

基于线粒体控制区的序列变异分析青海东部甘肃鼯鼠遗传多样性

蔡振媛^① 张 毓^② 都玉蓉^③ 苏建平^① 张同作^{①*}

① 中国科学院西北高原生物研究所 中国科学院高原生物适应与进化重点实验室 西宁 810001; ② 青海省林业厅 西宁 810008; ③ 青海师范大学生命与地理科学学院 西宁 810008

摘要: 甘肃鼯鼠 (*Myospalax cansus*) 是一种终年营地下独居生活的小型掘土类动物。本文通过测定 mtDNA 的控制区部分序列 (530 bp) 变异, 分析青海东部地区 8 个甘肃鼯鼠地理种群遗传多样性与遗传结构。158 个样本共发现 26 个变异位点, 定义了 39 种单倍型, 整体的平均单倍型多样性高 ($h = 0.9532$)、核苷酸多样性低 ($\pi = 0.00636$)。歧点分布和中性检验均说明青海东部甘肃鼯鼠种群在历史上存在着快速扩张的事件。基于邻接法构建的网络关系图中, 单倍型呈星状分布, 没有按地理位置形成对应类群。基因流 (N_m) 数据显示多数地理种群间基因交流贫乏, AMOVA 结果显示种群内与种群间遗传变异分别为 48.82% 和 51.18%, 遗传分化明显。IBD 分析表明, 甘肃鼯鼠的遗传分化与地理距离呈正相关, 说明距离隔离对甘肃鼯鼠种群分化具有重要作用。甘肃鼯鼠的这种遗传多样性与种群遗传结构特点, 可能是地下生活方式靠挖掘迁移带来的较小扩散能力的结果。

关键词: 甘肃鼯鼠; 线粒体控制区; 遗传多样性; 种群遗传结构

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Genetic Diversity and Population Structure of Gansu Zokors (*Myospalax cansus*) in Eastern Qinghai Inferred from Mitochondrial D-loop Sequences

CAI Zhen-Yuan^① ZHANG Yu^② DU Yu-Rong^③ SU Jian-Ping^① ZHANG Tong-Zuo^{①*}

① Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001; ② Qinghai Forestry Bureau, Xining 810008; ③ School of Life and Geography Sciences, Qinghai Normal University, Xining 810008, China

Abstract: Gansu zokors (*Myospalax cansus*) are small, solitary, subterranean rodents that inhabit the Loess Plateau in China. The genetic diversity and population genetic structure of *M. cansus* were determined by

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* 通讯作者, E-mail: zhangtz@nwipb.cas.cn;

第一作者介绍 蔡振媛, 女, 工程师; 研究方向: 动物学; E-mail: caizhenyuan@nwipb.cas.cn.

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analyzing the sequence variation of a 530 bp fragment of the mitochondrial D-loop region in 158 natural individuals from eight locations in eastern Qinghai (Fig. 1, Table 1). Total DNA was extracted following the Joe and David method from 0.3 g of ethanol-fixed tissue. The D-loop sequence was amplified using primers FR (5'-TACCATCCTCCGTGAAACCA-3') and RV (5'-CTAATAATAAGGCCAGGACC-3'), and PCR was performed in a 50 μ l reaction volume. PCR products were purified and directly sequenced in both strands of the DNA using forward and reverse primers for amplification on a MegaBACE 1000 DNA Analysis System. Sequences were recorded on both strands with an overlap of 70%. The sequences were checked and aligned using Clustal X with default settings and refined manually. Genetic diversity was estimated with Arlequin 3.10, using two different diversity indices: Haplotype diversity (h) and Nucleotide diversity (π). To estimate the extent of genetic differentiation among populations, pairwise genetic distances (F_{ST}) were calculated using Arlequin 3.10, and their significance was estimated by performing 10 000 permutations among individuals and populations. The same program was also used to calculate the gene flow (N_m), which is based on F_{ST} estimates, equivalent to the effective number of migrants between populations per generation. Analysis of molecular variance (AMOVA) was carried out to examine the significance of population structure. The phylogeographical pattern was examined by constructing haplotype networks using the median-joining network approach performed in Network 4.6.1.1. The hypothesis of neutral evolution was tested by Tajima's D function test and Fu's F_s -test with 10 000 permutations using Arlequin 3.10. Mismatch distributions of pairwise substitutional differences among haplotypes were also examined using Arlequin 3.10. The IBD 1.52 algorithm was used to test the correlation between genetic distance and geographical distance. The genetic distances were expressed by F_{ST} among populations, excluding the PA1 population which showed high genetic departure. The geographical distances (km) were estimated as straight-line distances between any pair of locations. The correlation between N_m and geographical distance was also estimated. Overall, 26 polymorphic sites were identified and 39 haplotypes were defined (Table 1 and 2). The number of haplotypes (N_h) and polymorphic sites (N_p) were also shown in Table 1. Haplotype diversity varied from 0.257 1 to 0.874 5, while nucleotide diversity varied from 0.000 82 to 0.005 20 (Table 1). Genetic diversity estimates revealed extensive haplotype diversity (0.953 2) and limited nucleotide diversities (0.006 36) in all populations (Table 1). Interestingly, the mismatch distribution analysis showed a unimodal pattern, reflecting a sudden population expansion (Fig. 3). A significantly large negative value of Fu's F_s was found when the total haplotypes were analyzed ($F_s = -22.10$, $P < 0.01$), which indicated a recent population expansion, as suggested by the mismatch distribution analysis. The estimated time of population expansion was 0.19 - 0.077 Mya, mostly corresponding to the interglacial period (0.17 - 0.021 Mya) before the last glacial maximum (LGM). The median-joining network was star-like throughout the studied range of *M. cansus*, showing that most individuals from different populations were highly interconnected with each other and they did not exhibit reciprocal monophyly (Fig. 2). All populations' pairwise F_{ST} values were statistically significant ($P < 0.01$), ranging from 0.249 12 (HZ1-LD2) to 0.775 70 (DT1-PA1) (Table 3), indicating that all populations were significantly differentiated from one another. The values of N_m based on F_{ST} estimates in Table 4 showed that the levels of gene flow were relatively low. Among 28 values, only three were greater than 1 and the smallest was only 0.144 58. It was consistent with the significant population differentiation. The differentiation was confirmed by the percentage of variation among populations and within populations

