Genetic Bottlenecks Driven by Population Disconnection

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Abstract: Connectivity among populations plays a crucial role in maintaining genetic variation at a local scale, especially in small populations affected strongly by genetic drift. The negative consequences of population disconnection on allelic richness and gene diversity (heterozygosity) are well recognized and empirically established. It is not well recognized, however, that a sudden drop in local effective population size induced by such disconnection produces a temporary disequilibrium in allelic frequency distributions that is akin to the genetic signature of a demographic bottleneck. To document this effect, we used individual-based simulations and empirical data on allelic richness and gene diversity in six pairs of isolated versus well-connected (core) populations of European tree frogs. In our simulations, population disconnection depressed allelic richness more than heterozygosity and thus resulted in a temporary excess in gene diversity relative to mutation drift equilibrium (i.e., signature of a genetic bottleneck). We observed a similar excess in gene diversity in isolated populations of tree frogs. Our results show that population disconnection can create a genetic bottleneck in the absence of demographic collapse.

Keywords: allelic richness, bottleneck, connectivity, European tree frog, gene diversity, Hyla arborea, isolation

Cuellos de Botella Genéticos Producidos por la Desconexión de la Población

Resumen. La conectividad entre poblaciones juega un papel crucial en el mantenimiento de la variación genética a escala local, especialmente en poblaciones pequeñas afectadas por la deriva genética. Las consecuencias negativas de la desconexión de la población sobre la riqueza alélica y la diversidad genética (heterocigosidad) están bien reconocidas y establecidas empíricamente. Sin embargo, no está bien reconocido que una disminución repentina en el tamaño poblacional efectiva inducida por tal desconexión produce un desequilibrio temporal en las distribuciones de frecuencias alélicas que es comparable con la firma genética de un cuello de botella demográfico. Para documentar este efecto, utilizamos simulaciones basadas en individuos y datos empíricos de la riqueza alélica y diversidad genética en seis pares de poblaciones aisladas de Hyla arborea versus poblaciones bien conectadas (núcleo). En nuestras simulaciones, la desconexión poblacional deprimió la riqueza alélica más que la heterocigosidad y por lo tanto resultó en un exceso temporal de diversidad genética en relación con el equilibrio por deriva mutacional (i.e., firma de un cuello de botella genético). Observamos un exceso similar en la diversidad genética en poblaciones aisladas de H. arborea.

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Introduction

Population connectivity is important for the maintenance of genetic variation. In structured population systems, isolated demes have less genetic variation than the system as a whole. Local mutations are their only potential source of genetic novelty (Frankham et al. 2002), so standing genetic variation is reduced by selection and drift at a rate that depends largely on local effective population size (Hartl & Clark 1997). The consequences of a loss of population connectivity are pronounced in small populations. The large decrease in genetic variation induced by drift may affect their long-term evolutionary potential (Franklin 1980; Frankham 2005). Genetic drift also promotes the accumulation of deleterious mutations (genetic load), which, in combination with inbreeding, can lower the average fitness of individuals (Lande 1988, 1995; Frankham 2005).

The influence of connectivity on the dynamics of allelic richness has been documented extensively, as have the processes of loss of gene diversity (i.e., heterozygosity expected from allelic frequencies, assuming Hardy–Weinberg equilibrium) that follow population isolation. Nevertheless, the sudden disconnection of a population from neighboring demes has another potential consequence that has not been well recognized: creation of a temporary disequilibrium in allelic frequency distributions due to a genetic bottleneck.

Theory predicts that population bottlenecks are followed by a loss of allelic richness and gene diversity until a new equilibrium is reached or diversity recovers through population growth (Nei et al. 1975). For highly variable loci, the rarest alleles are expected to disappear quickly, whereas gene diversity will be lost more slowly (e.g., Nei et al. 1975; Allendorf 1986; Leberg 1992). Therefore, there is a transitory excess of gene diversity after the bottleneck relative to the value expected on the basis of the number of alleles in the population. This temporary genetic signature has been observed in experimental and wild populations and it provides a means of detecting recent reductions in effective population size (Cornuet & Luikart 1996).

