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ORIGINAL ARTICLE

Genetic diversity decreases as population density declines: Implications of temporal variation in mitochondrial haplotype frequencies in a natural population of *Tscherskia triton*

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Abstract

Although the spatial genetic differentiation that occurs in animal populations has been extensively studied, information on temporal variations in genetic structure and diversity is still lacking, especially for animals with oscillating populations. In the present study, we used the mtDNA D-loop sequence to assess the temporal genetic variation in samples from six successive years for the greater long-tailed hamster, *Tscherskia triton.* Sampling was carried out between 1998 and 2003 in cropland on the North China Plain, China. A total of 108 individuals were analyzed. The temporal samples showed a high level of genetic diversity. Substantial genetic changes in haplotype frequencies over time were detected for the hamster population. Random genetic drift and migration are likely to be the major factors responsible for the observed temporal pattern. The genetic diversity of the hamster population was higher in years with higher population density, and lower in years with lower population density. The result supports our hypothesis that genetic diversity decreases when population density declines in animals whose population oscillates greatly between years. The combined effects of inbreeding and genetic drift caused by reproduction, dispersal and population size might play important roles in the observed changes in genetic structure and diversity between years.

Key words: genetic diversity, mtDNA D-loop, population structure, temporal genetic differentiation, *Tscherskia triton.*

INTRODUCTION

Analysis of spatio-temporal components of the genetic structure of a species is important to fully understand factors that affect the genetic variability and relationships among its populations. However, most studies of genetic population structure typically aim at geographical differentiation, and information on variation in genetic structure and diversity over time is scarce. Monitoring temporal fluctuations allows the degree of stability of the spatial structuring within a population to be ascertained (Scribner *et al*. 1997). Some short and long-term dynamics in the temporal stability of genetic population structures

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have been identified (Blanco *et al*. 2005; Ayllon *et al*. 2006); however, few studies have investigated the temporal changes in genetic population structure in a rodent population (Gaines *et al*. 1978; Zimmerman 1988). It has been reported that when population size declines drastically, genetic variability is lost, leading to an increased rate of inbreeding in subdivided or patchy populations (Wang & Caballero 1999) or small populations of endangered species (Frankham 1996). But it is still not clear how genetic diversity varies in populations of animals with continuous distributions and oscillating densities.

Inter-annual variations are the result of basic demographic processes such as survival, recruitment, emigration and immigration. A challenge in current population ecology is to identify the demographic machinery underlying inter-annual variation (Stenseth *et al*. 1998; Yoccoz & Mesnager 1998). Population fluctuations may influence genetic structure because demographic processes differ greatly between years. In population peak years, the dispersal rate of animals would be high, but the reproduction rate would be low, and vice versa. Dispersal is important for ensuring gene flow among different locations, thus reducing genetic differentiation among sites, but it is also important for increasing genetic diversity by introducing new alleles, and by reducing inbreeding. High reproduction rates will increase the average relatedness of a local population, and thus increase the probability of inbreeding. Thus, for a given local population, high dispersal and low reproduction will result in higher genetic diversity for high population densities, and low dispersal and high reproduction will result in low genetic diversity for small populations. Furthermore, during the low-density stage, a population would face high levels of genetic drift, which would further accelerate the decrease in the genetic diversity of local populations. Therefore, our hypothesis predicts that genetic diversity would decrease when population density declines for animals with oscillating populations.

The greater long-tailed hamster, *Tscherskia triton*, is one of the major rodent pests in farmlands of the North China Plain. It is distributed from northern China to Korea and the adjacent areas of Russia (Zhang & Wang 1998; Luo *et al*. 2000). It generally occupies farmlands, grasslands, and valleys near croplands, and lives solitarily throughout the year (Wang *et al*. 1996). The life history and ecology of this species have been extensively investigated (Wang *et al*. 1996; Zhang & Wang 1998). The hamster can survive a year in the wild. The breeding season of this species lasts from March to August. Adult females reproduce one to three times in one breeding season, with the litter size ranging from 9 to 11. Populations of the hamsters are characterized by marked instability in population size, with density often varying fivefold or more from year to year (Zhang & Wang 1998).

The purpose of the present study was to examine the genetic changes over 6 successive years in a natural population of the greater long-tailed hamster in cropland of the North China Plain, and to test our hypothesis that the genetic diversities of the hamster populations are positively correlated with population densities across years.