in AMOVA analysis, which were 51.18% and 48.82%, respectively (Table 5). The Mantel tests, excluding the PA1 population, revealed a significant negative correlation between genetic flow (N_m) and geographical distance ($r = -0.598$, $P = 0.001$, Fig. 3a), and a significant relationship between the genetic distance (F_{ST}) and geographical distance ($r = 0.608$, $P < 0.05$, Fig. 3b) among populations, suggesting that distance isolation played a remarkable role in genetic differentiation. The data suggest that the weak dispersal ability of subterranean animals has shaped the peculiar population genetic diversity and genetic structure of Gansu zokors.

Key words: Gansu zokors (*Myospalax cansus*); Mitochondria D-loop region; Genetic diversity; Population genetic structure

遗传多样性是生命进化和物种进化的基础 (施立明等 1993), 反映了一定空间范围内局部种群基因平均组成和丰富度水平 (Hedrick 2000), 决定一个物种进化潜力和抵御不良环境能力的主要因素。遗传组成、种群动态、基因流、遗传分化和遗传结构等是种群遗传多样性研究的重要内容, 这些研究对我们更好地了解物种的形成、进化机制、适应分化和扩散规律等具有重要意义 (Eriksson et al. 2004, Zhang et al. 2005, Mora et al. 2006, Meng et al. 2007, Ci et al. 2009, Du et al. 2010, Tang et al. 2010, 徐秀丽等 2013)。

甘肃鼯鼠 (*Myospalax cansus*) 隶属于啮齿目 (Rodentia) 鼯形鼠科 (Spalacidae) 鼯鼠亚科 (Myospalacinae) 鼯鼠属 (Norris et al. 2004), 主要分布于陕西、甘肃、宁夏南部、青海东部等黄土高原、森林草原地带, 是典型的地下掘土类动物, 其觅食、繁殖等绝大部分行为都在地下进行 (樊乃昌等 1982, 曹志东等 1994), 常常伴随高耗能的挖掘活动, 过高的挖掘能量代价严重限制其长距离的迁移活动, 从而扩散率明显低于地上活动动物 (魏万红等 1997)。动物的高扩散能力不一定带来高水平的基因流 (Palumbi et al. 1997, Hoelzel 1998, Waples 1998), 但低迁移能力很可能使种群间基因流受限, 而限制性的基因流会产生高水平的种群分化以及明显的种群遗传结构模式 (Duffy 1993, Duran et al. 2004a, b)。可以预测, 甘肃鼯鼠作为典型的地下掘土类动物, 低扩散水平会对其

遗传多样性和种群遗传结构产生重要影响。

控制区是线粒体的主要非编码区, 包含 mtDNA 转录与复制的调控元件, 也称为 D-loop 区。该区基因所受进化压力较小, 其遗传变异相比编码序列更大, 是线粒体 DNA 分子进化速度最快的区域, 平均进化速率是线粒体其他基因的 10 倍 (Greenberg et al. 1983), 成为种群结构分析和基因流研究非常有效的遗传标记 (Stacy et al. 1997, Aars et al. 1998, Mora et al. 2007, Ren et al. 2013)。此外, 该标记也适用于小地理尺度的种群结构研究 (Hirota et al. 2004, 蔡振媛等 2007), 而且控制区的序列分析通常会产生很多单倍型, 从而能够反映种群动态历史 (李增超等 2005, 陈光照等 2011, 徐秀丽等 2013)。

目前, 有关地下啮齿动物种群遗传多样性的研究很少, 本文通过测定青海东部地区甘肃鼯鼠 mtDNA 的控制区 3'端 530 bp 序列变异, 分析甘肃鼯鼠地下生活方式下低扩散能力等因素对其种群遗传多样性的影响。

1 材料与方 法

1.1 样本采集

从青海东部的大通县小寺村 (DT1)、互助县花园村 (HZ1)、乐都县下北山林场 (LD1)、乐都县辛家庄村 (LD2)、民和县新建村 (MH1)、民和县隆治村 (MH2)、平安县沙沟村 (PA1)、平安县寺台村 (PA2) 8 个地点, 利用弓箭法分别采集甘肃鼯鼠 23 只、12 只、20 只、15 只、