Although this bottleneck-detection approach has been useful in a large number of case studies, all of these studies, to our knowledge, focused on demographic bottlenecks (i.e., reductions in census population size). Genetic and demographic bottlenecks are often undifferentiated in the literature, presumably because the word bottleneck was originally used in the context of demographic collapses (e.g., Nei et al. 1975; Brookfield 2001) and because the genetic information has been, in this context, used most often as a tool to detect a demographic bottleneck (Cornuet & Luikart 1996; Luikart & Cornuet 1998).

The tools used to detect bottlenecks that are based on gene-diversity excess rely on a number of assumptions, including prebottleneck mutation–drift equilibrium and isolation of the population under study (i.e., no immigration before or after the bottleneck event, Cornuet & Luikart 1996). Violations of these assumptions have been discussed previously, and two conclusions have been drawn. First, genes from individuals entering a population after a collapse can quickly negate the excess gene diversity produced by the bottleneck (Keller et al. 2001; Ramakrishnan et al. 2005; Busch et al. 2007). Second, consistently low levels of immigration may hamper the detection of a bottleneck, whereas consistently high levels of immigration may produce a false genetic signature of a bottleneck (Cornuet & Luikart 1996; Luikart & Cornuet 1998; Pope et al. 2000; Leblois et al. 2006).

Thus, the importance of population connectivity in the context of bottlenecks has focused on the effect of immigrants on the inference of demographic bottlenecks. A decline in immigration rate, however, may result in a genetic bottleneck in the absence of demographic collapse. Such genetic bottlenecks are systematically, but perhaps erroneously, interpreted as evidence of substantial reductions in population size. This point is important because different causes of a genetic bottleneck may have different effects on probabilities of population persistence.

We used simulated genotypic data to illustrate the dynamics of a genetic bottleneck effect expected from disconnection of populations, and we tested for this effect in populations of the European tree frog (Hyla arborea). Amphibians are good biological models for the investigation of the consequences of losses of demographic and genetic connectivity because they are extremely sensitive to habitat fragmentation (Beebee & Griffiths 2005). The European tree frog is widespread in Europe, but is threatened over a large part of its distribution by recent anthropogenic fragmentation of its habitat and isolation of its populations (Carlson & Edenham 2000; Arens et al. 2006; Angelone & Holderegger 2009). We compared geographically isolated populations of tree frogs with well-connected populations in five European regions to examine whether and how population disconnection affects gene diversity and allelic richness.

Palabras Clave: aislamiento, conectividad, cuello de botella, diversidad genética, Hyla arborea, riqueza alélica
Methods

Tree Frog Sampling

We sampled tree frogs in five regions in Western Europe (Fig. 1) approximately 150–600 km from each other that were occupied by distinct tree frog metapopulations. Within each region, we sampled tree frogs in an isolated and in a well-connected breeding site during breeding season. Isolated populations were at the edge of a metapopulation and at least 5 km from the nearest neighboring deme (a distance large enough to drastically reduce immigration, if not to prevent it completely) (Stumpel & Hanekamp 1986; Fog 1993; Vos et al. 2000; Andersen et al. 2004). By contrast, core populations were near the center of each metapopulation, where they were surrounded by many neighboring demes. We sampled all regions in either 2006 or 2007, except for region 2, where we sampled two distinct pairs of core and isolated populations (2a in 2006 and 2b in 2007, Fig. 1). Thus, in total we sampled 12 populations (six pairs of core and isolated populations).

We collected adult tree frogs by hand. We determined the sex and used cotton swabs to take samples of buccal cells of each captured individual before release (Pidancier et al. 2003; Broquet et al. 2007). We stored the swabs in a freezer (−20°C or −80°C) for subsequent genetic analyses.