MATERIALS AND METHODS

Study area and sample collection

All individuals of *T. triton* included in the present study were collected between 1998 and 2003 in the vicinity of Raoyang County (38°08'N, 115°41'E), Hebei Province, China. This arid area comprises agricultural land, and is part of the North China Plain. Approximately 70% of Raoyang County is arable land. Hamsters were captured by using wooden kill-traps. Eight trapping plots were set in farmlands in the vicinity of Raoyang County. The distance between plots was generally 50-100 m. Each plot consisted of two trapping lines, and the distance between trapping lines was 25–30 m. Twenty-five traps spaced 5 m apart were set along each trapping line. Traps baited with fresh peanuts were set in the late evening and collected in the morning. Samples were collected in autumn during the six sampling sessions. Population densities were calculated as: trap success (T, %) = total number of captured hamsters/total number of traps × 100%. Trapping success for 1998–2003 are shown in Table 1.

Laboratory procedures

A total of 108 adult greater long-tailed hamsters were sampled for genetic analysis at the same biological locality, Raoyang County. Muscle tissues were preserved in 95% ethanol, and stored at –20°C for subsequent examination. Genomic DNA was isolated by using standard phenol–chloroform extraction protocols. Mitochondrial D-loop region amplifications were performed in accordance with the method of Xie and Zhang (2005). Amplifications were performed using 35 cycles of 45 s at 94°C, 55 s at 58°C, and 1 min at 72°C with a final 10-min extension step at 72°C in a PTC-100 Thermal Cycler (MJ Research, Watertown, MN, USA). Amplicons were purified using the QIAquick PCR Purification kit (Qiagen, Hilden, Germany). All samples were sequenced using BigDye reagents with an ABI 377 Automated Sequencer (Applied Biosystems, Foster City, CA, USA).

Data analyses

Sequences were initially aligned using CLUSTAL W software (Thompson *et al*. 1994), with further manual correction. Haplotype diversity (*H1*), haplotype number (*H2*) and nucleotide diversity (π) values were calculated for temporal samples of the study population taken in different years to measure DNA polymorphism. The statistical significance of the correlation between trap successes and genetic diversity values was tested using SPSS 10.0 for Windows. Analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was conducted using Arlequin software (Schneider *et al*. 2000). Pairwise F_{ST} values (Weir & Cockerham 1984) and exact tests of population differentiation were estimated (Raymond & Rousset 1995). Tajima's D procedure (Tajima 1989) and Fu's *Fs* neutrality test (Fu 1997) were applied. The effective female population size (*Nef*) and migration rate (*m*) were estimated using MLNE software (Wang & Whitlock 2003).

RESULTS

The temporal samples of the *T. triton* population in Raoyang County had a high level of genetic diversity. Among temporal samples, haplotype diversity (*H1*) varied from 0.795 to 0.906, and nucleotide diversity (π) varied from 0.0093 to 0.0100 (Table 1). A significantly positive correlation between haplotype diversity (*H1*) and trap success (T) was observed among samples collected from the 6 study years ($r = 0.87$, $P = 0.02$; Fig. 1a). The number of haplotypes (*H2*) tended to be positively correlated with trap success during the study period ($r = 0.77$, $P = 0.07$; Fig. 1b). There was no significant correlation between nucleotide diversity and trap success. These results suggest that there is higher genetic diversity in years with abundant hamsters than in years in which hamsters are less abundant.

Table 1 Genetic diversity and trap success of greater long-tailed hamster samples from differentyears

Year	Sample size	Trap success(%)	Gene diversity	No.of haplotypes	Nucleotide diversity
1998	19	1.84	0.866	11	0.0093
1999	19	2.86	0.906	10	0.0100
2000	20	1.09	0.879	12	0.0095
2001	17	0.59	0.824	9	0.0097
2002	13	0.05	0.795	6	0.0100
2003	20	0.25	0.858	8	0.0100

