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# Genetic differentiation and speciation in prealpine *Cochlearia*: Allohexaploid *Cochlearia bavarica* Vogt (Brassicaceae) compared to its diploid ancestor *Cochlearia pyrenaica* DC. in Germany and Austria

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**Abstract.** Significant geographic partitioning of genetic variation within *Cochlearia bavarica* was found within populations from Allgäu and SE Bavaria (Germany) exhibiting significant genetic differentiation. It has been demonstrated that allohexaploid *C. bavarica* evolved via hybridization between diploid *C. pyrenaica* and tetraploid *C. officinalis*. Presently, only *C. pyrenaica* is distributed throughout inland Central Europe. It has been concluded that *C. bavarica* is of inter- or postglacial origin, and its speciation was not influenced by human activities.

Isozyme analysis revealed that there is a correlation between interpopulational genetic distances and geographic distances among *C. bavarica* populations from both regions, and which is not the case for *C. pyrenaica* in Germany and Austria. Only high alpine *C. excelsa* is significantly differentiated among the diploid taxa analysed here. Geographically structured distribution of alleles and their frequencies in *C. bavarica* populations could not be explained with the distribution of these alleles in *C. pyrenaica*. The presented findings favour disruption of a former wider distribution area rather than migration of *C. bavarica* or a polytopic origin of this species.

**Key words:** Brassicaceae, *Cochlearia*, isozyme analysis, migration, phylogeography, polytopic origin.

## Introduction

Polyploidisation is thought to represent an important speciation mechanism in angiosperms. Several studies on evolution of polyploid complexes have demonstrated that recurrent formation of allo- and autopolyploid plant species are the rule rather than the exception. However, if polyploidisation plays an essential role in plant speciation processes one could assume that multiple and independent formations of a polyploid plant occurs more frequently than recognised so far. Within the Brassicaceae family polyploidisation is widespread and plays an important role on all taxonomic levels from tribes to ecotypes (e.g. Urbanska et al. 1997, Franzke et al. 1998, Koch et al. 1998a, Koch et al. 1998b, Koch and Hurka 1999, Koch et al. 1999, Koch and Al-Shehbaz 2000, Franzke and Hurka 2000, Koch and Al-Shehbaz 2002). Multiple independent formation of allopolyploids have been suggested for two *Tragopogon* species (Soltis et al. 1995, Cook et al. 1998), two *Cardamine* species (Franzke and Mummenhoff 1999), *Arabidopsis suecica* (Mummenhoff and Hurka 1995, O'Kane et al. 1997), and within the cruciferous genera *Yinshania* (Koch and

Al-Shehbaz 2000) and *Draba* (Koch and Al-Shehbaz 2002). Molecular studies have shown that some autopolyploids arose independently in *Coincya* (Leadlay and Heywood 1990), *Heuchera grossulariifolia* (Segraves et al. 1999) or *Grindella camporum* (McLaughlin 1986).

The genus *Cochlearia* L. section *Cochlearia* comprises several polyploid species which are of alloploid or autotetraploid origin. The polyploid complex is of pleistocenic origin (Koch et al. 1999) and there is only little morphological differentiation among the different taxa. In two recent studies molecular markers were used to analyse the origin of these polyploids (Koch et al. 1996, 1998a). In these studies it has been shown that tetraploid *C. officinalis* L., distributed along the northern coasts of Europe and showing a broad range of ecological and morphological differentiation in Scandinavia (Nordal and Stabbetorp 1990), originated from ancestors of a diploid coastal species, namely *C. aestuaria* (Lloyd) Heywood. It is unknown if this occurred via auto- or allopolyploidisation. Octoploid *C. anglica* L. is restricted to the coasts of northern France to southern Sweden. Most likely aestuarian ecotypes of tetraploid *C. officinalis* served as ancestral gene pool to establish octoploid *C. anglica*. A third coastal polyploid is represented by the hexaploid *C. danica* L. This species is the only annual and colonises coastal sand dunes. Its origin is still unclear, but hybridisation and introgression among the coastal species is a common process (Koch et al. 1996).

A second group of polyploids is restricted to continental inland stations. *Cochlearia polonica* Fröhl. and *C. tatrae* Borb. of Poland and Slovakia, respectively, are hexaploid taxa which have most likely originated via allopolyploidization from extinct inland *C. officinalis* and *C. pyrenaica* (Koch et al. 1998a). Both species are highly endemic as it is the case for *C. bavarica* Vogt.

*Cochlearia bavarica* is also hexaploid. Its distribution is restricted to two areas in Bavaria, Germany (Fig. 1). The allopolyploid origin from *C. pyrenaica* and *C. officinalis* ancestors is very likely (Koch et al. 1996, 1998a). It has been speculated that *C. bavarica* has been recently constituted via hybridisation between *C. pyrenaica* and cultivated *C. officinalis* in the 17th or 18th century in Bavarian monastery gardens (Vogt 1985). However, several lines of evidence suggest that *C. bavarica* is of an inter- or postglacial origin without human influence. First, there is no direct evidence that *C. officinalis* was cultivated in monastery gardens in Bavaria. Descriptions of plants cultivated in monastery gardens which were used as salad early in the year do also fit to *Nasturtium* or *Cardamine*. Second, *C. bavarica* occurs in undisturbed habitats characterised by cold water springs with high carbonate concentrations. This habitat is similar to *C. pyrenaica* stands, but it differs completely from recent coastal sites where *C. officinalis* is growing. Third, *C. pyrenaica* does not occur close to *C. bavarica*, but it is rarely found in neighboring areas in southern Germany, Austria, Switzerland and the Pyrenees. Fourth, differing allozyme sets and allele frequencies of *C. bavarica* populations demonstrate two geographically isolated ranges of this taxon in southern Germany (Koch et al. 1998a).

In the present study intra- and interpopulational genetic differentiation has been analysed to elucidate the origin of *C. bavarica*. As outlined above a recent origin is not very likely, and thus three additional hypotheses are proposed to explain the disjunct gap with significant genetic differentiation. 1) The geographic and genetic disjunction is a result of migration and subsequent genetic differentiation, 2) *C. bavarica* evolved two times independently (multiple or polytopic origin), or 3) The observed disjunct distribution of genetic diversity is the result of extinction of populations between both actual distribution sites. Some of these hypotheses were supported by examples within *Cochlearia*. For example a strong correlation between latitudinal distribution and genetic diversity was observed in *Cochlearia danica* and *C. officinalis*, which has been explained by migration and postglacial range

