regularly flooded in winter. It produces several decumbent radiating shoots, 10–60 cm in diameter and reproduces entirely by seeds that lack specific dispersal devices but may be transported by water. Concerning the mode of pollination, statements range from 'insect and self pollination' (Frank & Klotz, 1990), 'pollination may be entomophilous, but cleistogamy occurs under unfavourable conditions' (Coker, 1962) to 'self pollination' (Oberdorfer, 1983; Vogel, 1997). *Corrigiola litoralis* has an atlantic–subatlantic–meridional–littoral distribution and occurs naturally in Portugal, France, Benelux, S. England, W. and N. Germany, W. Poland, locally along Mediterranean coasts, and in S. and E. Africa (Meusel *et al.*, 1965; Field, 1994; Vogel, 1997). It finds optimal habitat conditions along the Loire (France), an unregulated river with extensive sandbanks (Wisskirchen,

1995). In Germany, *C. litoralis* has its largest populations along the river Elbe where it is regularly found. It reaches its continental limit of distribution near the border of the Czech Republic (Fig. 1). Along the rivers Weser and Rhine, only small and ephemeral populations are present. Along German rivers plants remain rather small compared to the Loire populations (Wisskirchen, 1995). Because of water level regulation *C. litoralis* is declining or already extinct at many places, e.g. in western Germany it is now absent from half of all previously occupied grid squares (Haeupler & Schönfelder, 1988). Based on the species range, population sizes, and individual plant size, we refer to German populations as peripheral, whereas French populations are subcentral.

This study was undertaken to characterize levels of genetic diversity and genetic differentiation of *C. litoralis* within and among rivers. The rivers investigated represent subcentral and peripheral positions in the range of *C. litoralis*. It is hypothesized that: (i) small populations are depauperate in genetic diversity; (ii) peripheral populations show higher levels of homozygosity and inbreeding because of unfavourable conditions; (iii) genetic differentiation occurs mainly between and not within rivers, because gene flow predominantly occurs by seed dispersal and is mainly restricted to a single catchment area.

Methods

Plants were sampled from natural populations on sandy or stony river banks. In October 1997 between nine and 19 plants were sampled randomly from 15 populations along the rivers Elbe, Weser, Rhine and Loire (including Allier) (Fig. 1, Table 1). In large populations whole plants were sampled, whereas in small populations (e.g. at the Bonn site the total population size was $n = 9$) only single branches were sampled from plants remaining on the site. Samples were immediately frozen in liquid nitrogen, transferred to the laboratory and stored at $-42°C$ until analysis. Population size was obtained by direct counting or by estimation from habitat size and density.

Genetic variation was assessed using standard horizontal starch gel electrophoresis of isozymes from homogenized branches (including stems, leaves and flowers; Tris-HCl grinding buffer, Soltis *et al.*, 1983). Staining recipes followed Wendel & Weeden (1989). A total of 11 enzyme systems gave clearly resolvable banding patterns: ACOH, ADH, AAT, GPI, IDH, CAP, MDH, PGDH, PGM, SkDH, MNR (Table 2).

The number of multilocus genotypes (N_{gt}), percentage of polymorphic loci (P), observed mean number of alleles per locus (A_o), mean observed heterozygosity (H_o), and mean expected heterozygosity under Hardy–

