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Higher genetic diversity on mountain tops: the role of historical and contemporary processes in shaping genetic variation in the bank vole

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Glacial phases during the Pleistocene caused remarkable changes in species range distributions, with inevitable genetic consequences. Specifically, during interglacial phases, when the ice melted and new habitats became suitable again, species could recolonize regions that were previously covered by ice, such as high latitudes and elevations. Based on theoretical models and empirical data, a decrease in genetic variation is predicted along recolonization routes as a result of the consecutive founder effects that characterize the recolonization process. In the present study, we assessed the relative importance of historical and contemporary processes in shaping genetic diversity and differentiation of bank vole (*Myodes glareolus)* populations at different elevations in the Swiss Alps. By contrast to expectations, we found that genetic variation increased with elevation. Estimates of recent migration rates and a contrasting pattern of genetic differentiation observed at the mitochondrial cytochrome *b* gene and nuclear microsatellites support the hypothesis that higher genetic diversity at high elevation results from contemporary gene flow. Although historical recolonization processes can have marked effects on the genetic structure of populations, the present study provides an example where contemporary processes along an environmental gradient can reverse predicted patterns of genetic variation. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **118**, 233–244.

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INTRODUCTION

Quaternary climatic changes have provided a remarkable force for shaping the distribution and current geographical genetic structure of all living organisms (Hewitt, 2004). It has been estimated that numerous ice-sheet advances and retreats have occurred during the last 3 Myr (Williams *et al.*, 1998). Glacial phases forced species to disperse to new locations, whereas, during interglacial periods, they could expand and recolonize habitats that became suitable again (Hewitt, 2000).

This colonization dynamics had inevitable genetic consequences (Austerlitz *et al.*, 2000; Clegg *et al.*, 2002; Ehrich *et al.*, 2007). Because of the stochastic loss of alleles as a result of genetic drift in small populations (Nei, Maruyama & Chakraborty, 1975), each (re-)colonization event leads to a decrease of genetic diversity (i.e. bottlenecks; Allendorf & Luikart, 2007). As a consequence, populations living in glacial refugia typically harbour a higher genetic diversity than populations in newly colonized areas (Hewitt, 2004).

In mountainous regions, most species could only survive in ice-free areas at low elevations or at the periphery of mountain chains during the Quaternary glaciations (Taberlet *et al.*, 2008). After the retreat of glaciers, these refugial populations recolonized suitable habitats at higher elevations (Hewitt, 1996) and, during the recolonization process, genetic diversity was lost. In line with this idea, it was found that

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mitochondrial (mt)DNA diversity decreases with elevation in grater white-toothed shrew (*Crocidura russula*) populations in the Swiss Alps (Ehinger *et al.*, 2002). Similar patterns at nuclear microsatellites were observed in the American pika (*Ochotona princeps*) in the Sierra Nevada, Canada (Henry, Sim & Russello, 2012). Genetic diversity also decreased with increasing elevation in natterjack toad (*Bufo calamita*) populations in the Pyrenees (Oromi *et al.*, 2012). However, exceptions to this pattern exist. For example, Alberto *et al.* (2010) found an increase of nuclear genetic diversity with elevation in the sessile oak (*Quercus petraea*) in the French Pyrenees, most likely as a result of directional gene flow driven by preferential pollen dispersal from lower to higher elevations.

In the present study, we investigated patterns of genetic variation along an elevational gradient in bank vole populations (*Myodes glareolus*). This small rodent has a broad European distribution, from the British Isles and central-western Europe to Scandinavia and western Russia (Cook, Runck & Conroy, 2004). It inhabits deciduous woodlands and open grasslands (Corbet & Harris, 1991). Given its wide distribution, the bank vole has been used to identify main European glacial refugia and pathways of postglacial recolonization (Deffontaine *et al.*, 2005; Kotlík *et al.*, 2006; Colangelo *et al.*, 2012; Filipi *et al.*, 2015).

The recolonization of the Alps by bank voles started after the last glacial maximum (LGM; approximately 25 000–10 000 years before present; Kelly, Buoncristiani & Schlüchter, 2004). Today, the species can be found from 0 to > 2200 m a.s.l. (Müller *et al.*, 2010). Previous phylogenetic studies revealed that three mitochondrial lineages have colonized the Alps, likely originating from different glacial refugia: the Western, the Basal, and the Italian lineages (Deffontaine *et al.*, 2005; Kotlík *et al.*, 2006). In the present study, we investigated patterns of genetic diversity and differentiation at the mtDNA cytochrome *b* gene (*cytb*) and at nuclear microsatellite markers in bank vole populations from the Swiss Alps aiming to reveal how historical colonization and contemporary gene flow have shaped elevational patterns of genetic diversity in populations of a wild-living vertebrate. To our knowledge, this is the first study to compare the fine-scale genetic structure of two distinct genetic markers that evolve at different evolutionary rates along potential recolonization routes in bank voles. We predict that, as a result of the historical recolonization process, genetic diversity at mtDNA markers decreases with increasing elevation. Contemporary processes might either reinforce or counteract these historical patterns, leading to either stronger or weaker patterns of differentiation at nuclear microsatellite markers compared to mtDNA markers along elevational gradients.

MATERIAL AND METHODS

Study species and sampling locations

During the summer 2014, 348 individual bank voles (*M. glareolus*) were captured at 16 locations in the Swiss Alps (Kanton Graubünden) using live-traps (Longworth Mammal Traps, Anglian Lepidopterist Supplies). Capture, handling of animals, and tissue sampling complied with the current laws of Switzerland and were all performed under a license issued by the Department of Food Safety and Animal Health of the Kanton Graubünden, Chur, Switzerland (permit number 2012_17). Sampling sites are reported in Fig. 1 and listed in Table 1. Ear biopsies of each individual were collected and stored in 95% ethanol. Genomic DNA extraction was performed with the Biosprint 96 DNA Blood Kit (Qiagen), using the protocol for DNA purification from tissue.