Molecular Analyses

We used the technique of Berset-Brändli et al. (2007) to extract total genomic DNA from buccal cell samples. Individuals from both core and isolated populations in a given region were genotyped with the same set of markers. Markers ranged from 11 to 18 autosomal microsatellite loci, depending on the region (Supporting Information). The loci we used were identified previously by Arens et al. (2000) or Berset-Brändli et al. (2008a, 2008b).

Genetic Variation

We used two parameters to quantify genetic variation of each locus in each population: allelic richness (r) and gene diversity (H). Allelic richness is the number of alleles that are expected to be found at a locus for a fixed sample size n when genetic information is actually available for a larger number of gene copies N (El Mousadik & Petit 1996). Allelic richness is calculated as

$$r_n = \sum_{i=1}^{k} \left[ 1 - \left( \frac{N - N_i}{n} \right) / \left( \frac{N}{n} \right) \right],$$

(1)

where k is the number of distinct alleles observed at this locus and N_i is the number of occurrences of the ith allele among the N sampled genes. This metric allowed us to compare the number of alleles observed in core and isolated populations within regions despite unequal sample sizes. We set n within each region as the smallest number of gene copies typed in a population (r_n values from core and isolated populations could therefore be compared within a region, but not across regions). We calculated gene diversity at each locus as

$$H = 1 - \sum p_i^2,$$

(2)

where p_i is the frequency of allele i in the population.

We used paired Wilcoxon signed-rank tests to compare differences in allelic richness and gene diversity in core and isolated populations (loci were independent replicates). We analyzed each population pair independently and then combined these results with Fisher’s combined test statistic (Fisher 1954; Sokal & Rohlf 1995)

$$\pi = -2 \sum \ln p_j,$$

(3)

where p_j is the p value previously obtained for region j. We compared these results with a χ^2 distribution with degrees of freedom set to twice the number of independent tests (i.e., df = 12). This method allowed us to combine the results from different sets of samples for different loci. In addition, we compared the average r_n and H over all loci in core and isolated populations within each region and used regions as independent replicates to calculate the probability of equal r_n and H in core and isolated populations with a binomial test.
Departure from Mutation–Drift Equilibrium

We assessed departure from mutation–drift equilibrium at each locus by calculating gene diversity excess, \( \Delta H = H - E(H) \), which is the difference between the observed gene diversity \( H \) and the equilibrium gene diversity \( E(H) \) expected given the number of alleles observed \( (k) \) at a locus and the number of gene copies \( (N) \) sampled at this locus. At equilibrium under the stepwise mutation model the relation is (Kimura & Ohta 1975)

\[
k = \frac{\theta + \beta}{\beta} \left( 1 - \prod_{i=0}^{N-1} \frac{\theta + i}{\theta + \beta + i} \right),
\]

where

\[
\theta = \frac{1}{2} \left[ \frac{1}{(1 - H)^2} - 1 \right]
\]

and

\[
\beta = \theta \frac{1 - H}{H} - 1.
\]

We used this relationship to infer \( E(H) \) from \( k \) at each locus.

To investigate whether disconnection depresses allelic richness more than heterozygosity and results in an excess of gene diversity in isolated populations, we used a linear mixed model fit by maximum likelihood to test the effect of connectivity status (core or isolated) on \( \Delta H \). We set connectivity status as a fixed effect and geographic region and locus as random factors. We used two approaches to assess whether the fixed effect was substantial. First, we used a likelihood-ratio test based on the decrease in AIC (Akaike’s information criterion) that followed the inclusion of the focal variable into the partial model that contained all other variables (e.g., Crawley 2007). Second, we calculated the 95% highest posterior density interval of parameter estimates by sampling their posterior distribution through a Markov chain Monte Carlo method (10,000 iterations) (Bates et al. 2008; J. Goudet, personal communication).

The analytical computation of \( E(H) \) can be slightly biased for high mutation rates and effective population sizes (Kimura & Ohta 1975; Shriver et al. 1993; Schlotterer et al. 2004). This potential bias is unlikely to have affected our data on tree frogs given the typical size of tree frog populations and their typical microsatellite mutation rates, but we nonetheless repeated the analyses with unbiased values of \( E(H) \) simulated in the software Bottleneck (Cornuet & Luikart 1996) under the stepwise mutation model.