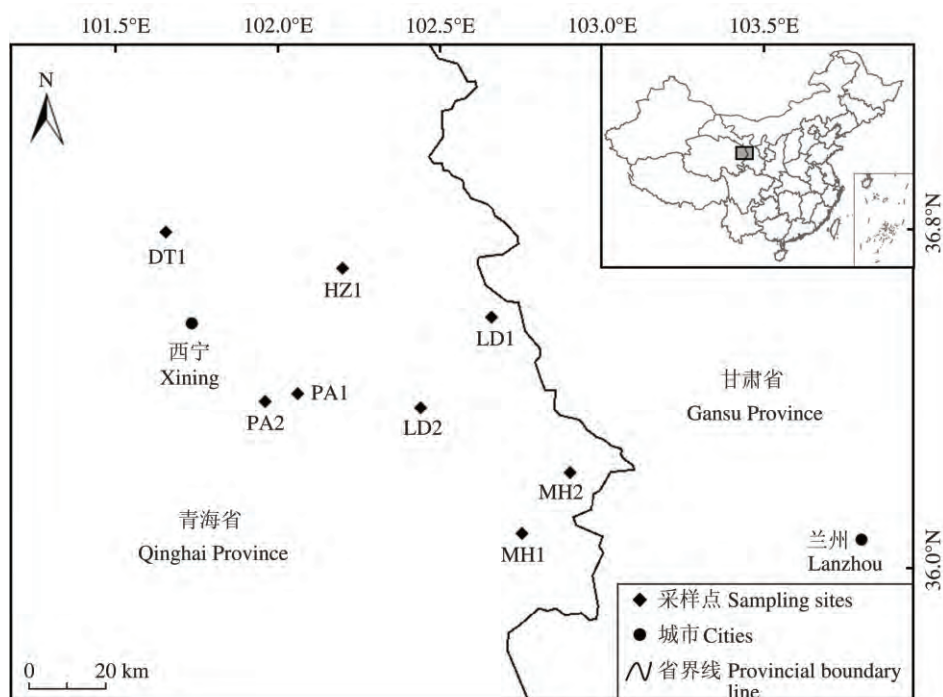


图 1 甘肃鼢鼠采样地点图

Fig.1 Map showing the locations of 8 populations of *Myospalax cansus* examined herein

DT1. 小寺村; HZ1. 花园村; LD1. 下北山林场; LD2. 辛家庄村; MH1. 新建村; MH2. 隆治村; PA1. 沙沟村; PA2. 寺台村。

DT1. Xiaosi village; HZ1. Huayuan village; LD1. Xiabeishan forest farm; LD2. Xinjiashuang village; MH1. Xinjian village; MH2. Longzhi village; PA1. Shagou village; PA2. Sitai village.

22 只、22 只、21 只、23 只，共计 158 只（图 1）。野外采集后立即分别取肝和肌肉组织各 2 g 左右，置于 95% 乙醇中固定保存（蔡振媛等 2006）。

1.2 总 DNA 提取、PCR 扩增与序列测定

参照 Sambrook 等（2001），用蛋白酶 K 和苯酚从哺乳动物细胞中分离高分子质量 DNA 的方法提取基因组总 DNA，部分步骤加以改进。

参照地下鼢鼠超种（Reyes et al. 2003）控制区的扩增引物、反应体系及条件进行扩增。引物序列为 FR（5'-TAC CAT CCT CCG TGA AAC CA-3'）和 RV（5'-CTA ATA ATA AGG CCA GGA CC-3'），在生工生物工程（上海）股份有限公司合成。扩增片段的长度为 650 bp 左右。反应体系 30 μ l，包括 40 ~ 60 ng 模板 DNA，10 mmol/L Tris-HCl（pH 8.3），2.5 mmol/L

MgCl₂，50 mmol/L KCl，0.5 mmol/L dNTPs，正反向引物各 0.5 μ mol/L，1 U *Taq* DNA 聚合酶。反应程序，95 $^{\circ}$ C 预变性 10 min；95 $^{\circ}$ C 变性 45 s，52 $^{\circ}$ C 复性 45 s，72 $^{\circ}$ C 延伸 90 s，30 个循环；72 $^{\circ}$ C 后延伸 7 min。扩增产物用 1.5% 的琼脂糖凝胶电泳检测。经检测扩增良好的产物用柱式 PCR 产物纯化试剂盒[生工生物工程（上海）股份有限公司]进行纯化。纯化后用 1.5% 的琼脂糖凝胶电泳检测，经检测纯化良好的产物用作进一步测序反应。

以纯化后的扩增产物为模板，单条扩增引物为引物，进行测序反应。反应体系为 10 μ l，包括 40 ng 左右模板，2 μ l ET，0.5 μ mol/L 引物。反应条件，95 $^{\circ}$ C 预变性 8 s；95 $^{\circ}$ C 变性 15 s，50 $^{\circ}$ C 复性 15 s，60 $^{\circ}$ C 延伸 90 s，循环 31 次；60 $^{\circ}$ C 续延伸 90 s。测序反应产物利用醋酸铵、乙醇纯化去除未参加反应的单核苷酸。纯化后产物

