Contents lists available at ScienceDirect



Biological Conservation



journal homepage: www.elsevier.com/locate/bioc

Gene flow counteracts the effect of drift in a Swiss population of snow voles fluctuating in size



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ARTICLE INFO

Article history: Received 9 March 2015 Received in revised form 8 June 2015 Accepted 13 June 2015 Available online xxxx

Keywords: Chionomys nivalis Genetic drift Genetic monitoring Immigration Population genetics

ABSTRACT

Genetic monitoring has emerged as a useful tool to better understand evolutionary processes acting within and among natural populations. Longitudinal studies allow the examination of temporal changes in neutral genetic patterns in relation to demographic data, which is particularly interesting in populations that undergo large fluctuations in size. Taking advantage of eight years (2006–2013) of genetic survey data (18 microsatellite loci) from a snow vole (*Chionomys nivalis*) population in the Swiss Alps, we explore whether and how gene flow and selection shape temporal variability in genetic diversity by counteracting the effect of genetic drift, and thereby maintain the high levels of heterozygosity observed in this population. Using simulations and empirical data, we show that effective population size is small, and that genetic drift would lead to a marked decline in genetic diversity. However, this force is counterbalanced by the restoring effect of immigration. In agreement with the predictions of neutral genetic theory, we found a strong, positive association between genetic diversity and population size, which suggests positive density-dependent dispersal. This is also supported by the observed changes in genetic composition over time. Meanwhile, selection for heterozygosity was weak, overriding the effect of drift only in one out of eight years. Altogether, our results highlight the importance of gene flow as a significant evolutionary force in shaping genetic patterns in the wild, and as a crucial process for the maintenance of genetic diversity in small populations.

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1. Introduction

Genetic variability is diminished through two major genetic processes: genetic drift (i.e., inbreeding under random mating) and inbreeding due to non-random mating (Frankham et al., 2002; Briskie and Mackintosh, 2004; Bouzat, 2010). In particular the effect of drift is considered to be a major threat to the viability and persistence of small and fragmented natural populations (Frankham, 1996; Allendorf et al., 2013). Genetic monitoring, i.e., the quantification of temporal changes in population genetic metrics (Schwartz et al., 2007), makes it possible to identify the moment when a population reaches a critical threshold and demands appropriate management actions to counteract the causes and consequences of reduced genetic variation (Frankham, 2010; Allendorf et al., 2013). More in general, it constitutes a useful tool for studying ecological and microevolutionary processes (Hoban et al., 2014).

The amount of drift a population experiences is not proportional to its census size, but rather its so-called effective population size (Ne)

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(Wright, 1978; Nei et al., 1975). Thereby, knowledge of the effective size of a population provides insight into the rate at which a population losses genetic variation. The effective population size is not only reduced by unequal sex ratios and variance in reproductive success, but also by fluctuations in population size, where years with small population sizes have disproportionally large effects (Motro and Thomson, 1982; Whitlock, 1992; Palstra and Ruzzante, 2008). However, non-stable demographic conditions disrupt the equilibrium between drift and immigration, which complicates the relationship between genetic variation and *Ne* (Vucetich and Waite, 2000).

The negative effect of genetic drift can be overcome by gene flow, even when it is rare or limited. Indeed, just a few immigrants can have a strong impact on the genetic diversity of a population (Madsen et al., 1999; Keller et al., 2001; Johnson et al., 2010; Hedrick et al., 2014). Two good examples of the positive impact of immigration on the viability of isolated populations come from wolf (*Canis lupus*) populations in Scandinavia (Vilá et al., 2003) and Isle Royale, Canada (Adams et al., 2011). In both cases, the arrival of a single immigrant led to the rescue and recovery of these bottlenecked populations (Vilá et al., 2003; Adams et al., 2011). The positive effect of immigration on genetic variability is the result of immigrants introducing novel alleles into the pool of local alleles, which can increase heterozygosity and offset the

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potentially negative effects of inbreeding (Keller and Waller, 2002; Westemeier et al., 1998; Marr et al., 2002). Hence, a rapid accumulation of new alleles through immigration can contribute to the maintenance of high levels of genetic variability in fluctuating populations (Ehrich and Jorde, 2005; Rikalainen et al., 2012; Gauffre et al., 2014). Furthermore, in addition to being different, the alleles introduced by immigrants may increase in fitness by increasing heterozygosity (i.e., overdominance effect), as only more heterozygous individuals may be able to successfully disperse into a new population (e.g., Selonen and Hanski, 2010; García-Navas et al., 2014). However, immigrant individuals may exhibit lower viability and fecundity if they express genes that are not adapted to local conditions (local maladaptation or outbreeding depression; Lynch, 1991; Hansson et al., 2004).

From the above it follows that for small populations, the arrival of immigrants is crucial not only in demographic terms (Ward, 2005; Schaub et al., 2010), but also in genetic terms (Hansson et al., 2000; Ortego et al., 2007). However, it has to be emphasized that immigration and gene flow cannot necessarily be equated, as the amount of gene flow will depend on the immigrants' capacity to spread their genes at their new location (Saccheri and Brakefield, 2002). Furthermore, the effect of immigration on genetic variability may interact with density, as evidenced by studies on arvicoline rodents (e.g., Ehrich and Jorde, 2005). For example, if gene flow increases with density, and because it is the absolute rather than the relative number of migrants that shapes genetic variation (Slatkin, 1985; Yu et al., 2010), genetic variability is predicted to increase with density (Charnov and Finnerty, 1980). On the other hand, if immigration shows negative density-dependence, the effect of increased immigration in low-density years may counteract or outweigh the effect of the increased amount of genetic drift in those years, resulting in the relationship between density and genetic diversity being zero or negative (Lambin and Krebs, 1991). Both models also make different predictions in terms of temporal genetic differentiation and genetic structure; the model of Charnov and Finnerty (1980) predicts the existence of temporal instability in genetic composition due to a loss of genetic variability at low density and low immigration, whereas the model of Lambin and Krebs (1991) does not predict significant temporal differentiation.

