Genetic polymorphism in populations of voles and shrews from the Kronotsky Reserve (Kamchatka Peninsula, Russia)

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Abstract
Genetic polymorphism in populations of voles and shrews from the Kronotsky Reserve (Kamchatka Peninsula, Russia). We studied genetic polymorphism of four mammal species *Myodes rutilus*, *Myodes rufocanus*, *Sorex isodon*, *Sorex caecutiens* from four localities, the Valley of Geysers, Uzon volcanic caldera, the Death Valley, and the Shore of Kuril Lake. In total, 172 individuals were genotyped using the inter–simple sequence repeat technique. We observed the lowest polymorphism in shrews *S. caecutiens*. In this species, 68.8% of bands were polymorphic, and Nei’s genetic diversity (*h*) was 0.27, while these values in *S. isodon* were 81.3% and 0.29, respectively. Populations of *M. rufocanus* were the most polymorphic among the studied species (*P* = 91.4, *h* = 0.34). Polymorphism in *M. rutilus* from Kamchatka (*P* = 87.2, *h* = 0.29) was similar to that from Western Siberia. In addition, we found a high genetic differentiation of rodent populations. The interpopulation component of genetic variability was about 30–40% (*Gst* = 0.31 in *M. rutilus* and 0.39 in *M. rufocanus*). Gene flow among populations of *M. rutilus* from Kamchatka was two times lower than that of populations of the species from taiga ecosystems in Siberia.

Key words: *Myodes*, *Sorex*, Differentiation of populations, Genetic variability, ISSR markers, Kamchatka Peninsula

Resumen
Polimorfismo genético en poblaciones de topillos y musarañas de la Reserva de Kronotski (península de Kamchatka, Rusia). Estudiamos el polimorfismo genético de cuatro especies de mamíferos *Myodes rutilus*, *Myodes rufocanus*, *Sorex isodon* y *Sorex caecutiens* de cuatro localidades: el valle de los Géiseres, la caldera volcánica Uzon, el valle de la Muerte y las orillas del lago Kuril. Genotipamos un total de 172 ejemplares utilizando la técnica de intersecuencias simples repetidas. Observamos el polimorfismo más bajo en la musaraña *S. caecutiens*. En esta especie, el 68,8% de las bandas resultaron polimórficas y la diversidad genética de Nei (*h*) fue de 0,27, mientras que en *S. isodon* estos valores fueron de 81,3% y 0,29, respectivamente. Las poblaciones de *M. rufocanus* fueron las más polimórficas entre las especies estudiadas (*P* = 91,4; *h* = 0,34). El polimorfismo de *M. rutilus* de Kamchatka (*P* = 87,2; *h* = 0,29) fue similar al de Siberia Occidental. Además, descubrimos una elevada...
diferenciación genética entre poblaciones de roedores. El componente interpoblacional de variabilidad genética fue del 30–40% ($G_{ST} = 0,31$ en *M. rutilus* y 0,39 en *M. rufocanus*). El flujo genético entre las poblaciones de *M. rutilus* de Kamchatka fue dos veces más bajo que entre las poblaciones de especies de los ecosistemas de la taiga siberiana.

Palabras clave: *Myodes*, *Sorex*, Diferenciación de poblaciones, Variabilidad genética, Marcadores ISSR, Península de Kamchatka

Resum
Polimorfisme genètic en poblacions de talpons i musaranyes de la reserva de Kronotski (península de Kamtxatka, Rússia). Vam estudiar el polimorfisme genètic de quatre espècies de mamífers *Myodes rutilus*, *Myodes rufocanus*, *Sorex isodon* i *Sorex caecutiens* de quatre localitats: la vall dels Guèisers, la caldera volcànica Uzon, la vall de la Mort i les ribes del llac Kuril. Vam genotipar un total de 172 exemplars mitjançant la tècnica d’interseqüències simples repetides. Vam observar el polimorfisme més baix en la musaranya *S. caecutiens*. En aquesta espècie, el 68,8% de les bandes van resultar polimòrfiques i la diversitat genètica de Nei ($h$) va ser de 0,27, mentre que en *S. isodon* aquests valors van ser de 81,3% i el 0,29, respectivament. Les poblacions de *M. rufocanus* van ser les més polimòrfiques entre les espècies estudiades ($P = 91,4$; $h = 0,34$). El polimorfisme de *M. rutilus* de Kamtxatka ($P = 87,2$; $h = 0,29$) va ser similar al de Sibèria Occidental. A més a més, vam descobrir una elevada diferenciació genètica entre poblacions de rosegadors. El component interpoblacional de variabilitat genètica va ser del 30–40% ($G_{ST} = 0,31$ en *M. rutilus* i 0,39 en *M. rufocanus*). El flux genètic entre les poblacions de *M. rutilus* de Kamtxatka va ser dues vegades més baix que entre les poblacions d’espècies dels ecosistemes de la taigà siberiana.