利用 Megabase 500 Automated sequencer 进行序列分析。利用正反引物进行双向测序, 片段重合大于 70%。

1.3 数据分析

利用软件 Chromas 和 Clustal X (Thompson et al. 1997) 对测序结果进行序列比对。比对后的序列通过 AWA 程序 (中国科学院西北高原生物研究所高原生物适应与进化重点实验室), 统计单倍型。采用 Arlequin 3.10 (Excoffier et al. 2005) 统计种群单倍型多样性 (h)、核苷酸多样性 (π), 测算种群间的基因流 N_m , 估算种群间遗传分化指数 F_{ST} (重复次数 10 000), 并进行 Tajima's D 、Fu's F_s 中性检验 (重复次数 10 000), 错配分布 (Mismatch) 分析 (自举值为 500), 及分子方差分析 (AMOVA)。利用公式 $\tau = 2\mu kT$ 进行扩张时间估算, τ 是错配分布的模型, 由 Arlequin 3.10 软件分析获得, μ 表示每个位点每代的突变速率, k 表示分析的片段长度, T 表示种群扩张时间 (Rogers et al. 1992)。突变速率采用文献报道的哺乳动物 mtDNA 的突变速率, 即每个位点每百万年发生 2% ~ 5% 的突变 (Wilson et al. 1985, Biju-Duval

et al. 1991, Irwin et al. 1991, Martin et al. 1993, Klicka et al. 1997, Avise et al. 1998, Niu et al. 2004, Tang et al. 2010)。利用 Network 4.6.1.1 (Bandelt et al. 1999) 中 median-joining 算法绘制单倍型之间的网络关系图。通过软件 IBD1.52 (Bohonak 2002) 检测地理隔离对甘肃麝鼠的影响程度, 用 F_{ST} 值表示遗传距离, 地理距离采用采样点间的球面距离, 同时也检测了基因流 N_m 与地理距离的相关性。由于平安县沙沟村 (PA1) 种群表现出很高的遗传偏差, 检测未包含 PA1 种群。

2 结果

2.1 遗传多样性

本研究测定了 8 个地理种群, 共 158 只甘肃麝鼠的线粒体 DNA625 ~ 626 bp 的序列, 包括 D-loop 区 3'端序列 (530 bp)、tRNA_{phe} (67 bp)、部分 12S rRNA 5'端序列 (29 bp)。仅对 D-loop 的 530 bp 序列进行后续分析。在 8 个地理种群 158 个样本中检测到 26 个变异位点, 定义了 39 种单倍型 (表 1, 2)。分别命名为 H1 ~

表1 甘肃麝鼠采样点信息以及遗传多样性指数

Table 1 Locations and diversity measures for the populations of *Myospalax cansus* studied

采样地 Collection sites	地理坐标 Location	种群代码 Population code	样本数 Sample size (n)	单倍型数 (N_i) Number of haplotypes	多态位点数 Number of polymorphic sites (N_p)	单倍型多样性 (h) Haplotype diversity	核苷酸多样性 (π) Nucleotide diversities
小寺村 Xiaosi village	36.79374°N, 101.67355°E	DT1	23	8	11	0.525 7 ± 0.126 2	0.003 09 ± 0.002 11
花园村 Huayuan village	36.70393°N, 102.19648°E	HZ1	12	5	7	0.818 2 ± 0.070 3	0.005 15 ± 0.003 28
下北山林场 Xiabeishan forest farm	36.58183°N, 102.63758°E	LD1	20	9	10	0.852 6 ± 0.053 2	0.004 61 ± 0.002 91
辛家庄村 Xinjiazhuang village	36.35852°N, 102.42797°E	LD2	15	4	10	0.466 7 ± 0.147 8	0.003 77 ± 0.002 52
新建村 Xinjian village	36.04847°N, 102.72783°E	MH1	22	8	9	0.874 5 ± 0.034 6	0.005 23 ± 0.003 21
隆治村 Longzhi village	36.19891°N, 102.87027°E	MH2	22	3	4	0.675 3 ± 0.049 1	0.005 10 ± 0.003 28
沙沟村 Shagou village	36.39377°N, 102.06366°E	PA1	21	2	2	0.257 1 ± 0.110 4	0.000 97 ± 0.000 95
寺台村 Sitai village	36.37430°N, 101.96724°E	PA2	23	5	7	0.703 6 ± 0.058 3	0.002 38 ± 0.001 74
合计 Total			158	39	26	0.953 2 ± 0.005 8	0.006 36 ± 0.003 63

H39, GenBank 号为 GU936827 ~ GU936865。核苷酸多样性为 0.000 82 ~ 0.005 20, 单倍型多样性为 0.257 1 ~ 0.874 5, 整体的核苷酸多样性较低, 多数种群的单倍型多样性较高, 只有平安县沙沟村 (PA1) 种群表现出较低的单倍型多样性 (0.257 0)。

2.2 种群分化和遗传结构

甘肃麝鼠 8 个地理种群间遗传分化指数 F_{ST} 从 0.249 12 (HZ1-LD2) 到 0.775 70 (DT1-PA1) (表 3), 统计上都是差异极显著

($P < 0.001$), 表明种群间分化明显。根据遗传分化指数 F_{ST} 计算出种群间的基因流 N_m 为 0.144 58 ~ 1.507 19 (表 4), 28 个值中只有 3 个大于 1, 且多数值较低, 说明种群间基因流水平很低, 这与种群间存在显著的遗传分化结果一致。甘肃麝鼠 8 个地理种群进行分子变异分析 (AMOVA), 51.18% 的遗传变异存在于种群间 (表 5), 也表明青海东部甘肃麝鼠地理种群间分化明显。