In addition to the role of gene flow in counteracting the negative effect of genetic drift on genetic variation, selection can play a significant role in shaping population genetic patterns as well. If heterozygotes have a higher fitness than homozygotes due to the negative effects of homozygosity at (linked) genome-wide distributed functional loci (i.e., inbreeding depression; Kempenaers, 2007), selection favouring heterozygotes may affect population genetic diversity. One of the most common effects of reduced genetic diversity is a decreased probability of survival, either during early-life stages or later in life (e.g., Kruuk et al., 2002; Mainguy et al., 2009). The selection against relatively homozygous individuals this generates may contribute to the maintenance or increase of heterozygosity over time (Bensch et al., 2006; Kaeuffer et al., 2007; Nietlisbach et al., 2015). However, although there is a growing literature showing evidence for a positive association between heterozygosity measured at putatively neutral microsatellite markers and fitness-related traits (e.g., Da Silva et al., 2009; reviewed in Chapman et al., 2009), the magnitude and direction of heterozygosity-fitness correlations may depend on the environmental conditions (Fox and Reed, 2011). In this vein, recent studies highlight that inbreeding depression may be accentuated under stressful and unpredictable conditions (Da Silva et al., 2005; Lesbarrères et al., 2005; Brouwer et al., 2007; Auld and Relyea, 2010), and accordingly, the strength of selection for heterozygosity may vary across years (Forcada and Hoffman, 2014).

Here, we study temporal changes in genetic variability in a small and relatively isolated population of European snow voles (*Chionomys nivalis*) over a period of eight years (2006–2013), which represents one of the few long-term individual-based study populations of rodents.

This time interval comprises a decrease in population size by around 40%, and a subsequent recovery period. Thereby this population provides a good model to investigate the relationship between genetic variability and demography. Specifically, we quantify the importance of the three evolutionary forces that have the potential to influence genetic variation over relatively short time periods (drift, gene flow and selection) in shaping temporal changes in genetic variability. Furthermore, we test whether these changes are density-dependent, and whether they show a pattern of genetic differentiation over time. Thereby, we provide insight into the mechanisms contributing to the maintenance of high genetic diversity in populations with erratic (i.e., non-cyclic) density fluctuations.

2. Methods

2.1. Study species

The European snow vole is a relatively large-sized (up to ~13 cm) microtine rodent whose distribution is restricted to mountainous regions of southern and eastern Europe (Pyrenees, Alps, Apennines, Carpathians) and south-western Asia, and which for this reason is considered a glacial relict species (Amori, 1999; Yannic et al., 2012). It inhabits high-mountain biotopes (mostly above the tree line; >1000 m), showing an overwhelming preference for rocky environments (scree and boulder-covered slopes) (Luque-Larena et al., 2002). This habitat preference leads to highly isolated populations and a naturally fragmented distribution (Castiglia et al., 2009). The snow vole has a promiscuous mating system in which both males and females mate with multiple partners (Luque-Larena et al., 2004). Females show territorial behaviour but tolerate the presence of relatives; daughters usually remain in their natal area, resulting in matrilineal female clusters. Males have overlapping home ranges, which can encompass the territories of several females (Luque-Larena et al., 2004). The reproductive period is from May to August, during which they produce one or two litters of one to five pups each (Janeau and Aulagnier, 1997). The average snow vole lifespan is 12-13 months, which means that most individuals do not survive the first winter (Janeau and Aulagnier, 1997; Pérez-Aranda, 2009). Unlike other microtine species, snow voles do not show strong population cycles (Yoccoz and Ims, 1999).

2.2. Study area

The study was carried out in the Swiss Alps, near the Churer Joch (Churwalden, canton of Graubünden; 46°48′ N, 9°34′ E; 2.030 m.a.s.l.). The study area (approx. 5 ha in size) consists of a west-exposed scree slope with sparse vegetation (high altitude shrubs) surrounded by meadows to the south and to the north, bedrock to the east and a coniferous forest to the west (see Supporting information for more details). Based on this, and the fact that our study site comprises most of the suitable habitat that can be found in this area, we assume this population to be ecologically fairly isolated. Nevertheless, there is another suitable habitat in close proximity (app. 400 m), which is inhabited by snow voles and which may act as a source of immigrants.

2.3. Live trapping

For eight consecutive years (2006–2013), snow voles have been live-trapped between mid-June and early October in a standardised manner. To this end, the study area is overlaid with a 10×10 m grid consisting of a total of 559 cells. A catch-and-release trap (Longworth model, Penlon Ltd, Oxford, UK) filled with hay and baited with apple, hamster food and peanut butter is placed in each cell. Animals captured for the first time are ear-clipped (2 mm diameter, thumb type punch, Harvard Apparatus, Massachusetts, USA) and individually marked by implanting a PIT tag (ISO transponder, Tierchip Dasmann, Tecklenburg, Germany) under the skin of the neck. Ear biopsy samples were

preserved in 95% ethanol + 5% TE buffer and stored at —20 °C until DNA extraction (see below). At each capture we recorded trapping location, identity, weight (to the nearest 1 g using a Pesola scale), sex and age. The latter is inferred based on weight (juveniles typically are <34 g), colour (juveniles are darker) and genital development. We conducted 20–30 capturing sessions per year, with 4 sessions necessary to complete one run through the entire study site. Traps (100–150 per day depending of the time of the year and the part of the study plot) were set up during the day, opened at dusk and checked around sunrise.

