Intraspecific range dynamics and niche evolution in *Candidula* land snail species

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Received 5 June 2005; accepted for publication 1 March 2006

The range dynamics of a species can either be governed by the spatial tracing of the fundamental environmental niche or by adaptation that allows to occupy new niches. Therefore, the investigation of spatial variation in the realized environmental niche is central to the understanding of species range limit dynamics. However, the study of intraspecific niche variation has been neglected in most phylogeographical studies. We studied the spatial distribution of the realized environmental niche in three land snail species of the genus *Candidula*, integrating phylogeographical methods, morphometrics, and spatial biodiversity informatics. The phylogeographical analyses showed significant range expansions in all species. These expansions were accompanied in *Candidula gigaxii* by a shift in the realized environmental niche, the species *Candidula unifasciata* followed its ancestral niche during expansion while the climate changed in the area of origin and *Candidula rugosiuscula* tracked the ancestral environmental conditions. The significant niche shifts were associated with potentially adaptive changes of shell morphology. We propose our presented approach as a practicable framework to test hypotheses on intraspecific niche evolution.

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ADDITIONAL KEYWORDS: biodiversity informatics – morphometrics – phylogeography – species range limits.

**INTRODUCTION**

Whether a reproducing population of any given species can be found at any particular site depends on two circumstances: (1) whether the site offers the organism the possibility to survive and reproduce successfully given the local abiotic and biotic characteristics (i.e. the site matches the fundamental niche of the species) and (2) whether the site could in principle be reached via dispersal in the species’ evolutionary history together with the requirement that this actually happened. The geographical range of a species, defined as the area where stably reproducing populations of the respective species can be found (Gaston, 1996), is therefore a result of the interplay of these two requirements (Holt, 2003). However, the range of a species is not static. It can shift, expand or contract in time and space as environmental conditions change and species evolve (Gaston, 1998). The range dynamics of a species can consequently be governed by two processes: (1) spatial tracing of the environmental niche without adaptation, including extinction if the niche vanishes or (2) adaptation to ecological conditions outside the ancestral niche, either allowing to colonize areas not yet occupied or to remain in the ancestral area despite changing conditions. Inferring the relative contribution of these processes is thus crucial for the understanding of a species’ range.

Theoretical studies predict intraspecific niche conservatism as a result of the interplay of dispersal, selection, and demographic asymmetries (Holt & Gaines, 1992; Holt, 1996; Kirkpatrick & Barton, 1997; Case & Taper, 2000). However, empirical evidence for both niche conservatism and niche evolution is scarce and equivocal (Anderson, Laverde & Peterson, 2002;
Peterson et al., 1999; Peterson & Holt, 2003), even though the role of these processes for the species range limits touches central questions of biogeography, speciation, community ecology, and conservation biology (Losos & Glor, 2003; Wiens & Donoghue, 2004). The empirical studies of niche evolution mentioned above mostly involved the analysis of the present day ranges of sister taxa. A largely unexplored issue is the interplay between intraspecific range changes and niche evolution. In the approach presented here, we combine phylogeographical methods to infer past range changes with biodiversity informatics and fieldwork to estimate the realized environmental niche in three land snail species of the genus Candidula (Hygromiidae, Helicoidea).

Land snails are particularly suited to study this issue for three reasons. First, their restricted dispersal capacity and intraspecific gene-flow tend to preserve phylogeographical patterns (Pfenninger, Poasada & Magnin, 2003b; Ross, 1999; Wilke & Duncan, 2004; Pinceel et al., 2005). Second, the (post)Pleistocene climate changes have led to substantial range dynamics in many species as evidenced in a recent comparative study of the Western-Palaearctic Helicoidea (Pfenninger, 2004). Third, land snail shells are often preserved in a subfossil state (Goodfriend, 1992), which allows cross-validation of phylogeographical hypotheses and the dating of events (Cruzan & Templeton, 2000).

On one hand, the presence of land snails seems to be determined rather by abiotic than biotic interactions. A particular geological underground is often a necessary prerequisite for the occurrence of land snail species (Goodfriend & Stipp, 1983). Survival, activity, and successful reproduction of snails depend strongly on absolute temperatures and available humidity, as well as the range and temporal co-occurrence of both factors (Arad, 2001; Backeljau, Baur & Baur, 2001; Cook, 2001; Gomot de Vaufleury, 2001). Thus, the fit of the ecological niche to the local long-term climate is a prerequisite for the persistence of a viable land snail population. Structural features of the habitat govern the microclimate, which in turn influences spatial and temporal activity patterns such as hibernation, aestivation, or egg deposition (Cook, 2001). On the other hand, the distribution of land snails seems rarely limited by food availability because most taxa are not specialized on particular food sources (Speiser, 2001). No other biological interactions are deemed crucial for the presence or absence of land snails at a particular place.