表3 甘肃麝鼠8个地理种群间遗传分化指数 (F_{ST}) (下三角) 与地理距离 (km) (上三角)
Table 3 The measure of genetic differentiation (F_{ST}) (below diagonal) and geographical distances (km) (above diagonal) between populations of *Myospalax cansus*

	DT1	HZ1	LD1	LD2	MH1	MH2	PA1	PA2
DT1		48.7	89.4	83.1	126.3	126.4	56.7	54.3
HZ1	0.517 40*		42.2	43.5	87.9	83.1	36.9	42.6
LD1	0.555 00*	0.399 29*		30.8	60.7	47.2	55.6	64.3
LD2	0.461 19*	0.249 12*	0.252 72*		44.4	44.1	33.0	41.4
MH1	0.521 58*	0.337 02*	0.339 83*	0.339 74*		21.2	71.1	77.7
MH2	0.615 02*	0.462 91*	0.425 11*	0.348 40*	0.384 21*		75.9	83.5
PA1	0.775 70*	0.688 54*	0.506 49*	0.661 11*	0.612 91*	0.720 31*		9.10
PA2	0.622 26*	0.423 37*	0.374 89*	0.282 83*	0.538 29*	0.519 48*	0.709 09*	

DT1. 小寺村; HZ1. 花园村; LD1. 下北山林场; LD2. 辛家庄村; MH1. 新建村; MH2. 隆治村; PA1. 沙沟村; PA2. 寺台村。

* 示甘肃麝鼠种群间的遗传分化指数 (F_{ST}) 差异极显著 ($P < 0.001$)。

DT1. Xiaosi village; HZ1. Huayuan village; LD1. Xiabeishan forest farm; LD2. Xinjiazhuang village; MH1. Xinjian village; MH2. Longzhi village; PA1. Shagou village; PA2. Sitai village.

* Means great significant difference of F_{ST} in eight populations of *Myospalax cansus* ($P < 0.001$).

表4 甘肃麝鼠8个地理种群间基因流 (N_m)
Table 4 Matrix of population pairwise migration rates (N_m) values between populations of *Myospalax cansus*

	DT1	HZ1	LD1	LD2	MH1	MH2	PA1
HZ1	0.466 37						
LD1	0.400 90	0.772 24					
LD2	0.584 14	1.507 19	1.478 48				
MH1	0.458 63	0.983 59	0.971 33	0.971 73			
MH2	0.312 98	0.580 12	0.676 18	0.935 13	0.801 38		
PA1	0.144 58	0.226 17	0.487 19	0.256 30	0.315 77	0.194 14	
PA2	0.303 53	0.681 01	0.833 71	1.267 82	0.428 87	0.462 51	0.205 13

DT1. 小寺村; HZ1. 花园村; LD1. 下北山林场; LD2. 辛家庄村; MH1. 新建村; MH2. 隆治村; PA1. 沙沟村; PA2. 寺台村。

DT1. Xiaosi village; HZ1. Huayuan village; LD1. Xiabeishan forest farm; LD2. Xinjiazhuang village; MH1. Xinjian village; MH2. Longzhi village; PA1. Shagou village; PA2. Sitai village. $N_m = (1 - F_{ST}) / (2 F_{ST})$.

表5 甘肃麝鼠线粒体D-loop序列分子变异分析 (AMOVA) 结果

Table 5 Analysis of molecular variance (AMOVA) for the D-loop sequences of *Myospalax cansus*

变异来源 Source of variation	自由度 Degrees of freedom	平方和 Sum of squares	方差组分 Variance components	方差比例 Percentage of variation
种群间变异 Among populations	7	133.241	0.924 03	51.18
种群内变异 Within populations	150	132.187	0.881 25	48.82
总计 Total	157	265.428	1.805 28	100.00
固定指数 Fixation indices		$F_{ST} = 0.511 85 (P < 0.001)$		