Sequencing of *CYTB*

We sequenced a fragment of 1100 bp of *cytb sensu* Kotlík *et al.* (2006) to estimate *cytb* variation in bank vole populations within and across locations and assign individuals to the previously described *cytb* lineages (Deffontaine *et al.*, 2005; Kotlík *et al.*, 2006). Amplifications were performed in a total volume of 10 μL containing 0.2 μL of JumpStart Taq DNA Polymerase (Sigma-Aldrich), 0.3 μL of each primer at 300 nM of each primer (LCLE 1: 5′-CCCTCTAAT-CAAAATCATCAA-3′ and MCLE 3: 5′-TTTCATTTCTGGTTTACAAGAC-3′) and 10 ng of DNA. The polymerase chain reaction (PCR) amplification protocol consisted of an initial denaturation step at 94 °C for 1 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 90 s, with a final elongation step at 72 °C for 10 min. PCR products were purified and sequenced in both directions on an ABI Prism 3730 capillary sequencer (Applied Biosystems) using Big Dye terminator, version 3.1 chemistry (Applied Biosystems). Raw sequences were edited and aligned using GENEIOUS, version 5.6.6 (Kearse *et al.*, 2012). All 348 samples were successfully sequenced at *cytb*.

Microsatellite genotyping

We additionally genotyped bank voles at eight autosomal microsatellites, divided into two multiplexed sets (set 1: Cg16E2, Cg13F9, Cg7E5, Cg17A7, Cg3A8; set2: Cg4F9, Cg8A5, Cg1F11; Rikalainen

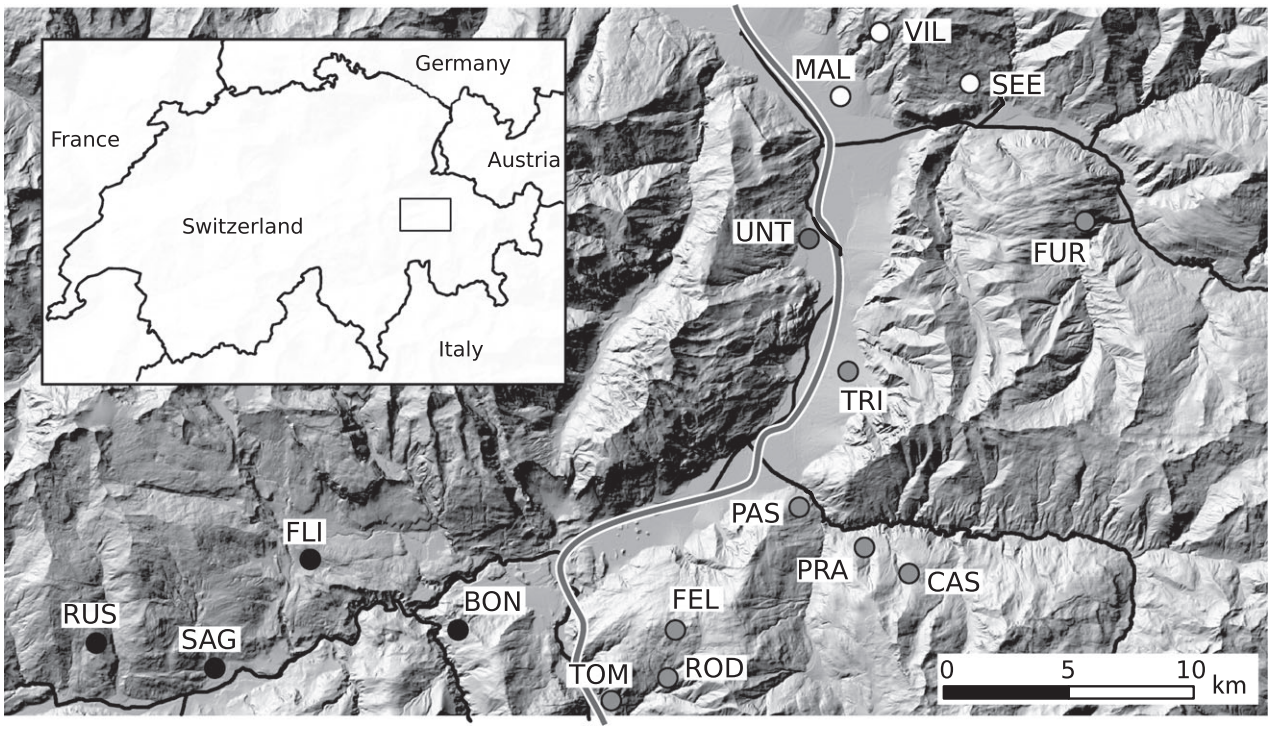

Figure 1. Location of sampled bank vole populations in the Swiss Alps. Sampling locations are indicated by circles. Circles of different colours (i.e. black, grey, white) represent the three homogeneous genetic groups identified by STRUCTURE analysis, according to mean *Q*-value observed in each population. Main rivers and roads are shown in black and grey, respectively. Acronyms refer to the sampling localities listed Table 1.