Climatic variation is known to be an effective selective agent for shell evolution in snails (Goodfriend, 1986; Hellberg, Balch & Roy, 2001). In Candidula, interspecies shell variation among Candidula unifasciata and Candidula rugosiuscula was attributed to differential adaptation to their respective, climatically differentiated habitats (Pfenninger, Eppenstein & Magnin, 2003a). Significant changes in shell morphology may thus be good indicators of an evolutionary response to environmental conditions.

Here, we aim to understand the processes that shaped the present day distributions of three Candidula species. In particular, we focus on two questions: (1) are inferred range expansions associated with shifts in the realized environmental niche and (2) are potential niche shifts reflected in morphological evolution?

MATERIAL AND METHODS

STUDIED TAXA AND THEIR DISTRIBUTION

The three selected taxa, C. unifasciata (Poiré 1801), C. rugosiuscula (Michaud 1831) and Candidula gigaxii (L. Pfeiffer 1857) are small land snails (less than 1 cm in diameter) that display substantial variation in shell morphology (Gittenberger, 1993; Pfenninger & Magnin, 2001). They occur on exposed, dry grass and scrublands, fallows, roadside verges and field margins with sparse vegetation cover, and exclusively on calcareous, alkaline soils or rocks (Magnin, 1993; Bahl et al., 1996). A molecular phylogeny based on mitochondrial 16S, cytochrome c oxidase subunit I and nuclear ITS-1 genes showed that C. unifasciata and C. rugosiuscula are sister species, with C. gigaxii as the closest relative (Pfenninger et al., 2003a).

The distribution ranges of the taxa were approximated using the spatial distribution of 498 Candidula populations (398 populations of C. unifasciata, 85 C. gigaxii populations, and 20 sites of C. rugosiuscula). To this end, the malacological collections of the Senckenberg-Museum, Frankfurt, the Naturkunde Museum, Bern, the private collection of A. Bertrand, and our own collections were examined for Candidula samples. This approach depends critically on the unequivocal species identification. The conchological distinction of C. gigaxii is unproblematic. The morphologically distinct, yet similar C. unifasciata and C. rugosiuscula can be reliably distinguished using mitochondrial 16S rDNA sequences (Pfenninger & Magnin, 2001). In the south east of France, the two species never co-occur in the same population and have distinct ranges due to different climate preferences (Pfenninger et al., 2003a). To avoid potential misidentifications, all populations considered here from this area were molecularly identified. As demonstrated by an extensive molecular survey (Pfenninger & Poasada, 2002), the occurrence of C. rugosiuscula is restricted to this area.

Sites that could not be unequivocally located with the information accompanying the samples were excluded from the analysis. The samples used
reflected the assumed distribution ranges very well (Kerney, Cameron & Jungbluth, 1983), as well as the population density in the different regions.

**PHYLOGEOGRAPHICAL ANALYSES**

Phylogeographical patterns were inferred from the spatial distribution of mitochondrial 16S rDNA haplotype variation for the species *C. gigaxii* and *C. rugosiuscula*. This comparatively short marker sequence has proven its suitability for phylogeographical inference in land snails due to its hypervariable loop regions (Ross, 1999; Guiller et al., 2001; Pfenninger et al., 2003b). For *C. unifasciata*, we relied on the inferences of a published phylogeographical analysis (Pfenninger & Posada, 2002). The *C. rugosiuscula* sequences used in the present study were previously reported by Pfenninger et al. (2003a), accession numbers AY238630-702. Ninety-three individuals of *C. gigaxii* were sequenced for this study. Sampling sites and number of individuals sequenced are shown in Table 1. Details of the molecular genetic analyses, including isolation, primers, and polymerase chain reaction conditions, are provided elsewhere (Pfenninger et al., 2003b). Haplotype sequences were deposited in GenBank (Accession numbers AY966496-588) and were aligned manually after an initial alignment with the Clustal W option (Thompson et al., 1997) in the computer program SequenceNavigator (Perkin Elmer, Applied Biosystems). The geographical distribution of the haplotypes is given in Tables 2, 3.

