Does interspecific competition affect the vertical habitat segregation of *Apodemus argenteus* and *Apodemus speciosus*?: Experimental evaluation by removal and food supplementation

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**Abstract.** A proximate factor causing the vertical habitat segregation of two sympatric wood mice, *Apodemus argenteus* and *Apodemus speciosus*, was analyzed by removal, and food supplementation, experiments. *A. argenteus* became actively arboreal during summer and autumn, while *A. speciosus*’ arboreal activity was negligible throughout the year, causing seasonal habitat segregation between the two species. Removal experiments showed that the arboreal habits of *A. argenteus* increased from summer to autumn on both removal and control grids, regardless of the removal of *A. speciosus*. Food supplementation, however, showed that the provision of additional food led to a rapid decrease in the arboreal habits of *A. argenteus*. These findings suggest that the arboreal habits of *A. argenteus* are not the result of interference competition between species, but are a behavioral response to temporal and spatial fluctuations in food availability.

**Key words:** *Apodemus*, arboreal habits, coexistence, foot-print recording technique, manipulation.

Two congeneric wood mice, *Apodemus argenteus* Temminck and *A. speciosus* Temminck are widely distributed in woodlands in Japan. In general, *A. argenteus* occurs mainly in mountainous terrain with dense vegetation cover, while *A. speciosus* is distributed in secondary forests in the lowlands (Miyao et al. 1963; Yoshida 1970), however, the distributions of the two species broadly overlap in many mountainous areas (Hiraiwa et al. 1957; Doi and Iwamoto 1982). Morphological and ecological similarities between the two species imply that they may be separated based on differences in their diet and habitat use, resulting from ecological competition. In fact, Shioya et al. (1990) found conspicuous microhabitat segregation between the two species where they are sympatric: *A. argenteus* preferred areas with deep litter and a rich overstory, while *A. speciosus* preferred areas where the forest floor was well covered with herbs including graminaceous plants. Sekijima (1997) also found that *A. argenteus* were increasingly arboreal from early summer to autumn, whereas *A. speciosus*’ level of arboreal activity was negligible throughout the year, and so concluded that seasonal vertical habitat segregation occurs between the two species. These studies suggest that the observed horizontal or vertical microhabitat segregation plays a role in limiting competition between the two species, however, because of the lack of experimental analysis, it has been unclear whether such habitat segregation resulted from interspecific interactions or other ecological factors.

The main purposes of this study were to demonstrate differential vertical habitat use of *A. argenteus* and *A. speciosus* over several years and to clarify proximate causes of habitat segregation by two manipulation experiments, namely selective removal of *A. speciosus* and food supplementation. The following two predictions were tested: (1) if interspecific competition causes *A. argenteus*’ arboreal habits, then removal of *A. speciosus* should decrease its frequency; (2) if *A. argenteus*, arboreal activity is a behavioral response to changing spatial distribution of food resources, food supplementation on the ground should reduce its arboreal activity.

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Methods

Study area
The study was conducted in a mixed deciduous forest (35°26’ N, 138°46’ E) at about 1200 m altitude in the foothills of Mt. Fuji, Yamanashi Prefecture, Japan. The vegetation in the study area consisted primarily of Japanese oak (*Quercus mongolica* var. *grosserrata*, *Q. serrata*), red pine (*Pinus densiflora*), and Japanese larch (*Larix kaempferii*), with well-developed understories of shrubs (e.g. *Prunus incisa*, *Actinidia arguta*, *Helwingia japonica*, *Deutzia crenata*, *Corylus sieboldiana*). In the herb stratum, a creeper (*Schisandra chinensis*) was dominant, with more than 80% coverage at almost all trap stations during summer.

Of the six small mammals captured during the study, the two woodland mice, *A. argenteus* and *A. speciosus* predominated (82%). The other small mammals were two species of voles (*Microtus montebelli* and *Eothenomys smithii*) and two species of insectivores (*Urotrichus talpoides hondonis* and *Crocidura dsinezumi chisai*).

Trapping procedure
Three trapping grids, a control and two treatment grids (Grid-A and Grid-B) were established. Their locations were selected to minimize differences in habitat characteristics among grids. The control grid was central and the two treatment grids were set on either side of the control grid. Distances between the control grid and Grid-A and Grid-B were 180 m and 210 m, respectively, which were sufficient to preclude routine inter-grid movements of mice. Each grid contained 10 × 7 arrays of trap stations set at 10 m intervals. A large Sherman live trap baited with sunflower seeds was placed at each trap station.