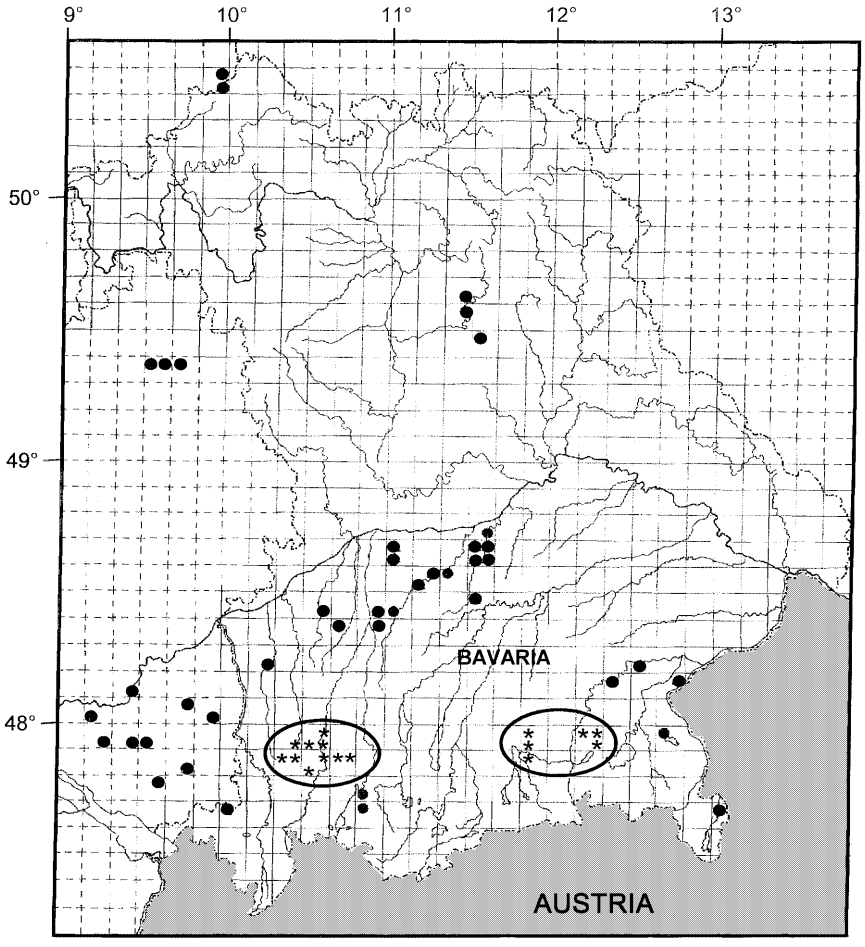


**Fig. 1.** Distribution of *Cochlearia pyrenaica* (●) and *Cochlearia bavarica* (★) in Bavaria, Baden-Württemberg and Hessen (Germany). Data points were compiled from records in herbaria and actual populations cited in Vogt (1985) and/or visited by the author

extension (Koch et al. 1998a). Extensive gene flow even over ploidy levels has been demonstrated for *C. officinalis* and *C. anglica* (Koch et al. 1998a). In order to test the hypothesis of multiple origin versus migration or disruption of a former continuous distribution, isozyme analyses were performed to evaluate the genetic structure of the *C. bavarica* populations and one putative parental taxon, *C. pyrenaica* from Germany and Austria.

## Material and methods

**Plant material.** Thirty-six populations, comprising *C. bavarica* and *C. pyrenaica* including closely related diploid *C. excelsa* and *C. macrorrhiza* were studied (Table 1). *Cochlearia excelsa* and *C. macrorrhiza* from East Austria were included, as the taxonomical status of these taxa is unclear; e.g. designating them as varieties under *C. pyrenaica* or a broader defined *C. officinalis* has been suggested (see Pobedimova 1970, Schultze-Motel 1986). Previously, *Cochlearia bavarica* and *C. pyrenaica* populations from Germany have been analysed using isozymes in order to elucidate systematics and evolution of the polyploid complex *Cochlearia* (Koch et al. 1998a). In this analysis isozyme data of *C. bavarica* (11 populations) and *C. pyrenaica* (8 populations) were not evaluated at the population level. The original data were reevaluated and compared to populations from Austria (12 populations of *C. pyrenaica*, one population of *C. macrorrhiza*, four populations of *C. excelsa*). In Austria nearly all extant *Cochlearia* populations have been analysed to reveal total genetic diversity, and only a few single individual "populations" have not been analysed. *Cochlearia* populations in Austria are highly endangered, for example only one *C. macrorrhiza* population of two individuals survived in the year 2000. *Cochlearia excelsa* is restricted to

**Table 1.** Collection data and sources of *Cochlearia* accessions examined for isozyme analyses. Detailed information about *Cochlearia bavarica* accessions nos. 14 to 25 and *C. pyrenaica* accessions nos. 80 to 89 can be viewed in Koch et al. (1998a). In total 607 individuals have been analysed

| | |
|---|---|
| *C. bavarica* (Germany) | 180 individuals analysed in total |
| pop. nos. 14–25: | for details refer to Koch et al. (1998a) |
| *C. pyrenaica* (Germany) | 160 individuals analysed in total |
| pop. nos. 82–89: | for details refer to Koch et al. (1998a) |
| *C. pyrenaica* (Austria) | 175 individuals analysed in total |
| Lassingbachtal, LOWER AUSTRIA, 860 m, N47°50′–E15°24′, 20 individuals | |
| Molterboden, LOWER AUSTRIA, 910 m a.s.l., N47°50′–E15°24′, 10 individuals | |
| Utreichsberg, LOWER AUSTRIA, 880 m a.s.l., N47°50′–E15°25′, 15 individuals | |
| Hauptretzhof south Türnitz, LOWER AUSTRIA, 510 m a.s.l., N47°53′–E15°28′, 15 individuals | |
| Kumpfmühle Halbach valley, LOWER AUSTRIA, 555 m a.s.l., N47°56′–E15°41′, 20 individuals | |
| Sieben Quellen near Mürzsteg, LOWER AUSTRIA, 800 m a.s.l., N47°41′–E15°35′, 15 individuals | |
| Niederalpl, west Mürzsteg, LOWER AUSTRIA, 950 m a.s.l., N47°40′–E15°24′, 15 individuals | |
| Brunntalgraben, west Mürzsteg, LOWER AUSTRIA, 810 m a.s.l., N47°40′–E15°28′, 15 individuals | |
| Dobrein, LOWER AUSTRIA, 820 m a.s.l., N47°40′–E15°27′, 5 individuals | |
| Haringbachgraben, STYRIA, 880 m a.s.l., N47°32′–E15°05′, 15 individuals | |
| Gams near Hieflau, STYRIA, 625 m a.s.l., N47°40′–E14°46′, 15 individuals | |
| Lehen near Lunz a.S., LOWER AUSTRIA, 580 m a.s.l., N47°50′–E15°01′, 15 individuals | |
| *C. macrorrhiza* | 12 individuals analysed in total |
| Moosbrunn near Vienna, 200 m a.s.l., LOWER AUSTRIA, N48°00′–E16°26′, 2 individuals | |
| (additional 10 individuals grown from seed material obtained from the Botanical Garden Berlin–Dahlem, originated from Moosbrunn near Vienna as well) | |
| *C. excelsa* | 80 individuals analysed in total |
| Turrach, Dieslingsee, STYRIA, 2100 m a.s.l., N46°51′–E13°56′, 20 individuals | |
| Turrach, Eisenhut, STYRIA, 2350 m a.s.l., N46°51′–E13°56′, 20 individuals | |
| Seckauer Zinken 1, STYRIA, 2320 m a.s.l., N47°20′–E14°44′, 20 individuals | |
| Seckauer Zinken 2, STYRIA, 2250 m a.s.l., N47°20′–E14°44′, 20 individuals | |

two high-alpine areas with several subpopulations, respectively.

