Mitochondrial DNA variation along an altitudinal gradient in the greater white-toothed shrew, Crocidura russula

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SHORT COMMUNICATION

Mitochondrial DNA variation along an altitudinal gradient in the greater white-toothed shrew, *Crocidura russula*

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Abstract

The distribution of mitochondrial control region-sequence polymorphism was investigated in 15 populations of *Crocidura russula* along an altitudinal gradient in western Switzerland. High-altitude populations are smaller, sparser and appear to undergo frequent bottlenecks. Accordingly, they showed a loss of rare haplotypes, but unexpectedly, were less differentiated than lowland populations. Furthermore, the major haplotypes segregated significantly with altitude. The results were inconsistent with a simple model of drift and dispersal. They suggested instead a role for historical patterns of colonization, or, alternatively, present-day selective forces acting on one of the mitochondrial genes involved in metabolic pathways.

Keywords: genetic drift, historical events, metabolism, metapopulation, range expansion, selection

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Introduction

Natural populations are often fragmented into more or less isolated demes. Fragmentation influences several aspects of population biology, especially the amount and distribution of neutral genetic polymorphism. Drift is expected to decrease levels of polymorphism within subpopulations, and to increase differentiation among them. At equilibrium, the classical relationship (Wright 1931) applies under island-model assumptions:

$$F_{ST} \approx \frac{1}{4Nm + 1} \quad (1)$$

where F_{ST} measures the differentiation among subpopulations, N is the size of each of these subpopulations, and m the rate of gametic dispersal among them (so that $2Nm$ measures the effective number of diploid immigrants per generation).

However, equation 1 is unlikely to hold true whenever populations fluctuate (Whitlock & McCauley 1999). Although extinction and recolonization processes should always cause drastic losses of genetic variance (Whitlock & Barton 1997), their effects on the distribution of variance are less straightforward, depending on the patterns of colonization and dispersal. Wade & McCauley (1988) showed that, if all the k colonists of an empty locality stem from one single random patch (the propagule-pool model), then genetic differentiation among subpopulations always increases with extinction rate. If, however, these k colonists stem from different random patches (the migrant-pool model), then differentiation (F_{ST}) will either increase or decrease with extinction rate, depending on whether the number of colonists (k) is smaller or larger than the number of effective immigrants ($2Nm$).

In this study, we asked whether the amount and distribution of genetic polymorphism among subpopulations of the greater white-toothed shrew (*Crocidura russula*) would reflect the disequilibrium dynamics of populations in marginal habitats. This species expanded in historical times from North Africa to south-western Europe (Catzeflis *et al*. 1985; Vogel & Maddalena 1987), and is still expanding (Frank 1984; Cosson *et al*. 1996; Kraft 2000; Vogel *et al*. 2002). In Switzerland, *C. russula* commonly occurs at low altitude (400–600 m; Genoud 1982, 1995), even though populations are genetically subdivided because of its anthropophilic habits (F_{ST} = 5–6% over a 16-km transect, consistent with an exchange rate of 4–5 individuals per generation under island-model assumptions; Balloux *et al*. 1998). Individuals are found regularly outside human settlements in lowland during summer (Genoud 1982, 1995), confirming connections among subpopulations.

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The species becomes more sparse and localized under the cooler conditions that prevail at higher altitudes (600–1000 m; Genoud 1982, 1995). Populations above 1000 m are rare, as only six are registered in the Swiss Fauna Data Bank (http://www.cscf.ch/). Anthropophily is obligatory above 600 m (Genoud 1995), because overwinter survival is not possible without access to sources of warmth and food (invertebrates), such as those provided by compost piles, stables and farms in rural habitats. Further adaptations to winter include communal nesting and the ability to enter daily torpors when temperature drops or food becomes scarce (Vogel *et al*. 1979; Genoud 1985). The long-term study of a montane population (750 m) yielded no capture outside human settlements despite extensive trapping (3600 trap nights; Genoud & Hausser 1979), suggesting that dispersal among villages is rare. That study also documented population bottlenecks owing to overwinter mortality, that led to a local extinction (Genoud & Hausser 1979). Vogel (1999 and personal communication) introduced breeding pairs in nine empty sites (isolated farms) at even higher altitudes (980–1383 m) in June 1996. Although successful reproduction took place during the summer, all populations but one went extinct by the following winter.