Before manipulation, live-trappings were conducted on the three grids monthly from April 1987 to August 1988, except for four months from November 1987 to February 1988, in order to confirm the species composition and the population density of the two species in the three grids. In the control grid, live-trapping continued until October 1990 to monitor population characteristics such as density, reproductive condition, home range size, and habitat use without manipulation. A trapping session consisted of five consecutive nights and traps were checked every morning. All mice captured were individually toe-clipped and ear-punched for identification. Station number, species, sex, reproductive condition, pelage color, and body mass were recorded for each capture. Captured mice were then released at their capture point.

Population density was estimated monthly as the minimum number of animals known to be alive on each grid during each trapping session. Home range size was estimated from a minimum convex area connecting the capture points.

Evaluation of arboreal habitat use
The footprint recording method, unlike the live-trapping method, is an effective tool for evaluating the frequency of arboreal activity of mice without restraining their activity. To evaluate the arboreal activity of the two *Apodemus* species, tracking-tunnels (7 cm wide × 19 cm long × 7 cm high), for footprint recording, were attached to the trunk of the tree nearest to each trap station at a height of 1.5–2 m above the ground.

The method used was a paper-and-ink technique making use of a chemical reaction (King and Edgar 1977; Shida 1989) based on a two-component dye system, in which one component is in the ‘ink’ and the other is sprayed onto the straw paper. The ink used contained 80 g of ferric nitrate, 120 g of polyethylene glycol, and 70 ml of water. A solution of 5% tannic acid in 75% ethanol was sprayed onto paper. After being dried, the paper was cut to the size required for the tracking-tunnels. Then, a tray lined with ink infiltrated flannellette sheeting (the ink pad) and the sprayed paper was prepared and set up in the tracking tunnels.

When a mouse enters a tracking-tunnel and moves from the ink pad to the paper, transferring even very little ink on its feet produces indelible, sharply defined, blue-black footprints on the paper. A small amount of peanut butter was dabbed into the tracking-tunnels to attract mice passing near the tunnel. Footprint recordings were conducted for one night immediately after each live-trapping session. The tracking papers were set up in the evening and collected the following morning.

For the forefoot this was:

\[Z_1 = 0.830X_1 - 1.579X_2 - 3.123X_3 - 0.196X_4 - 3.161X_5 + 33.011\]

And for the hindfoot this was:
$Z_2 = -0.592X_6 - 1.476X_3 - 5.303X_8 - 4.103X_9 + 85.834$

where $X_1, X_2, ..., X_9$ represents the distance between the footprints (Fig. 1). Footprints were identified as those of *A. argenteus* if $Z_1$ or $Z_2 < 0$ and as *A. speciosus* if $Z_1$ or $Z_2 > 0$. These functions explained 100% of the discriminant probabilities in both forefoot and hindfoot. The arboreal activity of the two species was evaluated as the percentage of the total number of tracking-tunnels (PT) utilized by each species.

**Removal experiment**

To clarify any effect of *A. speciosus* on the arboreal habits of *A. argenteus*, experimental removal of *A. speciosus* (with two replications) was conducted from April to September 1989. The two treatment grids (Grid-A and -B) were assigned as removal grids for *A. speciosus* and thereafter renamed Removal-A and Removal-B. In the control grids, trapping was done at the same interval, but without the removal of any individuals. In the two removal grids, all *A. speciosus* captured during the removal period were removed from the grids. A Sherman live trap baited with sunflower seeds was set at each trap station for two consecutive nights per trapping session.

The pre-removal period was from 28th April to 3rd June. In the removal grids, removals of *A. speciosus* were conducted from 3rd June to 25th September. Twelve removal sessions were set during the removal period. The interval between removal sessions was approximately one or two weeks. Three footprint recordings were taken during the pre-removal period and seven during the removal period.

**Food supplementation**

Food supplementation experiments with two replicates were conducted from 22nd August to 30th September 1988. Two treatment grids (Grid-A and -B) were assigned as food supplementation grids and renamed FS-A and FS-B. The pre-feeding period was from 22nd August to 20th September. Food supplementation began on 20th September and continued daily until 26th September on FS-A and 27th September on FS-B when *A. argenteus* became actively arboreal. In FS-A, 20 g sunflower seeds were supplied daily at each trap station on the ground from 20th until 23rd September, and thereafter the amount of food supplementation at each station was raised to 40 g per day because the effect of supplementation on the arboreal habits of *A. argenteus* was declining gradually. Similarly, in FS-B, 20 g oats were supplied daily at each station on the ground from 20th to 24th September, and thereafter raised to 40 g per day.