Mark-recapture analyses (not shown) revealed high among-session recapture probabilities for both age classes (adults: $92.4\% \pm 1.1$; juve-niles: $81.1\% \pm 3.0$). Goodness-of-fit tests provide no evidence for the existence of heterogeneity in trapability, indicating that most individuals present in a given year are caught at least once. Indeed, only two capturing sessions are required to capture 97% of juvenile individuals. Thus, apparent year-to-year survival and population size can be obtained without mark-recapture modelling.

2.4. Microsatellite genotyping and descriptive statistics

Genomic DNA was extracted from tissue samples using magneticparticle technology (BioSprint 96 DNA Blood Kit, Qiagen, Limburg, Netherlands). A total of 885 individuals were genotyped at 18 microsatellite markers, distributed over three multiplex panels, specifically designed for the snow vole. Thirteen markers have been previously described (Wandeler et al., 2008), while the remaining five (*Chni19*, *Chni21*, *Chni22*, *Chni24*, *Chni25*) are unpublished markers (see Table S1). In addition, voles were sexed by genotyping the male-specific *Sry* gene (Gubbay et al., 1990). Amplification products were run on an ABI 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA) and genotypes were scored using GENEMAPPER 4.1 (Applied Biosystems).

Microsatellite markers were tested for departure from Hardy– Weinberg (HW) equilibrium, both considering all loci together and each locus individually, using the Markov chain method as implemented in Genepop on the Web (http://genepop.curtin.edu.au/; Rousset, 2008). We also used Genepop to test for average heterozygote deficit or excess in relation to HW expectations using one-sided tests.

2.5. Parental assignment and detection of immigrants

We were able to assign the great majority of juveniles and adults captured for the first time to a father and a mother using a maximum likelihood model implemented in COLONY 2.0 (Jones and Wang, 2010) and a Bayesian approach implemented in the R package MasterBayes (Hadfield et al., 2006). Parentage analyses were performed for each year separately using a pool of candidate parents that included all adults sampled in that year or in the preceding year (with the exception of 2006, the first year of the study).

First-generation immigrants were identified by means of two approaches. First, we defined potential immigrants as those individuals whose parents could not be identified (pedigree-based approach). Second, we performed assignment tests implemented in the program GENECLASS2 (Piry et al., 2004) to detect long-distance immigrants ("genetic outliers") (marker-based approach). We evaluated the assignment precision using the Monte Carlo resampling procedure of Paetkau et al. (1995) (n = 10000), which computes the likelihood an individual originated from a given population. We only considered as longdistance immigrants those individuals assigned to the study population with a probability < 0.05. Using this criterion, more than half (55%) of the individuals with unknown parents were identified as immigrants in the assignment test; the remaining ones were not genetically differentiated from the study population and may have entered the study area from more nearby locations (i.e., short-distance immigrants). None of the long-distance immigrants identified on the basis of genetic data had known ancestors.

2.6. Genetic diversity estimates

We calculated annual estimates of expected (*He*) and observed (*Ho*) heterozygosity (Nei, 1987), using GenAlEx 6.5 (Peakall and Smouse, 2012). We also computed allelic richness (*Ar*) by employing the rarefaction procedure (El Mousadik and Petit, 1996) implemented in HP-RARE 1.1 (Kalinowski, 2005) to correct for unequal sample sizes among years. Individual genetic diversity was estimated using the homozygosity by loci (*HL*) index (Aparicio et al., 2006). *HL* weighs the contribution of loci depending on their allelic variability, giving more weight to highly polymorphic loci. *HL* values were computed using CERNICALIN, an Excel macro written by J.M. Aparicio (available at: https://sites.google.com/site/joaquinortegolozano/) and transformed (heterozygosity = 1 - HL) to reflect heterozygosity.

2.7. Genetic differentiation over time and denso-dependency

We tested for changes in heterozygosity over time in adults and juveniles separately using general linear mixed models. The first model included adult heterozygosity as a dependent variable, and study year, sex and origin (immigrant/resident) as explanatory factors. Because some individuals were observed in more than one year, individual identity was fitted as a random factor to avoid pseudoreplication. The second model included offspring heterozygosity as a dependent variable and year of birth, offspring sex and parental origin (i.e., if they are descendants of immigrants or not) as explanatory terms. Maternal and paternal identities were included as random effects to control for the nonindependence of heterozygosity of half and full-sibs. Immigrant juveniles (n = 22) were not included in this analysis (see Results). We used Akaike's Information Criterion corrected for small sample sizes (AIC_c) to refine the model by backwards stepwise deletion, with better fitting models resulting in lower AIC_c values (Burnham and Anderson, 2002). The relative importance of each variable was assessed based on the sum of AIC_c weights of those models with \triangle AIC_c \leq 2 in which the variable was present. AIC_c weights were computed using the functions available in the R package AICcmodavg (Mazerolle, 2011).

