Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

Genetic diversity of the subterranean Gansu zokor in a semi-natural landscape

G. H. Lin[1,2,*], Z. Y. Cai[1,*], T. Z. Zhang[1,2], J. P. Su[1] & S. J. Thirgood[3]

1 Key Laboratory of Adaption and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China
2 Graduate School of Chinese Academy of Sciences, Beijing, China
3 Macaulay Institute, Craigiebuckler, Aberdeen, UK

Keywords
habitat composition; geographic information systems; genetic diversity; human activities; subterranean rodents.

Correspondence
J. P. Su, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China.
Email: jpsu@nwipb.ac.cn

*These two authors contributed equally to this article.

Editor: Jean-Nicolas Volff

Received 7 November 2007; revised 18 January 2008; accepted 24 January 2008

doi:10.1111/j.1469-7998.2008.00423.x

Abstract

We studied the relationship between genetic diversity of the subterranean Gansu zokor *Myospalax cansus* and habitat variability in the Loess Plateau, Qinghai Province, China. We used a combination of geographic information systems and molecular techniques to assess the impact of habitat composition and human activities on the genetic diversity of zokor populations in this semi-natural landscape. Although they occurred relatively infrequently in the landscape, woodland and high-coverage grassland habitats were the main positive contributors to the genetic diversity of zokor populations. Rural residential land, plain agricultural land and low-coverage grassland had a negative effect on genetic diversity. Hilly agricultural land and middle-coverage grassland had little impact on zokor genetic diversity. There were also interactions between some habitat types, that is, habitat types with relatively better quality together promoted conservation of genetic diversity, while the interaction between (among) bad habitat types made situations worse. Finally, habitat diversity, measured as patch richness and Shannon's diversity index, was positively correlated with the genetic diversity. These results demonstrated that: (1) different habitat types had different effects on the genetic diversity of zokor populations and (2) habitat quality and habitat heterogeneity were important in maintaining genetic diversity. Habitat composition was closely related to land use thus emphasizing the importance of human activities on the genetic diversity of subterranean rodent populations in this semi-natural landscape. Although the Gansu zokor was considered to be a pest species in the Loess Plateau, our study provides insights for the management and conservation of other subterranean rodent species.

Introduction

Human activities are widely believed to be the major cause of the current global biodiversity crisis (Meffe & Carroll, 1997; Borgerhoff-Mulder & Coppollilo, 2005). The biodiversity of the planet is rapidly being depleted through unsustainable consumption, habitat modification, climate change and a myriad of other direct and indirect human impacts (Frankham, 2005). Genetic diversity is considered to be important for species viability in a variety of environments, and the conservation of genetic diversity has become a fundamental concern in conservation biology (Frankham, 1996). Numerous studies have investigated genetic diversity in natural populations (e.g. Arnaud *et al*., 2003; Knaepkensa *et al*., 2004; Neumann *et al*., 2004; Epps *et al*., 2006). Very few studies, however, have assessed the genetic diversity of subterranean rodent species (Reyes, Nevo & Saccone, 2003; Karanth *et al*., 2004), or investigated how the genetic diversity of these taxa is influenced by habitat composition.

Genetic diversity represents the average composition and richness of genes in local populations within appropriate geographic scales (Hedrick, 2000). In semi-natural environments with a high conservation value (Part & Soderstrom, 1999), natural and man-made patch types with different habitat conditions might make different contributions to the genetic diversity of local populations. For example, Neville, Dunham & Peacock (2006) indicated that trout populations had a higher genetic variability in a good-quality habitat than in a poor-quality habitat. Modeling studies also indicate that patches with different carrying capacities can influence the levels of genetic diversity within populations (Gibbs, 2001).