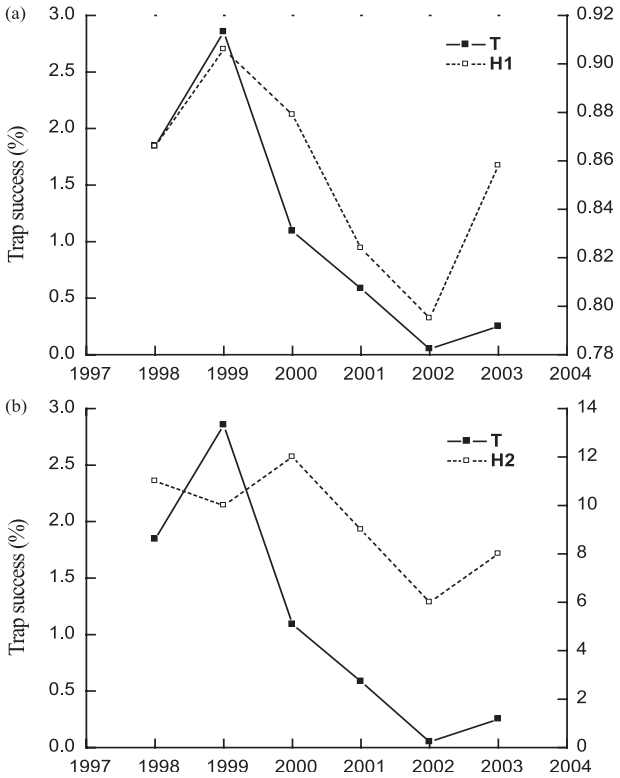

Figure 1 Relationship between genetic diversity (*H1*) or number of haplotypes (*H2*) and trap success (T) for a population of the greater long-tailed hamster between 1998 and 2003. (a) Haplotype diversity (*H1*). (b) Number of haplotypes (*H2*).

Pairwise F_{ST} values for the six temporal *T. triton* samples are shown in Table 2. A total of five of 15 population pairs differed significantly with respect to genetic structure. The largest genetic difference was found between the 2002 sample and the other five temporal samples. Hierarchical gene diversity analysis performed using all 6 temporal samples as a unit showed that the total genetic variability within samples was 96.5%, and the genetic variability among temporal samples was 3.5% ($P < 0.01$).

Tajima's *D* for all samples was –0.563 ($P = 0.35$), ranging from –1.460 ($P = 0.05$) to 0.931 ($P = 0.84$) for individual temporal samples. For all individuals the value of *Fs* was –3.829 ($P = 0.15$), varying between –3.181 ($P = 0.07$) and 1.193 ($P = 0.72$) for individual temporal samples. The null hypothesis of neutral evolution of the mtDNA D-loop sequence could not be rejected, suggesting that the D-loop region in this population was not under strong selection.

Table 2 Pairwise values of F_{ST} among temporal greater long-tailed hamster samples

	1998	1999	2000	2001	2002	2003
1998	0					
1999	0.017	0				
2000	-0.030	0.013	0			
2001	0.009	0.050*	0.019	0		
2002	0.095*	-0.014	0.087*	0.078*	0	
2003	0.002	0.029	0.038	0.051	0.111*	0

*Significant differences between two samples ($P < 0.05$).

The estimated effective population sizes of the hamster populations were low, but the estimated migration rate (*m*) was very high. The maximum likelihood effective female population size (*Nef*) was equal to 15 (95% CI: 9–49) and the estimated migration rate (*m*) was 0.999 (95% CI: 0. 785–1) for the annual samples.

DISCUSSION

The temporal samples of *T. triton* in the study population had substantial genetic structure. Hierarchical analysis showed that temporal samples were significantly genetically differentiated. A similar pattern was evident in the exact test. However, no population-specific haplotype was observed at a high enough frequency to allow direct temporal population identification. Moreover, significant changes in genetic composition were observed over time in the present study, despite the fact that the observed F_{ST} values were low. Similar genetic variation over time in rodent populations has been found in previous studies. Gaines *et al*. (1978) demonstrated genetic changes over population cycles of the prairie vole (*Microtus ochrogaster*). Zimmerman (1988) observed temporal genetic changes in a population of the pocket gopher (*Geomys bursarius*) over 10 years.

Three evolutionary forces are usually invoked to explain temporal variation in allelic frequencies: selection, random genetic drift, and migration. Previous experimental and empirical studies have revealed the effects of selection on temporal variations in genetic structure (Gyllenstein 1985; Mueller *et al*. 1985; Barker *et al*. 1986). However, the sequence used in the present study, the D-loop region, was not under direct selection in the studied population (Tajima 1989; Fu 1997). In the present study, no effect of direct selection was detected.