Paraules clau: *Myodes*, *Sorex*, Diferenciació de poblacions, Variabilitat genètica, Marcadors ISSR, Península de Kamtxatka

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**Introduction**

Boundary semi–isolated populations of common animal species are of great interest to population and evolutionary genetics. They are regarded as the cutting edge of evolution due to the peculiarity of the gene pool and ability for rapid evolutionary transformations. Many species of small mammals from the Far East form genetically well–differentiated populations or even endemic species (Naitoh and Ohdachi, 2006; Kovaleva et al., 2014). Genetic features of such forms allow us to consider them as separate conservation management units.

The wildlife of the Kamchatka Peninsula is unique due to the natural features of the environment and the protection of the Kronotsky Reserve and the South Kamchatka Sanctuary. The study of genetic diversity of animals is of particular interest for monitoring and protecting the genetic resources of the region.
Voles (Myodes spp.) and shrews (Sorex spp.) are common representatives of Palearctic fauna. They are widespread in Siberia and they also inhabit the Kamchatka Peninsula. Myodes (Rodentia) and Sorex (Eulipotyphla) are distinct orders. From an evolutionary point of view, shrews are a more ancient group than voles. These groups are also very different in their ecology, occupying different trophic niches in ecosystems. Despite this difference, in ecological studies they are often considered as a single group named micromammals. However, in evolutionary and environmental studies, they show very different patterns of distribution and population dynamics. Furthermore, as they have different adaptive and evolutionary potentials their conservation status also differs.

The genetic structure of Myodes populations from the Far East region has been studied using allozyme markers (Kawata and Ueda, 1984; Frisman et al., 2002; Primak and Zasypkin, 2011), mitochondrial markers (Iwasa et al., 2000, 2002; Abramson et al., 2012) and microsatellites (Iwasa et al., 2000; Matson and Baker, 2001). Pereverzeva and Primak (Pereverzeva et al., 2014; Pereverzeva and Primak, 2016; Pereverzeva et al., 2018) found a fairly high level of genetic polymorphism in Myodes rutilus (Pallas 1779) from the Far East, especially in mainland populations, whereas the island populations of this species showed a reduced level of polymorphism and a unique gene pool. Vole populations from islands were highly differentiated between themselves and in their comparison with mainland populations (Primak and Zasypkin, 2011; Pereverzeva et al., 2014). Besides, populations of Myodes rufocanus (Sundevall 1846) from some islands played an important role in enriching the gene pool of mainland populations due to historical interpopulation exchanges (Iwasa et al., 2000).

Data on the genetic polymorphism of mammal populations of the Kamchatka peninsula are available in only a few papers (Iwasa et al., 2000; 2002; Frisman et al., 2002; Haring et al., 2011; Ohdachi et al., 2012). This may be due to difficult access to the territory and the general lack of study fauna in this area. However, these data are of great interest due to the unique natural habitats of animals on the peninsula. The specific climatic conditions, high seismic activity, and isolation supports the use of animal populations from Kamchatka as models to study mammalian adaptation to extreme environmental conditions. In addition, these data can contribute to the development of recommendations to protect the biodiversity of the peninsula. The purpose of our research was to study the genetic variability and differentiation of small mammal population from the Kronotsky Reserve. The first hypothesis was that the micromammal populations of the Kamchatka peninsula are highly differentiated genetically from populations of Siberia and have lower polymorphism due to isolation. The second hypothesis was that polymorphism estimates would be different for voles and shrews.

**Material and methods**

We collected mammals in the Kronotsky Reserve and the South Kamchatka Sanctuary, the Kamchatka Peninsula, Russia, in July–August 2015–2016. In the Kronotsky Reserve, we caught mammals in three localities: in the Valley of Geysers (54.436 N, 160.136 E), in the Uzon volcanic caldera (54.512 N, 159.916 E), and in Death Valley (54.468 N, 160.189 E). The distance between these places is no more than 15 km but they differ in biotopic conditions and species composition. See table 1 for details. In the South Kamchatka Sanctuary, we caught mammals on the shore of Kuril lake (51.485 N, 157.041 E) (fig. 1).

Capture was carried out using Gero traps, and in some areas, the cylinder–day method was used. We processed a total of 2,359 trap–days and 396 cylinder–days. We treated the animals according to the regulations of the Ministry of Health of the Russian Federation (Order 755 of August 12, 1977). If the animals were alive when removed from the traps, they were immediately euthanized by placing them in a box with cotton wool moistened with chloroform.
Table 1. Locations and numbers of animals studied: Mrt, Myodes rutilus; Mrf, Myodes rufocanus; Ssd, Sorex isodon; Scc, Sorex caecutiens.