Leaf material was harvested directly in the wild, stored at 4 °C for transport on ice and frozen within 10 hours in liquid nitrogen for subsequent storage at −80 °C until further investigations.

Numbers of individuals collected and investigated are listed in Table 1.

Vouchers from representatives of all populations are deposited at the herbarium WHB (University for Agricultural Science Vienna).

**Electrophoresis and enzyme assay.** Three isozyme systems were assayed: Aspartate aminotransferase (AAT, E.C. 2.6.1.1), phosphoglucomutase (PGM, E.C. 2.7.5.1) and leucine aminopeptidase (LAP, E.C. 3.4.11.1). Detailed protocols of extract preparation and experimental procedure are described in Koch et al. (1998a). For these three isozymes representing nine loci the detailed distribution of the corresponding alleles in the different species can be viewed in Koch et al. (1998a) and herein are not shown again. The same designation for loci and alleles have been used for Austrian *Cochlearia* to enable direct comparisons. Enumeration of German *Cochlearia* populations is identical (*C. bavarica*: acc. nos. 14 to 25 and *C. pyrenaica*: acc. nos. 82 to 89) to Koch et al. (1998a).

Additional isozyme data of *C. bavarica* from Paschke (2001) have been evaluated. They used a starch-gel system and resolved additional allozymes from aconitase (ACO, E.C. 4.2.1.3), isocitrat-dehydrogenase (IDH, E.C. 1.1.1.41), 6-Phosphogluconat-dehydrogenase (6PGDH, E.C. 1.1.1.49), phosphogluco isomerase (PGI, E.C. 5.3.1.9) and shikimi-dehydrogenase (SKDH, E.C. 1.1.1.25).

**Genetic analysis.** For the diploid species genotype frequencies were calculated using BIOSYS-1 (Swofford and Selander 1989) to estimate the direct-count heterozygosity across all loci ($H_{obs}$), the expected heterozygosity under Hardy-Weinberg

equilibrium across all loci ($H_{exp}$), and genetic distances between populations according to Nei (1978). Genetic differentiation of populations was investigated by F-statistics (Wright 1965, 1978; Nei 1977) using the BIOSYS-1 software package. The total heterozygosity ($H_T$), the average heterozygosity among subpopulations ($H_S$), and the fixation index ($F_{ST}$) were determined as well as $F_{IS}$, the inbreeding coefficient.

For hexaploid *C. bavarica* a genotypic characterisation was not possible. Therefore, we used allele frequencies as basis for measurements of genetic distances between populations. Genetic differentiation was analysed using the presence and absence of diagnostic alleles. However, as *C. bavarica* is highly self-incompatible high levels of heterozygosity and populations in Hardy-Weinberg equilibrium were expected. The step WRIGHT78 was performed in BIOSYS-1 using the NOHRCHY option in order to obtain a value for genetic differentiation. Each population ("deme") was treated as a subdivision of the total set of populations, which is the only possible non-hierarchical analysis employing F-statistics when genotype frequencies are not available. The resulting $F_{DT}$ value can be used compared to $F_{ST}$ derived from genetic data of the diploids.

Correlation between geographic distance and the corresponding genetic distance among populations have been analysed to evaluate genetic isolation by distance. For statistical analysis the SPSS software package was used. UPGMA cluster analyses based on genetic distances among populations (derived from allele frequencies within populations) were performed to generate a phenogram showing additional geographic structuring of genetic variation among Austrian diploid *Cochlearia* populations using BIOSYS-1 (Swofford and Selander 1989). Koch et al. (1998a) have shown that isozyme-based phenograms separated *C. bavarica* into two significant differentiated groups and, furthermore, that there is no significant geographic structuring of German *C. pyrenaica* populations at all. Therefore, no additional phenograms including German *Cochlearia* populations were presented.

## Results

**Allelic variation in *Cochlearia bavarica* and *C. pyrenaica*.** No new alleles have been found compared to Koch et al. (1998a). However, for *C. macrorrhiza* and *C. excelsa* two and three additional alleles were detected, respectively (Table 2). The distribution of the observed alleles among the different taxa and populations is shown in Table 2. Allele frequencies for *C. bavarica*, *C. pyrenaica*, *C. excelsa* and *C. macrorrhiza* are summarised in Table 3. Genotype frequencies of diploid *Cochlearia* are not shown and are available upon request.

*Cochlearia bavarica* populations have five alleles which were not present in diploid *C. pyrenaica* (Table 2, indicated by a double-cross). All five alleles are present in tetraploid *C. officinalis*, one of the putative parents of *C. bavarica* (Koch et al. 1998a). Populations of *C. bavarica* from the two different areas (SE Bavaria and Allgäu) differed among each other by five alleles. Three out of the five alleles are present in *C. officinalis* but not in *C. pyrenaica* (*Aat2-7*, *Aat3-3*, *Pgm3-5*). The remaining two alleles are found in *C. officinalis* as well as in *C. pyrenaica* (*Lap1-2*, *Lap1-3*).

The results from Paschke (2001) are noteworthy (Table 4) because they found the allele *Pgm2-2* in populations of *C. bavarica* from both distribution areas, a finding that differs from Koch et al. (1998a). Furthermore, five additional alleles from additional isozymes have been found which distinguish both distribution areas (*Aco1-3*, *Aco2-2*, *Idh1-1*, *Idh1-2*, *6PGDH*). The two alleles which were found in *C. bavarica* as well as in *C. pyrenaica* (*Lap1-2*, *Lap1-3*) showed no geographical distribution pattern in *C. pyrenaica* (neither when presence-absence nor when allele frequencies were considered). Two of the three alleles (only LAP, AAT and PGM were considered) which originated from *C. officinalis* are rare in both *C. officinalis* and *C. bavarica* (*Aat3-3* and *Pgm3-5*) with an average allele frequency below 0.05%. There are no data available for allelic distribution of IDH, 6PGDH and ACO isozymes in *C. pyrenaica* and *C. officinalis*.