Five footprint recording sessions were made during...
the pre-feeding period and seven during the feeding period. To monitor changes in the number of the two species during the food supplementation experiment, live-trapping was conducted for three consecutive nights before and after food supplementation.

**Results**

Vegetation and mice densities in the three experimental grids

The tree densities (number of trees per ha) in the three grids were: 326 in the control grid, 298 in Grid-A, and 336 in Grid-B, and the proportional cover of the four principal tree species in the study areas, i.e. *Pinus densiflora*, *Larix kaempferii*, *Quercus mongolica* var. *grosserrata* and *Q. serrata*, was 92% for the control, 89% for Grid A and 85% for Grid B.

The densities of *A. argenteus* and *A. speciosus* in the three grids from April 1987 to August 1988 before manipulation increased simultaneously from spring to autumn, and this tendency was common among the three grids (see Fig. 2). No significant differences were recognized in the densities of each species among the three grids (Freedman’s test, NS for the two species).

Vertical habitat use in two *Apodemus speciosus* and *Apodemus argenteus*

In the control grid, a total of 3,500 footprint recording papers were set from August 1988 to October 1990. Of these, 2,250 papers recorded the movements of *Apodemus* spp. Discriminant functions revealed that 2,247 papers had recorded *A. argenteus* and only three *A. speciosus*. Throughout the study period, the percentage of tracking-tunnels used by *A. argenteus* was 64.3%, while that by *A. speciosus* was only 0.1%.

There was clearly seasonally related change in arboreal activity in *A. argenteus* with most activity recorded from July to October, reaching over 90% in August and September in each year (Fig. 3A). In contrast, arboreal activity by *A. speciosus* was rare, and recorded only twice in September 1988 and once in August 1989 (Fig. 3B).

In both sexes of *A. argenteus* there was a significant negative correlation between arboreal activity and average home range size (Spearman signed ranks test; $r = -0.466, P < 0.05$ for males; $r = -0.633, P < 0.005$ for females), suggesting that there was a complementary relationship between the arboreal and terrestrial activity in *A. argenteus* (see Fig. 4).

The effects of removing *A. speciosus* on the arboreal habits of *A. argenteus*

The density of *A. argenteus* in the control grid increased gradually from April to September and reached a peak in early September, and was mirrored by the densities in the two removal grids (see Fig. 5). The peak densities of *A. argenteus* during the removal of *A. speciosus* were 56 in Removal-A and 34 in Removal-B, although peak times differed between the grids. The densities of *A. speciosus* in the control grid were consistently lower than those of *A. argenteus*, although the seasonal tendency was similar to that of *A. argenteus*.

The PTs of *A. argenteus* in the control grid rose rapidly from late July to late September and reached 100% in mid-September (Fig. 5D). The PTs of *A. argenteus* in Removal-A and -B also increased from late July to late September as well as in the control grid,
regardless of removing *A. speciosus* (Figs. 5E and 5F). During the removal of *A. speciosus*, there was no significant difference in the PTs of *A. argenteus* between the control and either removal grid (Wilcoxon matched-pairs signed-ranks test, NS for both combinations).

**Effects of food supplementation on the arboreal habits of *A. argenteus***

During the pre-feeding period, there were no significant differences in the PTs of *A. argenteus* between the control and either treatment grid (Wilcoxon matched-pairs signed-ranks test, NS for both combinations) (see Fig. 6). After food supplementation, the PTs of *A. argenteus* in both FS-A and FS-B declined drastically (Figs. 6B and 6C) and were significantly lower than that in the control grid (*P* < 0.01 for FS-A, *P* < 0.01 for FS-B, using Wilcoxon matched-pairs signed-ranks test). Thereafter the PTs of *A. argenteus* increased gradually, reaching 55% in FS-A and 42% in FS-B. Further, additional food supplementation caused a further decrease in the PTs of *A. argenteus* (Figs. 6B and 6C).

During food supplementation, a conspicuous increase in the number of *A. speciosus* immigrants was observed in both FS-A and FS-B, unlike the control grid (Table 1). *A. argenteus* showed no evident change in the number of immigrants during the food supplementation in any of the grids.

**Fig. 3.** Seasonal changes in the minimum number known to be alive (MNA) in *Apodemus argenteus* and *A. speciosus* and the percentage of the tracking-tunnels utilized by each species relative to the total number of the tracking-tunnels (PT) in the control grid.