Subsequently, we tested for temporal genetic differentiation by estimating F_{ST} values between all pairs of years using F_{STAT} v.2.9.3.2 (Goudet, 2002). We also tested for the existence of an isolation-by-time pattern (sensu Hendry and Day, 2005), a temporal equivalent of isolation-by-distance (Maes et al., 2006; Ortego et al., 2011). To that end, we analyzed the association between a matrix of genetic distances (pairwise F_{ST}) between populations and a matrix of temporal distances (number of years elapsed between populations) by means of a Mantel test. Additionally, the pattern of population differentiation over time was visualized using a Principal Coordinates Analysis (PCoA) (see Supporting information).

To quantify the role of density in shaping genetic variation, we tested the predictions made by the models of both Charnov and Finnerty (1980) and Lambin and Krebs (1991) (see Introduction). We quantified the association between yearly estimates of genetic diversity (*Ho, He, Ar*) and population size (measured either as the number of adults or as the total number of individuals) on the other by means of linear least squares regression. All statistical analyses were carried out using R 3.2.0 (R. Development Core Team, 2012) with the exception of nonparametric tests, which were performed using PAST (folk.uio.no/ ohammer/past/).

2.8. The strength of genetic drift vs. immigration

We followed two approaches to test for the effect of immigration versus genetic drift on the temporal dynamics of genetic variability observed in the study population. First, we compared the observed annual mean expected heterozygosity based on the entire data set to the expected heterozygosity of a subset excluding all immigrant lineages (both short-distant and long-distant immigrants and their descendants). Second, we simulated the fate of a closed population with similar characteristics (same initial allelic frequencies and demographic parameters) to the study population using the software BOTTLESIM 2.6 (Kuo and Janzen, 2003). We used the "multilocus with variable population size" module, which allows setting the number of adults and juveniles present each year in the population. Based on our knowledge of the biology of the snow vole in general and this population in particular, we modelled the reproductive system as dioecy with random mating (which is the option that best fits the mating system of the species), a mean lifespan of 1 year, and 75% generation overlap (i.e., at least three overlapping generations). Neither selection nor mutations were accounted for in the model (Kuo and Janzen, 2003). The simulation was run with 1000 iterations.

2.9. The strength of genetic drift vs. selection

To elucidate the relative influence of selection on the observed temporal dynamics of genetic variability, we analyzed the relationship between individual heterozygosity and two fitness measures: number of recruiting offspring (an estimate of annual reproductive success) and survival probability. To test our ability to detect heterozygosity-fitness correlations (HFC) in this population using our set of markers, we estimated the degree of identity disequilibrium, which was found to be substantial and statistically significant. For more details, see Supporting Information.

First, we explored the relationship between individual heterozygosity and number of offspring recruited using generalized linear models. We constructed a model for each year of the study including number of recruiting offspring as a response variable (Poisson distribution with a log-link function) and heterozygosity, sex, origin, and body mass as predictors. Second, we tested for the existence of differences in genetic diversity between individuals (both adults and juveniles) that survived to the following breeding season and those that did not, by using a logistic regression framework where survival success (survived or not) was included as a binary response variable. We constructed a model for each year of the study by fitting heterozygosity as an explanatory variable, together with sex, age (adult/juvenile), and their interaction. Model selection was based on an AIC approach as described above.

In addition to the statistical tests outlined above, we estimated the directional selection differential (*S*) (Lande and Arnold, 1983; Kingsolver et al., 2001) for heterozygosity based on observed differences in this trait before and after survival selection. The selection differential S is equal to the difference between the mean of the individuals surviving to the next year and the mean of all individuals in the population (which is equivalent to the covariance between relative fitness and heterozygosity). We compared the observed level of selection (computed for each year and for the whole period) with the strength of genetic drift (i.e., the effective population size), which was determined from genotypic data using both the linkage disequilibrium (LD) and the heterozygote excess (HE) method. The premise of LD method, the most widely single-sample method, is that the magnitude of random associations of alleles at different gene loci is determined by Ne, the number of individuals sampled, and the recombination rate between loci (Hill, 1981). The HE method is based on the following principle: when the effective number of breeders in a population is small, the allele frequencies will be different in males and females, leading to an excess of heterozygotes in the progeny with respect to HW expectations (Pudovkin et al., 1996). This method estimates Ne with no bias and fair precision in polygamous or polygynous populations (Luikart and Cornuet, 1999). These analyses were performed using the software NEESTIMATOR 2.01 (Do et al., 2014). In addition, we obtained a demographic estimate of Ne for each year following Kimura and Crow (1963) as

 $Ne = N_i k_i - 1/k_i - 1 + (Vk_i/k_i)$

where *N* is the number of all sexually mature individuals of sex *i*, *k* is the mean number of offspring over all individuals of sex *i*, and Vk_i is the variance in reproductive success of sex *i*. We calculated the harmonic mean of the estimated annual *Ne* values, from both genotypic and demographic data, over the study period.

Finally, we computed narrow-sense heritability (h^2) estimates of heterozygosity from the linear regression coefficient of offspring on mid-parent values (Falconer and MacKay, 1996). This allowed us we to ascertain whether in our study system heterozygosity can respond to selection as has been shown in previous studies (Mitton et al., 1993).

3. Results

3.1. Temporal patterns in population size

Population size decreased continuously over the first six years (slope: -18.75, p < 0.01, $R^2 = 0.72$; Fig. 1a; Table 1a). During the years of lowest density (2010–2011), the number of juveniles was similar to the number of breeding adults (Fig. 1a). From 2011 onwards, the population size started increasing again, mainly attributable to an increased number of juveniles (Fig. 1a). Offspring production per adult decreased from 2006 to 2011, with the exception of 2009, when the mean number of captured offspring per adult was twice that in other years (Table 1a).