Austrian populations of diploid *Cochlearia* showed only 69% of the alleles found in German *Cochlearia* populations, which might reflect the relict status of these populations in East Austria (Table 2).

**Table 2.** Distribution of the alleles at the analysed loci for aspartate aminotransferase (*Aat*), leucine aminopeptidase (*Lap*) and phosphoglucomutase (*Pgm*) among taxa of section *Cochlearia* analysed herein. *Cochlearia bavarica* is split according to the disjunct areas in southern Germany

| | *Aat* | | | | | | | | | | | *Pgm* | | | | | | | | *Lap* | | | | | | | | | Σ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | 1 | | | 2 | | | | | | 3 | | | | 1 | 2 | | 3 | | | | 1 | | | | 2 | 3 | | | |
| Alelle | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | |
| *C. bavarica* (SE Bavaria) | + | + | – | + | + | # | + | – | # | # | + | – | + | + | – | [1] | + | + | – | – | – | – | + | + | + | + | – | + | + | – | 22 |
| *C. bavarica* (Allgäu) | + | + | – | + | + | # | + | – | # | – | + | – | + | + | – | – | + | + | – | + | – | # | + | – | – | + | – | + | + | – | 20 |
| *C. pyrenaica* GERMANY | + | + | – | + | + | – | + | – | – | – | + | – | + | + | – | – | + | + | – | + | – | – | + | + | + | + | – | + | + | – | 20 |
| *C. pyrenaica* AUSTRIA | + | + | – | + | + | – | + | – | – | – | – | + | – | + | + | – | + | – | – | + | – | – | – | – | + | + | – | + | + | – | 16 |
| *C. macrorrhiza* | + | – | – | – | + | – | – | – | – | – | + | – | – | + | + | – | + | – | – | + | – | – | – | – | + | – | – | – | + | – | 12 |
| *C. excelsa* | + | + | – | – | + | – | – | – | – | – | + | – | – | + | + | – | – | + | – | – | – | – | – | – | + | – | – | + | + | – | 12 |
| [2]*C. officinalis* | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | + | + | + | + | – | + | + | + | + | – | + | + | – | 29 |

Alleles present: +, # (# means "alleles from *C. officinalis* not found in *C. pyrenaica*"), alleles missing: –
[1] Detected by Paschke (1997); [2] Data for *C. officinalis* compiled from Koch et al. (1998)

## Population structure and genetic differentiation

***Cochlearia pyrenaica*, *C. excelsa* and *C. macrorrhiza*.** The mean direct-counted heterozygosity of the eight populations from Germany is 0.153 (SD 0.098) compared to 0.164 (SD 0.097) as expected under Hardy-Weinberg-equilibrium (HWE). These values demonstrate that despite self-compatibility the genetic population structure of *C. pyrenaica* is similar to that of an outbreeder. This could simply be explained by the fact that flowers are visited very frequently by pollinators. Furthermore, flower architecture of all three diploid taxa decreases the likelihood of transferring pollen from stamen to pistils of the same flower.

The same was observed for Austrian *Cochlearia* taxa (Table 4). F-statistics (Table 5) similarly support these findings. The inbreeding coefficient ($F_{IS}$) is nearly zero for all diploid taxa, implying nearly permanent outcrossing. Furthermore, $F_{ST}$ values are above 0.25 and indicate significant genetic differentiation of populations relative to the total (Wright 1978).

There is no geographic structuring of genetic diversity among German *C. pyrenaica* populations. Considering only Austrian *Cochlearia* populations some biogeographical differentiation can be observed (Fig. 2). The UPGMA cluster analysis of genetic distance between populations separated *C. excelsa* from *C. pyrenaica*, while four *C. pyrenaica* populations (marked with a circle in Fig. 2) are combined to one single cluster, all of which correlate with geographical distribution.

No additional structuring of genetic diversity is detectable, and *C. macrorrhiza* is integrated into *C. pyrenaica*.

***Cochlearia bavarica*.** *Cochlearia bavarica* is highly self-incompatible. The breeding system maximises genetic diversity, and mean heterozygosity should have values equal to these expected under Hardy-Weinberg equilibrium. However, because of missing genotype frequencies it was not possible to test this hypothesis, and because of the hexaploid genome constitution, gene diversity can not

be compared directly to *C. pyrenaica*. Nonetheless, genetic diversity measured as total number of different alleles (24 alleles) is higher than in allohexaploid *C. polonica* (15 alleles) and *C. tatrae* (20 alleles) (Koch et al. 1998a, Table 4), and it is even higher than in *C. pyrenaica*. (21 alleles). The breeding system has likely prevented populations from genetic erosion and loss of alleles since the formation of *C. bavarica*. The F-statistics performed in a non-hierachical way using each population (''deme'') as a subdivision of the total set of populations resulted in a $F_{DT}$ value equal 0.257, indicating great genetic differentiation between populations. The corresponding values for each region (Allgäu and SE Bavaria) calculated separately are similar to each other (0.081 and 0.109, respectively), indicating that most of the genetic variation is distributed between the two areas, rather than within regions.

**Geographic distribution of genetic variation.** Strong genetic differentiation by distance was observed for *C. bavarica* ($r = 0.8278$, $P < 0.0001$, Fig. 3a). However, this is only due to the high amount of differentiation between the two distribution areas. The analysis of these regions separately revealed no significant differentiation by distance. No genetic differentiation according to geographical distances could be detected for *C. pyrenaica* in Germany ($r = 0.070$, $P = 0.76$) or Austria ($r = 0.043$, $P = 0.72$) (Fig. 3b and c). If all Austrian diploid populations were combined within one data set (Fig. 2d) there was some correlation between genetic and geographic distances ($r = 0.2952$, $P = 0.0005$), but this is because of genetically and geographically isolated *C. excelsa* (Fig. 2). *Cochlearia excelsa* is the only species of the four taxa under study of high alpine regions, and it represents a relict in presumably mostly unglaciated alpine regions of the SE Alps with a high amount of relictual and endemic plant species. The maximum extent of the Würm glaciation is indicated in Fig. 2 (information redrawn from Tribsch 2000, ''Maximum extent of the Würm-ice sheet'' in Dobeš and Vitek, eds.).

## Discussion

The breeding system (self-incompatibility) in *C. bavarica* and the population biology of *C. pyrenaica* have maintained high levels of genetic differentiation in both species and high levels of genetic differentiation between populations relative to the total. The data presented here support the assumption that there was only minor loss of genetic variation in both species. Even in East Austria, which has a far more complex glacial and postglacial history than Bavaria, no major structuring of genetic variation in relation to geographic distribution could be observed (with the exception of *C. pyrenaica* populations in the West-East directed Dobrein valley system with several creeks and the adjacent Mürz river valley, Fig. 2).