Weinberg equilibrium (H_e) were calculated using Nei's (1978) unbiased heterozygosity. The number of multilocus genotypes, N_{gt}, is sensitive to sample size per population, but as correlation between them was nonsignificant, N_{gt} was used in further analyses. Means of the diversity statistics were obtained by averaging population estimates within countries or over all populations. Species-level estimates were
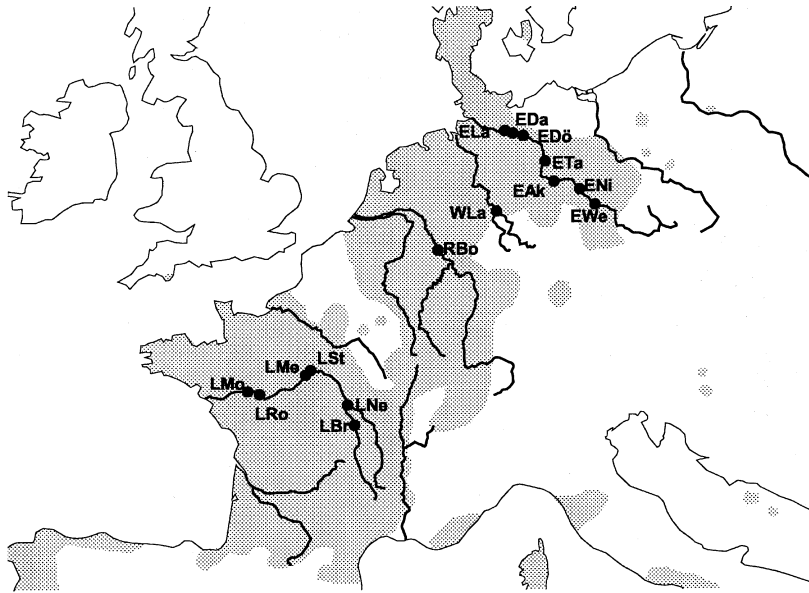
Fig. 1 Sampling sites and species range (shaded area, after Field, 1994) of *Corrigiola litoralis*. For abbreviation of sites, see Table 1.

Table 1 Genetic variability at 17 allozyme loci in 15 populations of *Corrigiola litoralis*

River Site	Site name	Population size	Sample size	N_{gt}	%P	A_o (SE)	H_o	(SE)	H_e	(SE)
Elbe										
EWe	Wehlen	100	16	1	0.0	1.0 (0.0)	0.000	(0.000)	0.000	(0.000)
ENi	Niederlommatzsch	100	9	1	0.0	1.0 (0.0)	0.000	(0.000)	0.000	(0.000)
EAk	Aken	>1000	13	1	0.0	1.0 (0.0)	0.000	(0.000)	0.000	(0.000)
ETa	Tangermünde	>1000	12	2	5.9	1.1 (0.1)	0.005	(0.005)	0.005	(0.005)
EDö	Dömitz	>1000	11	1	0.0	1.0 (0.0)	0.000	(0.000)	0.000	(0.000)
EDa	Darchau	>1000	12	2	5.9	1.1 (0.1)	0.000	(0.000)	0.017	(0.017)
ELa	Lauenburg	>1000	19	3	5.9	1.1 (0.1)	0.003	(0.003)	0.018	(0.018)
Weser										
WLa	Langwedel	9	9	2	5.9	1.1 (0.1)	0.000	(0.000)	0.022	(0.022)
Rhine										
RBo	Bonn	9	9	1	0.0	1.0 (0.0)	0.000	(0.000)	0.000	(0.000)
Mean Germany				1.6	2.7	1.0 (0.0)	0.001	(0.001)	0.007	(0.003)
Loire/Allier										
LBr	Bressolles	>1000	17	7	11.8	1.2 (0.1)	0.024	(0.017)	0.050	(0.035)
LNe	Nevers	>10000	19	6	11.8	1.1 (0.1)	0.012	(0.012)	0.046	(0.033)
LMe	Meung s.L.	<50	10	6	11.8	1.1 (0.1)	0.018	(0.013)	0.058	(0.040)
LSt	St.-Laurent	200	11	4	11.8	1.2 (0.1)	0.000	(0.000)	0.053	(0.038)
LRo	Les Rosiers	>10000	10	8	23.5	1.4 (0.2)	0.024	(0.018)	0.089	(0.044)
LMo	Montjean	>10000	10	5	17.6	1.2 (0.1)	0.012	(0.012)	0.057	(0.033)
Mean France				6.0	15.0	1.2 (0.1)	0.015	(0.004)	0.059	(0.006)
Population mean				3.3	7.6	1.1 (0.0)	0.007	(0.002)	0.028	(0.007)
Species estimate				20	29.4	1.5 (0.2)	0.007	(0.005)	0.065	(0.035)

N_{gt}, number of multilocus genotypes; %P, percentage polymorphic loci; A_o, mean number of alleles (SE); H_o, observed heterozygosity; H_e, expected heterozygosity (Nei, 1978) under Hardy–Weinberg equilibrium.

obtained by pooling all individuals across populations for calculation. Because sample sizes per population were very small for performing chi-squared tests, significance of deviations from Hardy–Weinberg equilibrium expected under panmictic conditions was tested with the conservative 'Probability-test' of GENEPOP (Raymond & Rousset, 1995). Tests were pooled across sites and across loci using Fisher's method. Significance of F_{ST} values was assessed with a probability test using a Markov chain method (Raymond & Rousset, 1995).