Here we investigate the amount and distribution of genetic polymorphism in the hypervariable control region of mitochondrial DNA (mtDNA) among 15 shrew populations in an altitude gradient. This marker should be informative of both drift and migration patterns, as dispersal is female biased in this species (Favre *et al*. 1997). Owing to longer and colder winters, high-altitude populations should undergo more fluctuations and extinction–colonization events than lowland populations, so that we expected levels of genetic polymorphism within sites to decrease with altitude. Highland populations are also smaller, more scattered, and the environment more hostile, which should reduce the level of immigration ($2Nm$). Since, furthermore, immigrants are diploid individuals (not haploid gametes) and, because of their limited dispersal ability, likely to stem from neighbouring localities (Balloux *et al*. 1998), dispersal patterns should match more closely the propagule than the migrant pool model. We therefore expected genetic differentiation to increase with altitude.

Materials and methods

Field work

The study area (Fig. 1) is situated in western Switzerland, between Lake Leman (374 m) and the Jura mountains (highest elevation 1718 m). The 15 sites analysed cluster

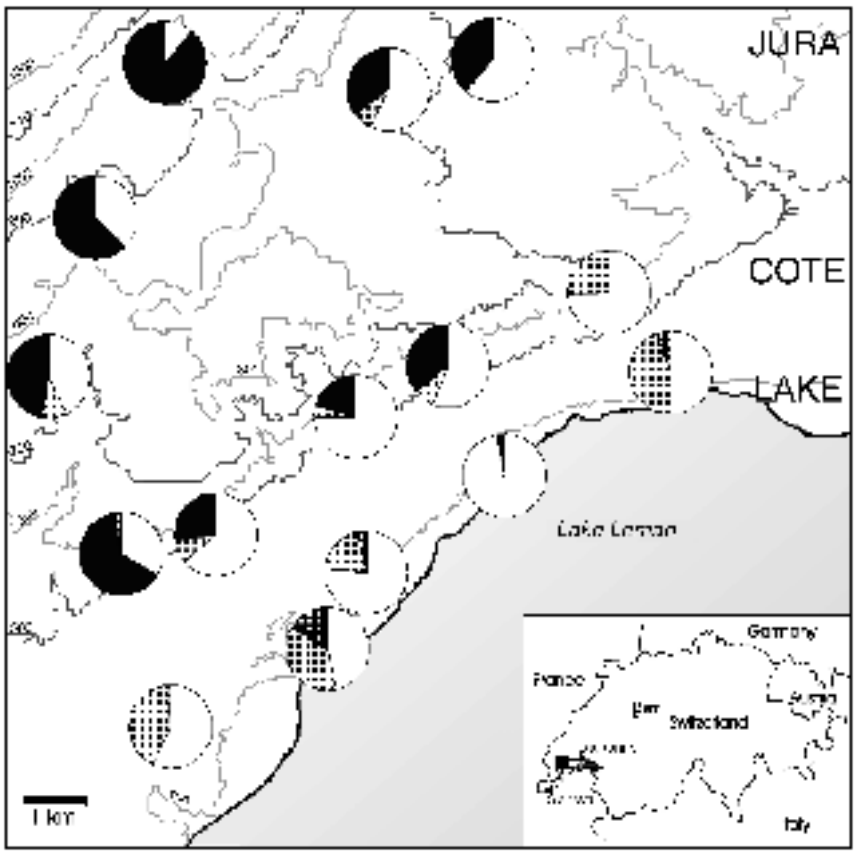

Fig. 1 Geographic situation of the 15 sites sampled, with altitude isoclines. Haplotype distribution shows a marked geographical pattern, *H1* (black) being much more common in highland (north-west) than is lowland populations. *L1* is given in white, other L haplotypes are shown as black dots on white ground, and other H haplotypes as white dots on black ground.

