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Assessing the Utility of Genetic Data as a Monitoring Tool: A Case Study of Eastern Red Bats (Chiroptera: Vespertilionidae: *Lasiurus borealis*)

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Abstract

High levels of bat and bird mortalities have been documented at wind energy facilities; particularly hard-hit among bats are the tree-roosting migratory species Lasiurus cinereus, L. borealis*, and* Lasionycteris noctivagans*, which together compose approximately 79% of affected bats. Traditional mark-recapture monitoring methods have proven ineffective for these species due to the fact that these bats roost in small numbers, fly very high, and are difficult to catch. Thus it is hard to tell what effect these deaths at wind energy facilities are having on population numbers. Genetic data may provide a means of monitoring populations when demographic methods are unsuitable. We used coalescent-based simulations to determine the efficacy of genetic data as a monitoring tool for short-term changes in population size. Simulations were run under demographic models parameterized using mitochondrial DNA sequence data and microsatellite genotypes from the eastern red bat,* Lasiurus borealis*. DNA sequence data and microsatellite genotypes were simulated in both panmictic and structured populations using the computer program,* ms*, and analyzed using statistical software (*microstat*) to interpret the results.* ms *is a coalescent-based program that simulates genetic data under specific population models that are parameterized by initial population size, rate of decline, time since the onset of decline, mutation rate of the chosen molecular marker, and pattern of population structure. Initial estimates of these parameters were taken from previous studies on* L. borealis *(initial population size = 3.3 million individuals, rate of decline = –1% per year, mitochondrial mutation rate = 10-5 substitutions per gene per generation, no significant population structure). Simulations were allowed to run from 1 to 1000 generations following the initial onset of population decline to determine the timescales necessary to observe significant loss of genetic diversity under biologically realistic conditions. Loss of genetic diversity was assessed using summary statistics including the number of segregating sites, nucleotide diversity, and Tajima's* D *for DNA sequence data; analogous measures for microsatellite data included average heterozygosity, $\hat{\theta}_P$, and Cox's Δ. We found that direct measures of diversity (segregating sites and average heterozygosity) are much more informative for detecting population declines than neutrality tests such as Tajima's* D *and Cox's Δ. Between the two types of markers, microsatellites provided more power to detect population declines over shorter timescales (hundreds of generations for microsatellites as opposed to thousands of generations for sequence data). These results demonstrate that even quickly-evolving microsatellite data are unlikely to be useful for the type of year-to-year comparisons needed by monitoring agencies. We conclude that genetic data do not appear to be a useful metric for monitoring red bat population declines due to wind turbine-associated deaths. We emphasize that these conclusions are limited to the population parameters examined in this study, specifically those for eastern red bats facing population declines from wind turbines. Similar questions in other species (e.g., little brown bats facing local extirpation from white-nose syndrome) should be addressed using models appropriately parameterized for those systems.*

Introduction

One application of conservation genetics is the analysis of molecular data to determine the amount of genetic variation present in an endangered population (Hedrick & Miller 1992). High levels of genetic variation are typically associated with healthy populations, and many conservation efforts place great emphasis on maintaining or increasing genetic diversity in threatened populations (O'Brien et al. 1983). Conservation agencies are increasingly relying on genetic monitoring of threatened and endangered populations with the assumption that these data are very quickly responsive to population size changes while requiring relatively small sample sizes and minimally invasive sampling techniques (Luikart et al. 1998).

Molecular markers have proven to be useful for detecting population bottlenecks (Garza & Williamson 2001). Luikart et al. (1998) showed that a loss of alleles and decrease in variance at microsatelite loci can signal population bottlenecks with greater statistical power than more commonly used

measures such as losses in heterozygosity. However, Luikart et al.'s (1998) analyses assumed an extremely severe bottleneck in a single panmictic population and evaluated only microsatellite data. Such power analyses may be limited in their applicability; ideally, the use of genetic data as a monitoring tool should be evaluated on a case-by-case basis (Hedrick & Miller 1992). Specifically, population parameters such as the initial population size, the degree to which populations are structured across the landscape, the level of gene flow connecting structured populations, and the rate of population decline must be considered in order to judge the utility of genetic monitoring (Hedrick 2001).

Polymorphism studies can be conducted using relatively small datasets (Nordborg 2001). The coalescent approach is useful in such studies due to the practicality of modeling the genealogy backward in time (Nordborg 2001; Hudson 2002). This approach traces lineages of alleles from a sample of the current population back to their most recent common ancestor, and imposes mutations upon these models (Harding 1998). Parameters such as effective population size, rates of population growth or decline, and patterns of population structure can be inferred from the shape of the resulting coalescent genealogies and the timing of genealogical coalescent events. Such coalescent methods prove particularly useful in power analyses for conservation genetic studies, since simulation software can be utilized to evaluate evolutionary histories specific to a species of interest. Here, we use such analyses to evaluate the utility of genetic monitoring techniques for a bat species threatened by wind turbines in North America.

Wind power is among the fastest growing sectors of the energy industry (Pasqualetti et al. 2004). However, the low cost and infinite renewability (U.S. Department of Energy 2005) of wind power are coupled with unexpected consequences (Morrison & Sinclair 2004; Kunz et al. 2007b). The presence of wind turbines is documented as having a negative effect on some bird and bat species. Bird fatalities have been estimated around an average of 2.19 fatalities per turbine per year in the U.S. for all species (Erickson et al. 2001), while more recent reports of bat fatalities range from 18.5 to 69.6 carcasses per turbine per year (reviewed in Kunz et al. 2007b).