et al., 2008; Tschirren *et al.*, 2011) to estimate fine-scale genetic variation and genetic structure across locations. The amplification was carried out in a total volume of 10 μL, including 5 μL of 2 × Qiagen Multiplex PCR Master Mix (Qiagen), 1 μL 10 × primer mix and 20 ng of genomic DNA. The amplification protocol consisted of an initial denaturation step at 95 °C for 15 min, followed by six cycles (set 1) or three cycles (set 2) of 94 °C for 30 s, 56 °C minus 1 °C per cycle for 90 s, and 72 °C for 60 s; then 27 cycles (set 1) or 30 cycles (set 2) at 94 °C for 30 s, 51 °C (set 1) or 53 °C (set 2) for 90 s, and 72 °C for 60 s. A final extension step at 70 °C for 15 min concluded the protocol. Labelled PCR fragments were run on an ABI Prism 3730 capillary sequencer (Applied Biosystems). Fragment length was determined with GENEMAPPER, version 3.7 (Applied Biosystems) using an internal lane standard (LIZ). All samples were successfully genotypes for all eight microsatellite markers. Microsatellite profiles were initially tested for the possible presence of null alleles, allele drop-out or scoring errors with MICRO-CHECKER, version 2.2.3 (Van Oosterhout *et al.*, 2004). GENEPOP, version 3.4 (Raymond & Rousset, 1995) was used to test for linkage disequilibrium for each pair of microsatellite loci and for locus-by-locus deviation from Hardy–Weinberg equilibrium. Sequential Bonferroni correction was used to control for multiple testing (Rice, 1989).

Diversity and divergence of *cytb*

A *cytb* haplotype network, representing genealogical relationships between sequences, was built using TCS, version 1.21 (Clement, Posada & Crandall, 2000). The *cytb* haplotypes were assigned to mitochondrial lineages using reference sequences from (Kotlík *et al.*, 2006). We calculated *cytb* haplotype diversity (h), nucleotide diversity (π), and the mean number of pairwise difference (*MNPD*) for each population with ARLEQUIN, version 3.5 (Excoffier & Lischer, 2010). Pairwise F_{ST} comparison of haplotype frequencies calculated with Arlequin 3.5 (Excoffier & Lischer, 2010) was used to assess *cytb* genetic differentiation between populations.

Microsatellite diversity, divergence, and population structure

For the microsatellite markers, we quantified genetic variation for each population by calculating observed (H_O) and expected (H_E) heterozygosity, allelic richness

Table 1. Genetic diversity, migration rates, and bottleneck test for the 16 sampled bank vole populations

		GPS Coordinates				Mitochondrial DNA (*cytb*)				Nuclear microsatellites					
Site	Label	North	East	Altitude (m)	*N*	N_H	*h*	*MNPD*	π	N_A	A_R	H_O	H_E	*m*	*M*-ratio
Bonaduz	BON	46.799	9.352	944	18	6	0.758	3.352	0.0030	9.3	8.06	0.81	0.82	0.255	0.69
Feldis	FEL	46.789	9.453	1673	25	7	0.843	2.307	0.0021	10.5	8.35	0.85	0.84	0.291	0.71
Rodels	ROD	46.760	9.425	630	21	3	0.266	0.438	0.0004	9.1	7.76	0.82	0.80	0.179	0.70
Tomils	TOM	46.772	9.453	1144	20	4	0.657	0.794	0.0007	9.0	7.44	0.82	0.81	0.230	0.67
Ruschein	RUS	46.795	9.169	1454	20	5	0.736	2.070	0.0019	9.6	8.07	0.86	0.82	0.304	0.69
Sagogn	SAG	46.783	9.233	693	37	3	0.106	0.162	0.0001	9.5	7.20	0.81	0.80	0.103	0.70
Flims	FLI	46.827	9.280	1138	16	3	0.691	1.316	0.0012	8.7	7.94	0.84	0.83	0.303	0.65
Furna	FUR	46.940	9.702	833	11	2	0.181	2	0.0018	7.6	7.63	0.88	0.80	0.319	0.74
Vilan	VIL	47.019	9.583	1774	17	6	0.794	3.411	0.0031	9.6	8.37	0.88	0.83	0.321	0.73
Malans	MAL	46.992	9.557	560	33	4	0.715	2.268	0.0020	10.6	8.01	0.77	0.83	0.121	0.71
Seewis	SEE	46.996	9.636	1106	13	5	0.692	2.410	0.0022	7.7	7.43	0.79	0.81	0.308	0.64
Praden	PRA	46.817	9.589	1582	22	4	0.748	1.844	0.0017	10.8	9	0.84	0.86	0.239	0.76
Passug	PAS	46.840	9.538	732	23	2	0.474	1.422	0.0013	9.5	7.82	0.73	0.80	0.189	0.71
Castiel	CAS	46.826	9.569	1094	17	9	0.843	3.267	0.0030	9.2	8.14	0.89	0.84	0.319	0.74
Trimmis	TRI	46.882	9.559	762	29	7	0.837	2.359	0.0021	9.2	7.31	0.81	0.80	0.163	0.67
Untervaz	UNT	46.937	9.546	528	26	7	0.846	4.720	0.0043	10.5	8.29	0.83	0.84	0.205	0.75

Sampling localities and the corresponding acronym, GPS coordinates, and number of samples collected (*N*) are reported. Mitochondrial DNA diversity is described by number of haplotypes (N_H), haplotype diversity (*h*), mean number of pairwise difference (*MNPD*), and nucleotide diversity (π). Nuclear microsatellites genetic diversity is described by number of alleles (N_A), allelic richness (A_R), and observed (H_O) and expected (H_E) heterozygosity. Migration rates (*m*) and the the *M*-ratio are also reported.

(A_R), and number of alleles (N_A) with FSTAT, version 2.9.3.2 (Goudet, 1995). Pairwise F_{ST} values between populations were calculated with ARLEQUIN, version 3.5 (Excoffier & Lischer, 2010). Pearson's correlation coefficient (*r*) was used to test whether genetic diversity calculated at *cytb* (*h*, π, and *MNPD*) and at nuclear microsatellites (N_A, A_R, H_O, and H_E) was correlated with elevation using R, version 3.0.3 (R Core Team, 2014).