According to the LD method, the estimated effective population size over the 2006–2013 period (average value) was about 58 individuals. Both genetic methods (LD and HE) provided very similar estimates of *Ne* (Table 1a). Estimates of *Ne* based on demographic data were much lower than those based on genetic data (mean *Ne/N* ratio = 0.30; Table 1a). Estimates obtained from both methods were however strongly correlated (LD vs. HE and HE vs. Demo; p < 0.01 in both cases). Finally, all *Ne* estimates were significantly associated with census population size (Table 1a). Overall, effective population size remained relatively constant during the period 2006–2010, while from 2011 onwards it decreased by 35% (Table 1a).

3.2. Temporal patterns in genetic variation

Overall, the studied population showed a high level of genetic variability: annual mean observed heterozygosity ranged between 0.70 and 0.92, and expected heterozygosity ranged between 0.811 and 0.833. Furthermore, allelic diversity was high (four to twenty three alleles; see Table S1), and mean allelic richness varied between 11.11 and 12.05 across years (Table 1b). Even though adult heterozygosity did not differ significantly among years ($F_{7,415} =$ 0.52, p = 0.79), we detected a slight but non-significant decrease in mean adult heterozygosity over time (slope: -0.001, p = 0.48, $R^2 = 0.08$; Fig. 1c). However, this negative trend does reach statistical significance excluding the year 2011 (slope: -0.002, p = 0.029, $R^2 = 0.64$). Offspring heterozygosity did not differ significantly among cohorts after controlling for parental identity (cohort: $F_{7,143} = 1.02$, p = 0.42; father identity: Z = 1.82, p = 0.03; mother identity: Z = 1.63, p = 0.05). However, Fig. 1b shows that temporal variation in mean offspring heterozygosity can be split into two distinct phases: a decrease during 2006–2010 (slope: -0.007, p =0.028, $R^2 = 0.84$) and an increase from 2010 onwards (slope: $0.007, p = 0.37, R^2 = 0.39$).

 $F_{\rm ST}$ values between pairs of years ranged from 0.002 to 0.013 (all *p*-values > 0.05 with the exception of the following pairwise comparisons: 2006 vs. 2012, 2006 vs. 2013, 2007 vs. 2013), and were positively correlated with temporal distance between populations measured in years (Mantel test, r = 0.79; p = 0.010). That is, population pairs more distant in time had higher $F_{\rm ST}$ values (Fig. 2a).



Fig. 1. (a) Temporal changes in population size (*total number*: dashed line; *number of juveniles*: grey line; *number of adults*: black line) and effective population size (estimated using the LD method; dotted line); (b) allelic richness (*Ar*: light-grey line) and expected heterozygosity (*He*: black line); and (c) observed heterozygosity (*Ho*) (*adults*: black line; *juveniles*: grey line). *Ar* and *He* are displayed together with the census population size estimates (dotted line and empty dots) as standardised values (*z*-scores) for the sake of illustration.

3.3. Population size and genetic variation

When testing for density-related changes in genetic diversity, we found that *He* and *Ar* fluctuated concomitantly with population size (Table 1, Fig. 1b); both genetic indices showed a positive relationship with either total population size or number of adults (all *p*-values < 0.05, see Supporting Information). There was no significant relationship between *Ho* and any estimate of population size (Table 1). Whereas there was no evidence for departures from HW equilibrium in the first 5 years (Table 1b), we found a heterozygote excess in the last three years (Table 1; 2011: p = 0.016; 2012: p = 0.022; 2013: p = 0.046).

3.4. Immigration

A total of 89 individuals were identified as either short (45%) or long-distance (55%) immigrants; 43% of these immigrants had no offspring, either because they were juveniles at the moment of capture and did not recruit as breeders, or because they were unable to obtain a mate (i.e., floaters). The proportion of male immigrants was significantly higher than the proportion of female immigrants (males: 66%, females: 34%; binomial test, p = 0.002), which is in

agreement with the male-biassed dispersal pattern typically exhibited by mammals (Greenwood, 1980).

Fifty-one immigrant individuals had descendants and 14 of them introduced novel alleles into the population. The number of immigrants arriving into the study population progressively decreased from 2006 to 2010 (Fig. 3a) and was positively (but not significantly) associated with annual density (Table 1a). However, the proportion of immigrants continued to decline as population size increased again (Table 1a). This means that, absolutely speaking, immigrants were more common in high-density years, but that this increase was not proportional to the increment in population size. Meanwhile, the number of effective immigrants (i.e., those that reproduced successfully) ranged from three to ten (modal value: 7 individuals per year) over the study period. We did not find significant differences in heterozygosity between immigrant and resident adults (estimate: -0.008 ± 0.01 ; $F_{1,423} = 0.61$, p = 0.43). Yet, immigrant juveniles (mean \pm SE: 0.870 \pm 0.01, n =22) and descendants of immigrants (mean \pm SE: 0.846 \pm 0.01, n =153) tended to be more heterozygous compared to those with both parents born within the study area (mean \pm SE: 0.832 \pm 0.01, n = 401) $(F_{1,421} = 3.73, p = 0.053 \text{ and } F_{1,655} = 3.19, p = 0.07, \text{ respectively}).$

When removing all immigrant lineages from the original data set, we found a substantial decline in H_e over the study period, especially during the last years (entire data set vs subset excluding immigrants:

Table 1

Overview of annual values of (a) demographic and (b) genetic parameters.

