**Taxonomic Reexamination among Three Local Populations of Anderson’s Red-backed Vole: Evidence from Crossbreeding Experiments**

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**Abstract.** In order to reexamine the taxonomic position of Anderson’s red-backed voles in question from the Kii Peninsula, the reproductive compatibility among local vole populations from the Kii Peninsula, Nagano and Fukushima was examined by crossbreeding experiments. Fertility of hybrids was ascertained until F4 in five cross types between Kii Peninsula female and Nagano male voles and to F3 in four cross types of Nagano females with Kii Peninsula males. The resulting F5 and F4 hybrids matured normally (in Line I). Moreover, F1 hybrids resulting from one cross type involving a Kii Peninsula female and a Fukushima male, and in one cross type involving a Fukushima female and a Kii Peninsula males, also, showed healthy growth (in Line II). Thus, the reproductive compatibility between both populations (in Lines I and II) was confirmed. Such reproductive compatibility among these three local populations of the Anderson’s red-backed vole strongly supports the belief that all belong to one species, *Eothenomys andersoni*.

**Key words**: Crossbreeding; Anderson’s red-backed vole; Fukushima population; Nagano population; Kii Peninsula population

In order to classify allied animals, it is indispensable to clarify morphological, ecological and physiological resemblances and differences between them and to grasp synthetically their mutual relation. Therefore, when there is a considerable doubt about populations classified by differences in their distribution and/or small morphological disparities, studies from various disciplines are advantageous resolving the problem. In particular, crossbreeding experiments, to investigate whether it is possible to exchange genes without disadvantage, is an important method of resolving a degree of affinity between populations.

With respect to the classification of Anderson’s red-backed voles inhabiting Honshu, there have so far been four differing opinions, based solely upon morphological characters. (1) Jameson (1961) and Imaizumi (1979) regarded them to be three separate species of *Aschizomys* (i.e., *A. andersoni* of the Tohoku district, *A. niigatae* of the Chubu district, and *A. imaizumii* of the Kii Peninsula). (2) Aimi (1980) considered them to be one species *Eothenomys*
andersoni. (3) Corbet and Hill (1986) also considered them one species, but Clethrionomys andersoni. (4) Kawamura (1988) considered them to be one species which he described as Phaulomys andersoni. In particular, the Kii Peninsula vole population, which is isolated from populations inhabiting central and northern Honshu was originally described as a full species Clethrionomys imaiizumii by Jameson (1961), then regarded as a subspecies of the Anderson's red-backed vole C. andersoni imaiizumii by Aimi (1967) because of its morphological characters, and then later described by Aimi (1980) as Eothenomys andersoni, belonging to another genus. The classification of the voles concerned has much remaining to be investigated, particularly because previous taxonomic studies were based on a few specimens and only on morphological parameters. As part of a study on the affinity between Anderson's red-backed voles from the Kii Peninsula and those from central Honshu (Mt. Yatsugatake), comparison of the occlusal pattern of molars, of skull characters, and of karyotypes (conventional, G-, and C-band patterns) of both populations has revealed them to be basically identical (Kitahara unpublished).

In order to reexamine the taxonomic status of these local populations of Anderson's red-backed vole, crossbreeding experiments were carried out among voles from the Kii Peninsula, Nagano and Fukushima. The purpose of this study is to examine the reproductive compatibility among Anderson's red-backed voles from three populations, and to discuss the taxonomic positions of these populations.

**Materials and Methods**

The voles used in this study were four males and seven females of the second generation captive bred Anderson's red-backed voles originating in Owase City, Mie Prefecture on the Kii Peninsula in December 1988; four males and three females caught on Mt. Yatsugatake in July and September 1990; and one male and two females from Mt. Azuma, Fukushima Prefecture (Shirozu, Fukushima City) caught in December 1992. These voles were housed individually in breeding cages, and mainly given hay and a commercial diet (ZC-1, Funabashi Nojo Co., Ltd., Chiba), supplemented occasionally with fresh grass and oats, and water ad libitum. Voles were weighed to check their physical condition nearly every seven days. The wild-caught voles from Nagano and Fukushima were reared for about three weeks, and then mated with the Kii Peninsula voles after confirming the descent of testes in males and the presence of cornified epithelial cells in females (see Table 1). Gravid females were inspected every day for deliveries so that their litters were discovered within 24 hr of birth. The number of young on the day when they were found was regarded as the litter size and differences between litter sizes were examined by Student's t-test.
Table 1. Crossbreeding results among Anderson's red-backed voles from the Kii Peninsula, Nagano and Fukushima populations.