We determined the most likely number (*K*) of genetic clusters using STRUCTURE, version 2.3.4 (Pritchard, Stephens & Donnelly, 2000). The admixture model, which allows for mixed ancestry of individuals, was applied using the LOCPRIOR options that consider sampling location information. We ran 10 independent analyses consisting of 1 000 000 iterations after a burn-in period of 250 000; all *K* values between 1 and 16 were explored. Likelihood at different *K* values (Pritchard *et al.*, 2000) and its rate of change (Δ*K*, Evanno, Regnaut & Goudet, 2005) were compared to determine the most plausible number of genetically homogeneous groups using STRUCTURE HARVESTER (Earl & VonHoldt, 2012). In addition, genetic structure was examined using the factorial correspondence analyses of individual differentiation in GENETIX, version 4.05.2 (Belkhir, 1999) and the principal coordinates analysis of population pairwise F_{ST} in GENALEX, version 6.501 (Peakall & Smouse, 2012).

Estimates of contemporary migration rates between populations were obtained using a Bayesian approach implemented in BAYESASS, version 3.0.3 (Wilson & Rannala, 2003), running 10 independents Markov chain Monte Carlo (MCMC) simulations with different seed values. Each analysis consisted of 1 000 000 iterations after a burn-in of 100 000. MCMC chains were sampled every 1000 iterations. Running parameters (Δ*A* and Δ*F*, both set at 0.50) were regulated to guarantee parameter acceptance as specified in the BAYESASS manual. After checking the convergence of the 10 simulations, the run with the highest likelihood was used for further analysis. Migration rates of populations were calculated as 1 – the fraction of individuals in a population that derive from the same population.

Moreover, we estimated the demographic dynamics of bank vole populations using the *M*-ratio test, which calculates the ratio between the number of alleles and the allelic range. This ratio is expected to be lower than 0.68, as a result of stochastic loss of rare alleles, in a population that suffered from a bottleneck (Garza & Williamson, 2001). Comparison between the observed *M*-ratio with the *M*-ratios obtained by 10 000 simulations under demographic

stability was used to test the statistical significance of our results using M_P_VAL (Garza & Williamson, 2001). As input, we used the population-mutation parameter ($\theta = 4N_E\mu$, where N_E in the effective population size and μ is the mutation rate), the proportion of mutations larger than a single step (p_g), and the mean size of multi-repeat mutations (Δg). We tested different values of θ (i.e. 1, 2, and 5), whereas p_g and Δg were set to 3.1 and 0.22, respectively, sensu Peery *et al.* (2012).

Quantifying the effects of geographical distance and elevation on historical and contemporary genetic differentiation

We used a multiple matrix regression with randomization analysis (MMRR) to quantify the relative influences of geographical distance and elevational difference on genetic differentiation between populations at both mitochondrial and microsatellite markers (Wang, 2013). We used pairwise F_{ST} values as a measure of genetic differentiation between populations for both mtDNA and nuclear microsatellites. Geographical distance and elevational difference between sites were calculated in km with R. Because elevational difference and mean elevation were correlated (Mantel test: Spearman's rank correlation = 0.34, $P = 0.008$), mean elevation between sites was also included in the analysis. We also used linear F_{ST} [$F_{ST}/(1 - F_{ST})$] and log-transformed (log km) geographical distances to test for genetic differentiation in a two-dimensional habitat. However, because we obtained similar results using linear and log-transformed distances, we only report the explanatory variables in km for simplicity. We predicted that genetic differentiation increases with elevational difference between sites because distances with higher elevational differences are more physiologically costly to cover. By contrast, genetic differentiation may increase or decrease with mean elevation depending on whether dispersal/gene flow increases or decreases along the elevational gradient. Finally, we used the standardized residuals of the linear regression of microsatellites-based genetic differentiation against the mtDNA-based genetic differentiation as a response variable in the MMRR to quantify the relative influences of geographical distance, elevational difference, and mean elevation on contemporary genetic differentiation. Normality, homoscedasticity and linearity were checked graphically based on models including each explanatory variable separately and one model including all variables simultaneously. The MMRR was performed with the code written by Wang (2013) and significance was tested with 1000 randomizations in R.

RESULTS

Diversity and divergence of *cytb*

Thirty-four *cytb* haplotypes were identified in the sampled bank voles, 31 of which have never been observed before (see Supporting information, Table S1). Most *cytb* haplotypes belonged to the Western bank vole lineage *sensu* Kotlík *et al.* (2006), whereas, in one population (FUR), we found that 10 out of 11 samples belonged to the Basal lineage (Kotlík *et al.*, 2006). Genealogical relationships between *cytb* haplotypes are shown in Fig. 2, highlighting an overall high level of genetic variation at *cytb* and confirming a substantial degree of divergence between the Western and the Basal lineages (six fixed mutations). Genetic variability at *cytb* for each population is reported in Table 1.

Microsatellite diversity and divergence

There was no indication for a systematic occurrence of null alleles at the analyzed microsatellite markers. Less than 4% of the performed tests (five out of 128) detected a deviation from Hardy–Weinberg equilibrium and no consistent deviations were observed across loci or populations. After correcting for multiple testing, only two out of 128 tests were significant for deviation from Hardy–Weinberg equilibrium. Similarly, only 11 out of the 448 tested locus pairs showed significant linkage and, again, patterns of linkage were not consistent across markers or populations. All microsatellites were therefore used for further analyses.