Patterns of genetic variation within and among populations were analysed using hierarchical *F*-statistics (Wright, 1965, 1978) with sites, countries (Elbe + Weser + Rhine populations = Germany; Loire populations = France) and the total as hierarchical levels. Additionally, differentiation of populations was assessed by UPGMA-cluster analysis of genetic distances (Nei, 1972) between sites. Population genetic analyses were performed with GENEPOP (Raymond & Rousset, 1995) and BIOSYS (Swofford & Selander, 1989). Cluster analysis was performed with NTSYS 1.80 (Rohlf, 1983). Outcrossing rate was calculated as $t = (1 − F)/(1 + F)$, where *F* is Wright's fixation index (Nei & Syakundo, 1958), assuming that populations are in inbreeding equilibrium, i.e. *F* is constant over generations. Only populations and loci that showed polymorphism were used in the calculation of outcrossing rate. The effects of population size and country on genetic diversity statistics were tested with ANCOVA using countries (Germany, France) as groups and population size as a covariate. In this analysis, population size was log-transformed and percentage polymorphic loci was arcsin-transformed.

Results

Genetic diversity

A total of 187 individuals was analysed for electrophoretic variability of 11 enzymes encoded by 17 putative genetic loci (Tables 2 and 3). At the species level *C. litoralis* showed a low level of genetic diversity, with only five out of 17 (29%) loci being polymorphic: *GPI-2*, *PGM*, *IDH-1*, *SkDH* and *ACOH-1* (Table 3). Applying a 0.99 or 0.95 criterion, only three loci (18%) were polymorphic. Species-wide estimates of mean number of alleles per locus and genetic diversity were $A = 1.5 ± 0.2$ (SE), and $H_e = 0.065 ± 0.035$ (SE), respectively.

French and German populations showed marked differences in their levels of genetic diversity. Along the Loire, all populations were polymorphic and all alleles but one of the polymorphic loci were detected. In contrast, in Germany only four downstream populations of the Weser and the Elbe were polymorphic, each at one single locus (*GPI-2*). In France a total of 16 multilocus genotypes was found in 77 individuals, whereas only four multilocus genotypes were detected in 111 individuals in Germany. Average gene diversity was $H_e = 0.059 ± 0.006$ for French and $H_e = 0.007 ± 0.007$ for German populations.

Corrigiola litoralis was highly homozygous: 109 out of 111 plants from nine German populations were homozygous at all 17 loci analysed. Only two plants from different populations were heterozygous at locus *GPI-2* in German sites ($H_o = 0.001$). In the Loire populations, a higher level of heterozygosity was detected ($H_o = 0.015$, Table 1). *Corrigiola* is far from being at Hardy–Weinberg equilibrium, because out of

Table 2 Enzyme systems included in the analysis

Enzyme system (EC no.)	Buffer system†	Locus (number of alleles)
Aconitate hydratase (4.2.1.4)	TCE	*ACOH-1* (2), *ACOH-2* (1)
Alcohol dehydrogenase (1.1.1.1)	TCE	*ADH-1* (faint bands), *ADH-2* (1)
Aspartate aminotransferase (2.6.1.1)	TBE	*AAT-1* (1), *AAT-2* (1), *AAT-3* (1)
Cytosol aminopeptidase (3.4.11.1)	TBE	*CAP-1* (1), *CAP-2* (1)
Glucose-6-phosphate isomerase (5.3.1.9)	S4	*GPI-1* (smeared bands), *GPI-2* (4)
Isocitrate dehydrogenase (1.1.1.42)	TCE	*IDH-1* (2), *IDH-2* (1)
Malate dehydrogenase (1.1.1.37)	S4	*MDH-1* (1), *MDH-2* (1)
Menadione reductase (1.6.99.-)	TBE	*MNR* (1)
Phosphoglucomutase (5.4.2.2)	TCE	*PGM* (3)
Phosphogluconate dehydrogenase (1.1.1.44)	S4	*PGDH* (1)
Shikimate dehydrogenase (1.1.1.25)	TCE	*SkDH* (2)