Tabla 1. Localizaciones y número de animales investigados: Mrt, Myodes rutilus; Mrf, Myodes rufocanus; Ssd, Sorex isodon; Scc, Sorex caecutiens

| The Reserve                  | Locations         | Mrt | Mrf | Ssd | Scc |
|------------------------------|-------------------|-----|-----|-----|-----|
| The Kronotsky Reserve        | Valley of Geysers | 67  | 14  | 23  | 4   |
|                              | Uzon Volcanic Caldera | –   | 27  | 3   | 10  |
|                              | Death Valley      | 4   | –   | –   | –   |
| South Kamchatka Sanctuary    | Kuril Lake Shore  | 18  | 2   | –   | –   |
| Total sample size            |                   | 89  | 43  | 26  | 14  |

The total sample size was 172 individuals: 89 Myodes (= Clethrionomys) rutilus (Pallas 1779), 43 Myodes (= Clethrionomys) rufocanus (Sundevall 1846), 26 Sorex isodon (Turov 1924), and 14 Sorex caecutiens (Laxmann 1788).

We extracted total genomic DNA from muscle tissue fixed in 70% ethanol using the alkaline lysis technique (Bender et al., 1983). To estimate the polymorphism of such different taxonomic groups as voles and shrews, we used the nonspecific inter–simple sequence repeat (ISSR) markers that allow us to obtain amplification products of genetic material in different groups of organisms (Zietjiewicz et al., 1994). In particular, five primers (AG)$_8$C, (GT)$_8$C, (AC)$_8$T, (TC)$_8$C, and (TG)$_8$A were used for (SSR)–anchored polymerase chain reaction amplification. We carried out the amplification in 25 μl of reaction mixture containing PCR buffer (0.01 M Tris–HCl, 0.05 M KCl, 0.1% triton X–100), 4 mM MgCl$_2$, 0.2 mM of each dNTPs, 1 μl of total DNA solution, 2.5 mM of primer and 0.2 unit/μL of Taq–polymerase, with the following PCR conditions: 94°C 7 min; then 94°C 30 sec, 52(56)°C 45 sec, 72°C 2 min (40 cycles); 72°C 7 min. ISSR–fragments were separated by 2% agarose gel electrophoresis with Tris–EDTA–Borate buffer. The sizes of the fragments were determined using 100 bp DNA molecular weight markers.

We used POPGEN software (Yeh et al., 1999) to compute the following population genetic characteristics: the percentage of polymorphic bands (P), the observed ($n_a$) and the effective number of alleles ($n_e$), Nei's gene diversity ($h$), Nei's original measures of genetic identity ($I$), the genetic distance ($D$), gene flow ($N_m$), and the interpopulation component of genetic variability ($G_{ST}$).

Results

The ISSR polymorphism we recorded from four species of small mammals is presented in table 2. Populations of the gray red–backed vole M. rufocanus were the most polymorphic among the studied species. The percentage of polymorphic bands (P) in this species was 91.4, and Nei's gene diversity was 0.34. The polymorphism in populations of the northern red–backed vole M. rutilus from Kamchatka was also high (fig. 2). The proportion of polymorphic bands in this species was 87.2, and Nei's gene diversity was 0.29.

The variability observed in the shrew S. caecutiens was lower than in the voles (table 2). In this species, 68.8% of bands were polymorphic, and Nei's gene diversity ($h$) was 0.27 while these values in S. isodon were 81.3% and 0.29, respectively.
We found a high genetic differentiation among vole populations in Kamchatka. The genetic distances among _M. rutilus_ populations varied from 0.112 at a microgeographic scale to 0.279, between two parts of the reserve studied. In _M. rufocanus_, genetic distances were 0.113 and 0.487, respectively. Populations of _M. rufocanus_ in Kamchatka were characterized presenting the greatest genetic distance (table 3).

The interpopulation component of genetic variability was about 11–39% in _M. rufocanus_ ($G_{ST} = 0.116–0.391$) and 20–31% in _M. rutilus_ ($G_{ST} = 0.198–0.306$). Gene flow was especially low in _M. rufocanus_.