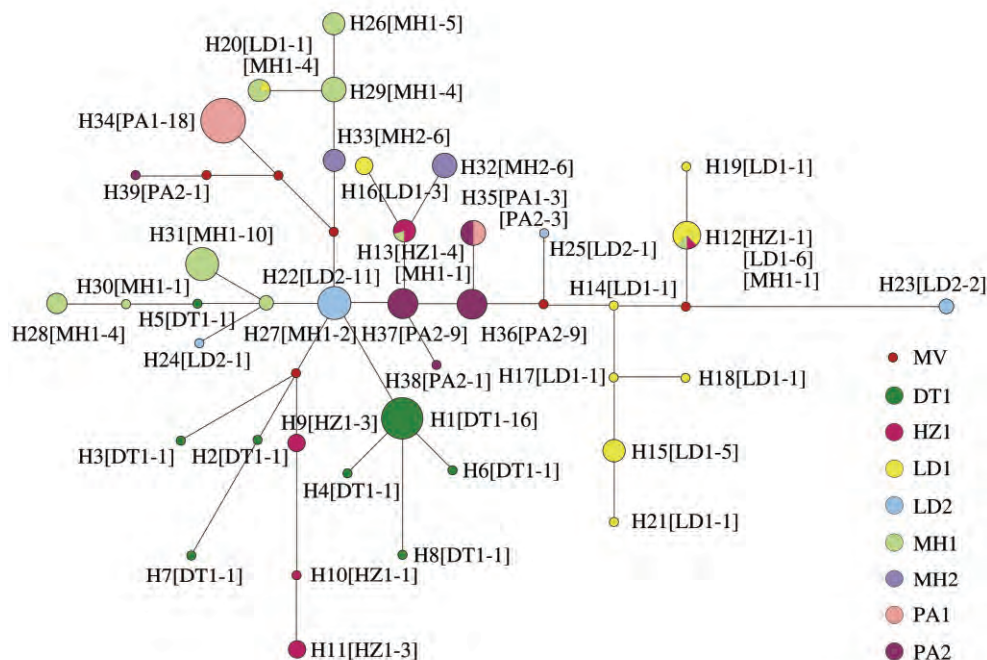


图2 8个甘肃麝鼠种群D-loop序列39个单倍型间的网络亲缘关系图

Fig. 2 Median-joining network showing genetic relationship among 39 mtDNA D-loop haplotypes in eight *Myospalax cansus* populations

DT1. 小寺村; HZ1. 花园村; LD1. 下北山林场; LD2. 辛家庄村; MH1. 新建村; MH2. 隆治村; PA1. 沙沟村; PA2. 寺台村。

DT1. Xiaosi village; HZ1. Huayuan village; LD1. Xiabeishan forest farm; LD2. Xinjiazhuang village; MH1. Xinjian village; MH2. Longzhi village; PA1. Shagou village; PA2. Sitai village.

每个圆圈代表一种单倍型, 圆圈大小与单倍型频率呈正比, 图中H1 ~ H39表示单倍型1 ~ 39, 圆圈旁标注了该单倍型分布的种群及频率, 例如, H1[DT1-16], 表示单倍型1在种群DT1中出现16次。红色圆点 (MV) 代表缺失单倍型, 最短线代表一步突变。

A cycle means a haplotype, the relative sizes of the circles in the network are approximately proportional to the frequencies of each haplotype. H1 - H39 means haplotype 1 to haplotype 39. The characters and numbers beside the circles represent the population and frequency of haplotype distributed. For example, H1[DT1-16] means haplotype 1 occurs 16 times in population DT1. The red dots (MV) represent missing haplotypes (not sampled or extinct). The shortest lines represent one mutational step.

基于39个单倍型构建的median-joining网络关系图(图2)中, 并没有按照各自的地理

种群聚合而形成明显的单系群, 而是各地理种群彼此交织在一起, 呈星状分布, 相邻单倍型

间突变在 4 步以内。

2.3 种群历史

对所扩增的青海东部 158 只甘肃麝鼠个体线粒体部分序列进行错配分布分析以及 Tajima's D 、Fu's F_s 中性检验, 来调查其种群历史动态。青海东部甘肃麝鼠单倍型和碱基差异呈单峰分布 (图 3), 预示其经历过种群扩张。错配分布分析 $\tau = 4.105$ ($P < 0.001$), 通过估算, 种群扩张发生在距今约 0.190 ~ 0.077 百万年前。Fu's F_s 值为 -22.10 ($P < 0.001$), 显示该地区甘肃麝鼠近期经过种群扩张事件, 与错配分布结果一致, 而 Tajima's D 值为 -0.78 ($P = 0.23$)。

2.4 IBD 检验

通过软件 IBD1.52 进行检测, 青海东部甘肃麝鼠 7 个地理种群 (DT1、HZ1、LD1、LD2、MH1、MH2、PA2) 间的基因流与地理距离呈显著的负相关 ($r = -0.598$, $P = 0.001$, 图 4a), 而地理距离与遗传距离呈显著的正相关 ($r = 0.608$, $P < 0.05$, 图 4b)。

3 讨论

3.1 遗传多样性与种群动态

一般情况, 数量巨大而稳定的种群能够长

期维持高水平的遗传变异, 而经历严重瓶颈效应的种群, 会因遗传漂变导致大量遗传变异的丢失。本研究中, 青海东部地下啮齿动物甘肃麝鼠的线粒体 D-loop 区呈现出单倍型多样性高、核苷酸多样性低的特点, 整体单倍型多样性高达 0.953 2, 核苷酸多样性仅有 0.006 36。与其他地理种群不同, 沙沟村 PA1 种群二者都很低 ($h = 0.257 1$, $\pi = 0.000 97$), 推测该地理种群近期可能经历了瓶颈事件, 经向当地林业部门咨询, 该采样点 2000 ~ 2001 年进行了连续高强度的灭鼠行动。

种群这种高水平的单倍型多样性和低水平的核苷酸多样性的特点经常归因于种群扩张, 小的有效种群迅速增长, 经一段时间保留了新的突变 (Avise et al. 1984, Watterson 1984, Nicolas et al. 2008, Sun et al. 2012)。种群动态 (增长、衰减、稳定) 的遗传过程可以通过错配分布模式等多种方法进行分析 (Tajima 1989, Rogers et al. 1992, Tajima 1996, Fu 1997, Ramos-Onsins et al. 2002)。错配分布呈现多峰型, 反映出基因树形状是高度随机的, 表明此种群动态是稳定的、平衡的; 错配分布呈现单峰型, 表明此种群近期曾经发生过种群扩张