Unless otherwise stated, we performed all analyses in R (version 2.6.1, R Development Core Team 2007) with packages for the analysis of genotypic data (HIERFSTAT, Goudet 2005) and for mixed-effects models (Ime4, Bates et al. 2008).

Simulations

We used a backward generation-by-generation coalescent algorithm developed by Leblois et al. (2009) to simulate multilocus genotypic data of a well-connected population that suddenly becomes isolated. Our simulated focal population had a constant carrying capacity of 100 diploid individuals, with a life cycle composed of birth, regulation (i.e., random removal of individuals in excess of the carrying capacity), gamete production, gamete dispersal, and panmictic reproduction within demes. We simulated data for 10 independent microsatellite loci evolving under a generalized stepwise mutation model (GSM, Pritchard et al. 1999) with variance in the number of repeat units set to 0.36 and a mutation rate of \( 5 \times 10^{-4} \) (mutation settings according to Leblois et al. 2006). The resulting genotypic data were used to calculate \( \Delta H \) for the focal populations under different simulation scenarios. We started with a situation in which the focal population was part of a regular grid of 25 populations connected through stepping-stone migration (with initial migration rate \( M_{ini} \) set to 0.1). Each population was thus connected to its four immediate neighbors with migration probability \( M_{ini}/4 \) (to avoid edge effects the grid was organized as a theoretical torus, e.g., Rousset 2004:33).

We then analyzed \( \Delta H \) after 10, 50, 100, 250, 500, 1000, and 2000 generations following a sudden decrease in dispersal rate (final migration \( M_{fin} \) set to zero for all populations). To investigate the sensitivity of \( \Delta H \) trajectories to various initial and final conditions of population connectivity, we varied either the initial migration rate (\( M_{ini} = 0.001, 0.005, 0.01, 0.05; M_{fin} = 0 \)) or the final migration rate (\( M_{fin} = 0.001, 0.005, 0.01, 0.05; M_{ini} = 0.1 \)). We plotted results either as heterozygosity excess (\( \Delta H \)) as a function of time or as measured heterozygosity (\( H \)) as a function of the expected value (\( E[H] \)). In such a plot, isolated populations at equilibrium are expected to lie on the diagonal, and \( \Delta H \) is measured as the vertical distance to this diagonal.

Each simulation scenario was replicated 200 times (a number of replicates that proved sufficient to ensure stabilization of the standard deviation of \( \Delta H \) across replicates, data not shown). We ran the simulation algorithm and calculated \( \Delta H \) values in the software IBDSim (Leblois et al. 2009).

Results

Simulations

The evolution of \( \Delta H \) with complete population disconnection exhibited the genetic signature of a bottleneck even for the smallest initial migration rate (\( M_{ini} = 0.001 \)) (Fig. 2a). Although the census size of the focal population was kept unchanged, \( \Delta H \) rose rapidly after population disconnection and reached its highest value (\( \sim 0.045 \))
after approximately 50 generations. It then slowly returned to its equilibrium value. Results for \( M_{\text{ini}} \) within 0.005–0.1 were qualitatively similar. The highest value of \( \Delta H \) was 0.11 (Fig. 2a). Additional simulations showed that \( \Delta H \) trajectories for higher migration rates (\( M_{\text{ini}} = 0.2 \) and 0.3) were similar to the case where \( M_{\text{ini}} = 0.1 \) (data not shown).

The \( \Delta H \) trajectory with partial disconnection had similar patterns only for small values of final migration (\( M_{\text{fin}} = 0.001 \)) (Fig. 2b). With less severe disconnection (\( M_{\text{fin}} \geq 0.005 \), the equilibrium \( \Delta H \) value exceeded zero (ca. 0.035), which masked the effects of reduction in connectivity. Similar results were obtained when starting values of \( M_{\text{ini}} \) were >0.1 (data not shown).