To infer range expansions from the haplotype data, we relied on three conceptually different approaches. The first approach is based on the idea that range expansions should be accompanied by a demographic population growth. This growth is expected to leave traces in the shape of gene-trees compared to the neutral expectation of coalescent theory. Tajima’s *D*

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**Table 1. Sampling sites for phylogeographical analyses**

| Abbreviation | Sampling site               | Latitude | Longitude | *Candidula gigaxii* (N) | *Candidula rugosiuscula* (N) |
|--------------|-----------------------------|----------|-----------|------------------------|------------------------------|
| STB          | St Baume                    | 43°20.37N | 05°38.87E | 4                      |                              |
| LOU          | Montagne de la Loube        | 43°22.16N | 05°57.68E | 5                      |                              |
| MAX          | St Maximin                  | 43°24.50N | 05°49.25E | 5                      |                              |
| SIM          | Simiane                     | 43°25.03N | 05°25.25E | 12                     |                              |
| THT          | Thoronet 2                  | 43°26.88N | 06°16.3E  | 4                      |                              |
| THO          | Thoronet 1                  | 43°27.47N | 06°16.39E | 8                      |                              |
| MEY          | Meyreuil                    | 43°29.18N | 05°30.06E | 3                      |                              |
| MTC          | Montagnac                   | 43°29.88N | 03°35.43E | 3                      |                              |
| ARB          | Les Arbois                  | 43°30.00N | 05°17.54E | 3                      |                              |
| COU          | Coudoux                     | 43°33.89N | 05°14.58E | 4                      |                              |
| LAN          | Lançon                      | 43°34.40N | 05°07.07E | 6                      |                              |
| QTE          | Quatre Termes               | 43°37.01N | 05°12.29E | 5                      |                              |
| REG          | Regasse                     | 43°39.30N | 06°08.00E | 5                      |                              |
| LAM          | Lambesc                     | 43°39.37N | 05°10.67E | 3                      |                              |
| PUY          | Le Puy St Reparade          | 43°39.65N | 05°22.77E | 7                      |                              |
| SPA          | St Paul                     | 43°40.30N | 05°54.11E | 3                      |                              |
| ROG          | Rognes                      | 43°41.18N | 05°18.79E | 5                      |                              |
| MAU          | Mausanne                    | 43°41.71N | 04°50.55E | 4                      |                              |
| VIT          | Vitrolles                   | 43°48.46N | 05°35.60E | 3                      |                              |
| TAR          | Tarascon                    | 43°50.46N | 04°44.22E | 7                      |                              |
| MBN          | Montauban                   | 44°10.93N | 01°32.11E | 3                      |                              |
| STP          | St Paul                     | 44°18.97N | 04°49.45E | 5                      |                              |
| LOR          | Loriol                      | 44°56.10N | 04°51.36E | 2                      |                              |
| ROU          | Roussilion                  | 45°16.12N | 04°57.53E | 7                      |                              |
| SIQ          | Sieq                        | 45°52.10N | 00°11.30W | 2                      |                              |
| MOR          | Mornant                     | 46°35.76N | 04°42.75E | 5                      |                              |
| STQ          | St Quentin                  | 47°15.08N | 01°00.66E | 6                      |                              |
| CVT          | Cravant                     | 47°40.75N | 03°41.51E | 6                      |                              |
| TRY          | Troyes                      | 48°17.85N | 04°08.17E | 3                      |                              |
| VER          | Verdon                      | 49°09.23N | 05°23.21E | 4                      |                              |
| FRY          | Freyburg                    | 51°09.98N | 11°47.09E | 2                      |                              |
| SAG          | Salzgitter                  | 52°05.86N | 10°22.01E | 4                      |                              |

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(Tajima, 1989) and $D^*$ and $F^*$ statistics (Fu & Li, 1993) examine the shape of gene-trees to test for deviation from a selectively neutral coalescent process in a population of constant effective size. The $F$-statistic (Fu, 1997) detects an excess of low-frequency alleles expected in the course of a population expansion (Schneider & Excoffier, 1999). Significant negative departures of these statistics from zero are interpreted either as evidence of selective sweeps (Fu, 1997) or population expansions (Fu, 1997; Slatkin & Hudson, 1991). Positive deviations may indicate a long-lasting population subdivision (Fu, 1997; Slatkin & Hudson, 1991). All calculations were performed with DnaSP, version 4.0 (Rozas et al., 2003).