Subterranean rodents are a widely distributed group of species that live primarily underground and are highly adapted to that environment (Lacey, Patton & Cameron, 2000). The Gansu zokor *Myospalax cansus* is an important rodent species in Loess Plateau in China, distributed in parts of Qinghai, Gansu, Shaanxi and Ningxia provinces (Northwest Plateau Institute of Biology Site, The Chinese Biodiversity Information Center, http://www.haibei.org). As a

typical solitary subterranean rodent species, Gansu zokor live their whole lives underground (Chu, Li & Li, 2007). The high energy costs of digging underground restrict the movement of subterranean rodents (Seymour, Withers & Weathers, 1998), thus making these species particularly sensitive to the habitat characteristics of local landscapes. In semi-natural landscapes, habitat characteristics such as vegetation coverage and land use may influence the persistence of local zokor populations (Hu, 2005; Jiang, Wang & Xue, 2005), and this might subsequently influence the genetic diversity of local zokor populations. In this paper, we use geographic information systems (GIS) and molecular techniques to examine the relationship between the habitat composition of the heavily impacted semi-natural landscape of the Loess Plateau in Qinghai Province and the genetic diversity of local populations of Gansu zokor.

Methods

Zokor sampling

The sampling sites were located in the Hehuang Watershed on the Loess Plateau in Qinghai Province in western China. This region has been subjected to a major reforestation project and trees have been planted in abandoned farmland since 2000 (Ye & Shu, 2006). Zokors eat the young trees and are considered to be a pest species. A zokor-eradication program was initiated by the local government and from April to June 2005, we followed the eradication teams and collected zokor carcasses. We collected ~200 zokor carcasses from each sampling site within an area of ~150 ha and recorded the global positioning system location from the center of the sampling site (Table 1). Approximately 20 zokors were randomly selected from each carcass collection for genetic analysis (unfortunately several samples were lost in the laboratory due to unknown reasons, and only 12 and 15 from HZ and LD2 were left, respectively). Muscle tissue samples were collected from each individual and preserved in 95% alcohol.

Molecular techniques

Total DNA was extracted following the Joe and David method (Sambrook & Russell, 2001) from 0.3 g ethanol-fixed tissue. The D-loop sequence was amplified with primers FR (TACCATCCTCCGTGAAACCA) and RV (CTAATAA TAAGGCCAGGACC), with the reference of the study of *Spalax ehrenbergi* (Reyes *et al.*, 2003). PCR was performed in a 50 μL reaction volume, with 40–60 ng of genomic DNA, 10 mmol L^{-1} Tris-HCl (pH 8.3), 2.5 mmol L^{-1} $MgCl_2$, 50 mmol L^{-1} KCl, 0.5 mmol L^{-1} dNTPs, primer FR and RV 0.5 μmol L^{-1}, respectively, and 1 U Taq polymerase. Thermocycling was conducted in a T-Gradient Thermoblock PCR machine (Biometra, Gottingen, Germany). After an initial denaturation step at 95 °C for 10 min, the reaction proceeded for 30 cycles as follows: 45 s at 95 °C, 45 s at 52 °C and 90 s at 72 °C. The PCR products were purified using a CASpure PCR Purification Kit following the protocol recommended by the manufacturer (Casarray, Shanghai, China), and directly sequenced using the same primers used for amplification of the D-loop mentioned above. Sequencing reactions were conducted in a Biometra thermocycler using a DYEnamic Dye Terminator Cycle Sequencing Kit (Amersham, Pharmacia Biotech Inc., Sunnyvale, CA, USA) following the manufacturer's protocol. Sequencing products were separated and analyzed on a MegaBACE 1000 DNA Analysis System (Amersham Pharmacia Biotech Inc.). Both strands of DNA were sequenced using forward and reverse primers. Sequences were recorded in both strands with an overlap of 70%.

Genetic analysis

The sequences were checked by eye and aligned using CLUSTAL W (Thompson *et al.*, 1997) and refined manually. Genetic diversity was estimated with Arlequin 3.10 (Excoffier, Laval & Schneider, 2005) using two different diversity indices:

(1) Haplotype diversity (H):

$$H = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} p_i^2\right)$$

(2) Nucleotide diversity (π_n):

$$\pi_n = \frac{\sum_{i=1}^{k} \sum_{j<i} p_i p_j \hat{d}_{ij}}{L}$$

Table 1 Distribution and frequency of each haplotype and genetic diversity list

Site	SZ	Private haplotypes	Shared haplotypes	HN	H	π_n
DT	23	h1(16), h2, h3, h4, h5, h6, h7, h8		8	0.5257	0.00274
HZ	12	h9(3), h10, h11(3)	h12, h13(4)	5	0.8182	0.00506
LD1	20	h14, h15(5), h16(3), h17, h18, h19, h21	h12(6), h20	9	0.8526	0.00420
LD2	15	h22(11), h23(2), h24, h25		4	0.4667	0.00383
MH1	22	h26(5), h27(2), h28(4), h29(4), h30	h12, h13, h20(4)	8	0.8745	0.00520
MH2	22	h31(10), h32(6), h33(6)		3	0.6753	0.00266
PA1	21	h34(18)	h35(3)	2	0.2571	0.00082
PA2	23	h36(9), h37(9), h38, h39	h35(3)	5	0.7036	0.00201