<table>
<thead>
<tr>
<th>Line and type of crosses</th>
<th>No. of pairs</th>
<th>No. of litters</th>
<th>No. of young</th>
<th>Mean litter size (±SD)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>♂</td>
<td>♀</td>
</tr>
<tr>
<td>I. Between Kii and Nagano populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kii (♀) × Nagano (♂)</td>
<td>8</td>
<td>15</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>(Kii × Nagano) F1 × (Kii × Nagano) F1</td>
<td>7</td>
<td>17</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>(Kii × Nagano) F2 × (Kii × Nagano) F2</td>
<td>7</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>(Kii × Nagano) F3 × (Kii × Nagano) F3</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>(Kii × Nagano) F4 × (Kii × Nagano) F4</td>
<td>6</td>
<td>10</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Kii (♀) × (Kii × Nagano) F1 (♂)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>(Kii × Nagano) F3 × (Nagano × Kii) F2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Nagano (♂) × Kii (♀)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Nagano × Kii) F1 × (Ngano × Kii) F1</td>
<td>7</td>
<td>11</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>(Nagano × Kii) F2 × (Ngano × Kii) F2</td>
<td>4</td>
<td>12</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>(Nagano × Kii) F3 × (Ngano × Kii) F3</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>II. Between Kii and Fukushima populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kii (♀) × Fukushima (♂)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Fukushima (♂) × Kii (♀)</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Ud: undetermined.

Results

The results of the breeding experiments on two lines of the Kii Peninsula population crossed with the Nagano population (Line I), and the Kii Peninsula population crossed with the Fukushima population (Line II), are given in Table 1. In Line I, seven cross types between Kii Peninsula females and Nagano males, and four types in Nagano females and Kii Peninsula males were attempted. In Line II one cross type between Kii Peninsula females and Fukushima males and vice versa was made. Litters were obtained from all of the 13 cross types and no reproductive disorder was noted.

Through five cross types between Kii Peninsula females and Nagano males (Line I), fertility was ascertained until F4, and F5 hybrids developed normally. Moreover, four cross types between Nagano females and Kii Peninsula males were also fertile until F3, and F4 hybrids matured normally. Furthermore, two litters were obtained from one cross type between a Kii Peninsula female and (Kii ♀ × Nagano ♂) F1 male, and a (Kii ♀ × Nagano ♂) F3 female and (Nagano ♀ × Kii ♂) F2 male, and they all developed normally. Although mean litter sizes among these 11 cross types of Line I varied from 2.5—3.87 (a range of 1—
5), none of the differences among them were significant \((p > 0.05)\).

In Line II, F1 hybrids were bred from one cross between a Kii Peninsula female and a Fukushima male, and between a Fukushima female and a Kii Peninsula male. They developed normally. Consequently, reproductive compatibility between the populations was confirmed. Moreover, mean litter sizes in these two cross types of Line II varied from 3.0 – 4.0 (a range of 2 – 5) and did not differ significantly from those of the 11 cross types of Line I \((p > 0.05)\), although admittedly sample sizes were small.

**Discussion**

1. **Reproductive Compatibility between Local Populations of Anderson’s Red-backed Voles from the Kii Peninsula, Nagano and Fukushima**