Wind turbines predominantly affect tree roosting migratory bats, including eastern red bats (*Lasiurus borealis*), hoary bats (*L. cinereus*), and silver-haired bats (*Lasionycteris noctivagans*), the three of which represent 72.8% of the annual bat fatalities reported for wind energy facilities in the United States (Table 1; Kunz et al. 2007b). Most of these fatalities in North America appear to be concentrated during the fall migration of the affected species (Cryan 2003). Because these species roost in low densities in relatively unpredictable locations, the population-level impact of these fatalities is currently difficult to assess and traditional demographic approaches have limited utility with these three species of bats (Cryan 2003; Kunz et al. 2007a). Because of the inefficiency of traditional approaches, such as the mark-recapture method and the limitations of such methods in inferring the demographics of bat populations, the use of molecular markers to estimate population parameters and demographic trends may be the best option for monitoring migratory tree-roosting bat populations and assessing the long-term importance of fatalities at wind power facilities. Our analyses will allow us to determine which marker type, loci, and analyses provide the most power for detecting recent population declines, such as those hypothesized in eastern red bats, and to make educated recommendations as to which methods should be pursued in future monitoring of this species. The immediate research objectives of the project were to:

- Assess the utility of genetic data as a monitoring tool for tracking population declines.

- Model genetic data and examine changes caused by manipulating population size, mortality rates, time since onset of decline, type of molecular marker used, and degrees of population structure.

Methods

Modeling of Lasiurus borealis *populations*

We used coalescent-based simulations to determine the efficacy of genetic data as a monitoring tool for short-term changes in population size. Simulations were parameterized based on DNA sequence data from the mitochondrial D-loop of the eastern red bat, *Lasiurus borealis* (Vonhof & Russell, unpublished data). To evaluate the utility of DNA sequence data, we modelled a haploid locus of 408 bp with a mutation rate $\mu = 2 \times 10\text{-}5$ substitutions/gene/generation and a generation interval of 5 years. To evaluate microsatellite data, we modelled a dataset of 20 diploid loci with a mutation rate $\mu = 10\text{-}5$ substitutions/locus/generation and the same generation interval of 5 years. To mimic population decline in *L. borealis* due to genetically-random mortality from wind turbines, we used a demography in which a single population initially sized at 3.3 x 106 individuals decreases by 1% per year with samples (n = 150) taken after 0, 5, 10, 50, 100, 500, and 1000 years (Fig. 2A). To examine the impact of population structure on the loss of genetic diversity in a declining population, we also considered a model in which a single population initially sized at 3.3 x 106 individuals first splits into two subpopulations of 1.65 x 106 connected by gene flow at a rate m = 0.01% Each subpopulation then decreases in size by 1% per year with samples (n = 150) taken after 0, 5, 10, 50, 100, 500, and 1000 years (Fig. 2B). Coalescent datasets (10,000 replicate datasets per demographic scenario) were simulated according to these demographic models using the software ms (Hudson 2002). For microsatellite data, variation simulated in ms was converted to microsatellite genotypes using the software microsat (M. Cox, unpublished code). Genetic variation was summarized as the number of segregating sites S, nucleotide diversity π, and Tajima's (1989) *D* using the software *ms_stats* (Hudson 2002) for DNA sequence data. For microsatellite data, genetic variation was summarized as average heterozygosity, θP, and Cox's Δ using the software *microstat* (M. Cox, unpublished code).

Results

Effect of marker type

We considered two types of molecular markers commonly used in studies of conservation genetics: mitochondrial DNA sequence data and autosomal microsatellite genotype data. These marker types vary in both mode of inheritance and in mutation rate, with mitochondrial DNA being maternally inherited and having a slower mutation rate than the biparentally inherited microsatellite loci. Our results indicate that microsatellite data are more effective than mitochondrial DNA sequence data for detecting population declines over relatively short time scales (Fig. 3-5).

Even though microsatellites appear to be markedly more responsive to demographic population declines, even these quickly evolving loci require hundreds of generations to manifest significant losses of genetic diversity (Fig. 3A). For example, average heterozygosity values remain relatively constant for at least 500 generations following the onset of population decline before declining significantly between 500 and 1000 generations. For mitochondrial DNA sequence data, we start to see a significant decline in simple diversity statistics (number of segregating sites, Fig. 3B) by 1000 generations following the onset of population decline.

Effect of summary statistic

We used multiple summaries of the data to evaluate which statistic(s) were most responsive to simulated population declines. These summary statistics fell into two general classes: direct measures of genetic diversity and neutrality tests. The former includes statistics such as average heterozygosity (Fig. 3A) and θP (Fig. 5A) for microsatellites and the number of segregating sites (Fig. 3B) and nucleotide diversity (Fig. 5B) for DNA sequence data, while the latter class includes Cox's Δ (Fig. 4A) for microsatellites and Tajima's *D* (Fig. 4B) for DNA sequence data.

Simple measures of genetic diversity such as average heterozygosity or segregating sites emerge as the most useful metrics for assessing population declines (Fig. 3). Although the simulated populations retained high levels of variation for long periods of time following the onset of population decline (on the order of 102 generations), average heterozygosity or segregating sites proved quite responsive to population losses totaling at least 60% of the initial population size. Statistics such as θP and nucleotide diversity (Fig. 5) represent the same direct measures of genetic diversity, but are scaled by the mutation rate; as such, they show the same overall pattern as heterozygosity and segregating sites.