DT, Datong County; HZ, Huzhu County; LD (1, 2), Ledu County; MH (1, 2), Minhe County; PA (1, 2), Pingan County. The numbers in the parentheses followed the haplotype names show the frequency of related haplotypes.

SZ, sample size; HN, the number of haplotypes identified; H, haplotype diversity.

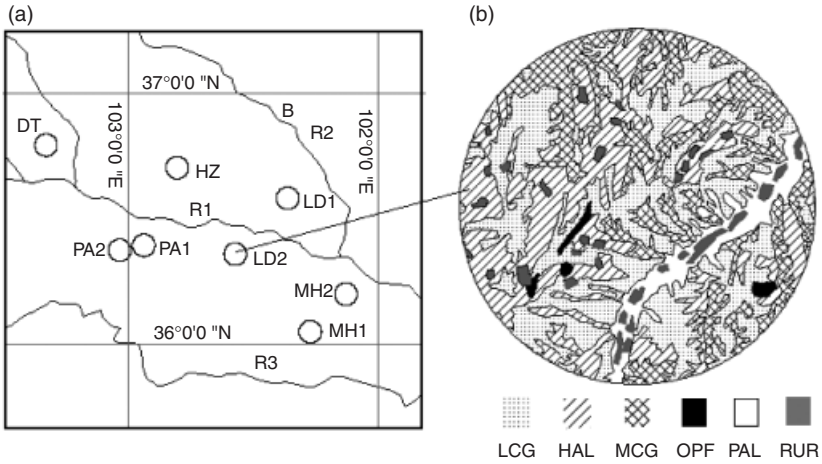

Figure 1 (a) Sampling sites in Hehuang Watershed, east of Qinghai Province. (b) An example (LD2) of the landscapes within a 5-km radius around. R1, Huangshui River; R2, Datong River; R3, Yellow River.

Table 2 Landscape types based on the Environment & Ecology Scientific Data Center of western China and our own study experiences

Patch type	Description
Arbor (ARB)	Natural or planted wood with cover rates higher than 30%
Shrubbery (SHR)	(Short) shrub with cover rates more than 40%
Open forest (OPF)	Wood with coverage between 10 and 30%, usually with grasses beneath
High-coverage grassland (HCG)	Either natural or semi-natural grassland with cover rates more than 50%
Middle-coverage grassland (MCG)	Either natural or semi-natural grassland with a cover rate of 20–50%
Low-coverage grassland (LCG)	Natural grassland with a cover rate of 5–20%
Bare land	With a vegetation cover rate below 5%
Rural resident (RUR)	With buildings, orchards or farmland mosaiced
Water body	Including river/canal; reservoir/pond; bottomland
Hilly agricultural land (HAL)	Part of hilly area used as agricultural land with few irrigating activities
Plain agricultural land (PAL)	Agricultural land in plain area with better irrigation

where n is the number of gene copies in the sample, k is the number of total haplotypes, $\hat{d}_{ij}$ is an estimate of the number of mutations having occurred because the divergence of haplotypes i and j, L is the number of loci and p_i and p_j are the sample frequencies of the i-th or the j-th haplotype, respectively (Excoffier *et al.*, 2005).