	Year								Test for density-dependence ^a		
	2006	2007	2008	2009	2010	2011	2012	2013	Coefficient ρ	t	р
(a) Demographic parameters											
Total number of individuals	215	232	158	190	159	82	99	122	-	-	-
Number of adults	79	80	67	57	78	40	40	47	-	-	-
Number of juveniles	136	152	91	133	81	42	59	75	-	-	-
Number of unsampled (ghost) parents	15	8	3	4	2	1	6	6	-	-	-
Number of immigrants	15	15	13	16	3	11	9	8	0.58 ¹	1.57	0.16
Proportion of immigrants (%)	19	10	15	17	4	17	22	17	-0.34^{1}	-0.88	0.41
Adult sex ratio (%) (prop. of females)	0.62	0.57	0.57	0.47	0.54	0.47	0.45	0.62	0.44 ¹	1.33	0.23
NeLD (linkage disequilibrium method)	55.1	57.2	54.3	38.1	58.7	31.7	25.3	32.1	0.92^{2}	9.89	< 0.001
NeHE (heterozygote excess method)	62.5	66.1	58.8	42.9	64.2	34.1	28.3	37.1	0.97 ²	13.79	< 0.001
NeDemo (demographic method)	28.8	28.4	19.5	19.0	34.7	12.9	15.0	12.2	0.83 ²	5.75	< 0.01
(b) Genetic parameters											
Observed heterozygosity (Ho)	0.844	0.837	0.826	0.831	0.832	0.829	0.836	0.829	0.39 ¹	1.01	0.35
Expected heterozygosity (He)	0.833	0.826	0.823	0.830	0.831	0.811	0.817	0.819	0.82 ¹	3.82	< 0.01
Number of alleles (K)	211	221	155	186	149	80	91	114	0.98 ¹	39.23	< 0.001
Allelic richness (Ar)	12.05	11.94	11.72	11.77	11.83	11.28	11.28	11.11	0.87 ¹	4.82	< 0.01
Hardy–Weinberg test ^b	HWE	HWE	HWE	HWE	HWE	HE	HE	HE	-	-	-

^a Correlation with ⁽¹⁾total number of individuals or ⁽²⁾number of adults was calculated using the linear least square regression model.

^b Test for Hardy–Weinberg equilibrium: HWE = Hardy–Weinberg equilibrium; HE = heterozygote excess.



Fig. 2. Correlation between genetic distance (F_{ST}) and time elapsed between sampling years.

t-test; *t* = 3.95, *p* < 0.005; Fig. 3b). In line with this, simulations conducted in BOTTLESIM showed that a virtual population with the same initial allele frequencies and the same density changes as the study population shows a marked decline in genetic diversity. Specifically, we observed a gradual decline in allelic richness and a rapid loss in heterozygosity during the period in which there was a drastic decline in population size (2009–2011). These simulations thereby show that genetic drift will erode the genetic variability of an isolated population undergoing the same density changes as the study population (Fig. 4). Furthermore, the discrepancy between the simulated and observed levels of genetic variation indicates that, in the absence of selection, the continuous influx of immigrants contributed substantially to lessening the effects of genetic drift (Fig. 4).

3.5. Selection

We did not find a significant effect of individual heterozygosity on annual reproductive success (number of offspring produced) in any year (this variable did not improve the model with the lowest AIC_c value; all p-values > 0.1). Body mass (in the case of males), but not origin (in either case), had influence on this variable in most years (p < 0.05 in six out of eight years). Similarly, survival was not influenced by individual heterozygosity in any year (this variable did not improve the model with the lowest AIC_c value; all p-values > 0.05), with the exception of 2010 (the best model was the one that included heterozygosity + age; heterozygosity: estimate on logitscale = 0.852 \pm 2.720, Wald stat. = 6.84, p < 0.01; age: β = 1.164 ± 0.269 , Wald stat. = 18.62, *p* < 0.001). Individuals that survived the 2010 winter (i.e., those present in 2011) were more heterozygous than those that did not (*total* survivors: 0.857 ± 0.117 , non-survivors: 0.811 \pm 0.078, adult survivors: 0.857 \pm 0.113 n =5, non-survivors: 0.829 \pm 0.107 n = 67, *juvenile* survivors: $0.857 \pm 0.062 \ n = 32$, non-survivors: $0.788 \pm 0.126 \ n = 49$). This coincides with the sudden increase in adult heterozygosity observed in 2011 (see Fig. 1b).

In line with the above, the selection differential (*S*) was positive in three of the seven years analyzed (Table 2), but significantly so only in 2010 (see above). For the whole period it was estimated to be 0.01. When considering each year separately, we found that genetic drift overwhelmed selection (i.e., 1/2Ne > S) in more than half of the years (Table 2). However, summed over the study period, we found the selection for heterozygosity to be higher than the effect of genetic drift (Table 2). Given this selection estimate (S = 0.010), genetic drift operationally overrides selection in this population when effective population size falls below 50 individuals. Heritability of heterozygosity ranged from 0 to 0.17 (mean \pm SE; $h^2 = 0.14 \pm 0.07$) in our study system.

