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Taxonomic re-examination of the striped field mouse, *Apodemus agrarius coreae* and *A. a. chejuensis*: Evidence from crossbreeding experiments (Mammalia: Rodentia)

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In order to re-examine the taxonomic status of the striped field mouse on Cheju Island, Korea, reproductive compatibility between local mouse populations from Cheju Island and Pusan was examined in crossbreeding experiments. Six types of crosses, including five in *A. a. chejuensis ♀×A. a. coreae ♂* and one in *A. a. coreae ♀×A. a. chejuensis ♂*, were attempted in this study. Of these, five pairs of *A. a. chejuensis ♀×A. a. coreae ♂* crosses succeeded in producing and rearing newborn youngs. However, the forty pairs comprising their F1 generations had a reproduction rate as low as 5% and thus clearly had difficulty in reproduction. Moreover, we could not obtain any newborn youngs from the ten pairs of the *A. a. coreae ♀×A. a. chejuensis ♂* crosses. The reproductive organs of F1 generation from the *A. a. chejuensis ♀×A. a. coreae ♂* cross were examined histologically. All the females were normal, while some abnormalities were observed in the males. Therefore, it was established that reproductive isolation exists between the two local populations. Consequently, these striped field mouse populations are defined as different species.

INTRODUCTION

The striped field mouse (*Apodemus agrarius*), widely distributing across the temperate region of the Eurasia (Corbet, 1978), belongs to *Apodemus*, Murinae, and includes twenty–two subspecies (Musser and Carleton, 1993). This species, in which many geographical morphometric variations are detected, is the most common among field rodents living in Korea (Hong and Lee, 1984). Thomas (1907) identified the striped field mouse from the Korean mainland and the striped field mouse from the Cheju Island as one subspecies of *Micromys a. manchuricus*, but he could not find out any differences in the external morphometric characters between the populations from these two areas. Thereafter, based on morphometric characters, this species has been classified into the following two to four subspecies; two subspecies of *A. a. pallescens* and *A. a. chejuensis* (Johnson and Jones, 1955), two subspecies of *A. a. manchuria* and *A. a. coreae* (Kuroda, 1934; Won, 1961), and four subspecies of *A. a. manchuria* in the extreme northern part, *A. a. coreae* throughout the major portion of the peninsular, *A. a. pallescens* in the coastal lowlands of southern and southwestern Korea, and *A. a. chejuensis* on the Cheju Island (Jones and Johnson, 1965). On the other hand, in recent years, cytogenetical and biochemical analyses have been carried out on *Apodemus* spp.
order to make it laboratory animal, resulting in detection of a polymorphism of transferrin within those four subspecies (Tsuchiya, 1984, 1985). In addition, some taxonomists insist that the striped field mouse from the Cheju Island, is one separate species from the results of the various analyses; i.e. the geographical variations of morphometric characters among three subspecies of the striped field mouse, A. a. coreae, A. a. pallescens and A. a. chejuensis (Koh, 1986), the morphometric and chromosomal analyses of A. a. coreae and A. a. chejuensis (Koh, 1987), the external and cranial characters of eight subspecies of the striped field mouse in Asia (Koh, 1991) and RFLPs variations of mitochondrial DNA in two subspecies of the striped field mouse, A. a. coreae and A. a. chejuensis from Korea (Koh and Yoo, 1992).

Taxonomy must be grounded on integrated conclusions which were clarified by investigations from various aspects. Where the populations from the two areas is uncertainly classified based on differences in their distribution and small morphological disparities and thus it is open to dispute, more extensive studies must be done. Among many techniques, a crossbreeding experiment is one of the most effective methods to assess the differences between species level. Studies on crossbreeding experiments have been reported for Apodemus species on their inter– or intra–specific breeding results of mainland and island populations (Jewell and Fullagar, 1965), and for Clethrionomys species on their interspecific breeding results between mainland populations (Grant, 1974). Two subspecies, A. a. coreae from the Korean mainland and A. a. chejuensis from the Cheju Island, have been separated from each other by the ocean for a long time and have been intensely discussed about their taxonomic status compared to the remaining two subspecies. The aims of this study are to determine the reproductive compatibility and to re-examine their taxonomic status by crossbreeding experiments.