We plotted the same data in the phase plane \( H \) versus \( E(H) \) (Fig. 3), with trajectories corresponding to either complete disconnection from a range of initial \( M_{\text{ini}} \) values (Fig. 3a) or to different reductions in connectivity from a \( M_{\text{ini}} = 0.1 \) value (Fig. 3b). Disconnection generally decreased \( E(H) \) more rapidly than \( H \), but this effect was masked when final connectivity values exceeded 0.001 due to a positive equilibrium \( \Delta H \) value (Fig. 3b).

**Empirical Data**

We genotyped 539 tree frogs (14–100 per population, mean approximately 44) (Supporting Information). For each region, allelic richness was on average lower in the isolated than in the core population (\( r_{\text{isolated}}/r_{\text{core}} \) averaged over loci ranged 0.54–0.94; Fig. 4a). This pattern was significant in regions 1–3 (Wilcoxon tests, all \( p < 0.05 \)) but not in region 4 (\( p = 0.18 \)) and region 5 (\( p = 0.14 \)). Combining results of these tests led to a highly significant effect of connectivity status (core or isolated) on allelic richness (Fisher’s procedure, \(-2 \sum j \ln p_j = 71.4, p < 10^{-9}\)). Furthermore, the probability of observing a higher allelic richness (averaged over loci) in all core populations under the null hypothesis of no difference in core versus isolated populations was \( p < 0.02 \) (binomial test: number of successes, six; number of trials, six).

Gene diversity was also lower in isolated than in core populations, although differences were less pronounced (\( H_{\text{isolated}}/H_{\text{core}} \) range 0.76–0.96; Fig. 4b) and variance among loci was higher. Differences between populations were only significant in region 2b (Wilcoxon tests, \( p = 0.02 \), range of \( p \) for all other regions 0.06–0.38). Nonetheless, when we combined all regions, core populations appeared significantly more diverse (Fisher’s procedure, \(-2 \sum j \ln p_j = 24.5, p < 0.05 \)). The binomial approach also demonstrated the higher gene diversity of core populations (\( p < 0.02 \)).

Isolated populations displayed a higher excess of gene diversity (\( \Delta H \)) than core populations in five out of six pairwise comparisons: \( \Delta H_{\text{isolated}} - \Delta H_{\text{core}} \) ranged from –0.006 to 0.31 (Fig. 4c). In our linear mixed model, the average expected difference in excess gene diversity between isolated and core populations was 0.125, a pattern that was highly significant in model comparisons (likelihood-ratio test based on decrease in AIC, \( p = 1.7 \times 10^{-9} \)) and in the 95% interval limits for highest posterior density obtained for the effect of connectivity status (highest posterior-density interval 0.09–0.16).
Figure 3. Trajectories of gene diversity (H) versus equilibrium gene diversity predicted by the number of alleles (\(E[H]\)) following a reduction in population connectivity for the same simulations as in Fig. 2 (solid circles, conditions before disconnection; open circles, H and E(H) values for increasing numbers of generations after disconnection). Isolated populations at mutation–drift equilibrium are expected to lie on the diagonal (\(H = E[H]\)), and \(\Delta H\) is the vertical distance of a dot to the diagonal. For clarity only data for generations 10, 50, 100, 250, and 500 are shown (stability is nearly achieved after 500 generations). In (a) populations are completely disconnected, and initial migration rate \(M_{\text{ini}}\) starts with different initial conditions (see text and Fig. 2a). In (b) populations have different levels of partial disconnection, starting from an initial migration rate \(M_{\text{ini}} = 0.1\).

Discussion

We found that the complete disconnection of a population-generated disequilibria in allelic frequencies akin to those that result from a reduction in census population size (demographic bottleneck). The temporary deficit of allelic richness (or excess of gene diversity) that resulted from the isolation of once-connected populations induced the genetic signature of a bottleneck. In our simulations, excess gene diversity peaked after about 50 generations, but was still marked after a few hundred generations. This genetic signal was discernable even when the focal population was only slightly connected before becoming completely isolated (Figs. 2a & 3a).