Habitat analysis

Habitat variability was assessed using the '1:100 000 Land Use Data in Qinghai Province' as the data source. These data were provided by Environmental and Ecological Science Data Center for West China, National Natural Science Foundation of China (http://westdc.westgis.ac.cn). We used ArcGIS 9.0 (Environmental Systems Research Institute) to convert the original vector coverage into a grid-style layer. As noted above, the spatial scale for the target 'gene pool' must be estimated appropriately, because too large or too small scales cannot accurately reflect the relationship between habitat conditions and genetic diversity of local populations. Based on the study of Wei *et al.* (1997), Sullivan, Sullivan & Hogue (2001), we estimated the annual dispersal of Gansu zokor to be 100 m. Because the heavy land use activities in this region was generally considered from 1949 when People's Republic of China was founded, the appropriate range that can reflect the influence of human activity on population genetic diversity might be 5.6 km (100 m × (2005−1949). However, the distance between the sites PA1 and PA2 was about 9.0 km; thus, in order to avoid too much of an overlap of these two sites, eclectically, a 5 km radius around each sampling center was used to analyze the habitat composition (Fig. 1).

Habitat composition and habitat diversity indices, including the percentage area of each habitat type, patch richness (number of patch type, PR) and Shannon's diversity index (SHDI) of each mosaic, were calculated using FRAGSTATS 3.3 (McGarigal *et al.*, 2002). In total, there were 11 patch types according to the '1:100 000 Land Use Data' in the eight landscape mosaics (see Table 2). However, bare land and three water types were ignored because they occurred very infrequently in the landscape and, more importantly, zokors were very unlikely to inhabit such places.

Statistical analysis

The direct correlations between habitat variables as well as between habitat variables and genetic diversity (including

Table 3 Composition (%) and diversity of habitat mosaics

Site	ARB	SHR	OPF	HCG	MCG	LCG	RUR	HAL	PAL	PR	SHDI
DT	0.00	0.00	2.84	0.00	16.41	42.01	2.02	18.22	18.41	6	1.46
HZ	0.00	25.35	0.00	3.65	43.91	14.14	1.22	3.57	7.90	7	1.48
LD1	11.89	0.00	23.10	8.66	27.86	0.03	0.11	28.35	0.00	7	1.53
LD2	0.00	0.00	0.93	0.00	20.25	37.86	3.58	30.47	6.92	6	1.40
MH1	5.03	13.74	11.08	16.14	13.14	0.88	0.38	34.07	5.42	9	1.82
MH2	0.00	0.00	0.00	0.00	7.44	37.58	0.97	44.01	8.24	5	1.18
PA1	0.00	0.00	0.00	0.00	34.82	41.95	1.66	7.96	13.40	5	1.27
PA2	0.00	2.79	0.00	0.04	12.03	58.79	3.45	12.99	9.84	7	1.28

DT, Datong County; HZ, Huzhu County; LD (1, 2), Ledu County; MH (1, 2), Minhe County; PA (1, 2), Pingan County; ARB, arbor; HAL, hilly agricultural land; HCG, high-coverage grassland; LCG, low-coverage grassland; MCG, middle-coverage grassland; OPF, open forest; PAL, plain agricultural land; PR, patch richness; RUR, rural resident; SHDI, Shannon's diversity index; SHR, shrubbery.

Table 4 Spearman's correlation matrix of different variables

Indices	TWL	HCG	MCG	LCG	RUR	HAL	PAL	PR	SHDI
HCG	0.855**								
MCG	0.240	0.114							
LCG	−0.599	−0.660§	−0.262						
RUR	−0.527	−0.634§	−0.048	0.833*					
HAL	−0.048	0.000	−0.690§	−0.333	−0.286				
PAL	−0.599	−0.689§	−0.119	0.857**	0.548	−0.405			
PR	0.858**	0.921**	0.037	−0.420	−0.321	−0.049	−0.581		
SHDI	0.946**	0.812**	0.310	−0.619	−0.452	0.000	−0.619	0.840**	
H	0.826*	0.939**	−0.119	−0.667§	−0.690§	0.190	−0.667§	0.865**	0.738*
π_n	0.790*	0.723*	0.214	−0.762*	−0.476	0.214	−0.738*	0.729*	0.881**

§$P<0.1$; *$P<0.05$; **$P<0.01$.