Table 1 Coordinates and altitudes of the 15 sites sampled. Also indicated are the number of haplotypes and individuals (*n*), the trapping effort needed to complete the sample (*eff*; number of nights, 150 traps each), the trappability (*trap*, number of captures per night), the number of captures per individual (*rec*) as well as the measures of haplotype (*h*) and nucleotidic (π) diversity

Transect	Village	East	North	Altitude	*L1*	*L2*	*L3*	*L4*	*H1*	*H2*	*H3*	*H4*	*H5*	*n*	*eff*	*trap*	*rec*	*h*	π
Jura	St George	509.58	151.96	930	2				16					18	11	4.75	1.8	0.21	0.0026
	Marchissy	508.38	149.36	830	7				12					19	7	6.86	1.4	0.49	0.006
	Bassins	507.44	146.55	750	9	2			10					21	10	9.75	2.3	0.61	0.0064
	Gimel	513.12	151.55	735	16	2			10					28	13	5.77	1.8	0.56	0.006
	Saubraz	514.77	151.95	680	11				7					18	6	10.7	2.3	0.5	0.0062
Côte	Bougy	516.72	148.12	560	14	5								19	3	26.7	1.8	0.41	0.0013
	Vincy	512.65	146.36	530	18	1			5					24	2	30.5	1.1	0.41	0.0044
	Begnins	508.78	143.96	530	11				21			1		33	3	16.7	1.1	0.5	0.0058
	Tartegnin	513.82	146.73	500	14	1		1	9					25	5	13.6	1.6	0.58	0.0064
	Luins	510.25	144.13	450	14	2			6					22	4	12.5	1.2	0.54	0.0053
Lake	Gland	509.25	141.18	430	14	1	9				1			25	3	24	1.3	0.58	0.004
	Perroy	517.52	146.88	420	9	8							1	18	3	17.3	1.4	0.58	0.0023
	Bursinel	512.59	143.44	420	22	6				1				29	2	26	1.1	0.39	0.0016
	Dully	512.14	142.77	420	14	10		1	1	4				30	2	36	1.1	0.67	0.004
	Rolle	514.91	145.26	390	33				1					34	1	49	1	0.059	0.0007
Total					208	38	9	2	98	5	1	1	1	363					

into three altitude classes (Table 1). The five sites close to the lake (390–430 m) have the warmest climate [215–235 days growing season (GS); 9.5–10.5 °C average annual temperature (AAT)]. The five localities among the vineyards of the Côte region (450–560 m) have a cooler climate (205–215 days GS, 8.5–9.5 °C AAT). Finally, the five localities in the Jura mountains (680–930 m) have a cold climate (190–205 days GS, 7.5–8.5 °C AAT; Schreiber *et al*. 1977).

Trapping took place in 1999 and 2000, from June to August (i.e. during population density peaks). At each site, 150 Longworth traps prebaited with *Tenebrio molitor* larvae were set for several nights until enough shrews (≈ 30 individuals) were captured. Trapping sessions, however, were stopped after a few nights if trapping success was low. We had to sample eight villages at the highest range (Jura transect), and six at the midrange (Côte transect) to obtain sufficient sample sizes for five sites per altitude class. Tissue samples were collected by toe clipping, then stored frozen at −20 °C. Individuals were released onsite after trapping.