All microsatellite loci were polymorphic. Overall genetic variation was high in all populations, with expected heterozygosity (H_E) ranging from 0.80 (ROD) to 0.86 (PRA) and the mean number of alleles per locus (N_A) ranging from 7.75 (SEE) to 10.88 (PRA) (Table 1).

After correcting for multiple testing, pairwise population differentiation (F_{ST}) was significant between all pairs of populations, with the highest and lowest value between MAL and SAG (0.102) and between FEL and CAS (0.027), respectively (see Supporting information, Table S2).

Genetic structure, migration rates, and demographic dynamics

Factorial correspondence analysis suggested a weak genetic substructure in bank vole populations at the fine geographical scale (see Supporting information, Fig. S1). Bayesian assignment analysis, using the methodology presented in Pritchard *et al.* (2000), identified $K = 8$ as the most likely number of

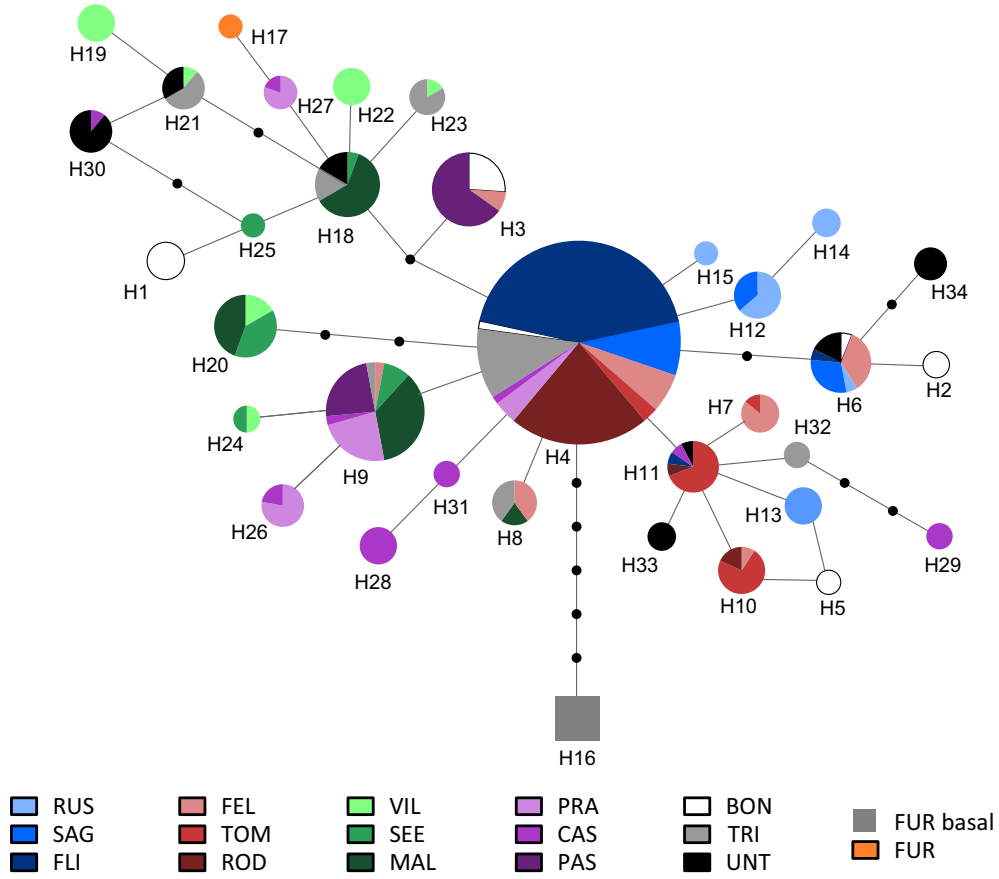

Figure 2. Haplotype network for the cytochrome *b* gene (*cytb*). Colours are indicative of the locations where the haplotypes were found. The circle sizes are proportional to haplotype frequency.

homogeneous genetic clusters, whereas it was $K = 3$ according to Evanno's method (ΔK, see Supporting information, Figs S2, S3; Evanno *et al.*, 2005). In agreement with the latter, principal coordinates analysis of pairwise population differentiation identified three genetic clusters (Fig. 3A).

Garza–Williamson's test did not provide evidence for a decline in effective population sizes because, among the few populations showing a *M*-ratio below 0.68, none of them was significantly different from what is expected under a scenario of demographic stability (Table 1).

As expected, recent migration rates between populations were relatively low (Table 1; Viitala, Hakkarainen & Ylönen, 1994; Kozakiewicz, Chołuj & Kozakiewicz, 2007). The estimated proportion of received and donated migrants for each population is reported in the Supporting information (Table S3). A strong positive and significant correlation between elevation and migration rate was found (Fig. 4, Table 2).

Relationship between genetic diversity and elevation

Nuclear genetic diversity (measured as observed and expected heterozygosity) and allelic richness were significantly positively correlated with elevation (Fig. 4, Table 2). A positive but nonsignificant correlation was also found between elevation and the number of alleles (N_A) (Fig. 4, Table 2).

For *cytb*, the relationships between genetic diversity indices and elevation were positive (Table 2), although only the relationship between *cytb* haplotype diversity and elevation reached statistical significance when accounting for differences in population size.