Neutrality tests such as Cox's Δ and Tajima's *D* appear to be ineffective for detecting or monitoring population declines over the time scales considered in this study (Fig. 4). These test statistics are expected to approach 0 for neutral loci in a very large population, such as was simulated in our constant population size scenario (i.e., time since the onset of population decline = 0). This expected pattern was observed in our simulations, with variation around the expected average test statistic value of 0. With increasing time since the onset of population decline, we observed little change in the neutrality test statistics except for a notable but statistically insignificant increase at 1000 generations.

Effect of population structure

Eastern red bats show no evidence of genetic population structure throughout their range (Vonhof & Russell, unpublished data). Although not true for eastern red bats, population structure is a common phenomenon among species of conservation concern; therefore, we evaluated the impact of a simple island model of structured populations on the rate of loss of genetic diversity (Fig. 3). These structured populations maintained comparable levels of genetic diversity for similar periods of time as unstructured panmictic populations. When population declines persisted for >500 generations, however, unstructured populations lost diversity at a significantly higher rate than structured populations.

Discussion

Previous research has supported genetic data as an effective means for monitoring population declines (Hedrick & Miller 1992; Luikart et al. 1998; Garza & Williamson 2001). However, these studies were limited in a number of ways, particularly in the demographic models and genetic markers that were evaluated. For large populations experiencing a relatively low rate of population decline such as the eastern red bat, our results indicate that genetic monitoring is not likely to be effective for time spans of <500 generations.

We found that direct measures of genetic diversity (segregating sites and average heterozygosity) are much more informative for detecting population declines than neutrality tests such as Tajima's *D* and Cox's Δ. Direct measures of diversity remained high for hundreds of generations, but they proved responsive to population losses above 60% of the initial population size. Tajima's *D* and Cox's Δ varied around an average of zero with statistically insignificant changes even after 1000 generations. Neutrality tests are often used in demographic studies as indications of population growth; the overabundance of singleton mutations associated with population growth tends to cause significantly negative values for these analyses (Russell et al. 2005). Our analyses suggest that the same test statistics are not useful for detecting population declines. One caveat to this conclusion stems from the particular neutrality tests that were examined in our study. Tajima's *D* has previously been shown to be lacking in statistical power for detecting population growth, whereas statistics such as Fu's (1997) *Fs* proved powerful enough to detect sudden population growth (Ramos-Onsins & Rozas 2006). Such differences leave open the possibility that our analyses with Tajima's *D* (and its microsatellite analog, Cox's Δ) were ineffective because we examined an inappropriate neutrality test. Future analyses should evaluate other neutrality tests to determine the true utility of neutrality tests as a measure of population declines.

Between the two types of markers, microsatellites provided more power to detect population declines over shorter timescales (hundreds of generations for microsatellites as opposed to thousands of generations for sequence data). Microsatellite data are highly variable and are commonly used for the assessment of short-term trends in population sizes (Hedrick 2001). Our study supports the conclusion that haploid DNA sequence data possess significantly less power for detecting population declines than microsatellite data; however, neither marker type appears to be useful for monitoring population declines on the yearly timescales typically required by monitoring agencies.

If the observed and projected growth of wind energy facilities are coupled with the estimates of bat fatalities at wind turbine facilities, it is expected that population decline in the eastern red bat is an immediate problem for conservationists (Pasqualetti et al. 2004; Kunz et al. 2007b). Given that our method is only significant on the order of 102 generations, these methods of genetic evaluation do not prove useful. We conclude that genetic data do not appear to be a valid metric for monitoring red bat population declines due to wind turbine-associated deaths.

For population declines persisting longer than 500 generations, structured population retained diversity to a greater extent than un-

structured populations. Although this may seem counter-intuitive, a review of genetic analyses in plant studies has shown that small structured populations tend to maintain higher levels of diversity in total than large panmictic populations (Ellstrand & Elam 1993). As the total population size decreases, subpopulations in a structured population may individually function as refugia for different samples of alleles, allowing a larger amount of genetic information to be preserved in total than in a single unstructured population. We emphasize that the simple island model of population structure evaluated in our study is probably not realistic for most species; unequal subpopulation sizes or asymmetric migration rates may alter these results.

We further emphasize that these conclusions are limited to the population parameters examined in this study, specifically those for eastern red bats facing population declines from wind turbines. The specific evolutionary history and population parameters of a species provide invaluable information when assessing the utility of a proposed conservation genetic study (Hedrick 2001). Similar questions in other species (e.g., little brown bats facing local extirpation from white-nose syndrome) should be addressed using models appropriately parameterized for those systems.

Acknowledgements

We thank Dr. Murray Cox, Massey University, for invaluable advice and access to unpublished code used in simulating and analyzing microsatellite data. Dr. Maarten Vonhof, Western Michigan University, provided access to unpublished DNA sequence data from *Lasiurus borealis*.