As a second approach to distinguish recurrent population processes from historical events, we used nested clade analysis (NCA; Templeton, 1998; Templeton, Routman & Phillips, 1995). NCA is a statistical approach that first tries to reject the null hypothesis of random association between haplotype variation and geography, and then interprets the significant patterns using explicit criteria that include an assessment of sampling adequacy. This approach uses the temporal information contained in a haplotype-tree or network to partition historical (e.g. fragmentation, range expansion) from recurrent (e.g. gene-flow, drift, system of mating) processes responsible for the observed pattern of genetic variation. The haplotype network of the 16S haplotypes of each species was inferred using statistical parsimony (SP; Templeton, Crandall & Sing, 1992). The SP network was constructed with the computer program TCS, version 1.06 (Clement, Posada & Crandall, 2000). The nesting design was manually overlaid on the SP network following the rules given in Crandall (1996) and Templeton (1998). The nested cladograms for the 16S haplotypes of C. gigaxii and C. rugosiuscula are presented in Figure 1. The program GeoDis, version 2.0 (Posada, Crandall & Templeton, 2000) was used to estimate the various NCA distance measures and to calculate their statistical significance by comparison with a null distribution derived from 10 000 random permutations of clades against sampling localities. Biological inferences from the observed patterns were drawn using the updated inference key given in Templeton (2004).

Third, a coalescence approach was used to calculate maximum likelihood estimates of various population parameters. In particular, estimates of gene-flow among populations can be obtained taking explicitly into account population structure and demographic processes. To infer past expansion events, we esti-

| Table 2. Haplotype distribution of Candidula gigaxii |
|-------------------------------------------|
|                 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | Σ  |
| SAG             | 4 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 4 |
| FRY             | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 |
| MTC             | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 3 |
| CVT             | 1 | 5 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 6 |
| STQ             | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 6 |
| MBN             | 1 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 3 |
| TRY             | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 3 |
| MOR             |   | 2 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 5 |
| ROU             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 7 |
| LOR             | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 |
| STP             | 1 | 1 | 1 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 5 |
| TAR             | 2 | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 7 |
| MEY             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 4 |
| COU             | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 4 |
| PUY             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 7 |
| VIT             |   | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 7 |
| ROG             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 5 |
| MAU             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 5 |
| REG             |   | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 4 |
| SPA             |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 5 |
| VER             |   | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 3 |
| SIQ             | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 4 |
| Σ               | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 24 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 41 | 4 | Σ  | 93 |

For definitions of sampling site abbreviations, see Table 1.
mated migration patterns using the software package MIGRATE, version 1.7.1 (Beerli & Felsenstein, 1999). The MIGRATE approach has advantages over equilibrium approaches because it takes into account historical and asymmetrical gene-flow (Beerli & Felsenstein, 2001). Because it is computationally unfeasible to estimate the migration rates among all populations investigated, they were pooled into geographical regions (Fig. 2). To obtain past gene-flow estimates, we used ten short chains with 2000 steps and 40 000 sampled genealogies, and three long chains with 5000 steps and 1000 000 sampled genealogies. In each run, the first 10 000 genealogies were discarded as burn-in. The analysis was repeated ten times with different start parameters (i.e. random seed, upper and lower bounds of both estimates of transition/transversion ratio and nucleotide composition). The resulting gene-flow and theta matrices differed by up to 50% in the quantity of the estimated parameters. However, qualitatively, they were virtually identical as demonstrated by pairwise correlation coefficients that were no lower than 0.97. Gene-flow estimates whose lower 95% confidence boundary was below $10^{-8}$ individuals per generation in all runs were considered as zero and were therefore not reported. MIGRATE was recently strongly criticised for its failure to recover the true gene-flow parameters from simulated data (Abdo, Crandall & Joyce, 2004). However, we were more interested in the general pattern (i.e. particularly the direction) of the historic gene-flow than in a precise parameter estimate. Because the performance to recover the direction and relative strength of gene-flow was not excoriated in the study by Abdo et al. (2004), we considered MIGRATE to comprise a valuable approach in this respect until proved otherwise. However, in combination with the population genetic approach and the NCA, which has the weakness of not assessing errors and directionality in its inferences (Knowles & Maddison, 2002), the coalescence analysis provides an independent and powerful tool for phylogeographical analysis.

ESTIMATION OF ENVIRONMENTAL AND STRUCTURAL HABITAT NICHE

The realized environmental niche can be estimated from presence-only data with high precision by extracting meaningful niche dimensions from spatial information on the distribution of environmental parameters. For each of the 498 Candidula populations, 19 biologically meaningful climatic parameters were extracted from the BIOCLIM (Nix, 1986) environmental layers with a spatial resolution of 2.5 min as implemented in the computer program DIVA-GIS, version 4.2 for spatial analysis of biodiversity (Hijmans et al., 2001). Principal component analysis
(PCA) axes were extracted from the BIOCLIM variables to examine divergence in environmental space between populations from the inferred area of origin and the expansion area.