When isolation remained incomplete (\(M_{\text{fin}} \geq 0.005\) in our simulations), the temporary excess in gene diversity was masked by the positive \(\Delta H\) induced by migration itself (Figs. 2b & 3b; Pope et al. 2000; Leblois et al. 2006). This positive \(\Delta H\) arose because immigrants only rarely contributed private alleles (thus local number of alleles \([k]\) was affected little). Instead, immigrants tended to average allelic frequencies over populations (i.e., locally rare alleles increased in frequency and locally frequent alleles decreased in frequency), which increased local gene diversity \(H\) (and thus \(\Delta H\)). Partial disconnection affected population genetics in this case (Fig. 3b), but \(H\) and \(E(H)\) were lost at similar rates so that \(\Delta H\) remained relatively constant.

These results call into question the most accurate interpretation of excess heterozygosity and genetic signatures of bottlenecks detected in case studies. Excess heterozygosity stemming from migration versus a genetic bottleneck can be distinguished because their effects on allelic richness and diversity differ. Migration increases both \(H\) and \(E(H)\) above the levels expected in an isolated population. In our case study of tree frogs, isolated populations displayed lower allelic richness and gene diversity; thus, we believe the observed excess heterozygosity stemmed from genetic bottlenecks (which had to be strong enough to outweigh the positive equilibrium of the \(\Delta H\) value expected in connected core populations).

The interpretation of genetic bottlenecks, however, is less straightforward. Population bottleneck primarily refers to substantial reductions in census population size, possibly followed by recovery (e.g., Brookfield 2001). Generally, genetic signatures are examined to detect such
Population Disconnection and Genetic Bottlenecks

Figure 4. Pairwise comparisons of (a) allelic richness ($r$), (b) gene diversity ($H$), and (c) excess gene diversity ($\Delta H$) in core versus isolated populations of European tree frogs (gray dots, a microsatellite locus typed in a core-isolated population pair; solid circles, average values are shown within each region). Locus-specific values for the two populations being compared are the x and y coordinates. The diagonal represents equality. Any point situated, for example, below the diagonal means the value of interest was lower in the isolated population of a given pair.

events, but, according to Luikart et al. (1998), a genetic bottleneck may also occur in the absence of a demographic bottleneck if variations in family size or sex ratio reduce effective population size but leave demography unchanged. We suggest that the complete disconnection of a population also leads to an effective genetic bottleneck that is accompanied by a temporary disequilibrium in the distribution of allelic frequencies.

Accurately interpreting the genetic signature of a bottleneck therefore requires knowledge of the history of the connectivity of the population under study (Comps et al. 2001; Leblois et al. 2006). Relative to the situation of a historically isolated population (for which the bottleneck tests were developed) (Cornuet & Luikart 1996), connectivity may affect interpretation of genetic bottlenecks in two ways. First, some constant level of immigration is suspected to have occurred before and after the presumptive demographic bottleneck. Although there is no straightforward way to assess the effect immigration may have on excess gene diversity (but see Pope et al. [2000] and Leblois et al. [2006] for simulation-based approaches), this situation has been discussed in the literature. A high immigration rate may result in false bottleneck signatures, whereas a low immigration rate may hamper the detection of a true bottleneck (Pope et al. 2000; Keller et al. 2001; Ramakrishnan et al. 2005; Busch et al. 2007). These outcomes can be acknowledged when interpreting observed patterns of excess gene diversity (e.g., Storz et al. 2002; Lindsay et al. 2008; Procházka et al. 2008; Barrientos et al. 2009). In tree frogs, for instance, gene flow may protect populations from the effects of genetic bottlenecks (Burns et al. 2004).