Appendix

Simulation code for single-population model
./ms 150 500000 -t 13.068 -eN 0 1
./ms 150 500000 -t 13.068 -eN 0 0.95099005 -eG 0 -33166.108317 -eN 1.51515E-06 1
./ms 150 500000 -t 13.068 -eN 0 0.904382075 -eG 0 -33166.108317 -eN 3.0303E-06 1
./ms 150 500000 -t 13.068 -eN 0 0.605006067 -eG 0 -33166.108317 -eN 1.51515E-05 1
./ms 150 500000 -t 13.068 -eN 0 0.366032341 -eG 0 -33166.108317 -eN 3.0303E-05 1
./ms 150 500000 -t 13.068 -eN 0 0.006570483 -eG 0 -33166.108317 -eN 0.000151515 1
./ms 150 500000 -t 13.068 -eN 0 4.31712E-05 -eG 0 -33166.108317 -eN 0.00030303 1

Simulation code for structured-population model
./ms 150 500000 -t 13.068 -I 2 75 75 330 -eN 0 1
./ms 150 500000 -t 13.068 -I 2 75 75 313.8267165 -eN 0 0.95099005 -eG 0 -33166.108317 -eN 1.51515E-06 1
./ms 150 500000 -t 13.068 -I 2 75 75 298.4460848 -eN 0 0.904382075 -eG 0 -33166.108317 -eN 3.0303E-06 1
./ms 150 500000 -t 13.068 -I 2 75 75 199.6520022 -eN 0 0.605006067 -eG 0 -33166.108317 -eN 1.51515E-05 1
./ms 150 500000 -t 13.068 -I 2 75 75 120.7906726 -eN 0 0.366032341 -eG 0 -33166.108317 -eN 3.0303E-05 1
./ms 150 500000 -t 13.068 -I 2 75 75 2.168259404 -eN 0 0.006570483 -eG 0 -33166.108317 -eN 0.000151515 1
./ms 150 500000 -t 13.068 -I 2 75 75 0.014246512 -eN 0 4.31712E-05 -eG 0 -33166.108317 -eN 0.00030303 1

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Table 1. Species composition of annual bat fatalities at wind energy facilities. Table reproduced from Kunz 2007b.

Species	Pacific Northwest	Rocky Mountains	South-Central	Upper Midwest	East	Total
Hoary bat	153 (49.8%)	155 (89.1%)	10 (9.0%)	309 (28.9%)	396 (28.9%)	1023 (41.1%)
Eastern red bat	-	-	3 (2.7%)	106 (20.3%)	471 (34.4%)	580 (23.3%)
Western red bat	4 (1.3%)	-	-	-	-	4 (0.2%)
Seminole bat	-	-	-	-	1 (0.1%)	1 (0.1%)
Silver-haired bat	94 (30.6%)	7 (4.1%)	1 (0.9%)	35 (6.7%)	72 (5.2%)	209 (8.4%)
Eastern pipistrelle	-	-	1 (0.9%)	7 (1.3%)	253 (18.5%)	261 (10.5%)
Little brown myotis	2 (0.7%)	6 (3.5%)	-	17 (3.3%)	120 (8.7%)	145 (5.8%)
Northern long-eared myotis	-	-	-	-	8 (0.6%)	8 (0.4%)
Big brown bat	2 (0.7%)	2 (1.1%)	1 (0.9%)	19 (3.6%)	35 (2.5%)	59 (2.4%)
Brazilian free-tailed bat	48 (15.6%)	-	95 (85.5%)	-	-	143 (5.7%)
Unknown	4 (1.3%)	4 (2.2%)	-	30 (5.7%)	15 (1.1%)	53 (2.1%)
Total	307	174	111	523	1371	2486

Figure 1. Bat carcass specimens collected at wind turbine sites. Species names are given in text: BigBr = big brown bat (Eptesicus fuscus), Myotis = Myotis spp., Hoary = hoary bat (Lasiurus cinereus), Red = red bat (L. borealis), and SilvH = silver-haired bat (Lasionycteris noctivagans). Figure reprinted from Howe et al. (2002).

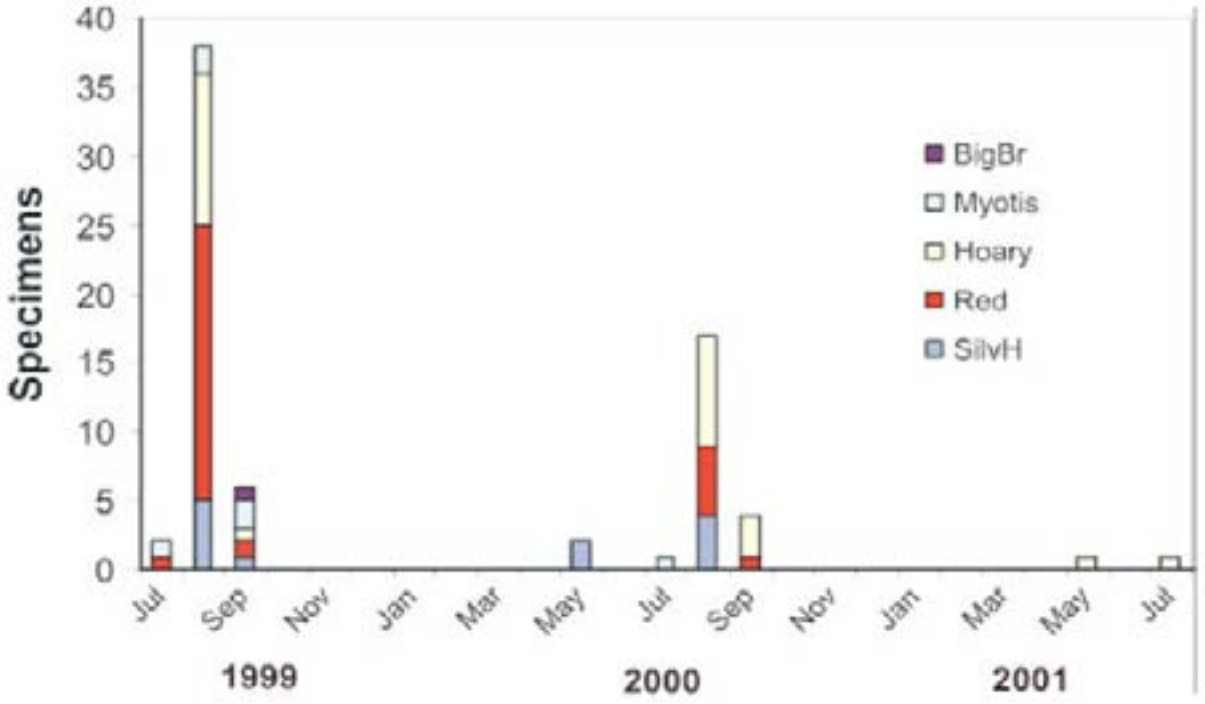

Figure 2. Demographic models of population decline. **A.** Single panmictic population. **B.** Structured population.