†S4: continuous Tris-Citrate pH 6.3; electrode: 0.223 M Tris, 0.086 M citric acid; gel: 0.008 M Tris, 0.003 M citric acid (Selander *et al.*, 1971). TCE: continuous Tris-Citrate-EDTA pH 7.0; electrode: 0.135 M Tris, 0.045 M citric acid, 1.3 mM EDTA; gel: 0.01 M Tris, 3.2 mM citric acid, 0.09 mM EDTA (Murphy *et al.*, 1996). TBE: discontinuous Tris-Borate-EDTA pH 8.6; stock solution: 0.90 M Tris, 0.5 M boric acid, 0.02 M EDTA; anode: 1:6 dilution; cathode: 1:4 dilution; gel: 1:19 dilution (Murphy *et al.*, 1996).

Table 3 Allele frequencies at polymorphic loci of *Corrigiola litoralis* L. For sites see Table 1

Locus	GPI-2				PGM			IDH-1		SkDH		ACOH-1	
Allele	a	b	c	d	a	b	c	a	b	a	b	a	b
EWe	—	1.00	—	—	—	1.00	—	—	1.00	1.00	—	1.00	—
ENi	—	1.00	—	—	—	1.00	—	—	1.00	1.00	—	1.00	—
EAk	—	1.00	—	—	—	1.00	—	—	1.00	1.00	—	1.00	—
ETa	—	0.96	—	0.04	—	1.00	—	—	1.00	1.00	—	1.00	—
EDö	—	1.00	—	—	—	1.00	—	—	1.00	1.00	—	1.00	—
EDa	—	0.83	0.17	—	—	1.00	—	—	1.00	1.00	—	1.00	—
ELa	—	0.82	0.18	—	—	1.00	—	—	1.00	1.00	—	1.00	—
WLa	—	0.78	0.22	—	—	1.00	—	—	1.00	1.00	—	1.00	—
RBo	—	1.00	—	—	—	1.00	—	—	1.00	1.00	—	1.00	—
LBr	—	0.74	0.26	—	0.06	0.24	0.71	1.00	—	1.00	—	1.00	—
LNe	0.16	0.84	—	—	—	0.47	0.53	1.00	—	1.00	—	1.00	—
LMe	0.40	0.60	—	—	—	0.65	0.35	1.00	—	1.00	—	1.00	—
LSt	0.09	0.55	0.36	—	—	0.82	0.18	1.00	—	1.00	—	1.00	—
LRo	0.10	0.70	0.20	—	0.05	0.45	0.50	0.90	0.10	1.00	—	0.85	0.15
LMo	—	0.70	0.30	—	—	0.80	0.20	1.00	—	0.90	0.10	1.00	—

18 single-locus tests for deviation from equilibrium performed from all populations, 12 were significant (data not shown). Only one test is expected to be significant by chance at the 5% significance level. All combined tests, either combining across populations or across loci, were significant. Mean F_{IS} of polymorphic loci was 0.755, indicating a low level (14%) of outcrossing (Table 4). Calculation of outcrossing rate for countries revealed 16% in French and 3% in German populations.

Measures of genetic diversity, N_{gt}, A, $\%P$, H_o and H_e, were significantly correlated with each other ($r \geq 0.658$, $P \leq 0.008$). N_{gt}, A, $\%P$ and H_e were significantly correlated with population size ($r \geq 0.477$, $P \leq 0.036$). However, ANCOVA revealed that the country level had significant effects on all genetic diversity statistics, whereas population size had no significant effect after controlling for country (Table 5). This indicated that decreased genetic diversity in small populations was mainly a result of overall reduced genetic diversity in German populations.