To characterize the habitat, the following meristic variables of all 129 Candidula populations visited during sampling for phylogeographical analyses were recorded: exposition, topography, cover of the soil, humidity, stratification, degree of artificialization, and anthropogenization, according to Labaune & Magnin (2001). We used a nonlinear principal component analysis (NPCA) to summarize habitat score variables of the seven habitat characteristics. NPCA was developed for the analysis of categorical data and can be used in a similar fashion as standard PCA.

**Shell shape**

The shells of 445 adult individuals (186 each for C. unifasciata and C. gigaxii and 73 for C. rugosiuscula) sampled for genetic analysis were scored for eight shell variables by digital image analysis.

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**Figure 1.** Statistical parsimony 16S rDNA haplotype networks of Candidula gigaxii (A) and Candidula rugosiuscula (B). Each numbered circle corresponds to a haplotype, with the lines between them comprising a single mutation step. The size of the circles is proportional to their frequency in the sample. Missing but logically necessary haplotypes are marked by a small circle. The haplotype with the highest probability of being the ancestral haplotype is marked with a square.
analysis, according to the procedure described by Pfenninger & Magnin (2001). Shell sculpture traits were measured according to ‘rib-spacing’ (i.e. the average distance between ribs), ‘coarseness’ (i.e. the average distance between a base line and the perimeter of the ribs), and ‘regularity of ribs’ (i.e. the coefficient of variation of inter-rib distances). Size independent shell shape (shape 1, 2, and 3) was assessed by the use of the first three relative warp scores of a thin plate spline analysis (Rohlf, 1992). This geometrical analysis of shape was based on 55 landmarks applied to an electronic image of the individual. The height and breadth of the shell were taken as measurements of size. The eight variables for all individuals from all taxa were used in a single PCA to summarize shell character differences.

**Statistical analysis**

Environmental and structural niche dimension PCA factor values of sampling sites and individual shell variation were compared by one-way analysis of variances (ANOVAs), grouped according to provenance from either the inferred area of origin or the expansion area. The analyses were carried out for each species separately.

**Results**

**Identification of range expansions**

Sequencing the mitochondrial 16S rDNA from 93 *C. gigaxii* individuals resulted in a fragment that was 372–373 bp in sequence length. We found 21 haplotypes with a mean overall distance of 0.002. The resolved SP haplotype network is shown in Figure 1A. In *C. rugosiuscula*, 320–321 bp could be used for analysis from 78 individuals. The 34 haplotypes found had a mean distance of 0.021 (Tables 2, 3). The resulting haplotype network is shown in Figure 1B.

In *C. gigaxii*, a significant negative departure from neutral expectations, indicating demographic population expansion, was detected in all the statistical tests (Fu’s $F = -6.18$, $P < 0.002$; $D^* = -3.13$ $P < 0.05$; $F^* = -3.05$ $P < 0.02$; $D = -1.52$, $P < 0.01$). NCA identified a continuous range expansion for the total cladogram of *C. gigaxii* (Table 4). This inference is corroborated by the coalescence gene-flow analysis that identified the populations in south-east France (Provence) as the source of the expansion (Fig. 2). Other groupings of the populations resulted in similar results; in particular, the inferred direction of the expansion was not affected by different pooling. The expansion proceeded from south-east France to the North of the present species range. Consequently, populations south of 44.5°N and between 3.2° and 7.2°E were considered to inhabit the area of origin for this species (Fig. 3).

In *C. rugosiuscula*, the evidence for a population expansion was less clear. Although Fu’s $F$ is highly significant ($-12.71$ $P < 0.001$), the other statistics failed to detect departures from the neutral expectation ($D^* = -1.1$, not significant; $F^* = -1.05$, not significant; $D = -0.74$, not significant). The overall dominating phylogeographical pattern in *C. rugosiuscula* was isolation-by-distance. However, on lower clade levels, some colonization events could be identified. The Eastern sites in the species range appeared to be colonized...
Table 4. Inferences of nested clade analysis Candidula gigaxii and Candidula rugosiuscula

| Clade       | $\chi^2$ | $P$     | Inference                                           |
|-------------|----------|---------|-----------------------------------------------------|
| Candidula gigaxii |         |         |                                                     |
| 1-1         | 166.1    | 0.0002  | Isolation by distance                               |
| 1-2         | 237.7    | 0.0005  | Isolation by distance with some long distance dispersal to the German populations |
| Total cladogram | 161.1    | 0.0000  | Contiguous range expansion                         |
| Candidula rugosiuscula |     |         |                                                     |
| 2-1         | 65.8     | 0.0150  | Contiguous range expansion towards STB             |
| 2-3         | 13.0     | 0.0004  | Long distance colonization of LOU                   |
| 3-2         | 18.0     | 0.0000  | Long distance colonization of MAX                   |
| 3-3         | 18.2     | 0.0006  | Isolation by distance with some long distance colonization to THT and THO |
| 4-1         | 44.0     | 0.0000  | Isolation by distance                               |
| 4-2         | 24.3     | 0.0000  | Isolation by distance                               |
| Total cladogram | 64.4     | 0.0000  | Isolation by distance                               |