4. Discussion

For eight consecutive years, we surveyed a small and relatively isolated snow vole population fluctuating in size. This provided us with a comprehensive examination of the factors shaping longitudinal patterns in genetic variability. We showed that effective population size is small, which would be expected to result in a substantial reduction of genetic variability over time through genetic drift. However, we found this effect is offset by the effect of immigration, which plays a key role in maintaining a high level of genetic diversity. In addition, at least in some years, selection favouring heterozygous individuals may contribute to the maintenance of heterozygosity. However, in most years it is too weak and effective population sizes too small to overcome the effect of drift (Bouzat, 2010). The high and relatively stable genetic diversity observed in this population is consistent with the results of



Fig. 3. (a) Temporal variation in the number of immigrants (black dots) and effective immigrants (grey dots) entering the study population and (b) averaged expected heterozygosity computed for the entire data set (empty dots) and a subsample excluding all immigrant lineages (filled dots).



Fig. 4. Simulated (filled dots) genetic diversity indices (*Ar*: allelic richness; *Ho*: observed heterozygosity; *Ae*: effective number of alleles; *He*: expected heterozygosity) for a virtual close population undergoing the same density changes as the study population in comparison with the observed data (empty dots).

previous studies on cyclic rodent populations, as well as population genetic theory (Ehrich et al., 2009; Pilot et al., 2010; Rikalainen et al., 2012; Gauffre et al., 2014).

4.1. Genetic drift

The smaller the population, the more likely the chance that events change allele frequencies, i.e., the stronger the genetic drift. The loss of particular alleles or genes, may contribute significantly to population extinction risk, since loss of genetic variation can lead to a decrease in fitness (reviewed in Reed and Frankham, 2003) and adaptive potential (e.g., Frankham et al., 1999; Pertoldi et al., 2007; Luquet et al., 2011; Biljsma and Loeschcke, 2012). However, the negative effect of genetic stochasticity may depend on the characteristics (e.g., life-history) of each population. In particular, genetic drift is expected to have a strong impact on populations with short-generation times. For example, Hailer

Table 2

Predicted magnitude of genetic drift (1/2Ne) on the basis of Ne estimates computed following three different genetic and demographic approaches (*LD*: linkage disequilibrium method, *HE*: heterozygote excess method, *Demo*: demographic method), and selection differential (*S*) on heterozygosity derived from the mean values of this trait before and after survival selection. Asterisks denote those years in which the effect of genetic drift was stronger than selection.

Year	Drift (1/2N	le)		Selection differential (S)				
	Ne (LD)	Ne (HE)	Ne (Demo)					
2006*	0.010	0.008	0.019	-0.030				
2007	0.009	0.007	0.018	0.017				
2008*	0.009	0.008	0.026	-0.014				
2009*	0.013	0.011	0.026	0.007				
2010	0.008	0.008	0.014	0.021				
2011*	0.015	0.014	0.033	-0.041				
2012*	0.020	0.014	0.040	-0.091				
Overall ^a	0.008	0.011	0.025	0.010				

^a Overall effect of genetic drift computed for the 2006–2012 period.

et al. (2006) showed that the long generation time (17 years) of whitetailed eagles (*Haliaeerus albicilla*) acted as an intrinsic buffer against the negative effect of drift during a major bottleneck event; a similar bottleneck would have lowered genetic diversity four times more in a population with an individual lifespan of 1 year, the mean generation time in our vole population (Hailer et al., 2006). Also, the effect of genetic drift becomes more difficult to predict under non-stable scenarios, in harsh and variable environments where populations undergo large fluctuations in numbers from year to year (Vucetich et al., 1997).

Here, we found that the effective population size was around 50 individuals (range: 25-66), which was 70-80% lower than the census size. In line with this, we detected a subtle and gradual decrease in genetic variability over the study period, coinciding with the observed decline in population size. Indeed, as expected from theory (Crow and Kimura, 1970; Soulé, 1987), we found a strong relationship between both Ar and He and population size (Figs. 1b and S2). Surprisingly, there are few other studies showing such a pattern across years (Ortego et al., 2007, but see Ehrich et al., 2009; Pilot et al., 2010; Devillard et al., 2011), and most evidence for the genetic consequences of population decline comes from studies comparing the genetic diversity of same-species populations or meta- and interspecific analyses (e.g., Frankham, 1996). Most likely as a result of the observed reduction in allelic richness, we detected a heterozygote excess in the last three years (2011–2013), when the Ne was reduced by about half compared to previous years. This is due to rare alleles being the first to get lost when small populations experience a sudden decline, and it can therefore be interpreted as a transient signature of decreasing effective population size (Maruyama and Fuerst, 1985; Luikart and Cornuet, 1998; Luikart et al., 1998). Nevertheless, changes in heterozygosity varied much less over time than expected based on the amount of drift the population experiences, which can be attributed to the restoring effect of immigration (see more below).

Although genetic diversity remained moderately high throughout the study period, the genetic composition of the population changed between the different phases it went through. This may be driven by changes in both kin structure (García-Navas et al., unpublished manuscript) and the influx of immigrants, which was higher in highdensity years (see also Berthier et al., 2006; Gauffre et al., 2014). In line with this, there was a significant correlation between pairwise F_{ST} and temporal distance between populations (measured in years), indicating the existence of a pattern of isolation-by-time. Our results thus are more consistent with the model proposed by Charnov and Finnerty (1980), which assumes positive density-dependent immigration and predicts the existence of significant genetic differentiation between the years before and after a genetic bottleneck. According to this model, gene flow may be enhanced in high-density years if local competition increases the likelihood of dispersing (Charnov and Finnerty, 1980).