MATERIALS AND METHODS

1. Crossbreeding experiment

In this study, thirteen A. a. coreae, consisted of six males and seven females, and twenty–five A. a. chejuensis, consisted of thirteen males and twelve females were used as the first generation. All the striped field mice used in this study were caught in either Mt. Whangreung, Pusan in the Korean mainland or Mt. Halla, Cheju Island at different periods, on August, 1994, March and November, 1995, and August, 1996. After capturing, each mouse was bathed in Negubon solution, so that external parasites were exterminated. The breeding experiments were conducted from September, 1994 to April, 1997. All the animals were housed as breeding pairs in 24×39×19cm cages made from polycarbonate. The cages floors were covered with wood chips and substantial straw was provided as nest materials. All the striped field mice were maintained on a 14-h L: 10-h D photoperiod with lights on at 08:30 hour, and the room temperature and humidity are 23°C and 60% respectively. Water and food were available ad libitum, the diet consisting of canary seeds, Chinese foxtail and Japanese millet seeds, flax seeds, sunflower seeds and a commercial diet (NMF; Oriental Yeast Co., Ltd., Tokyo). Mice used in the present experiments were as follows: A. a. chejuensis from 3 to 12 months old (weigh up to 35g in females, 45g in males); A. a. coreae from 3 to 12 months old (weigh up to 30g in females, 40g in males); hybrid F1 (A. a. chejuensis ♂×A. a. coreae ♀) up to 3 months old.
(weigh up to 35 g in both sexes). To facilitate breeding, females were taken into male's cages for three months. Pregnant females were checked every day so that newborn youngs were detected within one day after birth. Breeding occurred in monthly for the year. Litter sizes were recorded at this time.

2. Histological techniques

The reproductive organs of F1 generation (nine males and nine females) of A. a. chejuensis ♀×A. a. coreae ♂ were studied histologically. Each mouse was killed with diethyl ether and then the testes and cauda epididymides were removed from the males, and the ovaries, oviducts and uteruses were removed from the females. These tissues were fixed in Bouin's solution, dehydrated in a series of ethanol followed by xylene, and embedded in Paraplast Plus (Monject Co., Ltd., U.S.A.). Sections were cut to a thickness of 4–5 μm and stained with Delafield hematoxyline and eosin. The activity of spermatozoa in caudal epididymides of the males was recorded. Patterns of the development of the follicle cells and corpora lutea of the females was observed.

RESULTS

1. Crossbreeding experiments

In this study, eight types of crosses were examined, involving five types of crosses in the line of A. a. chejuensis ♀×A. a. coreae ♂, one type of cross in the line of A. a. coreae ♀×A. a. chejuensis ♂, one type of cross in the line of A. a. chejuensis ♀×A. a. chejuensis ♂ and A. a. coreae ♀×A. a. coreae ♂ (Table 1). Three of the five pairs of A. a. chejuensis ♀×A. a. coreae ♂ (type I) succeeded in producing and rearing newborn youngs. On the other hand, the forty pairs which were composed of the first generation bred at the rate as low as 5% and, thus, a clear difficulty was detected in their reproduction. Moreover, of the three pairs which were composed of the second generation, only one pair produced and reared a litter which included five newborn youngs (reproductive rate 33.3%, type III). The eight pairs of A. a. chejuensis ♀×the

<table>
<thead>
<tr>
<th>Types of crosses</th>
<th>No. of Successful No. of</th>
<th>Mean litter size</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of pairs</td>
<td>No. of litters</td>
<td>young litter size</td>
</tr>
<tr>
<td>I. A. a. chejuensis × A. a. coreae</td>
<td>5</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>II. (chejuensis×coreae)F1 × (chejuensis×coreae)F1</td>
<td>40</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>III. (chejuensis×coreae)F2 × (chejuensis×coreae)F2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IV. A. a. chejuensis × (chejuensis×coreae)F1</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>V. (chejuensis×coreae)F1 × A. a. chejuensis</td>
<td>10</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>VI. A. a. coreae × A. a. chejuensis</td>
<td>10</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>VII. A. a. chejuensis × A. a. chejuensis</td>
<td>23</td>
<td>19</td>
<td>60</td>
</tr>
<tr>
<td>VIII. A. a. coreae × A. a. coreae</td>
<td>11</td>
<td>8</td>
<td>23</td>
</tr>
</tbody>
</table>
first generation (F1) ♀ (type IV) and the ten pairs of the first generation (F1) ♀×A. a. chejuensis ♂ (type V) reproduced at 37.5% and 20% respectively. No any newborn youngs from the ten pairs in line A. a. coreae ♀×A. a. chejuensis ♂ (type VI) were obtained. The twenty three pairs of A. a. coreae ♀×A. a. chejuensis ♂ (type VII) and ten pairs of A. a. coreae ♀×A. a. coreae ♂ (type VIII) reproduced at 82.6% and 72.7% respectively.