Genetic analyses

Total DNA was extracted from frozen phalanges following a salt/chloroform procedure modified from Miller *et al*. (1988) by adding one step of chloroform/isoamylalcohol extraction (24:1). The second hypervariable domain (HVII) of the mitochondrial control region (D-loop) was then amplified using the primers L16517 (Fumagalli *et al*. 1996) and H00651 (Kocher *et al*. 1989). Reactions were done in a 25-μL volume containing 1 μg/μL BSA, 2.5 mM $MgCl_2$, 1 μM of each primer, 0.2 μM each dNTP, 1 unit *Taq* DNA polymerase (GibcoBRL) and 2.5 μL PCR buffer (GibcoBRL). The amplification programme (93 °C for 45 s, 45 °C for 45 s and 72 °C for 60 s, 35 cycles) was run on a DNA Thermal Cycler (Perkin Elmer, Norwalk, CT, USA).

The amplification of ≈ 1 kb polymerase chain reaction (PCR) products was checked by agarose (1%) gel electrophoresis. PCR products were purified using the QIAQuick kit (Qiagen), with a 30-μL dH_2O final elution volume. Sequencing was restricted to the single copy DNA between the primer L16517 and the R2 repeats (Fumagalli *et al*. 1996), yielding 325 bp sequence. Sequencing reactions were carried out in a 10-μL volume comprising 0.1 μM primer, 4 μL Dye mix (Perkin Elmer) and 5 μL PCR product. The sequencing programme was 3 min denaturation, 25 cycles of 96 °C for 20 s, 50 °C for 15 s, and 60 °C for 4 min Sequencing products were precipitated with ethanol, then run on a 6% polyacrylamide gel on an ABI 373 sequencer (Perkin Elmer). The sequences were aligned manually in SEQUENCHER Version 3.0 (Gene Codes Corp., Ann Arbor, MI, USA) and the haplotypes identified in MACCLADE Version 3.08 (Maddison & Maddison 1999).

Statistical analyses

In order to limit the pseudoreplication that might arise from sampling several individuals from the same family, all juveniles were excluded from the analyses unless they possessed a haplotype not found in any of the adults captured in the same or a neighbouring trap. The level of genetic polymorphism within sites was calculated both as haplotypic diversity *h* and as nucleotidic diversity π (Nei 1987) using ARLEQUIN Version 2.0 (Schneider *et al*. 2000). Hierarchical *F*-statistics were used to compute the genetic

differentiation among villages within the study area (F_{ST}), among villages within altitude classes (F_{SR}), and among altitude classes (F_{RT}) using AMOVA from ARLEQUIN 2.0. Non-hierarchical partitioning of variance among villages within classes (F_{SRi}) were also calculated for the three altitude classes *i* separately (FSTAT Version 2.9; Goudet 1995). The effect of geographical distance and altitude differences between villages on pairwise F_{ST} were assessed by partial Mantel tests (Manly 1991, 1997) using (FSTAT 2.9; Goudet 1995). All other statistics were calculated in s + 2000 (MathSoft Inc.).

Results

Trapping

The number of individuals caught per night declined dramatically with altitude, spanning two orders of magnitude (Kendall's $\tau = -0.667$, $n = 19$, $P = 0.0001$; Table 1). Trapping in three high-altitude villages, for instance, yielded one individual each in two nights (i.e. 300 trap nights). Consequently, the number of nights needed to acquire sufficient sample sizes increased with altitude (Kendall's $\tau = 0.386$, $n = 15$, $P < 0.0005$), reaching 6–13 nights per site in the Jura transect, compared with 1–3 nights per site in the Lake transect (Table 1). Individual recapture rate also increased with altitude (Kendall's $\tau = 0.505$, $n = 15$, $P = 0.008$), reaching 1.4–2.3 captures per individual in the Jura transect, vs. 1.0–1.4 in the Lake transect (Table 1). Larger proportions of local populations were thus sampled at higher altitudes.

Genetic analyses

Haplotype distributions . The nine haplotypes found were classified in two groups H and L, separated by one transversion and some transitions (Table 2). Global diversity was low: most (95%) individuals possessed one of three common haplotypes (Table 1), and the remaining 5% one of six rare haplotypes.