The second case refers to the situation we have outlined here: the population under study formerly was part of a network of populations exchanging migrants, but is no longer receiving immigrants. Although, to our knowledge, this situation has not been discussed explicitly in the literature, it may occur frequently. Signs of a genetic bottleneck may thus be interpreted incorrectly as a recent demographic crash. Population disconnection does not have the same causes as a demographic collapse, and maintaining populations in these two sets of circumstances may require different conservation strategies.

It is important to distinguish the observed patterns (signatures of genetic bottleneck) from the causes of the observed patterns (demographic bottleneck or other causes of decreased effective population size) in genetic studies of population bottlenecks. Yet, distinguishing among alternative causes may prove difficult because they are dependent. For instance, habitat fragmentation frequently affects census size of populations and their connectivity to other populations simultaneously. Moreover, immigrants not only contribute their genes to a population, they also increase local abundance. Isolated populations are therefore more susceptible to demographic fluctuations than connected populations (e.g., reviewed in Kawecki 2008).

The combined effects of disconnection and demographic stochasticity cannot be eliminated as a potential cause of the genetic patterns observed in our data on tree frogs. Nevertheless, we based our selection of study populations only on their geographical isolation or proximity.
to other populations. Because, as shown in our simulations, disconnection is by itself sufficient to produce a large excess of gene diversity, the most parsimonious interpretation of the excess gene diversity we observed was that our isolated populations recently experienced a genetic bottleneck that was associated with population disconnection. It is plausible, however, that some level of reduction in population size exacerbated the effect of disconnection in some or all of the frog populations we studied. Results of our simulations suggest the bottleneck signature is a long-term effect. The bottleneck peaked after about 50 generations (i.e., 150 years in the case of tree frogs); thus, the relevant empirical data that would have helped us assess the role of population demography were not attainable.

The effect of connectivity status (core vs. isolated) was significant overall, but it was not homogeneous: $\Delta H$ differed significantly among geographic regions, which we expected given the marked differences in history of fragmentation among the regions. For instance, the pair of core and isolated populations with the least difference in gene diversity excess (pair 5, Thur Valley, northeast of Zürich [Fig. 1]) was in a region that had relatively little fragmentation (Angelone & Holderegger 2009). By contrast, the pair with the highest difference in $\Delta H$ (pair 3, northwest of Lausanne) was part of a remnant metapopulation for which the reduction of connectivity between breeding sites was severe (Pellet et al. 2004; J.J. et al., unpublished data).

In contrast with the region factor, the effect of loci on $\Delta H$ patterns was not significant. Among-loci variations in allelic richness and gene diversity ($r$ and $H$, Figs. 4a-b) and in allelic frequency distributions (data not shown) nonetheless suggest there was a large variation in the mutation dynamics of the microsatellites we used in this study. It seems, for instance, unlikely that our microsatellites all fit the same mutation model. Although we used a stepwise mutation model to calculate gene diversity values expected at equilibrium, our conclusions were largely independent of this assumption because we compared locus-specific $\Delta H$ values among populations. To detect bottlenecks in case studies, by contrast, one generally calculates $\Delta H$ in a focal population (i.e., testing for an excess of gene diversity on the basis of equilibrium level expected for some assumed mutation model). It is important therefore to compare excess gene diversity with a range of mutation models, as illustrated by Cornuet and Luikart (1996). For instance, with the software Bottleneck, we obtained a significant excess of gene diversity in some isolated populations of tree frogs or a significant deficit in some core populations, depending on the mutation model assumed (data not shown).

A corollary result of our study was that isolated populations had reduced allelic richness and gene diversity. If these populations are not yet at mutation–drift equilibrium, as we suggest, one may expect these levels of genetic variation to decline further (possibly quite rapidly; Fig. 3) (Broquet et al. 2009) to the very low levels found in tree frog populations that have been isolated for a long time (Edenmann et al. 2000) unless connectivity can be restored.

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**Supporting Information**

Sample sizes and a list of microsatellite loci used for each population in the examination of gene diversity, allelic richness, and gene diversity disequilibria are available online (Appendix S1). The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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