4.2. Immigration

We simulated genetic variability over time for a closed population with a similar demographic history as our study population. Thereby we could directly quantify the effect of genetic drift, in the absence of immigration. Our results show that immigration can counterbalance the effect of genetic drift in populations experiencing a severe reduction in effective population size (Keller et al., 2001). We observed that in this virtual population, allelic richness declines faster than heterozygosity, which supports the notion that Ar is more sensitive than He to a reduction in population size, at least in the short term (Nei et al., 1975; Leberg, 2002). We also found that heterozygosity declines substantially when excluding all immigrants (and their first-generation descendants) from the data set, especially during the low-density period, when offspring with at least one immigrant parent accounted for a significant proportion of the population. This shows that gene flow constitutes an important mechanism for the maintenance of genetic diversity in this small population (e.g., Ortego et al., 2007; Rikalainen et al., 2012). On the whole, we found that over a guarter (27%) of realized immigrants brought novel alleles into the local gene pool. This can be particularly important for small populations, because the incorporation of new allelic variants might increase their adaptive potential (Willi et al., 2006) and contribute to alleviate inbreeding depression (Keller and Waller, 2002). Indeed, a widely accepted rule-of-thumb is that one immigrant per generation is sufficient to maintain some genetic variability within small populations (Mills and Allendorf, 1996; Newman and Tallmon, 2001; Wang, 2004). In addition, offspring born from crosses between individuals from genetically divergent population might benefit from hybrid vigour (i.e., heterosis; Crow, 1948), promoting a rapid spread of immigrant genomes (Saccheri and Brakefield, 2002). In accordance with this, we found that immigrant individuals produced slightly more heterozygous descendants when mating with locally born individuals in comparison to crosses between locally born individuals. This, together with the observed rate of effective immigration (about 7 individuals per year), suggests that gene flow can counter the negative effect of drift in this population (Ehrich and Jorde, 2005).

4.3. Selection

Long-term datasets have identified selection against homozygous individuals as a possible mechanism for the maintenance or increase of heterozygosity over time in declining or inbred populations (e.g., Kaeuffer et al., 2007). However, heterozygote advantage may only be apparent under adverse conditions (Keller et al., 1994; Brouwer et al., 2007). Here we failed to detect an effect of individual heterozygosity on number of offspring recruited. However, we found that snow voles that survived to the 2010 winter constituted a nonrandom subset of the population with respect to heterozygosity; more heterozygous individuals (both adults and juveniles) had higher survival prospects in comparison with more homozygous individuals. There were no significant differences found in heterozygosity between survivors and non-survivors for the remaining years. Meteorological data indicate that the 2010 winter was the coldest one, but the snow-cover period was not longer than in other years (see Supporting information). Thus, environmental data at hand cannot fully explain the decline observed in this population during the 2010–2011 period. Our results indicate that in most years the strength of selection identified in this population is relatively weak and would require an effective population size above 50 individuals to outweigh the effect of genetic drift. Indeed, this figure (Ne > 50) is frequently evoked in conservation biology as a benchmark to avoid severe loss of genetic variability in the short term (Harmon and Braude, 2010; but see Frankham et al., 2014; Franklin et al., 2014; García-Dorado, in press).

4.4. The interplay between drift, gene flow and selection

Our study shows that this population maintains a high level of genetic variability despite it having undergone a large fluctuation in size. Analyses of temporal genetic structure indicate that it has experienced significant genetic drift in recent years. Nevertheless, the negative impact of this microevolutionary force is being countered by the effects of immigration and selection. The importance of gene flow and (to a lesser extent) selection as buffers against the effect of random genetic drift is exemplified by the relatively large variation in heterozygosity observed between 2010 and 2011. The marked decline in heterozygosity observed in 2010 coincides with a decrease in the influx of immigrants, and the sudden increase observed in the subsequent year, can be explained by both the restoration of immigration and the fact that relatively heterozygous individuals were more likely to survive to the 2010 winter. Hence, our results support the view that the arrival of only a pulse of immigrants can overcome the effect of genetic drift, and that gene flow thereby has a strong impact on the persistence and evolution of natural populations (Keller et al., 2001; Saccheri and Brakefield, 2002; Frankham, in press). Our study also reinforces the notion that census population size constitutes a good proxy for genetic diversity (e.g., Miller and Waits, 2003). Thereby we believe that these findings are of great interest for the management of other mammal species exhibiting erratic density fluctuations. We conclude that information from long-term individual-based genetic studies is needed to help us understand the links between demographic processes and temporal patterns of genetic variability in natural populations (Pelletier et al., 2009). In particular, the combination of genetic and life-history data may be especially valuable for studies focused on microevolutionary processes in cyclic populations of rodents (Norén and Angerbjörn, 2014).

Acknowledgements

We would like to thank the many people who have provided assistance in the field; this paper would not have been possible without their help. Three anonymous reviewers made valuable comments on an earlier version of the manuscript. Our research was supported by grants from the "Basler Stiftung für Biologische Forschung", the "Claraz Stiftung", the University of Zurich and the Swiss National Science Foundation (SNF; ref. 31003A-141110). TB is supported by this SNF grant, and VGN is supported by a Forschungskredit of the University of Zurich (FK-14-103). This study was carried out in full compliance with the Swiss legislation following the guidelines for the use of free-ranging, wild animals in research, under a licence for trapping and handling (ear-clipping) animals from the Amt für Lebensmittelsicherheit und Tiegersundheit (Chur, Graübunden).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx. doi.org/10.1016/j.biocon.2015.06.021.

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