   The species is a unit consisting of a large, intercommunicating gene pool, whereas the individual is merely a temporary vessel holding a small portion of the contents of the gene pool for a short period of time (Mayr 1963). For the maintenance of species integrity, isolating mechanisms consisting of two categories, i.e., premating and postmating mechanisms have been proposed (Mayr 1963, Futuyma 1986). The former category, premating mechanisms, contains: seasonal and habitat isolation, and reproductive isolation by behavioral discrepancy and physical disparity. The latter category, postmating mechanisms, includes isolation by failure in fertilization, developmental disorder of fertilized eggs, hybrid inviability and hybrid sterility. Although crossbreeding experiments or artificial inseminations have been carried out with some mammals, there have been few fertile hybrids because of the various postmating isolating mechanisms. Examples of hybrids which die during embryogenesis are as follows: brown rats *Rattus norvegicus* × black rats *R. rattus* (Hiraiwa and Yoshida 1955, Yosida and Taya 1977), goats *Capra hircus* × sheep *Ovis aries* (Alexander et al. 1967), rabbits *Oryctolagus cuniculus* × hares *Lepus americanus* (Chang et al. 1969) and ferrets *Mustela furo* × minks *M. vison* (Chang et al. 1969). By contrast, crosses between horses *Equus caballus* × donkeys *E. asinus* (Benirschke et al. 1962), horses *Equus caballus* × zebras *E. grevyi* (King et al. 1966), Syrian hamsters *Mesocricetus newtoni* × golden hamsters *M. auratus* (Raicu and Bratosin 1968) and Shaw’s jirds *Meriones shawii* × Libyan jirds *M. libycus* (Lay and Nadler 1969), all produce hybrids which survive to adulthood, although males and/or females are sterile.

   In this study, offspring from 96 litters from a total of 11 breeding crosses of the Kii Peninsula population and the Nagano population (Line I) were reared in a healthy way, and their fertility was confirmed by F4 hybrids through five cross types between Kii Peninsula females and Nagano males, and by F3 hybrids through four cross types between Nagano females and Kii Peninsula males. Thus, it was established that postmating mechanisms do not exist between those populations. Moreover, the absence of postmating mechanisms was corroborated by the production of F1 hybrids also between the Kii Peninsula and Fukushima populations (Line II).
As to mean litter sizes obtained from crossbreeding experiments, the figures of 2.5-3.87 in the Kii Peninsula population and the Nagano population (Line I) were smaller in some degree than 3.93±0.12 (SD) within the Kii Peninsula population (Kitahara unpublished). However, the mean litter sizes in the former had a broad variation range (see Table 1) and did not differ significantly from those of the latter (p > 0.05).

2. The Taxonomic Position of the Anderson's Red-backed Vole from the Kii Peninsula

It must be always kept in mind that fertility or sterility of hybrids is not the sole criterion defining species (Futuyma 1986). Fertile offspring are obtained from natural hybridization of wood-mice Peromyscus leucopus with cotton-mice P. gossypinus (McCarley 1954, 1964), and from crossbreedings of coyotes Canis latrans with dogs C. familiaris (Mengel 1971), and Eurasian bank voles Clethrionomys glareolus with North American red-backed voles C. gapperi (Grant 1974) under laboratory conditions: in such species, it is premating mechanisms that have the most important role in considering the taxonomic position. As mentioned above, the reproductive compatibility was confirmed between the local vole populations from the Kii Peninsula, Nagano and Fukushima, although there is the geographical isolation as premating mechanisms among them (Jameson 1961, Aimi 1967, 1980, Miyao 1981, Shimizu 1987). Therefore, the local populations used here should be an identical species belonging to a morphocline, as pointed out also by Aimi (1980, 1981) and Miyao (1981).

In this connection, the geographical isolation of the Kii Peninsula population from the population north of Nagano seems to have resulted from the transition of vegetation types following climatic changes in the Japanese Islands during the relatively short period from the late Glacial to the post Glacial age, i.e., only 10,000 years ago (Yasuda 1974, 1980). It is thought that the retreat of subarctic forest northwards occurred concurrently with the warming of the Japanese Islands, and consequently a population of Anderson's red-backed voles was left as relic (Aimi 1967) in the isolated relatively cool region centering around Mt. Odaigahara on the Kii Peninsula and then adapted itself to the local climate. Despite the geographical isolation existing between these populations, they hybridized readily under experimental conditions.

Taking into account the similarity of the molar occlusal enamel pattern, the coincidence of skull characters, and the identity of karyotypes (Kitahara unpublished), the absence of reproductive isolation between the three local populations strongly supports the concept that they should be regarded as belonging to one species, Eothenomys andersoni as proposed by Aimi (1980).

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References


Crossbreeding in the Anderson's Vole