Genetic differentiation

Population subdivision is high as shown by four of five F_{ST} values being significantly different from zero and reaching 0.98 at locus *IDH* (Table 4). Mean F_{ST} was 0.585. As no differentiation was found between the German rivers Rhine, Weser and Elbe, they were grouped together as Germany vs. France (Loire). The country level was used as an additional level in a hierarchical analysis. Hierarchical *F*-statistics revealed that genetic variability was partitioned at 57% among

Table 4 *F*-statistics (Wright, 1965, 1978; Weir & Cockerham, 1984) for all populations of *Corrigiola litoralis*

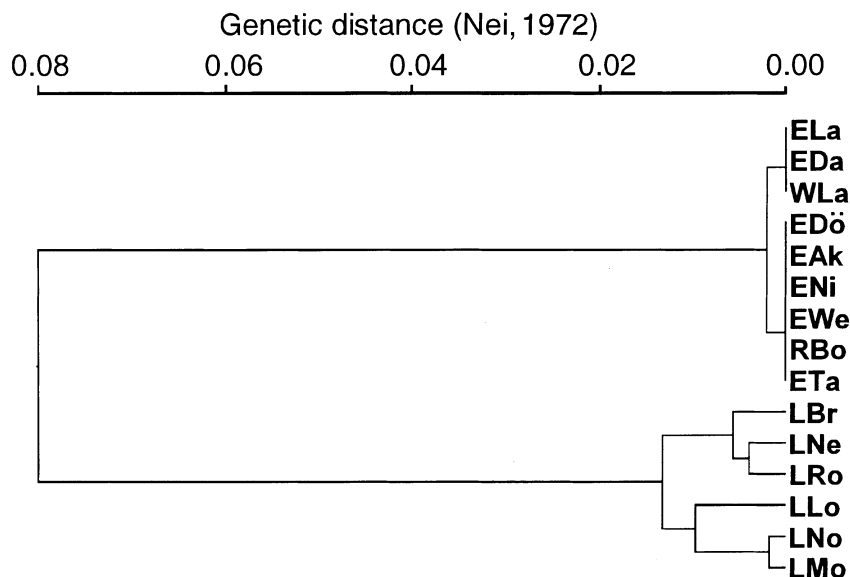| Locus	F_{IS}	F_{IT}	F_{ST}
GPI-2	0.855	0.870	0.108***
PGM	0.609	0.769	0.411***
IDH-1	1.000	1.000	0.980***
SkDH	1.000	1.000	0.024
ACOH-1	0.633	0.668	0.093*
Mean	0.755	0.898	0.585

Table 5 ANCOVA *F*-values testing for effects of country and population size on genetic diversity statistics of *Corrigiola litoralis* (for explanation see Table 1)

	Country	Population size
N_{gt}	47.01***	1.16
$\%P$	24.03***	2.25
A_o	8.39*	1.28
H_o	11.47**	0.14
H_e	46.84***	0.66

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

sites, 52% among countries and 11% among sites within countries ($F_{site/total} = 0.568$, $F_{site/country} = 0.105$, $F_{country/total} = 0.517$). Differences between countries were attributable to two alleles of *PGM* and one allele of *GPI-2*, which only occurred in Loire populations, and the *a*-allele and *b*-allele of *IDH-1*, which occurred nearly exclusively in French and German sites, respectively (Table 3).

Genetic distances according to Nei (1972) among populations ranged from 0.000 to 0.102. Mean genetic

Genetic distance (Nei, 1972)

| 0.08 | 0.06 | 0.04 | 0.02 | 0.00 |

ELa
EDa
WLa
EDö
EAk
ENi
EWe
RBo
ETa
LBr
LNe
LRo
LLo
LNo
LMo

Fig. 2 Phenogram of genetic distances calculated for 17 allozyme loci in 15 populations of *Corrigiola litoralis* L. from the Elbe (E**), Weser (W**), Rhine (R**) and Loire (L**).

distance was 0.010 and 0.001 within France and Germany, respectively, and 0.080 between France and Germany. The UPGMA cluster analysis of genetic distances (Fig. 2) emphasizes the geographical pattern of differentiation. Populations from the Loire and those from German rivers formed two distinct clusters. However, populations were not clustered according to geographical position along the respective rivers.