H, haplotype diversity; HAL, hilly agricultural land; HCG, high-coverage grassland; LCG, low-coverage grassland; MCG, middle-coverage grassland; PAL, plain agricultural land; PR, patch richness; RUR, rural resident; SHDI, Shannon's diversity index; TWL, total woodland.

haplotype diversity and nucleotide diversity) were analyzed using Spearman's rank correlations. It should be noticed that, the arbor tree, shrubbery and open forest occurred in no more than four mosaics. Based on their similar attributions, instead, the summation of these three types (total woodland, TWL) was used when testing their effects on genetic diversity. When calculating PR and habitat diversity, however, in order not to lose too much information, the original nine types were used (Table 3).

Because the habitat in every sampling site was a mixture of habitat types, it is suggested (kindly by the reviewer) that the potential effects of interactions among habitat types on genetic diversity should not be ignored. We used stepwise multiple linear regression analysis to test the effects of a combination of 2 or more habitat types on genetic diversity. Moreover, because there were high direct correlations between some independent variables (Table 4), we introduced the principle component regression analysis (PCRA) to circumvent the problem of multicollinearity (Massy, 1965). All statistical analyses were performed using SPSS 15.0 for Windows software. For the PCRA, we at first performed a factor analysis with Varimax rotation to show the structure of independent variables. We then introduced a program based on SPSS second time exploitation (Huang, Xiang & Liu, 2007), which combines a serial of necessary calculating processes of PCRA to show how the components can explain the genetic diversity variations.

Results

Genetic diversity

A total of 158 zokors in eight sampling sites were analyzed, and the amplified mtDNA fragment varied from 625 to 626 bp, containing the 3′-D-loop region (530 bp), the tRNAphe sequence (67 bp) and partly 5′-12S rRNA (29 bp). A total of 39 haplotypes were identified in the eight populations, including 27 variable sites (Table 1). The frequency and distribution for each haplotype are also given in Table 1.

Haplotype diversity varied from 0.2571 to 0.8745, while nucleotide diversity varied from 0.00082 to 0.00520. Neither haplotype diversity (Spearman's rank correlation, $N = 8$, $P = 0.955$) nor nucleotide diversity (Spearman's rank correlation, $N = 8$, $P = 0.298$) was related to the number of samples used for DNA analysis. It seems that although there were relatively very few individuals from HZ and LD2 analyzed, it did not cause obvious biases in representing the relative genetic diversity of different populations.

Table 5 Component matrix of the first three rotated (Varimax) components extracted in factor analysis

	Component		
Independent variable	1	2	3
TWL	0.642	−0.709	0.210
HCG	0.798	−0.551	−0.135
MCG	−0.063	−0.287	0.935
LCG	−0.525	0.828	−0.097
RUR	−0.192	0.847	−0.020
HAL	−0.035	−0.370	−0.909
PAL	−0.243	0.746	0.182
PR	0.962	−0.167	−0.043
SHDI	0.912	−0.303	0.016

HAL, hilly agricultural land; HCG, high-coverage grassland; LCG, low-coverage grassland; MCG, middle-coverage grassland; PAL, plain agricultural land; PR, patch richness; RUR, rural resident; SHDI, Shannon's diversity index; TWL, total woodland.

Habitat composition

The habitat composition of the eight sampling sites is given in Table 3. Middle-coverage grassland (MCG), low-coverage grassland (LCG) and hilly agricultural land were common in all eight sampling sites. Woodland occurred in six of the eight sampling sites but was common in only three sites (HZ, LD1 and MH1). Plain agricultural land and high-coverage grassland (HCG) occurred in seven and four of eight sites, respectively. Rural residential land occurred in all sites but was common in none.

Relationship between habitat composition and genetic diversity

Zokor populations from different habitat types showed different levels of genetic diversity (Table 1). Genetic diversity was positively correlated with the availability of TWL and HCG and negatively correlated with the availability of LCG, RUR and PAL (Table 4). MCG and HAL had no significant correlations with genetic diversity. Two measures of habitat diversity (PR and SHDI) were positively correlated with both haplotype diversity and nucleotide diversity.

Stepwise multiple linear regressions at the 95% confidence level ($P < 0.05$) generated three and two models for H and π_n, respectively. TWL alone explained (adjusted R^2, the same below) 55.5% of the haplotype diversity variation, while the combination of TWL and MCG and the combination of TWL, MCG and HCG explained 68.8 and 75.3% of the haplotype diversity variation, respectively. For π_n, LCG alone explained 58.5% of the variation, while the combination of LCG and RUR explained 72.5% of its variation.