Stochastic temporal fluctuations in allelic frequency due to genetic drift may be important in small populations (Waples 1990; Waples & Teel 1990). No finite population is genetically stable over time because of genetic drift. Shifts in gene frequencies are expected, particularly in organisms that have small or cyclically fluctuating populations (Vuorinen & Eskelinen 2005). The low value of *Nef* corresponded well with the small effective population size. In previous studies it has been determined that small effective population sizes lead to strong genetic drift (Waples 1990; Waples & Teel 1990). It has also been shown that the strong genetic drift in small populations results in significant changes of genetic composition over time within populations (Wang & Caballero 1999). Jorde and Ryman (1996) suggested that random genetic drift caused most temporal allele frequency shifts in natural brown trout populations. Levy and Neal (1999) suggested that genetic drift could be the primary force shaping chloroplast gene frequencies. Moreover, populations that experience large reductions in effective size can increase identity by descent, loss of molecular genetic variation, and increase in the importance of stochastic changes in population size or composition (Lande 1988). In our case, the number of haplotypes was reduced to approximately half in 2002, when the population density was lowest. Therefore, genetic drift might play an important role in altering genetic structure and diversity in temporal samples of the hamster population in years with low densities.

Migration among populations is another important population parameter driving the dynamics of genes in space and time. Dispersal is used to explain temporal variation among species (Viard *et al*. 1997). In the present study, a high migration rate was estimated for this population, despite the low estimated effective population sizes. Barton and Whitlock (1997) suggested that populations with small effective population sizes are subject to strong genetic drift, and so their levels of genetic variation are expected to be low and declining, unless this is balanced by gene flow. Østergaard *et al*. (2003) found that the high migration rate between small brown trout populations maintained the high levels of genetic variation observed. Theoretically, the distribution of neutral genes in subdivided populations is considered to be balanced by the forces of genetic drift and migration (Slatkin 1985). Genetic drift results in local differentiation, whereas migration may prevent the divergence of populations. The gene flow caused by individual dispersal should decrease the genetic differentiation among adult populations. Therefore, the integration of random genetic drift and migration might be the major factor responsible for the temporal pattern observed in the present study.

Hierarchical analysis and the exact test both indicated

that there was significant genetic change between temporal samples. This genetic variation in the temporal populations probably corresponded with fluctuations in the density of the greater long-tailed hamster population. The population density was relatively high in 1998 and 1999, but became very low in 2002 and 2003. The trap success in 2002 was extremely low. Correspondingly, the genetic diversity in 2002 was also the lowest during the study period. This result supports our hypothesis that genetic diversity is positively correlated with population abundance in animals with oscillating populations.

The observed positive correlation between genetic diversity and population density across years was probably caused by inbreeding and genetic drift, forces which also act on small isolated populations of many endangered species. In small populations, the opportunities for mating are restricted, even given random mating patterns. Thus, mating among relatives is common and the proportion of individuals that are homozygous at many loci increases (Gaggiotti 2003). Small populations tend to fix an appreciable fraction of the genetic load via genetic drift, resulting in elevated levels of among-population inbreeding (Keller & Waller 2002). Density fluctuations in small mammals reduce the effective population size substantially, which is expected to lower genetic diversity and cause inbreeding (Vuorinen & Eskelinen 2005). Populations of *T. triton* are characterized by marked instability in population size, with density often varying fivefold or more from year to year (Zhang & Wang 1998). Zhang and Wang (1998) also found that years in which the hamster was more abundant, there was a higher dispersal rate, and that in years in which there was lower abundance, there was a lower dispersal rate. Lower levels of dispersal in years with low densities might accelerate inbreeding and genetic drift in this hamster population. After population crashes, the population density of this hamster can be very low, with populations often occurring in small patches. It is very likely that mating among hamsters was very restricted in years with low population densities, which might have resulted in inbreeding and genetic drift, and thus low genetic diversity.

In conclusion, a population of the greater long-tailed hamster in Raoyang County exhibited substantial temporal changes in genetic structure from year to year. Random genetic drift and migration are likely to be the major factors responsible the observed temporal pattern. The genetic diversity of the hamster population decreases when a population declines. The integration of inbreeding and genetic drift caused by differences in reproduction and dispersal may play important roles in shaping the genetic structure of a hamster population over time.

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