Discussion

Genetic diversity at the species level

Corrigiola litoralis is highly inbreeding, which was confirmed by seed-set in plants in a growth chamber (Durka, pers. obs.). Even in subcentral populations allowing optimal growth, only few heterozygotes were found. Thus the notion of Coker (1962) that cleistogamy occurs under unfavourable conditions must be extended in that autogamy is the rule in *C. litoralis* even under favourable conditions. In three German populations showing some allozyme polymorphism, outcrossing rate was lower than in French populations, supporting the hypothesis that in peripheral populations inbreeding is increased.

Although selfing leads to individual homozygosity, selfing species are not necessarily poor in allelic variants (Allard *et al.*, 1968). *Corrigiola litoralis*, however, had low levels of overall diversity compared to other selfing species. The percentage of polymorphic loci (population level: 7.6%; species level: 29%) is far lower than the mean of selfing species (population level: 20%; species level: 42%; Hamrick & Godt, 1990). Limited allozyme

variation may reflect restricted habitat range or homogeneity of the environment (Hedrick *et al.*, 1976). Very low levels of genetic variability have been recorded in aquatic taxa (Barrett *et al.*, 1993). Triest (1991) suggests that this may partly reflect the generally more homogeneous substrates in water and wetlands compared to strictly terrestrial habitats. All populations investigated in this study originated from similar primary habitats on sandy or gravelly banks of rivers flooded regularly in winter. In time and space this is a very narrow niche in which genetic homogeneity is plausible. On the other hand, within-habitat heterogeneity with respect to soil water is very high on sandbanks and riverbanks varying from 0 to 2 m above water level. Additionally, soil substrate along the river varies from gravel in the upper reaches to sand in the lower reaches. Thus, there is considerable heterogeneity in the habitat of *C. litoralis*, which could give rise to genetic variability. However, habitat dynamics may override any small-scale habitat structure. *Corrigiola litoralis* is faced with regular dramatic perturbations of the habitat through flooding which disrupts any small-scale structure of, for example, topography. Additionally, although seeds of *C. litoralis* lack special dispersal devices and would, without flooding, stay beneath the dying plant, flooding leads to mixing of seed genotypes, thus preventing the build-up of small-scale genetic structure.

Although there is considerable heterogeneity of habitat conditions, both within and between populations, regular flooding may promote genetic homogeneity because all individuals of a population may face the same environment. This may be a general feature of the plant community in which *C. litoralis* grows. Several of these annual species (e.g. *Chenopodium* spp., *Polygonum*

lapathifolium, *Xanthium* spp.) are reported to display very low levels of genetic variability, at least as colonizers (Barrett & Shore, 1989). No perennial species is found together with *Corrigiola*. Interestingly, the plants from river margins for which considerable genetic variation has been reported, were outbreeding, insect-pollinated perennials (Ritland, 1989; Liao & Hsiao, 1998). Thus, the general observation that allozyme variability is lower in annual selfers than in perennial outcrossers (Hamrick & Godt, 1990) is confirmed for plants of river margins as well.

Intraspecific patterns of genetic differentiation

Genetic differentiation was found mainly among countries at a level of Nei distances of 0.08. This value corresponds to distances found between local races or subspecies, which typically range from 0.00–0.05 and 0.02–0.07, respectively (e.g. Levin, 1978; Helenurm & Ganders, 1985; Nei, 1987). Considering the geographical distribution of *C. litoralis* (Fig. 1), two subranges can be distinguished, namely (i) a north-eastern part including the Rhine, Weser and Elbe systems characterized by the *b*-allele of *IDH-1* and (ii) a south-western part including the Loire characterized by the *a*-allele of *IDH-1* and multiple alleles at *GPI-2* and *PGM*. Large-scale geographical patterns of intraspecific races fixed for alternative alleles have previously been documented in other selfing annual species, e.g. *Chenopodium fremontii* (Crawford & Wilson, 1977). The *b*-allele of *IDH-1*, which occurs throughout the German populations, was detected in a single individual along the Loire. Although sample sizes in the Loire populations were restricted to 10–19 individuals and may therefore underestimate rare alleles, in a total of 77 individuals the *b*-allele only occurred once. Thus, the *b*-allele of *IDH-1* is extremely rare in France and it might be argued that it is not identical to that detected in the German populations and represents a homoplasy.