In PCRA, the first three components were extracted in factor analysis, and they were able to represent most (the cumulative per cent of variance was 89.95%, higher than the general criterion of 85%) of the variation of all nine independent variables. The rotated components are shown in Table 5. However, only the first eigenvector was significantly correlated with H and π_n, and it explained (R^2) 57.6 and 65.2% of the variation of H and π_n, respectively.

Discussion

The main finding of our study was that the genetic diversity of Gansu zokors was strongly influenced by habitat composition and habitat diversity on the Loess Plateau. First of all, all the three statistic approaches (direct Spearman's rank correlation, multiple linear regression and PCRA) had shown that different habitat types had different effects on genetic diversity, as expected. The results that the combination of some habitat types could better explain genetic diversity variation proved our assumption that there were interactions among habitat types. It seems that the interaction between (among) habitat types with a relatively better quality promoted conservation of genetic diversity, while the interaction between (among) bad habitat types made the situations worse. Moreover, the results from PCRA definitely divided the independent variables into three groups: positive variables, including TWL, HCG, PR and SHDI; negative variables, including LCG, RUR and PAL; and neutral variables, MCG and HAL. Because the first component alone could still explain more than half of the variations of H and π_n, the results had emphasized the significance of positive variables (especially habitat heterogeneity variables, PR and SHDI) on genetic diversity.

Population size is one of the most important factors influencing genetic diversity (Frankham, 1996). Small populations with reduced reproductive fitness and higher inbreeding coefficients may lose genetic diversity through genetic drift (Vergeer *et al.*, 2003; Hensen & Wesche, 2006). Although very thick vegetation has been shown to reduce the population size of zokors (Jiang *et al.*, 2005), TWL and HCG appeared to provide enough food resources and suitable underground nesting sites (Fan & Gu, 1981) to retain a considerable population size that is important to maintain genetic diversity. In common with other studies (Hu, 2005), LCG with little food resources and dry soil that was hard to dig had low densities of zokors. The heavily human-influenced RUR and PAL did not provide a suitable habitat for zokors, and subsequently, in addition to LCG, exerted negative effects on the genetic diversity of zokor populations. MCG and HAL appeared to be an intermediate habitat for zokors and had neither a positive nor a negative impact on genetic diversity. It should be noticed that the combination of TWL, HCG and MCG was better at explaining the haplotype diversity variation. Although MCG did not have a high population-carrying capacity, we suggested that it could have provided a temporary refuge to help the animal to survive through bad situations. Although in our study we did not have exact data on zokor population size, we suggest that different habitat types had affected the genetic diversity of local populations through their influence on zokor population dynamics, consistent with studies on other taxa (Knaepkensa *et al.*, 2004).

Habitat heterogeneity is thought to be an additional important factor affecting genetic diversity (Powell, 1971).

The 'balancing selection hypothesis' suggests that different genotypes had a selective advantage in different environments, and as a result, populations in spatially variable landscapes maintain higher levels of genetic diversity (Hedrick, 2007). We used two indices of habitat heterogeneity (PR and SHDI) to describe the habitat composition attributes at the landscape scale (McGarigal *et al.*, 2002). The positive correlations between these two heterogeneity indices and both haplotype diversity and nucleotide diversity in this study suggested that the genetic diversity of subterranean rodent populations increased in response to habitat heterogeneity.

Human activities and, in particular, human modification of natural landscapes, is widely recognized as having a negative impact on the genetic diversity of organisms, especially for those species with a low dispersal rate (Arnaud *et al.*, 2003; Werth *et al.*, 2006). Our study on Gansu zokors lends some support to these findings. The area of RUR and PAL in our study had a negative impact on the extent of natural landscapes such as TWL and HCG, which provided a good habitat for zokors. RUR and PAL were negatively correlated with the genetic diversity of zokor populations, and we suggest that the area of these habitat types can be used as reliable indices of the extent to which human activities have modified this landscape and impacted on natural processes.