STB, St Paul; LOU, Montagne de la Loube; MAX, St. Maximin; THT, Thoronet 2; THO, Thoronet 1.

Figure 3. Point occurrence data used for Candidula gigaxii (grey squares), Candidula rugosiuscula (black circles), and Candidula unifasciata (white triangles). Inferred area of origin and expansion area for Candidula unifasciata and Candidula gigaxii are indicated.
through independent events, either by continuous range expansion or long-range dispersal (Table 4). These events were also reflected in the migration pattern, as reconstructed by coalescence analysis (Fig. 1B). Because the fossil record suggests that this area was not colonized before, these five populations were therefore considered to be the result of a range expansion.

The phylogeographical analysis of C. unifasciata reported by Pfenninger & Posada (2002) inferred a postglacial northerly corridor-migration through river valleys from populations south of 44.5°N and between 4.8° and 7.2°E (Fig. 3). In the present study, this area was considered to have harboured the ancestral populations in subsequent analyses.

ENVIRONMENTAL AND STRUCTURAL NICHE

The PCA on BIOCLIM variables extracted three meaningful (Eigenvalue > 1) niche dimensions, accounting for 81.3% of the total variation. Axis 1, accounting for 47.8% of the total variation, was a climatic gradient from warm and dry to cool and wet summers. Axis 2 (18.2%) opposed sites with annually variable temperatures and precipitation in the summer months to populations in areas with more uniform temperatures and main precipitation in the winter. The third axis (15.3%) distinguished between locations experiencing a small annual temperature range with a uniform precipitation regime throughout the year vs. populations exposed to a large annual temperature range with seasonally varying precipitation. Only one meaningful axis was extracted from the NPCA on habitat structure variables. It described a gradient from essentially undisturbed habitat (e.g. on mountain ridges) to heavily disturbed, anthropogenized areas (e.g. industrial fallows and road side verges).

In C. gigaxii, the realized environmental niche of the expansion populations differed significantly from those in the area of origin for all three niche dimensions (Fig. 4). Populations in the area of origin experienced a pronounced Mediterranean climate with summer drought, winter rain, and large seasonal temperature differences, whereas the expansion populations are under an Atlantic climate regime with year-round precipitation and little seasonal temperature differences. Even though there was a tendency to occupy more disturbed, anthropogenic-influenced habitat in the expansion area, this difference was not significant (Fig. 5).

The realized environmental niche of populations in the area of origin and the expansion populations did not differ significantly in C. rugosiuscula (Fig. 4). The same was true for the structural habitat niche dimension (Fig. 5).

There were also significant differences in the first two dimensions of the realized environmental niche between refugial and expansion populations in C. unifasciata. Because the number of samples in each class was highly unbalanced (364 expansion vs. 43 origin populations), we drew 20 random samples of size 43 from the expansion populations and performed an ANOVA for each data set. The results did not qualitatively differ from those presented here. For this species, the climate regime in the refugial area was significantly less Mediterranean compared to C. gigaxii, but still significantly more so than the continentally influenced climate in the expansion populations. Candidula unifasciata prefers the same rather undisturbed habitats in the expansion area as in the ancestral range (Fig. 5).

SHELL SHAPE EVOLUTION

Two meaningful PCA axes were extracted from the morphometric analysis, representing 52.2% of total shell variance. The first axis (33.8%) was a gradient of overall size, whereas axis 2 described the difference between smooth, globular shells with large aperture and thickly ribbed, depressed shells with small apertures.

The shells of adult C. gigaxii are significantly larger in the ancestral range whereas, in C. unifasciata, it is the opposite. Candidula rugosiuscula shells showed no significant size difference. The shell shape of both C. gigaxii and C. unifasciata is more depressed with a smaller aperture in the area of origin than in the secondarily occupied areas (Fig. 6).