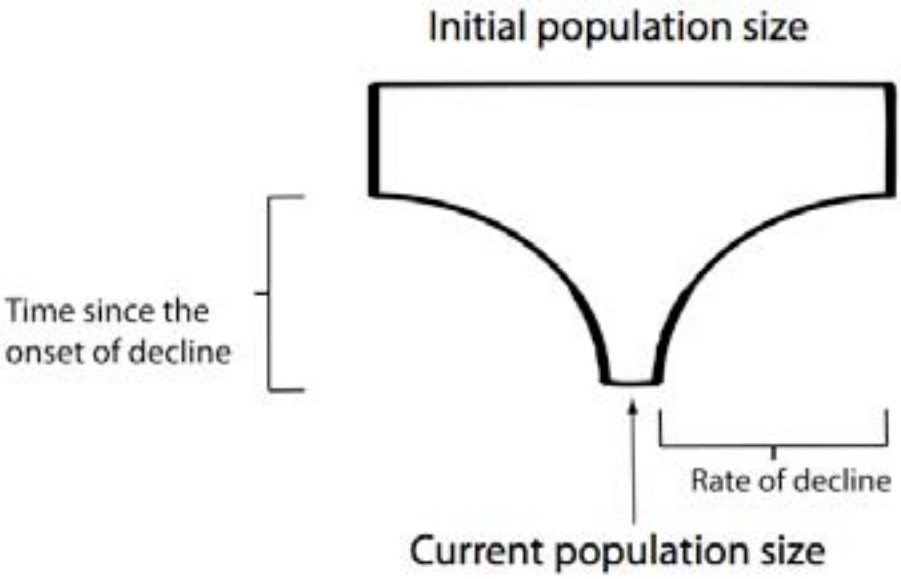

Figure 2A.

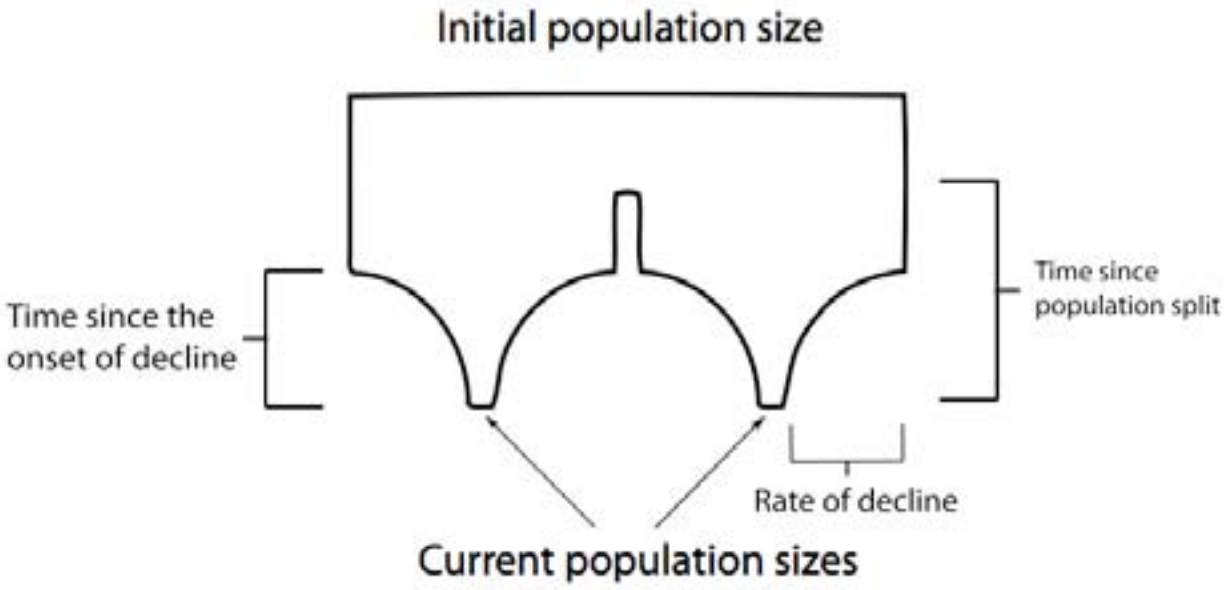

Figure 2B.

Figure 3. Decline in genetic diversity with increasing time since the onset of population size reduction. Data were simulated under demographies with (black) and without (blue) population structure. Average diversity statistics are shown with 95% CI (dashed lines). **A.** Diversity measured as average heterozygosity for 20 autosomal microsatellite loci. **B.** Diversity measured as the number of segregating sites in haploid (mitochondrial) DNA sequence data.