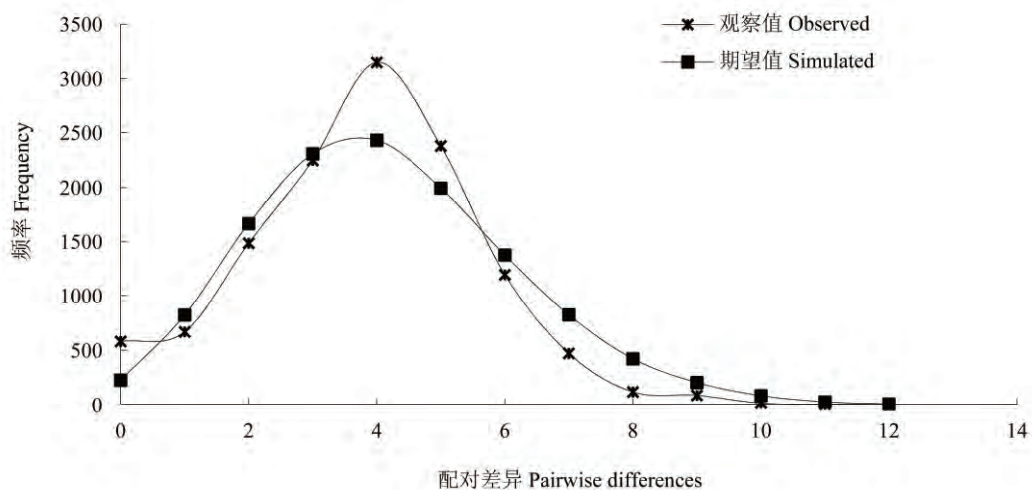


图3 基于D-loop部分序列 (530 bp) 的青海东部甘肃麝鼠整体种群歧点分布

Fig. 3 Mismatch distributions of the 530 bp D-loop sequences of the total *Myospalax cansus* populations in eastern Qinghai

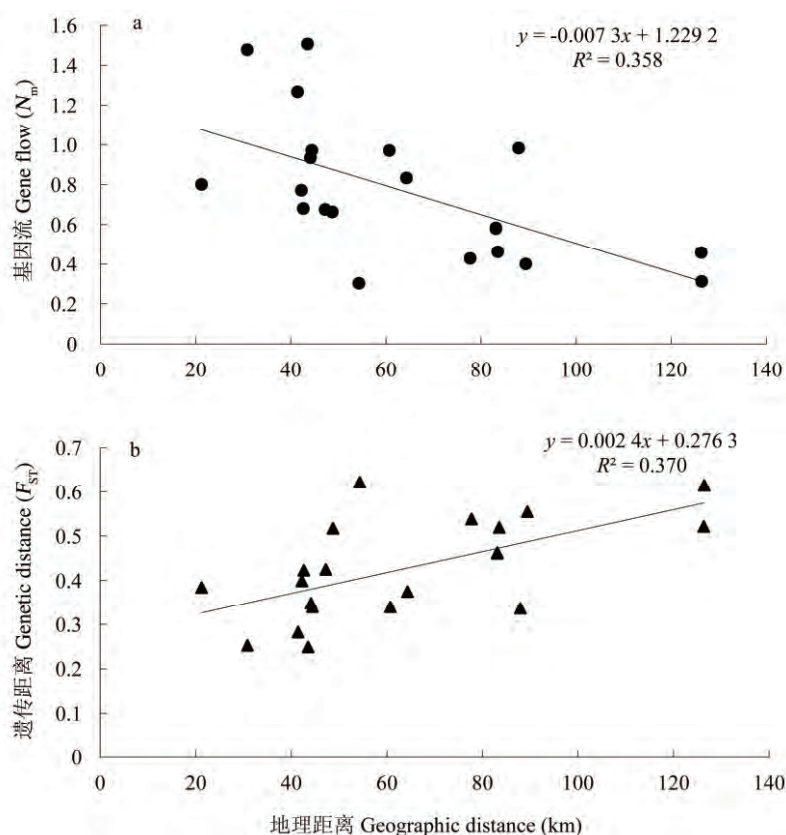


图4 甘肃麝鼠7个地理种群IBD检测结果

Fig. 4 Isolation by distance based on seven populations of *Myospalax cansus*

a. 基因流 (N_m) 与地理距离的相关性; b. 遗传距离 (F_{ST}) 与地理距离的相关性。

The measure of genetic distance (F_{ST}) and gene flow (N_m) are plotted against the geographic distance respectively between populations, PAI excluding for the high genetic departure. N_m was used in a, and F_{ST} was used in b.

(Rogers et al. 1992) 或邻近种群间伴随高水平的迁移发生分布区扩张 (Ray et al. 2003, Excoffier 2004)。Fu (1997) 发现 F_s 检验对种群扩张非常敏感, 种群扩张 F_s 会得到大的负值。与之相似, 种群扩张能使 D 值显著地负向偏离零。比较而言, Tajima's D 检验倾向估测古老突变, 能够揭示古老种群事件, 而 Fu's F_s 检验对近期的种群事件更敏感。本研究中, 青海东部甘肃麝鼠种群 F_s 值为 -22.10 ($P < 0.001$), D 值为 -0.70 ($P = 0.28$), F_s 值比 D 值更显著的偏离零, 表明青海东部甘肃麝鼠种群近期经历了种群扩张, 这与错配分布单峰型的结果一致。研究范围内的甘肃麝鼠 D-loop 区