Subterranean rodents are a widely distributed group, occurring on every continent, except Australia (Sherman, 2001). These rodents are typically viewed as pest species and are subject to systematic control programs in many countries (Zhang, Zhang & Liu, 2003). A number of subterranean rodent species are listed as endangered or vulnerable by IUCN (2006) including the Chinese zokor *Myospalax fontanierii*, Michoacan pocket gopher *Zygogeomys trichopus*, Oaxacan pocket gopher *Orthogeomys cuniculus*, greater mole rat *Spalax microphthalmus* and Magellanic tuco-tuco *Ctenomys magellanicus*. Subterranean rodents are considered to be important ecosystem engineers with major impacts on nutrient cycling, soil structure and vegetation composition (Smallwood, Geng & Zhang, 2001; Reichman & Seabloom, 2002; Zhang *et al.*, 2003; Wang *et al.*, 2005). Despite the potential importance of these species in ecosystem dynamics, little attention has been paid to studies of their ecology, management and conservation. While the Gansu zokor in the Loess Plateau is considered to be a pest species and subject to systematic control in support of reafforestation, our study might provide useful information for conservation and management applications on other subterranean rodent species.

Acknowledgements

This work was supported by the Chinese Academy of Science Innovation Program CXLY-2003-3. S.J. Thirgood was funded by the International Exchange Programme of The Royal Society of Edinburgh. We thank Dr X.M. Lian and L. Yu for helpful comments on the paper.

References

Arnaud, J.F., Madec, L., Guiller, A. & Deunff, J. (2003). Population genetic structure in a human-disturbed environment: a case study in the land snail *Helix aspersa* (Gastropoda: Pulmonata). *Heredity* **90**, 451–458.

Borgerhoff-Mulder, M. & Coppollilo, P. (2005). *Conservation: linking ecology, economics and culture*. Princeton: Princeton University Press.

Chu, Z.C., Li, J.G. & Li, Y.M. (2007). Analysis of digging efficiency of the forelimbs of three rodents. *Chin. J. Zool.* **42**, 17–20.

Epps, C.W., Palsboll, P.J., Wehausen, J.D., Roderick, G.K. & Mccullough, D.R. (2006). Elevation and connectivity define genetic refugia for mountain sheep as climate warms. *Mol. Ecol.* **15**, 4295–4302.

Excoffier, L., Laval, L.G. & Schneider, S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinformatics Online* **1**, 47–50.

Fan, N.C. & Gu, S.Q. (1981). The structure of the tunnel system of the Chinese zokor. *Acta Their. Sin.* **1**, 67–72.

Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Conserv. Biol.* **10**, 1500–1508.

Frankham, R. (2005). Stress and adaptation in conservation genetics. *J. Evol. Biol.* **18**, 750–755.

Gibbs, J.P. (2001). Demography versus habitat fragmentation as determinants of genetic variation in wild populations. *Biol. Conserv.* **100**, 15–20.

Hedrick, P.W. (2000). *Genetics of populations*, 2nd edn. Massachusetts: Jones and Bartlett Publishers.

Hedrick, P.W. (2007). Balancing selection. *Curr. Biol.* **17**, 230–231.

Hensen, I. & Wesche, K. (2006). Relationships between population size, genetic diversity and fitness components in the rare plant *Dictamnus albus* in Central Germany. *Biodivers. Conserv.* **15**, 1572–9710.

Hu, S.R. (2005). Occurrences of Gansu zokor and the control in Yan'an region. *Shanxi. For. Sci.* **3**, 24–25.

Huang, H.J., Xiang, J.W. & Liu, X.M. (2007). The direct execution of principal component regression – using second time exploitation of SPSS. *J. Gansu Sci.* **19**, 107–109.

IUCN (2006). Red list of threatened species. www.iucnredlist.org

Jiang, T.A., Wang, S.Q. & Xue, Z.D. (2005). Correlation between vegetation coverage and zokor population quantity. *Bull. Soil Water Conserv.* **25**, 24–27.

Karanth, K.P., Avivi, A., Beharav, A. & Nevo, E. (2004). Microsatellite diversity in populations of blind subterranean mole rats (*Spalax ehrenbergi* superspecies) in Israel: speciation and adaptation. *Bot. J. Linn. Soc.* **83**, 229–241.

Knaepkensa, G., Bervoetsb, L., Verheyenc, E. & Eensa, M. (2004). Relationship between population size and genetic diversity in endangered populations of the European bullhead (*Cottus gobio*): implications for conservation. *Biol. Conserv.* **115**, 403–410.