Considering this, three alternative explanations can explain the strong genetic differentiation in relation to geographical distances in *C. bavarica*: (1) *C. bavarica* has evolved twice independently, (2) *C. bavarica* migrated from one refugium to the other in postglacial times, or (3) a former, inter- or postglacial continuous distribution area with a gradient of genetic differentiation had become disrupted.

**Polytopic origin?** The first hypothesis assumes that the genetic variation of at least one parental taxon showed enough geographic differentiation to account for the differences in five alleles (considering only AAT, PGM, and LAP) or 11 alleles (considering data from Paschke (2001)) among the two distribution areas. However, *C. pyrenaica* showed no geographic partition of genetic variation. One would therefore assume that two genetically very distinct *C. officinalis* gene pools served as parental gene reservoir, although this assumption is unlikely for at least two reasons:

First, while there are no extant populations of *C. officinalis* in Central Europe, *C. officinalis* likely had a continuous distribution in inter- or postglacial times. For example, ''inland'' or ''fresh-water'' ecotypes are known from Scandinavia, which evolved from coastal *C. officinalis* during its postglacial colonisation. There

**Table 3.** Allele frequencies for three isozyme systems (aspartate aminotransferase AAT; phosphogluco mutase PGM, leucine aminopeptidase LAP) in *Cochlearia* populations under study. Population numbers correspond to Table 1

| | *Aat* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Locus* | 1 | | 2 | | | | | | 3 | | |
| *Allele* | 1 | 2 | 1 | 2 | 3 | 4 | 6 | 7 | 1 | 2 | 3 |
| *C. bavarica* | | | | | | | | | | | |
| 18 Glonn | 0.200 | 0.800 | 0.333 | 0.250 | | 0.033 | | 0.384 | 0.950 | | 0.050 |
| 23 Thalham | | 1.000 | | | 0.050 | 0.433 | 0.100 | 0.417 | 1.000 | | |
| 24 Vagen | | 1.000 | 0.033 | 0.100 | | 0.267 | 0.433 | 0.167 | 0.650 | 0.317 | 0.033 |
| 14 near Baiersried | 0.717 | 0.800 | 0.017 | 0.383 | 0.367 | 0.233 | | | 1.000 | | |
| 15 Eßmühle | 0.800 | 0.200 | | 0.517 | 0.483 | | | | 1.000 | | |
| 16 Friesenried | 0.550 | 0.450 | 0.150 | 0.400 | 0.233 | 0.217 | | | 1.000 | | |
| 17 Gfäll | 0.817 | 0.183 | | 0.450 | 0.517 | 0.033 | | | 0.933 | 0.067 | |
| 19 Immenthal | 1.000 | | | 0.284 | 0.533 | 0.183 | | | 1.000 | | |
| 20 Klessen | 0.788 | 0.212 | 0.017 | 0.590 | 0.317 | 0.076 | | | 0.970 | 0.030 | |
| 21 Ollarzried | 0.850 | 0.150 | 0.150 | 0.267 | 0.300 | 0.233 | 0.050 | | 1.000 | | |
| 22 Stoßberg | 0.550 | 0.450 | | 0.283 | 0.317 | 0.333 | 0.067 | | 1.000 | | |
| 25 Zadels | 1.000 | | 0.033 | 0.433 | 0.317 | 0.217 | | | 1.000 | | |
| *C. excelsa* | | | | | | | | | | | |
| Dieslingsee | 1.000 | | | 1.000 | | | | | 1.000 | | |
| Eisenhut | 0.525 | 0.475 | | 1.000 | | | | | 1.000 | | |
| Seckauer Tauern1 | 0.600 | 0.400 | | 1.000 | | | | | 1.000 | | |
| Seckauer Tauern2 | 0.389 | 0.611 | | 1.000 | | | | | 1.000 | | |
| *C. macrorrhiza* | | | | | | | | | | | |
| Moosbrunn | 1.000 | | | 1.000 | | | | | 1.000 | | |
| *C. pyrenaica* GER | | | | | | | | | | | |
| 82 Einbeck | 0.800 | 0.200 | | 0.300 | | 0.700 | | | 1.000 | | |
| 83 Horgau | | 1.000 | | 0.250 | | 0.750 | | | 1.000 | | |
| 84 Kelmis | | 1.000 | | 1.000 | | | | | 1.000 | | |
| 85 Oberalme | 0.866 | 0.134 | 0.232 | 0.768 | | | | | 0.500 | 0.500 | |
| 86 Obernhausen | | 1.000 | | 1.000 | | | | | 1.000 | | |
| 87 Waging a. S. | 0.900 | 0.100 | | 1.000 | | | | | 1.000 | | |
| 88 Taching | 1.000 | | | 1.000 | | | | | 1.000 | | |
| 89 Zusmarshausen | 0.400 | 0.600 | 0.200 | 0.750 | | 0.050 | | | 1.000 | | |
| *C. pyrenaica* AUS | | | | | | | | | | | |
| Lassingbach | 1.000 | | | 1.000 | | | | | 1.000 | | |
| Haringbach | 1.000 | | | 1.000 | | | | | 1.000 | | |
| Kumpfmühle | 1.000 | | 0.313 | 0.688 | | | | | 1.000 | | |
| Molterboden | 1.000 | | | 1.000 | | | | | 1.000 | | |
| Gams | 1.000 | | | 0.962 | | 0.038 | | | 1.000 | | |
| Lehen | 1.000 | | | 1.000 | | | | | 1.000 | | |
| Utreichsberg | 1.000 | | | 1.000 | | | | | 1.000 | | |
| Hauptretzhof | 1.000 | | | 0.389 | | 0.611 | | | 1.000 | | |
| Sieben Quellen | | 1.000 | | 0.636 | | 0.364 | | | 1.000 | | |
| Niederalpl | 0.250 | 0.750 | | 0.536 | | 0.464 | | | 1.000 | | |
| Brunntal | | 1.000 | | 0.300 | | 0.700 | | | 1.000 | | |
| Dobrein | | 1.000 | | 0.500 | | 0.500 | | | 1.000 | | |