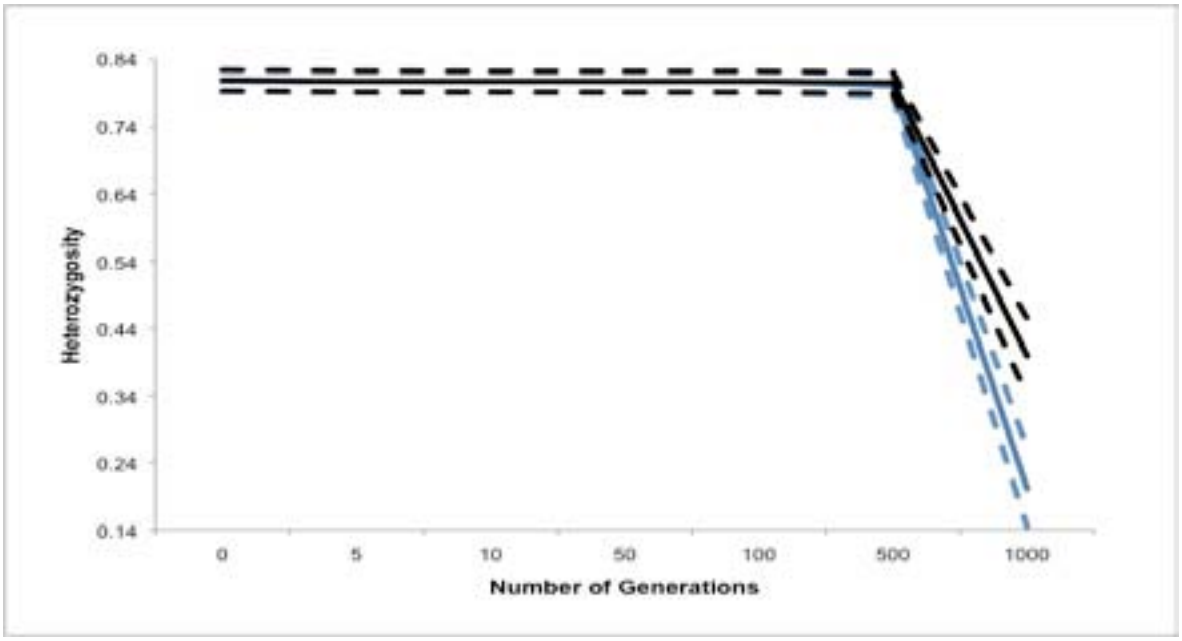

Figure 3A.

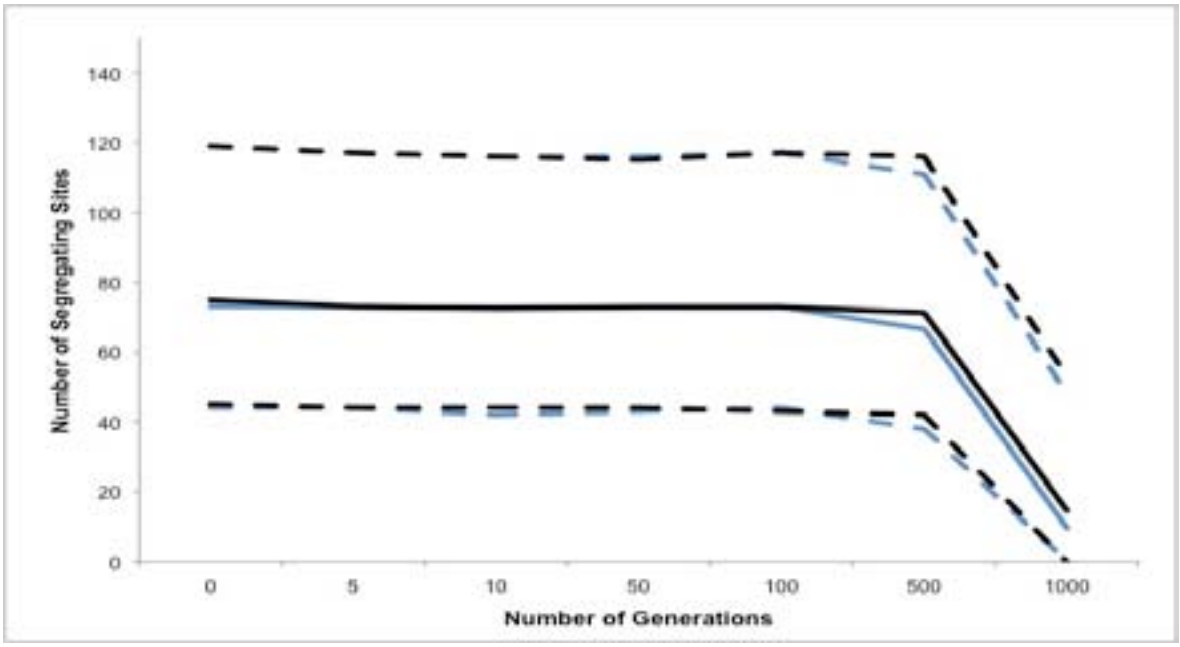

Figure 3B.

Figure 4. Change in neutrality tests with increasing time since the onset of population size reduction. Data were simulated under demographies with (black) and without (blue) population structure. Average test results are shown with 95% CI (dashed lines). **A.** Neutrality measured as Cox's Δ for 20 autosomal microsatellite loci. **B.** Neutrality measured as Tajima's D for haploid (mitochondrial) DNA sequence data.