Mean litter sizes were 4.77±1.02, 5.14±2.41, 4.80±0.44, 4.80±1.30, 4.60±1.29, 4.73±0.92 and 4.72±0.73 (range, 2–9), for type I, type II, type IV, type V, type VII, type VIII and in total, respectively. The significant difference among them was not detected (P>0.05).

The mean body weights at birth were 2.42 g±0.04 (N=36), 2.39 g±0.04 (N=36), 2.41 g±0.05 (N=5), 2.42 g±0.05 (N=24), 2.41 g±0.03 (N=17), 2.53±0.12 (N=59) and 2.31 g±0.09 (N=59) for type I, type II, type III, type IV, type V, type VII and type VIII, respectively.

2. Histological examination

The reproductive organs of F1 generation borne by A. a. chejuensis ♀×A. a. coreae ♂ were examined histologically. In each female, the primary and secondary follicle cells and Graafian follicles were observed (Fig. 1). On the other hand, concerning the males, the following abnormalities were detected: One had abnormal testes and cauda

Fig. 1. Photograph of the ovary in F1 hybrids from A. a. chejuensis (♀)×A. a. coreae (♂): Normal oogenesis in the ovary. An, antrum; Cl, corpus luteum; Co, cumulus oophorus; E, egg; Gf, Graafian follicle; i, infundibulum.
Fig. 2. Photographs of the testis and caudal epididymis in F1 hybrids from *A. a. chejuensis* (♀) × *A. a. coreae* (♂). a) The seminiferous tubules with Sertoli cell (Sc), spermatagonia (Sg), spermatocytes (Sc) and spermatids (St). b) The cauda epididymal tubules without sperm. Cd, cell debris; Ec, epithelial cell; Rb, round body.
Table 2. Spermatogenesis in F1 of Apodemus agrarius chejuensis (♀)×A. a. coreae (♂).

<table>
<thead>
<tr>
<th>Individual Number</th>
<th>Testes length (mm)</th>
<th>Spermatozoa in testes</th>
<th>Spermatozoa in caudal epididymides</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>absence</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>43</td>
<td>18.59</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>18.91</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>61</td>
<td>15.11</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>65</td>
<td>17.41</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>241</td>
<td>18.41</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>245</td>
<td>18.14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>247</td>
<td>19.72</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>249</td>
<td>18.73</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>19.76</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Tests length: length of major axis of right testis.
Spermatozoa in testes and caudal epididymides: , absence; +, rare;
++, medium; +++, many. Control data was obtained from sexually active males (90 days old) of A. a. coreae.

epididymides; in another one, spermatogenesis did not occur and no sperm was observed in its caudal epididymis, although the testes developed (Fig. 2); in another five males, the testes developed and a few sperm were recorded in testes but no sperm existed in the caudal epididymis; and in another two males, the testes developed, spermatogenesis occurred and sperm existed in the caudal epididymis as seen in normal males. All the histological results indicates that in F1 males the spermatogenesis is not completed and, thus, they have some problems in the reproductive organs (Table 2).