**Table 3** (continued)

| *Pgm* | | | | | | *Lap* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | | 3 | | | | 1 | | | | 3 | |
| 1 | 2 | 1 | 2 | 4 | 5 | 1 | 2 | 3 | 4 | 1 | 2 |
| 1.000 | | 0.233 | 0.767 | | | 0.133 | 0.367 | 0.133 | 0.367 | | 1.000 |
| 1.000 | | | 1.000 | | | 0.167 | 0.167 | | 0.666 | 1.000 | |
| 1.000 | | | 1.000 | | | | 0.500 | | 0.500 | 1.000 | |
| 0.467 | 0.533 | | 1.000 | | | 0.500 | | | 0.500 | 0.700 | 0.300 |
| 0.333 | 0.667 | 0.333 | 0.667 | | | 0.500 | | | 0.500 | 0.500 | 0.500 |
| 0.617 | 0.383 | 0.667 | 0.100 | | 0.233 | 0.500 | | | 0.500 | 0.500 | 0.500 |
| 0.061 | 0.939 | | 1.000 | | | 0.500 | | | 0.500 | 0.500 | 0.500 |
| 0.200 | 0.800 | 0.333 | 0.667 | | | 1.000 | | | | 0.500 | 0.500 |
| 0.212 | 0.788 | 0.333 | 0.667 | | | 0.500 | | | 0.500 | 0.500 | 0.500 |
| 0.233 | 0.767 | 0.333 | 0.200 | | 0.467 | 0.500 | | | 0.500 | 0.500 | 0.500 |
| 0.483 | 0.517 | 0.233 | 0.567 | | 0.200 | 0.600 | | | 0.400 | 0.500 | 0.500 |
| 0.300 | 0.700 | 0.700 | 0.300 | | | 1.000 | | | | 0.500 | 0.500 |
| 1.000 | | | 1.000 | | | | | 1.000 | | | 1.000 |
| 1.000 | | | 1.000 | | | | | 1.000 | | | 1.000 |
| 0.300 | 0.700 | | 1.000 | | | | | 1.000 | | 0.400 | 0.600 |
| | 1.000 | | 1.000 | | | | | 1.000 | | 0.556 | 0.444 |
| 0.833 | 0.167 | 0.167 | 0.167 | 0.667 | | | | 1.000 | | 0.167 | 0.833 |
| 1.000 | | 1.000 | | | | | 0.750 | | 0.250 | 0.750 | 0.250 |
| 0.250 | 0.750 | | 0.350 | 0.650 | | 0.450 | 0.550 | | | | 1.000 |
| 1.000 | | 0.050 | 0.950 | | | | | | 1.000 | | 1.000 |
| 0.720 | 0.280 | 0.378 | 0.622 | | | 0.222 | 0.611 | | 0.167 | | 1.000 |
| 0.550 | 0.450 | 0.550 | 0.450 | | | | | | 1.000 | | 1.000 |
| 0.550 | 0.450 | 1.000 | | | | | 0.650 | | 0.350 | 0.200 | 0.800 |
| 0.600 | 0.400 | 1.000 | | | | | | 1.000 | | | 1.000 |
| 0.450 | 0.550 | | 0.700 | 0.300 | | 0.850 | 0.050 | 0.100 | | 0.100 | 0.900 |
| 1.000 | | 1.000 | | | | | | | 1.000 | 1.000 | |
| 1.000 | | 1.000 | | | | | | | 1.000 | 1.000 | |
| 1.000 | | 1.000 | | | | | | 1.000 | | 1.000 | |
| 1.000 | | 0.400 | | 0.600 | | | | | 1.000 | 1.000 | |
| 1.000 | | 0.692 | | 0.308 | | | | | 1.000 | 1.000 | |
| 1.000 | | 0.500 | | 0.500 | | | | | 1.000 | 0.682 | 0.318 |
| 1.000 | | 1.000 | | | | | | | 1.000 | 0.350 | 0.650 |
| 0.667 | 0.333 | 1.000 | | | | | | | 1.000 | 0.222 | 0.778 |
| 0.273 | 0.727 | 0.955 | | 0.045 | | | | | 1.000 | | 1.000 |
| 0.857 | 0.143 | 0.821 | | 0.179 | | | | | 1.000 | 0.571 | 0.429 |
| 1.000 | | 0.900 | | 0.100 | | | | | 1.000 | 0.650 | 0.350 |
| 0.500 | 0.500 | 1.000 | | | | | | | 1.000 | 0.500 | 0.500 |

**Table 4.** Comparison of allele frequencies of alleles not shared between *C. bavarica* populations from the two disjunct areas in southern Germany. Data from Paschke (1997) and Paschke (2001) are also shown (*)

| | *C. BAVARICA* | | *C. PYRENAICA* | |
|---|---|---|---|---|
| Allele | SE Bavaria | Allgäu | GERMANY | AUSTRIA |
| *Aat2-7* | 0.322 (0.11) | 0.000 | ONLY *C. officinalis* | |
| | 0.171 (0.11)* | 0.000* | | |
| *Aat3-3* | 0.030 (0.02) | 0.000 | ONLY *C. officinalis* | |
| | locus not resolved* | | | |
| *Pgm2-2* | 0.000 | 0.677 (0.16) | 0.640 (0.24) | 0.142 (0.23) |
| | 0.354 (0.35)* | 0.864 (0.16)* | | |
| *Pgm3-5* | 0.000 | 0.100 (0.16) | ONLY *C. officinalis* | |
| | 0.000* | 0.032 (0.07)* | | |
| *Lap1-2* | 0.344 (0.14) | 0.000 | 0.326 (0.32) | 0.000 |
| | locus not resolved* | | | |
| *Lap1-3* | 0.044 (0.06) | 0.000 | 0.137 (0.32) | 0.083 (0.27) |
| | allele not resolved* | | | |
| *Aco1-3** | 0.317 (0.21) | 0.000 | UNKNOWN | UNKNOWN |
| *Aco2-2** | 0.000 | 0.114 (0.13) | UNKNOWN | UNKNOWN |
| *Idh1-1** | 0.000 | 0.134 (0.13) | UNKNOWN | UNKNOWN |
| *Idh1-2** | 0.000 | 0.130 (0.13) | UNKNOWN | UNKNOWN |
| *6-PGDH2-1** | 0.050 (0.122) | 0.000 | UNKNOWN | UNKNOWN |

are furthermore tetraploid *Cochlearia* on heavy metal enriched sites at inland stations in Great Britain (Koch et al. 1998a). A former continuous continental distribution is also supported by the fact that *C. officinalis* was also involved as parental taxon during the constitution of inland *C. tatrae* and *C. polonica* in Poland and Slovak Republic (Koch et al. 1998a).

Second, it has been demonstrated that there is a good correlation between northern distribution and total genetic variation (measured as alleles per population) in *C. officinalis*: i.e. number of alleles decreases with increasing northern distribution (one allele per two northern paralleles corresponding to approximately 230 km). This genetic erosion has been explained by migration and colonisation accompanied by genetic drift (Koch et al. 1998a). Taking this value of genetic depauperation during migration (one allele per 230 km) as an estimator for genetic differentiation of postglacial inland *C. officinalis*, the geographic distances between the two *C. bavarica* distribution areas (approximately 170 km) are not great enough to explain the genetic differences in present day *C. bavarica* with five alleles per 170 km, a seven-fold higher differentiation measured as loss of alleles per distance.

**Migration?** The migration model assumes extensive genetic drift which greatly exceeded that which has effected *C. officinalis*. Under the migration model one should be able to detect direction of migration measured as a gradient of decreasing genetic variation. However, this is not the case, as five alleles are unique to the Allgäu and SE Bavarian regions, respectively (Table 4). Furthermore, within-region population differentiation is comparable among both regions.