Genetic differentiation in *C. litoralis* between French and German populations was caused by loss of alleles at two loci and fixation of different alleles at another locus. Several mechanisms may have led to the observed differentiation: (i) selection, (ii) genetic drift, or (iii) founder-effects in postglacial colonization history.

(i) Peripheral populations are under strong directional selection. Selection may act on enzyme loci or on genes linked to them (Clegg, 1983). In the context of the overall level of genetic diversity, habitat disturbance was discussed above as a potential selective force leading to genetic uniformity. However, different levels of diversity in subcentral and peripheral populations are not interpreted as an effect of differential intensities of habitat disturbance. The habitat of *C. litoralis* has to be maintained by physical perturbation equally in subcentral and peripheral populations. Selective forces limiting the atlantic–subatlantic range of *C. litoralis* may be connected to features of continentality like decreasing minimum temperatures, lengthening of frost periods or frequency of late frosts. In fact, the range of *C. litoralis* seems to be limited by low temperature, because in Germany no populations are found below the −2°C mean January isotherm (Vogel, 1997). Thus, low temperature might select specific genotypes in peripheral populations. However, it is not clear how low temperature can affect *C. litoralis*, because it survives frost periods as seed and its growing season is limited by the time of subsiding and rising floods.

(ii) Peripheral populations are at higher risk of genetic drift than more central populations. The rate of genetic drift is specified by the population size and the selfing rate (Gregorius, 1991). Peripheral populations of *C. litoralis* were smaller and had a higher inbreeding rate than subcentral populations. Thus, genetic drift may have led to decreased genetic variability in peripheral German populations of *C. litoralis*.

(iii) Peripheral populations frequently go extinct and recolonization may take place by only a few individuals comprising a low number of genotypes. Declining genetic diversity towards the periphery of the species ranges has been found in many species (Lesica & Allendorf, 1995). For European species a decline in genetic diversity was found, for example, in *Alnus glutinosa* (King & Ferris, 1998), *Arabidopsis thaliana* (Kuittinen *et al.*, 1997), *Calluna vulgaris* (Mahy *et al.*, 1997), *Picea abies* (Lagercrantz & Ryman, 1990) and *Quercus* (Dumolin-Lapègue *et al.*, 1997). The decline of diversity in these species parallels south–north or east–west postglacial colonization routes and can be interpreted as the effect of repeated bottlenecks during range expansions (Hewitt, 1996). A similar scenario could be invoked for *C. litoralis* to explain the loss of alleles in German populations. Postglacial recolonization of the Rhine, Weser and Elbe river systems may have started from only a few genotypes originating from French rivers. This is supported by the fact that at the two loci *GPI-2* and *PGM*, the allele observed in Germany is the most common allele in France. However, the change of one fixed allele into the other at locus *IDH-1* requires the additional assumption of mutation at that particular locus since the time of colonization. Alternatively, a different source population for the current German populations might be postulated. To address this issue further, an extensive investigation of the *IDH-1* locus and other markers at the DNA level needs to be undertaken in the whole range of *C. litoralis*.

In recent decades, *C. litoralis* has increasingly been found on secondary habitats like railway embankments, industrial dumps and water reservoirs with changing water level (Vogel, 1997). In the Netherlands, *C. litoralis* is currently only known from railway embankments (Westhoff, 1968). However, populations from such secondary habitats were not included in this study. It would be of interest to know to what extent these populations resemble the genotypes from primary habitats along river margins. As *C. litoralis* is a declining species, secondary habitats might play a role in conservation of the species (Vogel, 1997).

This study has shown that at the species level *C. litoralis* displays very low allozyme variability as an effect of inbreeding. This effect may be further enhanced by a narrow niche in a habitat regularly homogenized by flooding. Central European populations of *C. litoralis* are genetically depauperate relative to more southwestern populations, probably as an after-effect of postglacial colonization history. Nevertheless, pronounced genetic differentiation caused by unique alleles makes peripheral populations of *C. litoralis* in central Europe important as evolutionarily significant units.

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