DISCUSSION

With few exceptions (Davis & Shaw, 2001), phylogeographical studies have relied on the tacit or explicit assumption of niche conservatism to explain inferred range expansions (Taberlet et al., 1998; Hewitt, 2004). However, several well-known examples show that local adaptation is possible in very short evolutionary time, even in the face of gene-flow (Berlocher, 2000; Grant & Grant, 1993; Schluter, Boughman & Rundle, 2001). Additionally, a certain variation in environmental conditions may be expected over larger distribution ranges. The crucial question is whether the environmental variation among the region of origin and expansion area is significantly larger than the within each region. In the case of non-overlapping environmental conditions, it can be assumed that the range expansion confronted the species with constraints that were as yet unexperienced. Here, we could show that range expansions in land snails can be accompanied by both significant ecological and morphological differences compared with the area of origin.
All three of the species ranges investigated showed signs of range dynamics in the phylogeographical analysis. The use of a single, relatively short, albeit highly variable marker gene for phylogenetic inference could be viewed as problematic. However, the congruence of results from three conceptually different methods suggests that the inferred range expansions have indeed taken place (Cruzan & Templeton, 2000). The inference of a range expansion of *C. gigaxii* is strengthened by the fossil record. In the expansion range of *C. gigaxii*, subfossil specimens were detected in deposits only from approximately 2000 years bp onwards whereas, in south-east France, the fossil record dates continuously back to the Pleistocene (F. Magnin, unpubl. data). It is therefore likely that the colonization of the Northern parts of the species range

![Figure 4. Analysis of variance comparisons of environmental niche estimates for the area of origin and expansion area. Vertical bars indicate 95% confidence intervals, boxes indicate the standard deviation.](image-url)
Figure 5. Analysis of variance comparisons of structural niche estimates for the area of origin and expansion area. Vertical bars indicate 95% confidence intervals, boxes indicate the standard deviation.

Figure 6. Analysis of variance comparisons of shell morphology for the area of origin and expansion area. Vertical bars indicate 95% confidence intervals, boxes indicate the standard deviation.
happened in historic times, perhaps as a consequence of the introduction of systematic agriculture. Timing the expansion of *C. rugosiuscula* is difficult. In the early Holocene, the expansion area was mostly covered with woods, which does not constitute suitable habitat. According to archaeological and pollen studies, an anthropogenic deforestation of the area started approximately 7500 years bp, probably creating the necessary open habitat structure for the colonization (F. Magnin, unpubl. data). The inferred range expansion of *C. unifasciata* (Pfenninger & Posada, 2002) is also backed up by fossil evidence. It must have happened soon after the onset of the Holocene climate warming because populations in the Rhine valley were already established 9000 years bp, as supported by the fossil record (Puissegur, 1978).

However, the extent of the range expansions was substantially different. Although both *C. gigaxii* and *C. unifasciata* increased the species range by at least a factor of ten, the territorial gain of *C. rugosiuscula* was less then 50%. The ancestral range of all species is the south east of France, which makes it likely that all speciation events also took place in this area (Pfenninger *et al.*, 2003a). We found significant differences in the realized environmental niche of *C. gigaxii* and *C. unifasciata* among the ancestral and expansion areas for three and two niche dimensions, respectively (Fig. 4). Notably, the 95% confidence intervals did not overlap in these cases, which shows that comparable climatic conditions are absent in the areas of origin and the expansion areas, respectively. Non-evolutionary reasons, such as biased sampling, geographical coincidence of unmeasured ecological variables or climatic variation below the data resolution, as well as evolutionary explanations, may account for the observed effects.

Sampling bias could arise if populations of a particular climate are systematically or randomly under- or over-represented in one area. This is unlikely in the present case, because (1) the sampling was extensive (Fig. 3) and (2) climate distributions in the respective areas of origin and expansion were relatively homogeneous (Fig. 4). Geographical coincidence of the entire species range with an unmeasured ecological variable, in particular the interaction with other species, can not be excluded. However, crucial biotic interactions are neither known for the species in question, nor for other land snails. Climatic variation below the scale considered (2.5 min × 2.5 min) could lead to an overestimation of the differences among areas. The populations in the northern part of the expansion area were mainly found at places that offer rather warm microclimates (e.g. on south facing slopes). However, all environmental niche dimensions are also determined by the precipitation regime and differences in seasonality that have only low local variability. The observed differences in large-scale climate may thus be mediated by the existence of favourable local microclimates in the expansion area, but can not equalize the pronounced difference between Mediterranean and Atlantic or continental climate conditions, respectively. In particular, the seasonal timing of favourable temperature and moisture conditions differs in a way among the areas such that life cycle stages (e.g. the reproduction period) are shifted for several months in *C. unifasciata* (M. Pfenninger, unpubl. data).