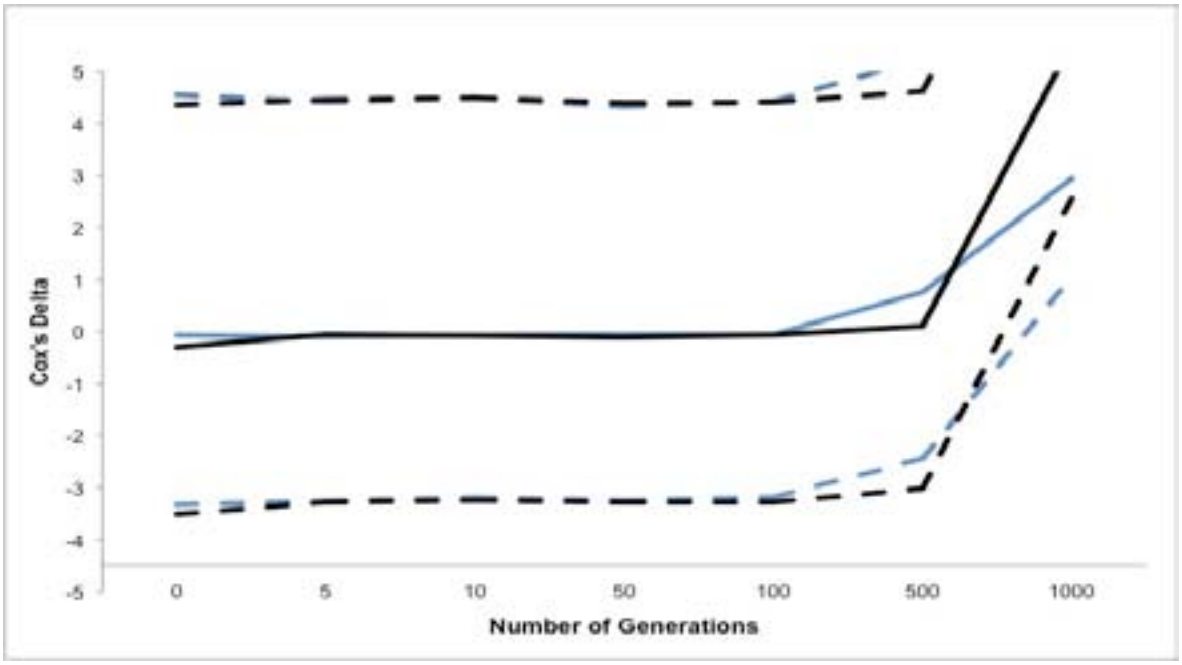

Figure 4A.

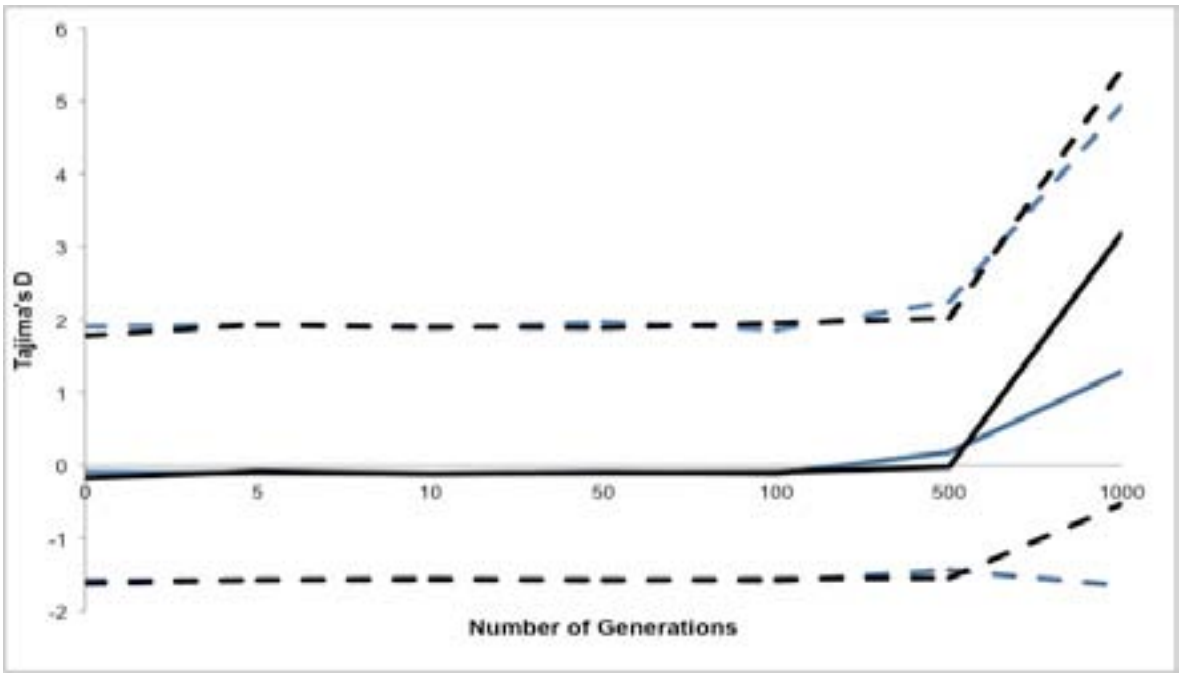

Figure 4B.

Figure 5. Decline in genetic diversity with increasing time since the onset of population size reduction. Data were simulated under demographies with (black) and without (blue) population structure. Average diversity statistics are shown with 95% CI (dashed lines). **A.** Diversity measured as average θP for 20 autosomal microsatellite loci. **B**. Diversity measured as average π for haploid (mitochondrial) DNA sequence data.