The number of haplotypes decreased with altitude, although the association was only marginally significant (Kendall's $\tau = -0.31$, $n = 15$, $P = 0.07$). Pooling sites within altitude classes showed that eight haplotypes occur in the Lake transect, five in the Côte transect, and three in the Jura transect (Table 1). Rare alleles were absent in the Jura transect, suggestive of past bottlenecks (Luikart *et al*. 1998). However, neither haplotype diversity (*h*) nor nucleotidic diversity (π) showed the expected decline with altitude (Table 1).

The two haplotype groups H and L segregated with altitude. Whereas group H is rare in the Lake transect (7%), it represents one third of individuals (34%) in the Côte transect, and over half (53%) in the Jura transect. A linear regression of the frequency of pooled H haplotypes on altitude explains 70% of the variance ($n = 15$, $P = 0.0001$). Interestingly, the H group is mostly represented by its rare alleles (*H2* to *H5*) in the Lake transect (7 of 9 occurrences), whereas these rare alleles are absent at higher altitudes (1 of 42 occurrences in the Côte transect, and 0 of 55 in the Jura transect).

F-*Statistics.* The pattern of differential haplotype distributions corresponded to high levels of genetic differentiation among sites within the study area ($F_{ST} = 0.244$, $P < 0.001$), a significant part of which was due to differentiation among altitude classes ($F_{RT} = 0.148$, $P < 0.001$). The differentiation of villages within altitude classes was also significant, but less so ($F_{SR} = 0.113$, $P < 0.05$), and differed according to altitude: nonhierarchical *F*-statistics showed that differentiation was highest in the Lake transect ($F_{SRL} = 0.183$), intermediate in the Côte transect ($F_{SRC} = 0.141$) and lowest in the Jura transect ($F_{SRJ} = 0.112$). Finally, partial Mantel test showed pairwise F_{ST} to correlate strongly with altitude difference between villages ($P = 0.0005$), but not with geographical distance ($P = 0.77$).

Haplotype Consensus	Polymorphic site: 196	215	221	252	263	271	272	316	GenBank Accession no.
	G	A	A	T	C	A	G	–	
H1	A	G	–	–	–	–	–	–	AF343009
H2	A	–	–	–	–	–	–	–	AF343010
H3	A	G		–	–	–	A	–	AF343011
H4	A	G	G	–	–	–	–	–	AF343012
H5	–	G	–	–	–	–	–	–	AF343013
L1	–	–	–	–	A	G	–	–	AF343014
L2	–	–	–	–	A	–	–	–	AF343015
L3	–	–	–	C	A	–	–	–	AF343016
L4	–	–	–	–	A	G	–	C	AF343017

Table 2 Consensus sequence and point mutations. The two families H and L are separated by one transversion (263) and some transitions. 316 is an insertion. The consensus haplotype was not found in the field. The last column provides GenBank access numbers

Discussion

The trapping returns are consistent with a marked decrease in population density with altitude. The extreme scarcity of shrews in three of eight suitable sites in the montane transect is also suggestive of strong bottlenecks. These patterns are unlikely to stem from behavioural differences: Genoud & Hausser (1979) showed by radioactive tracking that patterns of daily activity and home-range use do not differ between montane and lowland populations during the breeding season (as opposed to the winter season). Our results support their conclusions that montane populations of *Crocidura russula* are smaller and undergo more fluctuations than lowland populations.

According to our expectations, the number of haplotypes decreased with altitude. Absence of rare haplotypes characterized populations most likely to experience bottlenecks. Although bordering on significance, the test for association is probably conservative, given that larger proportions of the populations were sampled at higher altitudes.