Relative contributions of geographical distance and elevation to historical and contemporary genetic differentiation

To quantify the effects of geographical distance and elevation on genetic differentiation, only the 15

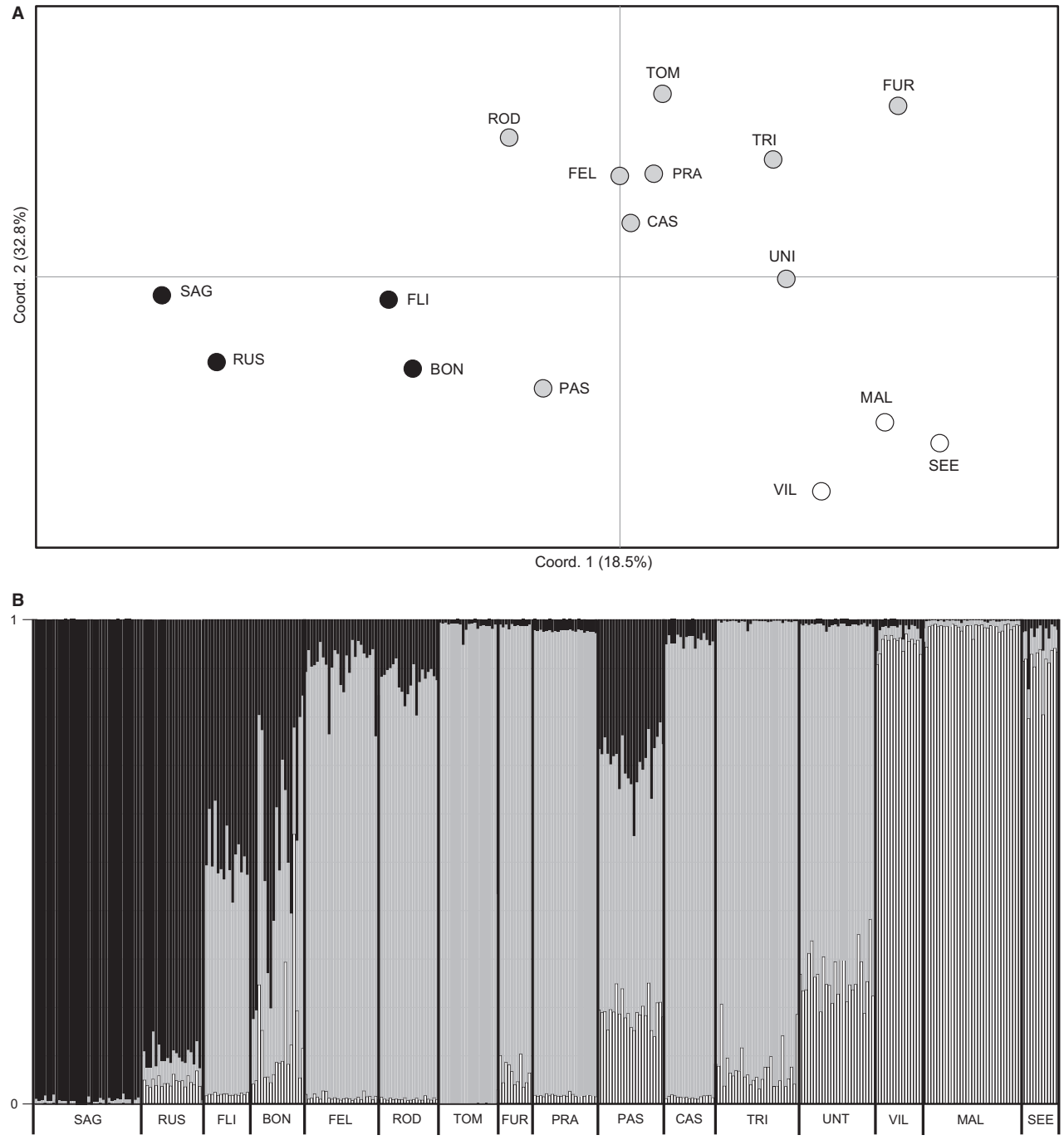

Figure 3. Genetic structure of bank vole populations based on microsatellite markers. Principal coordinate analysis of the pairwise F_{ST} between populations (A). Plot of individual proportion of ancestry for the three genetic clusters identified in STRUCTURE (B).

Western lineage populations were used to avoid a bias as a result of the single population mainly composed by individuals of the Basal lineage (FUR). Neither geographical distance ($\beta = 2.926 \times 10^{-3}$, pseudo t-value = 2.345, $P = 0.087$), elevational difference ($\beta = -4.736 \times 10^{-2}$, pseudo t-value = -1.227, $P = 0.330$), nor mean elevation ($\beta = 5.622 \times 10^{-5}$, pseudo t-value = 1.196, $P = 0.621$) explained a significant part of the mtDNA-based genetic differentiation (overall model: $r^2 = 0.08$, $P = 0.363$). By contrast, genetic differentiation at microsatellites was significantly associated with geographical

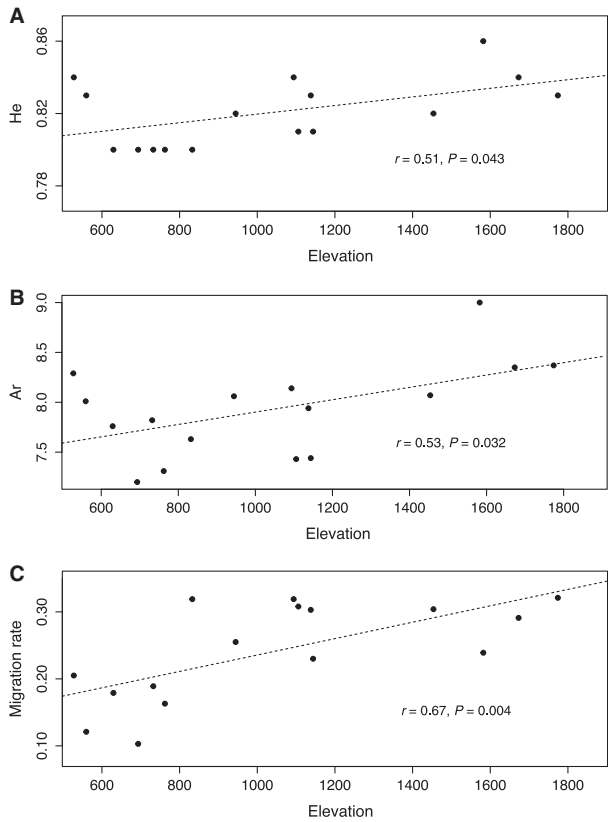

Figure 4. Correlations based on microsatellite markers between expected heterozygosity (A), allelic richness (B), and migration rate (C) with elevation.