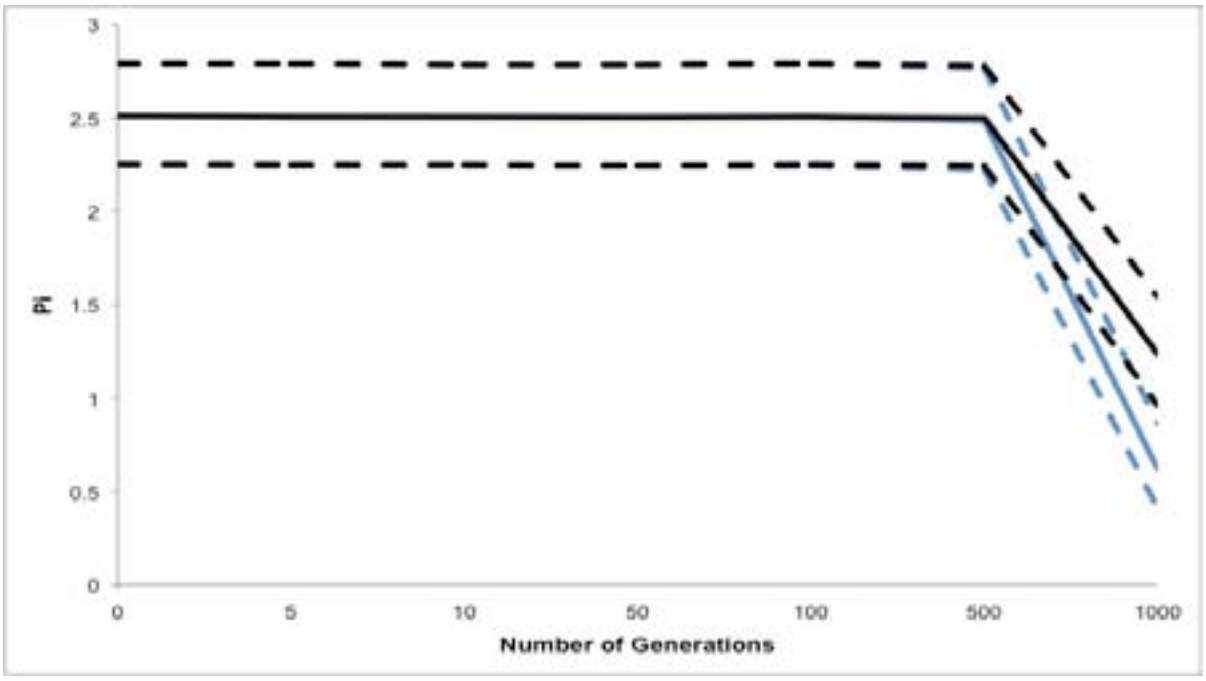

Figure 5A.

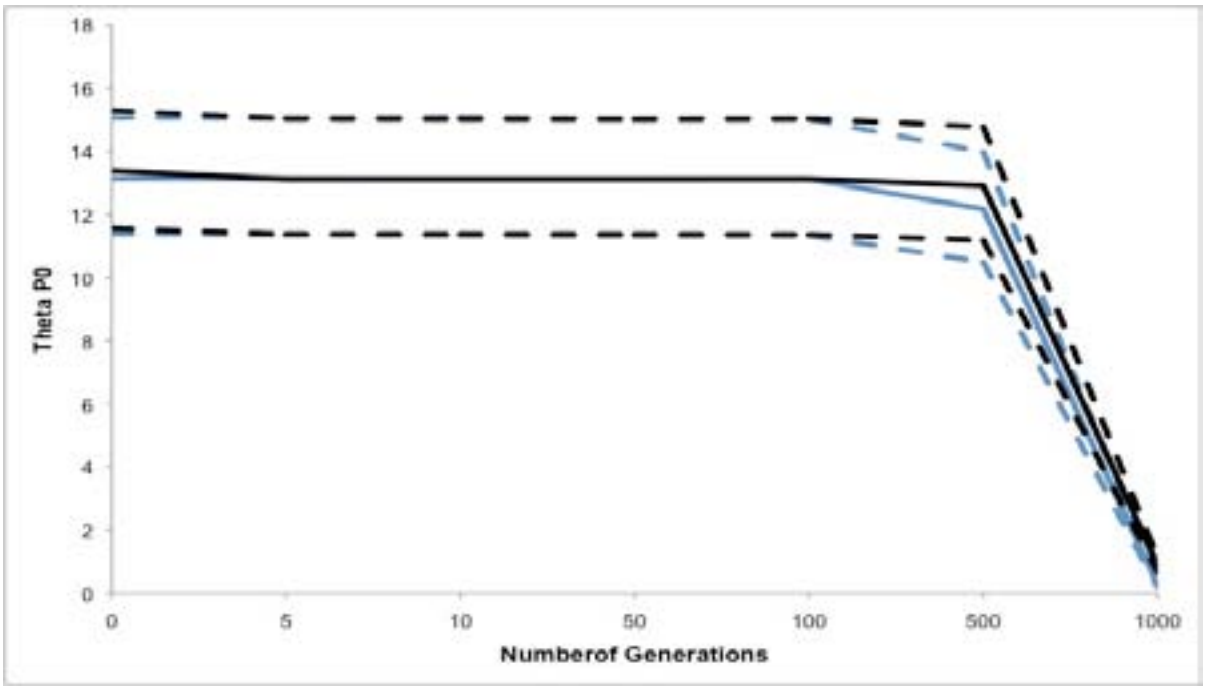

Figure 5B.