**Disruption and extinction?** It is thus more likely that *C. bavarica* is the result of a single ancient origin and has previously occupied a greater distribution area. These populations may have been genetically differentiated over geographic distance with subsequent extinction of geographically intermediate populations leading to the disrupted distribution area seen today.

However, this model similarly assumes genetic drift, although we can not distinguish

**Table 5.** Gene diversity and F-statistics of *Cochlearia* taxa under study

| | Mean heterozygosity | | | | | |
|---|---|---|---|---|---|---|
| | $H_S$ | HWE[a] | $F_{IS}$ | $F_{IT}$ | $H_T$ | $F_{ST}$ |
| German *C. pyrenaica* | 0.153 (0.098) | 0.164 (0.097) | 0.026 | 0.565 | 0.343 | 0.554 |
| Austrian *C. pyrenaica* | 0.117 (0.082) | 0.126 (0.085) | −0.117 | 0.497 | 0.260 | 0.550 |
| *C. macrorrhiza* | 0.111 (0.056) | 0.141 (0.075) | nc | nc | nc | nc |
| *C. excelsa* | 0.102 (0.031) | 0.113 (0.046) | 0.027 | 0.475 | 0.189 | 0.460 |
| SE Bavaria *C. bavarica* | | 0.214 (0.056) | | | | |
| Allgäu *C. bavarica* | | 0.313 (0.053) | | | | |

[a] expected under HWE
nc: not computed because of too few individuals

between loss of alleles through drift before populations disappeared, or enhanced drift effects accompanying the separation of the two distribution areas. A combination of both processes likely explains the data, with allelic diversity being more effectively decreased in *C. bavarica* compared to *C. officinalis*.

In support of this, there is a strong positive correlation between population size and genetic diversity (Paschke 2001, Paschke et al. 2002), and thus if migration took place (or even if population size varied to a greater extent) from a large population to smaller isolated colonies or islands of populations, it was repeatedly accompanied by a significant loss of genetic variation. There is furthermore a correlation between genetic diversity and habitat type (Paschke 2001) with highest diversity being found in open habitats (tundra-like), the likely "ancestral" habitat type. In contrast shadowed woodland habitats are characterised by decreased genetic diversity. Both findings might explain an increasing effect of genetic drift (and maybe selection) acting on populations from both distribution areas, resulting in increased levels of genetic erosion.

**Assumptions on Austrian diploid *Cochlearia*.** The isozyme data might indicate that *C. macrorrhiza* is a particular morphotype of *C. pyrenaica* rather than a separate lineage. However, *C. macrorrhiza* is not only characterised by enhanced root growth resulting in felt-like rootstocks, but also the infructescence is less elongated than in *C. pyrenaica* from Germany, Austria and France (Vogt 1985). Furthermore, *C. macrorrhiza* grows at lower elevations in Austria, at least 350 m, than *C. pyrenaica* (see Table 1). These findings could serve as an indicator for a separate status of *C. macrorrhiz*a, despite the sampling of only one population, which might have resulted in a strong statistical bias.

It has been shown that the distribution area of *C. pyrenaica* extends far more to eastern Europe. Populations of *C. pyrenaica* have been reported from the West Caparthians in Slovakia (Valachovič and Kochjarová 2000) and from the East Carpathians in Romania (Stefureac and Lungeanu 1976). Populations from Slovakia are diploid (Kochjarová, pers. comm.), whereas populations from Romania, named as *C. pyrenaica* DC. var. *borsaeana* Coman et Nyár. (Coman 1946), have been reported to be hexaploid (Lungeanu 1972). These observations might imply that *C. macrorrhiza* is one of the diploids bridging the alpine-carpathian disjunction. Future analysis of diploid populations from Austria and the Eastern part of Central Europe and Southeastern Europe using molecular markers with increased genotypic resolution (AFLPs – amplified fragment length polymorphisms) might provide more significant evidence for the status of *C. macrorrhiza*.

The decrease in genetic diversity of Austrian *C. pyrenaica* relative to German *C. pyrenaica* is remarkable (Table 2), assuming that