Table 2. Summary of correlations between different indices of genetic variation and elevation

	Variable	*r*	*P*
Mitochondrial DNA	*h*	0.422	0.116
	MNPD	0.105	0.707
	π	0.105	0.708
	N_H	0.262	0.326
	h/N	**0.553**	**0.032**
	MNPD/*N*	0.287	0.299
	π/N	0.286	0.299
	N_h/N	0.394	0.130
Nuclear microsatellites	*Nall*	0.150	0.577
	$\boldsymbol{H_O}$	**0.532**	**0.033**
	$\boldsymbol{H_E}$	**0.510**	**0.043**
	$\boldsymbol{A_R}$	**0.535**	**0.032**
	M	**0.671**	**0.004**

Significant correlations are indicated in bold.

distance ($\beta = 6.847 \times 10^{-4}$, pseudo *t*-value = 9.663, $P = 0.001$), although not with elevational difference ($\beta = -3.079 \times 10^{-3}$, pseudo *t*-value = −1.405, $P = 0.244$) or mean elevation ($\beta = -9.162 \times 10^{-6}$, pseudo *t*-value = −3.431, $P = 0.107$; overall model: $r^2 = 0.51$, $P = 0.001$). mtDNA-based genetic differentiation explained 18.4% of the variation at microsatellites-based genetic differentiation (MMRR: $P = 0.009$). The residual microsatellite-based genetic differentiation (after taking the genetic differentiation at mtDNA into account) was significantly associated with both geographical distance and mean elevation but not with elevational difference (geographical distance: $\beta = 0.065$, pseudo *t*-value = 8.969, $P = 0.001$; mean elevation: $\beta = -0.001$, pseudo *t*-value = −4.482, $P = 0.024$; elevational difference $\beta = -0.165$, pseudo *t*-value = −0.734, $P = 0.522$; $r^2 = 0.49$, $P = 0.001$). Including the genetic differentiation at mtDNA as an explanatory variable in the MMRR model rather than using residual values provided quantitatively similar results (geographical distance: $\beta = 6.148 \times 10^{-4}$, pseudo *t*-value = 9.267, $P = 0.001$; mean elevation: $\beta = -1.050 \times 10^{-5}$, pseudo *t*-value = −4.284, $P = 0.040$; elevational difference $\beta = -0.968$, pseudo *t*-value = −0.386, $P = 0.386$; mtDNA-based genetic differentiation: $\beta = 2.389 \times 10^{-2}$, pseudo *t*-value = 4.636, $P = 0.008$; $r^2 = 0.60$, $P = 0.001$).

DISCUSSION

Genetic variation in natural populations is the result of biological characteristics of the studied species (e.g. dispersal capability or breeding system) and their biogeographical history (e.g. colonization processes), as well as contemporary processes (linked to landscape features, demography or natural selection; for example, Avise, 2000; Hewitt, 2000). Therefore, when analyzing the genetic diversity of natural populations, all of these aspects must be taken into consideration. In the present study, we studied bank vole populations in the Swiss Alps aiming to assess how historical and contemporary factors have shaped genetic variability along an elevational gradient.

Previous phylogeographical work has identified several mitochondrial bank vole lineages in Europe, which have recolonized the continent from different refugia after the last glaciation (Deffontaine *et al.*, 2005, 2009; Kotlík *et al.*, 2006; Filipi *et al.*, 2015). In the present study, we confirmed that the Western and the Basal lineages co-occur in the eastern part of the Swiss Alps, although individuals from the Basal lineage were rare and restricted to a single location. Unexpectedly, the mitochondrial genetic differentiation between the two lineages was not found back at nuclear microsatellite markers. Indeed, when forcing STRUCTURE to assign populations to two genetic groups based on microsatellite data, the Basal population was not separated from the

Western populations. A number of processes can lead to such a mitonuclear discordance, including adaptive introgression of mtDNA, demographic disparities, sex-biased asymmetries, and even drift (Toews & Brelsford, 2012). All of these processes were observed in bank voles or closely-related species (Le Galliard *et al.*, 2012; Sutter, Beysard & Heckel, 2013; Boratyński *et al.*, 2014) and, with the data available, it is difficult to disentangle them in the present study.

Fine-scale genetic analyses based on microsatellite data revealed that all populations were weakly genetically differentiated, with the most likely number of homogeneous groups across populations being three. These three identified groups matched geographical features of the region, with a first group (MAL, VIL, and SEE) located in the northernmost part of the study area, a second group (RUS, SAG, FLI, and BON) located in the south-western part of the study area, and the third group (UNT, FUR, TRI, PAS, PRA, CAS, FEL, ROD, and TOM) located in the central part. The Rhein and Landquart rivers are likely barriers to gene flow between the 'northern' group and the other populations. Similarly, the highway in the Rhein valley may restrict gene flow between the 'central' and 'southern' groups. However, the populations of UNT and TRI, which are located on the two opposite sides of the valley, showed a very similar genetic composition. In addition to these two potentially important barriers to gene flow, the significant correlation between geographical distance and genetic differentiation suggests that geographical isolation contributes to the observed north–south differentiation.