单倍型 median-joining 网络图呈现星状结构, 这种星状拓扑树常常也是近期发生种群扩张的信号 (Slatkin et al. 1991, Kim et al. 2012, Sun et al. 2012, Ren et al. 2013)。通过估算, 种群扩张发生在大约 0.190 ~ 0.077 百万年前, 这与末次冰期前的间冰期 (0.170 ~ 0.021 百万年) 时间一致。间冰期较温暖的气候使冰盖退缩, 为甘肃麝鼠种群快速增长提供机遇, 种群扩张快速导致少数碱基位点的快速进化, 短时间内的变异积累提高了单倍型多样性, 而核苷酸多样性的提高需要更长时间的积累。

3.2 低扩散能力与种群遗传结构

基因分化和基因流是评估物种种群遗传结

构的重要指标。根据 Wright (1949) 关于遗传分化指数的大小和分化程度的解释, F_{ST} 值 0 ~ 0.05 表示低度遗传分化, F_{ST} 值 0.05 ~ 0.15 表示中度遗传分化, F_{ST} 值 0.15 ~ 0.25 说明遗传分化比较大。基于线粒体 D-loop 区序列的 AMOVA 分析结果显示, 青海东部甘肃鼯鼠地理种群间 F_{ST} 值为 0.511 85, 各地理种群间遗传分化指数 F_{ST} 值为 0.249 12 ~ 0.775 70, 表明青海东部甘肃鼯鼠各地理种群间存在明显的遗传分化, 说明这些地理种群间基因流贫乏, 这与基因流 N_m 结果一致。 $N_m < 1$, 表明群体间可能由于遗传漂变而发生分化, $N_m > 1$, 表明群体间的基因流水平较高, 群体间遗传分化较小。 取样地理种群间基因流 N_m 为 0.145 80 ~ 1.507 19, 28 个组合大多数 $N_m < 1$, 只有 3 个组合 (HZ1-LD2、LD1-LD2、LD2-PA2) $N_m > 1$, 表明青海东部多数甘肃鼯鼠地理种群间基因交流很弱, 产生明显遗传分化。

IBD 检测显示地理种群间基因流与地理距离显著负相关, 遗传分化指数 F_{ST} 与地理距离显著正相关, 这表明种群间地理隔离对基因流和遗传分化起重要作用, 研究地区甘肃鼯鼠种群间遗传分化中大约 37.0% 的变异可以由地理隔离解释, 这可能与甘肃鼯鼠的低迁移扩散能力有关。关于地下啮齿动物迁移扩散距离的数据很少, 一般认为地下啮齿动物的迁移扩散能力非常有限 (魏万红等 1997)。与其他地下动物相似, 地下高耗能的挖掘活动导致的非常有限的扩散能力也是甘肃鼯鼠重要的特点之一。限制性的扩散能力通常伴随着低水平的基因流, 基因流水平低的物种, 单倍型谱系分析往往会得到明显的地理分支 (Hunt 1993, Duran et al. 2004a, b, Eriksson et al. 2004)。

甘肃鼯鼠和高原鼯鼠 (*M. baileyi*) 是近缘物种 (Norris et al. 2004), 都是典型的地下啮齿动物。高原鼯鼠基于线粒体不同基因的系统发育分析都得到稳定的分支, 分别与采集的地理种群高度吻合, 种群间遗传变异占比大于 80%。 (蔡振媛等 2007, Tang et al. 2010)。本研究也

期望观察到甘肃鼯鼠地理种群间遗传差异远远大于地理种群内遗传差异, 但是 AMOVA 分析结果显示, 本研究区域内, 尽管遗传分化明显, 但甘肃鼯鼠遗传变异占比在地理种群内外相差不大。同时, median-joining 网络图并没有呈现出明显的地理分布格局。蔡振媛等 (2007) 的研究中发现高原鼯鼠种群间遗传分化中大约 79.6% 的变异可以由地理隔离解释, 地理隔离对高原鼯鼠的作用比对甘肃鼯鼠的作用更明显。这可能与高原鼯鼠生活于高寒地区 (年均气温 1.2°C), 冻土期长, 食物条件差, 迁移扩散能力受到更大的制约有关。此外, 本研究的区域范围较小, 故没能观察到甘肃鼯鼠明显的谱系地理结构。下一步应扩大甘肃鼯鼠采样范围, 更深入地开展研究, 从而获得更全面的遗传多样性信息。

青海东部甘肃鼯鼠的高单倍型多样性与低核苷酸多样性的遗传多样性特点提示其在末次冰期前的间冰期发生了种群扩张。而该地区甘肃鼯鼠种群间受限的基因流及高遗传分化水平的遗传结构特点, 则是地下生活方式挖掘迁移带来的较小的扩散能力作用的结果。然而, 由于本研究的区域仅占甘肃鼯鼠的分布范围相对狭窄的一部分, 全面了解甘肃鼯鼠的遗传多样性、种群动态及其影响因素, 尚需要扩大甘肃鼯鼠采样范围, 加入核基因等标记的分析, 更深入地开展研究。

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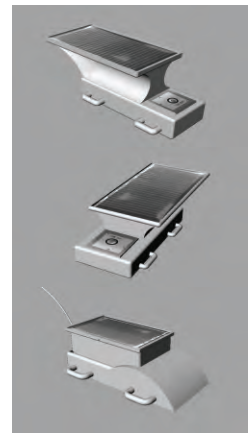
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邮箱: sales@blueoceanix.com

http://www.blueoceanix.com