Phenotypic plasticity could account for the observed geographical variation in *C. gigaxii* and *C. unifasciata*, as well as evolutionary innovation or both. Because it can be assumed that the observed climatic differences have a profound effect on all life cycle stages of land snails (Arad, 2001; Backeljau, Baur & Baur, 2001; Cook, 2001; Comot de Vaufleury, 2001), the non-overlap of climatic conditions in the expansion areas and the regions of origin argues for an adaptive explanation. Another point in favour of an evolutionary origin of the observed differences are the significant changes in the shell size and shape. Especially the shape differences constitute a selective advantage by minimizing the loss of humidity under water stress conditions because a small aperture minimizes the area of exposed surface and a flat shell allows to penetrate deeper into the vegetation or under stones (Goodfriend, 1986). The non-overlap of shape PCA confidence intervals shows that the respective morphotypes are either rare or absent in the respective area (Fig. 6). A rapid morphological shell evolution in the course of a postglacial range expansion was also shown for intertidal snails (Hellberg *et al.*, 2001). Interestingly, no such shell evolution was observed in *C. rugosiuscula*, where the range expansion took place within the borders of the ancestral niche (Fig. 6). However, only common garden or reciprocal transplant experiments could partition among evolutionary change and phenotypic plasticity.

The dating of the inferred expansions by the fossil record allows to forward hypotheses on the driving mechanisms behind them. In the early Holocene, climatic conditions in parts of south-east France resembled conditions much more similar to the conditions in the expansion areas today (Blondel & Aronson, 1999). The northwards spread of *C. unifasciata* thus most likely tracked the climatic and structural niche. In this species, the populations remaining in the ancestral area had to adapt to the reinstallation of a Mediterranean climate regime rather than the expansion populations to Northern conditions. By contrast, the colonization of the northern part of the species range in *C. gigaxii* occurred according to the fossil record when the present day climate distribution was already
in place. Here, the possibly anthropogenic dispersed populations (Dörge et al., 1999) had to cope with new environmental challenges.

At first sight, two out of three cases presented here contradict the theoretical expectation for niche conservatism over the species range (Kirkpatrick & Barton, 1997). The arguments in these studies were mainly based on the assumption of gene-flow from the centre of the distribution range to its periphery that prevents adaptation to local conditions. This assumption may not be valid for species with restricted dispersal capacities for which land snails are the proverbial example. Even on a local scale, the gene-flow within a continuously suitable habitat patch is limited by distance (Pfenninger, Bahl & Streit, 1996; Arnaud et al., 1999).

On a regional scale in a fragmented landscape, the recurrent gene-flow among populations is usually even more restricted (Arnaud et al., 2001; Arter, 1990; Pfenninger, 2002). This could be seen as contradictory to the above demonstrated ability to colonize large areas. However, long-range dispersal in land snails is mostly passive (Dörge et al., 1999) and, once a population is established, the neighbourhood population structure effectively prevents local extinction due to demographic reasons alone, even in small patches of habitat (Pfenninger & Bahl, 1997). Land snail populations are thus more or less independently evolving units that allow for local adaptation. Because this may be true for most invertebrate taxa with restricted dispersal capacities, this should be considered in future modelling studies on the evolution of species ranges. Moreover, the results gained from the three closely-related taxa initially inhabiting the same ancestral area suggest that it may be difficult to predict the reaction of a species on a global climate change event based on the reaction of close phylogenetic relatives. This finding is not in accordance with studies on organisms with much better dispersal capacities, such as birds, mammals, and butterflies (Peterson et al., 1999; Peterson, 2003).

The present work suggests that adaptation must be considered as the cause of species’ range dynamics. Within the proposed framework, the inference of range expansions using molecular markers serves as a basis for statistical comparisons of traits potentially limiting the distribution (e.g. morphology, physiology, parasitic load) among the identified ancestral and expansion populations. A desirable extension of the approach would be a test of the gained hypotheses with common-garden and reciprocal transplant experiments. Thus, niche conservatism should be regarded as a testable hypothesis and not as the most probable assumption in the study of species’ range limits dynamics in phylogeographical studies or predictive applications of ecological niche modelling.

ACKNOWLEDGEMENTS

The study was supported by DFG grant PF390/3-1 to M. P. We thank the curators Margret Gosteli and Roland Janssen for access to the museum collections and Alain Bertrand for his data on point occurrences. The suggestions of Jan Pinceel, Klaus Schwenk, Michael Hellberg, Michael Bell, David Posada, and three anonymous referees improved the manuscript.

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