In most previous studies, it was found that genetic diversity declines with increasing elevation (Ehinger *et al.*, 2002; Henry *et al.*, 2012; Oromi *et al.*, 2012; but see Alberto *et al.*, 2010), which is in congruence with the theoretical prediction that genetic diversity is lost during the (re-)colonization processes as a result of consecutive founder effects (Hewitt, 2004). Unexpectedly, we observed the opposite pattern in the present study: a consistently higher neutral genetic diversity at higher elevations. There are several non-mutually exclusive explanations for this finding.

First, some studies have proposed that ice-free habitats on mountain peaks (nunataks; Blytt, 1876) have acted as refugia during the Quaternary glacial phases (Schonswetter *et al.*, 2002). Under this scenario, re-colonization pathways after the LGM would be reversed (i.e. re-colonization starts from refugial populations at high elevation), and genetic diversity would thus be lowest at low elevation. Furthermore, under this scenario, genetic differentiation is predicted to be either unrelated to elevation or to increase with elevation because genetic differentiation is generally stronger among ancestral populations than among newly-colonized populations (Hewitt, 1996). However, although nunatak refugia might be a plausible scenario for some plant and invertebrate species (Stehlik, 2000; Lohse, Nicholls & Stone, 2011), this is unlikely for small mammals given their ecological requirements. Moreover, the negative correlation between genetic differentiation and elevation observed in the present study is in conflict with the predictions of this scenario.

Second, the high genetic diversity at high elevation might be explained by bottleneck events occurring in low-middle elevation populations; for example, as a result of anthropogenic influences (Theurillat & Guisan, 2001). Indeed, for many plant and animal species, anthropogenic impacts on habitats can have negative consequences for effective population sizes and genetic diversity (Frankham, 2005; Allendorf & Luikart, 2007). However, the genetic bottleneck test, which is based on the frequency distribution of allele sizes and therefore independent from the genetic diversity within populations (i.e. heterozygosity), did not provide evidence for population size reduction in any of the studied populations. Demographic processes are thus unlikely to explain the observed pattern.

Finally, higher gene flow at high elevation may explain the higher genetic diversity because gene flow maintains local genetic variation and counteract genetic drift by mixing alleles between populations. This higher gene flow could be historical: bank voles could have colonized high elevation habitats from different sides of the mountain (i.e. from different valleys), which would lead to the admixture of different ancestral genetic variants and thus increase genetic diversity. Alternatively, or in addition, higher gene flow at high elevation may be a result of higher dispersal activity. Indeed, although rodents generally disperse over short distances (< 100 m; Le Galliard *et al.*, 2012), dispersal strategies appear to be flexible and related to environmental heterogeneity (Dugger *et al.*, 2010; Le Galliard *et al.*, 2012; Santoro, Green & Figuerola, 2013). Environmental conditions vary dramatically along elevational gradients, with high elevation habitats being exposed to more extreme weather events, such as particularly cold winters or very dry periods during summer (Beniston & Stephenson, 2004; Beniston, 2005; Huggel *et al.*, 2010). Such weather events can affect resource availability, increasing the need of dispersal for high elevation populations. Indeed, we observed a positive correlation between migration rates and elevation, a negative correlation between genetic differentiation and elevation (after correcting for geographical distance and elevation difference), and an increase of genetic diversity with elevation. Taken together,

these results support the hypothesis that gene flow increases with elevation.

To further clarify the relative importance of historical and contemporary processes in shaping genetic differentiation of bank vole populations along the elevational gradient, we performed an MMRR analysis. We found that the relationship between elevation and genetic differentiation at microsatellites was significant after taking into account mitochondrial genetic differentiation, whereas such pattern was not observed on mitochondrial genetic differentiation. This demonstrates that only microsatellites exhibit a signal of higher gene flow at higher elevations. Although we cannot exclude the possibility that the signal of higher gene flow at high elevation might have been lost at mitochondrial DNA because of drift (Irwin, 2002), the pattern suggests that the increased gene flow at higher elevation is a contemporary rather than historical process.

In conclusion, counter to theoretical predictions and empirical findings in other species, we observed a higher, rather than lower, genetic diversity in bank vole populations at high elevation. Our analyses strongly suggest that this pattern is the result of an increase of contemporary gene flow (i.e. dispersal and/or immigration success) in high elevation habitat, probably because of ecological factors such as higher environmental instability. Interestingly the higher genetic diversity at high elevation could influence the long-term viability (Frankham, 2005; Charlesworth & Willis, 2009), as well as the evolutionary potential of high elevation populations. The latter might be of particular importance given that ecological alterations as a result of climate change are predicted to be strongest in high elevation environments (Beniston & Stephenson, 2004; Beniston, 2005). Overall, our findings show that contemporary processes can counteract or even reverse genetic footprints of historical recolonization.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Factorial correspondence analysis (*FCA*) of individual differentiation based on nuclear microsatellites.

Figure S2. Number of genetically homogeneous clusters identified by STRUCTURE according to $L(K)$ (A) and ΔK (B)

Figure S3. Plots of individual proportion of ancestry considering two and eight homogeneous genetic clusters.

Table S1. List of haplotypes found in the present study with their frequency and accession numbers. Labels refer to Fig. 2.

Table S2. Pairwise F_{ST} comparisons between populations based on eight nuclear microsatellites (below the diagonal) and based on the cytochrome *b* gene (*cytb*) (above the diagonal). All of the values were significant after sequential Bonferroni correction.

Table S3. Proportion of received and donated migrants for each population according to BAYESASS. Source populations are listed in columns, whereas sink populations are arranged in rows. Mean posterior distribution of migration rates higher that 0.1 are highlighted.