**Discussion**

Our data are the first estimates of genetic polymorphism using nuclear markers in small mammal species from Kamchatka. We identified high polymorphism in the vole _M. rutilus_. The findings of high polymorphism in this species are consistent with data obtained by other researchers using mitochondrial genetic markers (Pereverzeva et al., 2014). _M. rutilus_ is an absolute numerical dominant in the main biotopes in the vicinity of Kuril Lake and is abundant in the Valley of Geysers.
Table 2. Indicators of ISSR polymorphism in small mammal populations of the Kamchatka Peninsula: \( n \), sample size; \( N \), the number of polymorphic bands; \( P \), percentage of polymorphic bands; \( h \), Nei’s gene diversity; \( n_a \), observed number of alleles; \( n_e \), effective number of alleles.

| Species          | \( n \) | \( N \) | \( P (%) \) | \( h \) | \( n_a \) | \( n_e \) |
|------------------|--------|--------|------------|-------|--------|--------|
| Myodes rutilus   | 89     | 41     | 87.23      | 0.29  | 1.87   | 1.48   |
| Myodes rufocanus | 43     | 43     | 91.39      | 0.34  | 1.91   | 1.60   |
| Sorex isodon     | 26     | 39     | 81.25      | 0.29  | 1.81   | 1.50   |
| Sorex caecutiens | 14     | 33     | 68.75      | 0.27  | 1.69   | 1.50   |

Fig. 2. Gel electrophoresis profiles of ISSR–PCR fragments amplified using the (TC)8C primer: 1, 2, 7–10, specimens No. 278, 280, 286–289 of Myodes rutilus; 3–6, specimens No. 281, 283–285 of Myodes rufocanus from the Valley of Geysers; M, 100 bp DNA molecular weight marker (2% agarose gel, ethidium bromide staining).

Fig. 2. Electroforesis en gel de fragmentos ISSR–PCR amplificados utilizando el iniciador (TC)8C: especímenes 1, 2, 7–10, especímenes nº 278, 280, 286–289 de Myodes rutilus; 3–6, especímenes nº 281, 283–285 de Myodes rufocanus del valle de los Géiseres; M, marcador de peso molecular de ADN 100 bp (2% gel de agarosa, colorante de bromuro de etidio).
Table 3. Indicators of genetic differentiation of Myodes populations: I, Nei’s original measures of genetic identity; D, genetic distance; G<sub>ST</sub>, interpopulation component of genetic variability; N<sub>m</sub>, gene flow; * according to Zhigileva and Gorbacheva (2017).

| Compared groups | I     | D   | G<sub>ST</sub> | N<sub>m</sub> |
|-----------------|-------|-----|---------------|--------------|
| Populations of M. rufocanus of Kamchatka |       |     |               |              |
| Valley of Geysers – Uzon volcanic caldera, 15 km distance between them | 0.893 | 0.113 | 0.116 | 3.79 |
| Kronotsky Reserve – South Kamchatka Sanctuary | 0.614 | 0.487 | 0.391 | 0.78 |
| Populations of M. rutilus of Kamchatka |       |     |               |              |
| Valley of Geysers – Death Valley, 15 km distance between them | 0.894 | 0.112 | 0.198 | 2.03 |
| Kronotsky Reserve – South Kamchatka Sanctuary | 0.875 | 0.279 | 0.306 | 2.13 |
| Populations of M. rutilus of Western Siberia* | 0.825 | 0.192 | 0.158 | 2.66 |
| Populations of M. glareolus of Western Siberia* | 0.805 | 0.221 | 0.202 | 1.97 |

The level of ISSR–polymorphism of voles from Kamchatka differs little from the polymorphism of the populations of Myodes species from Western Siberia. The proportion of polymorphic bands on the same set of the ISSR markers in populations of M. rutilus from Western Siberia was 91.8%, Nei’s gene diversity was 0.34, and the effective number of alleles was 1.58 (Zhigileva and Gorbacheva, 2017). These data are similar to the mean values of these parameters in M. rutilus populations from Kamchatka (table 2). Evidently, living on the distribution border on the Peninsula does not affect the polymorphism of neutral genetic markers. However, the genetic differentiation of vole populations from Kamchatka is more pronounced than that in the ecosystems of Siberia. The indexes in populations from Kamchatka were higher than those in populations of Myodes species from Siberia (Zhigileva and Gorbacheva, 2017). Gene flow was two times less in Kamchatka than in Siberia (table 3). Gene flow in vole populations of the Kronotsky Reserve indicates that exchange of migrants may be empeded due to the landscape features of the reserve.

The voles from the Valley of Geysers and Uzon volcanic caldera demonstrated asynchronous changes in their numbers, which also confirms their belonging to different populations. This indicates significant landscape–geographical isolation between Uzon volcanic caldera and the Valley of Geysers, although the distance between them is about 15 km.

In the Kamchatka Peninsula, local populations of small mammals are strongly isolated because migration is obstructed by mountains. Their movements are also limited by geothermal fields, which they avoid. This isolation makes the local populations vulnerable and increases the risk of loss of genetic diversity. The data on high genetic differentiation among small mammal populations in Kamchatka should be taken into account when developing recommendations for protection of genetic resources in the peninsula.
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