DISCUSSION

1) Reproductive compatibility between A. a. chejuensis and A. a. coreae

It is difficult to know if morphologically different and geographically isolated populations of animals deserve subspecific or specific rankings, since the crucial test of interbreeding (Mayr, 1963) is not performed in nature. Breeding experiments can help to resolve the matter by revealing how much reproductive compatibility if any, exists. Extrapolations are then made on the basis of its converse, reproductive isolation, making due allowance wherever possible for the lack of correspondence between nature and the experimental situation (Grant, 1974). The pairs of A. a. coreae ♂×A. a. chejuensis ♂ produced no newborn youngs. In addition, the three of five pairs of A. a. chejuensis ♂×A. a. coreae ♂ produced and reared successfully twenty-two newborn youngs in total. By contrast, the pairs composed of F1 generation of A. a. chejuensis ♂×A. a. coreae ♂ produced a few newborn youngs and no reproduction following post-parturition estrus occurred among them, even if the newborn youngs grew successfully. The results of these crossbreeding experiments suggested that a reproductive isolation would have existed between the striped field mouse population from the Korean mainland and the Cheju Island. In general, reproductive isolation is
likely to contain the followings: firstly, (a) premating isolating mechanisms, which involving (i) seasonal and habitat isolation, (ii) reproductive isolation by behavioral discrepancy and (iii) physical disparity; and secondly, (b) postmating isolating mechanisms, which involving (i) isolation by failure in the fertilization, (ii) developmental disorder of fertilized eggs, (iii) hybrid inviability and (iv) hybrid inviability or sterility (Mayr, 1963; Futuyma, 1986). The species is a unit consisting of a large, intercommunicating gene pool, whereas the individuals is merely a temporary vessel holding a small portion of the contents of the gene pool for a short period of time (Mayr, 1963). 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. As examples of hybrids which die during embryogenesis, the follows combinations have been well known: black rats *Rattus rattus* × brown rats *Rattus norvegicus* (Hiraiwa and Yoshida, 1955; Yoshida and Taya, 1977), goat *Capra hircus* × sheep *Ovis aries* (Aixander et al., 1967), rabbit *Oryctolagus cuniculus* × hares *Lepus americanus* (Chang et al., 1968) 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 auratus* × golden hamster *M. auratus* (Raicu and Bratoson, 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.

2) The taxonomic Position of the Korean striped field mouse from Cheju Island

The failure in breeding in captivity does not mean directly the reproductive isolation in the wild (Rubinoff and Rubinoff, 1971). In genus *Apodemus*, this is more likely to occur because its breeding is not easy in captivity. Although it must be always kept in mind that fertility or sterility of hybrid is not the sole criterion defining species, the results of these crossbreeding experiments mean that an obvious reproductive isolation exists in the two subspecies, *A. a. coreae* and *A. a. chejuensis*. In other words, type VI pair seems to belong to (i) or (ii) of (b), and type I–V pairs are likely to belong to (iv) of (b). Fertile offspring are obtained from natural hybridization of wood mouse *Peromyscus leucopus* with cotton mouse *P. gossyinus* (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.

Cheju Island was formed by series of volcanic activities at the end of Tertiary period in Cenozoic era and in recent it was 12,000 years since this island had been isolated from the Korean peninsula by the increase of sea level. Moreover, such island as Wan, Koeje, Jin and Chuja formed about 7,000 BP when sea level rose to its present depth (Ohshima, 1990).

As defined by Mayr (1969) a biological species comprises “groups of actually or potentially interbreeding populations which are reproductively isolated from other such groups”. Jardine and Sibson (1971) stated that the distinct criteria for populations to be recognized as different species are: (a) dissimilarity in morphological attributes; (b)
differences in ecological and/or geographical range; (c) degree of interfertility; (d) cytological difference; and (e) differences of enzyme and proteins.

As mentioned above, the reproductive incompatibility was confirmed between the local mouse populations from the Cheju Island and the Korean mainland, there is the geographical isolation as pre-hatching mechanisms each other.

The results of the histological examination also suggest that a genetical variation would be existed, resulting in a speculation between the local populations from the Korean mainland and the Cheju Island. These lead to a conclusion that both populations of the striped field mouse from these two areas, which have been geographically separated from each other for a long time, have reproductive isolations between them. These data strongly support the specific division of "Apodemus chejuensis" occurring in the Cheju Island from Apodemus agrarius in the mainland proposed by Koh and Yoo (1992). These results are consistent with recent new evidences on the taxonomy of the striped field mouse in Korea.

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