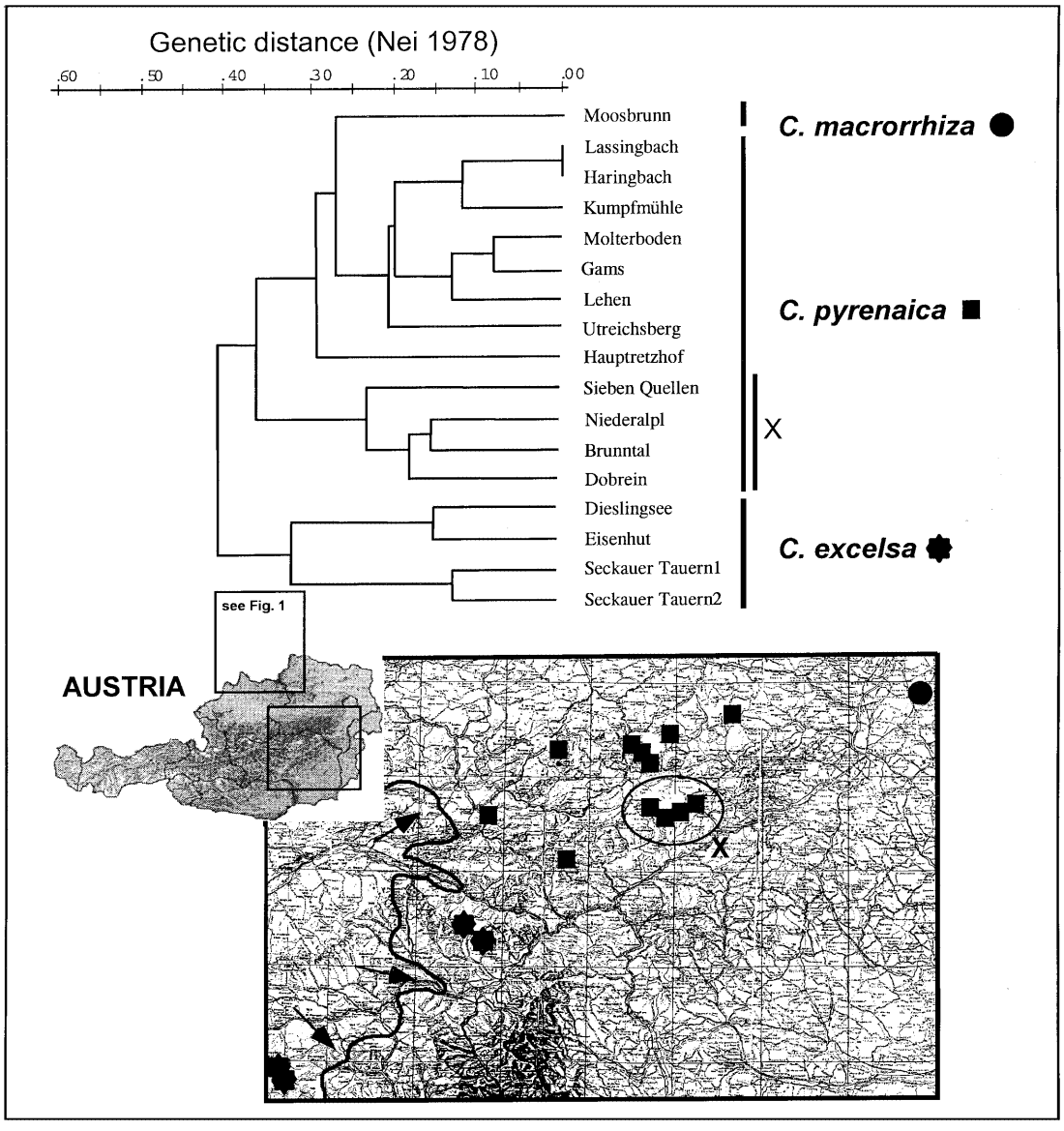


**Fig. 2.** Distribution of *Cochlearia pyrenaica* (■), *C. macrorrhiza* (●), and *C. excelsa* (✶) populations analysed herein. The phenogram has been calculated from allele frequencies among populations using Nei's (1978) unbiased genetic distance and the UPGMA method. X indicates populations from the East-West directed Dobrein valley system. The maximum extension of the last glacier (Würm glaciation) is indicated as solid line. The arrows indicate the direction of the glacier front line. The geographic position of the map in Fig. 1 is indicated

Austrian populations provided the source for the postglacial recolonization in Germany. As the data presented here cannot explain this unexpected distribution pattern of genetic variation, two explanations are possible: 1) additional source areas (e.g. the Pyrenees) were involved in postglacial recolonization, or 2) there was a major loss of genetic variation in Austrian populations after recolonization of Central Europe.

A second relictual area in the Pyrenees, where *C. pyrenaica* is still distributed, is

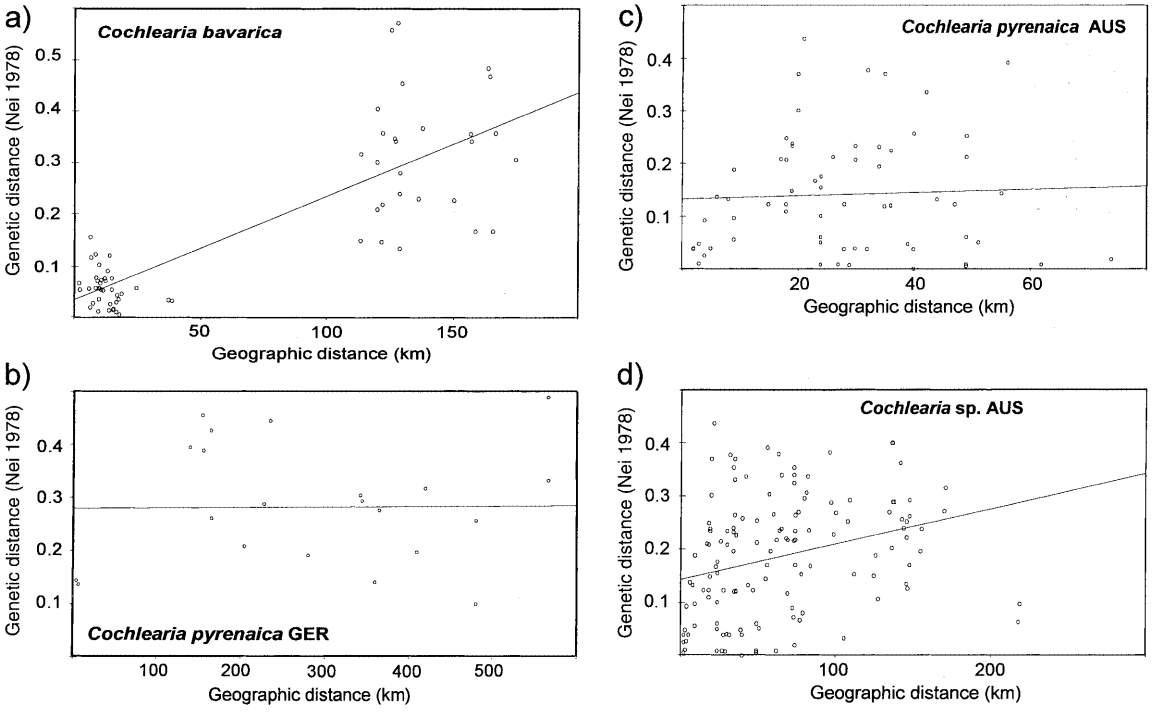


**Fig. 3.** Correlation of inter-populational genetic distances (Nei 1978) and corresponding geographic distances. **a** *Cochlearia bavarica*, **b** *Cochlearia pyrenaica* in Germany, **c** *Cochlearia pyrenaica* in Austria, **d** all diploid Austrian *Cochlearia* combined. For details refer to text

possible. Several molecular studies demonstrated that the Iberian peninsula served as one out of three main pleistocenic glacial refugia (e.g. Konnert and Bergmann 1995, Hewitt 1996, Comes and Kadereit 1998, Taberlet et al. 1998, Hewitt 1999, Sharbel et al. 2001). Moreover, the ancestral diploid taxon for most polyploid coastal species, namely *C. aestuaria*, is endemic to NW Spain (Koch et al. 1996, 1998a). Molecular data showed extremely low genetic differentiation among taxa of section *Cochlearia* considering ITS (nuclear internal transcribed spacer regions) and plastidic *trn*L intron sequences, which indicate a pleistocenic or holocenic origin for all taxa (Koch et al. 1999). The closest relatives from section *Glaucocochlearia* are also endemic to Spain and NW Africa and are much older.

The distribution and ecological differentiation of diploid *Cochlearia* such as *C. aestuaria* in Spain and high-alpine Austrian *C. excelsa*, indicate significant differentiation processes prior to the last maximum glaciation. The present-day distribution of *C. pyrenaica* in Austria in a small area of the SE lower Alps between appr. 550 to 1000 m can be regarded as a result of range size fluctuations since the last maximum glaciation about 20,000 years ago. However, according to Tribsch (2000) all *C. pyrenaica* populations in Austria are located outside the maximum ice shield of the last maximum Würm glaciation (see Fig. 2) and no recolonization took place. The most likely reason for this is the strict ecological preference to cold carbonate-rich water springs. These habitats are rare and therefore colonization is more unlikely than for many other habitats.

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