Lacey, E.A., Patton, J. & Cameron, G. (2000). *Life underground: the biology of subterranean rodents*. Chicago: University of Chicago Press.

Massy, W.F. (1965). Principal components regression in exploratory statistical research. *J. Am. Stat. Assoc.* **60**, 234–256.

McGarigal, K., Cushman, S.A., Neel, M.C. & Ene, E. (2002). *FRAGSTATS: spatial pattern analysis program for categorical maps*. Amherst: University of Massachusetts.

Meffe, G.K. & Carroll, C.R. (1997). *Principles of conservation biology*, 2nd edn. Sunderland: Sinauer Associates.

Neumann, K., Jansman, H., Kayser, A., Maak, S. & Gattermann, R. (2004). Multiple bottlenecks in threatened western European populations of the common hamster *Cricetus cricetus* (L.). *Conserv. Genet.* **5**, 181–193.

Neville, H.M., Dunham, J.B. & Peacock, M.M. (2006). Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. *Landscape Ecol.* **21**, 901–916.

Part, T. & Soderstrom, B. (1999). Conservation value of semi-natural pastures in Sweden: contrasting botanical and avian measures. *Conserv. Biol.* **13**, 755–765.

Powell, J.R. (1971). Genetic polymorphisms in varied environments. *Science* **174**, 1035–1036.

Reichman, O.J. & Seabloom, E.W. (2002). The role of pocket gophers as subterranean ecosystem engineers. *Trends Ecol. Evol.* **17**, 44–49.

Reyes, A., Nevo, E. & Saccone, C. (2003). DNA sequence variation in the mitochondrial control region of subterranean mole rats, *Spalax ehrenbergi* superspecies in Israel. *Mol. Biol. Evol.* **20**, 622–632.

Sambrook, J. & Russell, D. (2001). *Molecular cloning: a laboratory manual*, 3rd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Seymour, R.S., Withers, P.C. & Weathers, W.W. (1998). Energetics of burrowing, running, and free-living in the Namib Desert golden mole (*Eremitalpa namibensis*). *J. Zool. (Lond.)* **244**, 107–117.

Sherman, P.W. (2001). Bringing subterranean rodents to light. *Ecology* **82**, 1998–1999.

Smallwood, K.S., Geng, S. & Zhang, M.H. (2001). Comparing pocket gopher (*Thomomys bottae*) density in alfalfa stands to assess management and conservation goals in northern California. *Agr. Ecosyst. Environ.* **87**, 93–109.

Sullivan, T.P., Sullivan, D.S. & Hogue, E.J. (2001). Reinvasion dynamics of northern pocket gopher (*Thomomys talpoides*) populations in removal areas. *Crop. Prot.* **20**, 189–198.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* **25**, 4876–4882.

Vergeer, P., Rengelink, R., Copal, A. & Ouborg, N.J. (2003). The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *J. Ecol.* **91**, 18–26.

Wang, D.M., Zhou, X.C., He, K.N., Li, S.R., Shi, C.Q. & Chang, G.L. (2005). The potential productivity of the main species used in the Datong reafforestation project on former farmland, Qinghai Province. *Acta Ecol. Sin.* **12**, 2984–2990.

Wei, W.H., Wang, Q.Y., Zhou, W.Y. & Fan, N.C. (1997). The population dynamics and dispersal of plateau zokors after removing. *Acta Their. Sin.* **17**, 53–61.

Werth, S., Wagner, H.H., Holderegger, R., Kalwij, J.M. & Scheidegger, C. (2006). Effect of disturbances on the genetic diversity of an old-forest associated lichen. *Mol. Ecol.* **15**, 911–921.

Ye, P.L. & Shu, N.P. (2006). Effect and measure of reafforestation of farmland project in haidong region, Qinghai Province. *Pratacult. Sci.* **23**, 24–27.

Zhang, Y.M., Zhang, Z.B. & Liu, J.K. (2003). Burrowing rodents as ecosystem engineers: the ecology and management of plateau zokors *Myospalax fontanierii* in alpine meadow ecosystems on the Tibetan Plateau. *Mammal Rev.* **33**, 284–294.