By contrast, neither haplotype nor nucleotide diversity showed the expected decrease with altitude. Furthermore, levels of genetic subdivision were the opposite of our expectations, showing a decrease with altitude. Other observations are also difficult to explain by a simple neutral model of metapopulation dynamics. First, pairwise F_{ST} values were unrelated to geographical distance, but significantly related to altitude. Under a migration–drift scenario, this pattern would suggest that exchange occurs only among villages at the same altitude, independent of distance. It is unlikely that migrants disperse only along altitude isoclines. Second, the highly significant cline of haplotype frequencies with altitude was unexpected under a migration drift model. Spatial autocorrelations might arise under limited, short-distance dispersal, but allele frequencies should remain independent of ecogeographical variables.

The latter result suggests two alternative explanations for the observed pattern:

i) Historical events may have shaped the present-day haplotype distribution. On a large geographical scale, patterns of genetic differentiation may result from independent routes of colonization (e.g. Davison 2000). On a smaller geographical scale (i.e. closer to the one investigated here), area effects may result from the type of colonization process. During range expansion, rare long-distance colonists create isolated populations in advance of the main front, inducing spatial clustering of genotypes that can persist for hundreds of generations, or even longer if reinforced by scattered barriers to gene flow (Endler 1977; Nichols & Hewitt 1994; Ibrahim *et al*. 1996; Goodacre 2000). One adult pair of *C. russula* is enough to initiate a rapid colonization of empty sites (Vogel 1999), so that rare long-distance colonization could drive range expansion in this species. Isolated populations occur in the northern limit of its present-day distribution, some of which are recent (Frank 1984), whereas others may have existed for over a century (Roschen *et al*. 1984; Borkenhagen 1995).

ii) Selection could maintain the observed haplotype distributions. One haplotype (*H1*) or haplotypic family (H) would be favoured under the cooler conditions prevailing at high altitude (and possibly be counter-selected under warmer conditions). The target of selection could be any mitochondrial gene. Lack of recombination makes the mitochondrial genome particularly susceptible to genetic hitchhiking, which explains frequent departures from neutrality (Ballard & Kreitman 1994, 1995; Wise *et al*. 1998). Mitochondrial evolution is particularly rapid in warm-blooded animals (Majerus *et al*. 1996), presumably because the proteins coded in mitochondria are directly involved in metabolic pathways such as oxidative phosphorylation (cellular respiration) or ATP synthesis. Maternal inheritance of metabolic traits has been documented in mammals (e.g. York *et al*. 1997). Some mtDNA haplotypes affect oxygen uptake in humans (Dionne *et al*. 1991), as well as resting metabolic rate and body weight (Rowe & Ravussin 1994; Rowe *et al*. 1996). In shrews, the fine tuning of energetic budget and resting metabolic rate is important for winter survival (Genoud 1985). *C. russula* can enter daily torpor when temperature is low or food is scarce (Vogel *et al*. 1979; Genoud 1985). Such conditions occur frequently in the Jura transect, but rarely on the border of the lake, so that selective pressures on a mutation affecting the rate of recover after torpor (a process controlled by proteins from the inner mitochondrial membrane; Klaus *et al*. 1991) should vary with altitude. The reduced level of overall diversity is indeed consistent with the action of selective sweeps (though it may also result from the kin-structured bottlenecks that characterize propagule-pool colonization; Wade *et al*. 1994).

The best way to test these alternatives would be to identify a mechanistic connection between changes in the H family frequency and the action of the presumed selective agent (winter harshness). Indirect tests might, however, be conducted through the typing of neutral and independent nuclear microsatellite markers. The historical scenario predicts that the spatial patterns of nuclear markers should closely match those documented for cytoplasmic genes. Area effects in the banding and colour patterns of *Cepaea nemoralis*, for instance, have been shown to coincide closely with allele frequencies at neutral microsatellite markers, thereby strongly favouring a historical explanation (Davison

& Clarke 2000). The selection scenario, by contrast, predicts that isolation by altitude is restricted to mitochondrial haplotypes. The only patterns expected in nuclear genes would be the isolation by distance stemming from the limited dispersal rate of *C. russula* (as documented by Balloux *et al*. 1998), as well as a decrease in diversity with altitude due to the disequilibrium dynamics that characterize high altitude populations.

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