**Fig. 4.** A relationship between the percentages of the tracking-tunnels utilized by *A. argenteus* (PT) and their home range sizes on the ground. Here, the data that were collected in the control grid from August 1988 to October 1990 were used for the analysis.
Discussion

Propriety of experimental grids

Experimental manipulation in the field is essential in order clearly to understand the role of interspecific interactions in biocommunities. However, whether reliable results accrue from field manipulation experiments depends on experimental design, such as the configuration of experimental grids, the number of replications, and the style of manipulation. Particularly, the setting and the conditions of experimental grids have a considerable effect on the output of manipulation experiments, because it is impossible to regulate almost all parameters other than a targeted parameter (Hairston 1989). In this study, three grids, consisting of a control grid and two treatment grids as replicates, were established in mixed deciduous forests. The vegetation survey before manipulation showed that the three grids shared similar characteristics in terms of tree density and species composition. Furthermore, live-trapping before manipulation demonstrated that there were no remarkable differences in either the number or the composition of the two Apodemus species among the three grids. Based on these results, the three study grids were judged to provide appropriate conditions for the manipulation experiment.

Evaluation of arboreal habits

In this study, the footprint recording technique was applied to evaluate arboreal activity of mice. An advantage of this method over the live-trapping, is that it allows the evaluation of the frequency of a species’ activity without restraining that activity. The likely pitfall, is misidentification of footprints. For two Apodemus species with similar footprints, therefore, linear discriminant analysis, using the footprints of the two species taken previously in the laboratory, was applied to distinguish statistically between the footprints of the two species. The discriminant function obtained made it possible to accurately distinguish between the footprints of the two Apodemus species. To confirm whether
identification of the two species based on this function was reliable, a simultaneous evaluation of the arboreal activity of mice by both the footprint recording and the live-trapping methods was conducted in the control grid from August 1988 to August 1989 (Sekijima 1997). The frequencies of arboreal activity by mice found by the footprint recording method strongly correlated with those obtained by live trapping. Footprint recording, in combination with discriminant analysis, was judged to be an effective tool for evaluating the habits of two sympatric Apodemus species easily, reliably, and inexpensively.

Proximate factors causing seasonal habitat segregation of two A. argenteus and A. speciosus

Evaluation of the habitat use by mice from 1988 to 1990 showed convincingly that A. argenteus is a semi-arboreal species, whereas A. speciosus is terrestrial. A. argenteus became increasingly arboreal from summer to autumn, whereas A. speciosus was only rarely arboreal at any time of year, thus contributing to seasonal habitat segregation between the two species.

To clarify the proximate factor causing the arboreal habitat shift of A. argenteus, two predictions were tested by two manipulation experiments, i.e. removal of A. speciosus and food supplementation. The first prediction was that interspecific competition causes the habitat shift of A. argenteus from summer to autumn. If interspecific competition causes the arboreal behaviour of A. argenteus, then removal of A. speciosus should reduce arboreal activity by A. argenteus during the manipulation. However, the arboreal activity of A. argenteus in the two removal grids increased from summer to autumn, regardless of the removal of A. speciosus, thus contradicting the first prediction. Furthermore, remarkable increases in arboreal activity were observed simultaneously in the same season (i.e. from late August to September) in all three grids, although the peak densities and timing of peak densities of A. argenteus populations differed among the grids. These results indicated that a seasonally dependent factor, rather than interspecific competition, affected the arboreal habitat shift of A. argenteus.

The second prediction was that the arboreal activity of A. argenteus reflects a behavioral response to temporal and spatial changes in resource availability. If A. argenteus shifts to increased arboreal activity in response to changes in food resources, then food supplementation on the ground should reduce their arboreal activity. In accordance with the second prediction, food supplementation led to a rapid decrease in the arboreal activity of A. argenteus.
of *A. argenteus*. Furthermore, this manipulation provided an additional interesting finding. The arboreal activity of *A. argenteus*, which was reduced by food supplementation, subsequently increased despite food supplementation continuing (Fig. 6). Thereafter, increased food provided on the ground reduced the arboreal activity of *A. argenteus* once more. Live trapping before and after food supplementation showed that more *A. speciosus* than *A. argenteus* immigrated into the treatment grids during food supplementation. The phenomenon observed here can be interpreted as follows: the provision of superfluous food on the ground promoted the immigration of *A. speciosus*, contributing to strong exploitative competition between the two species on the ground, and consequently the activity mode of *A. argenteus*, which had shifted to the ground following food supplementation, shifted again into the trees.

In conclusion, during short-term manipulation within a restricted area such as in this study, there was no apparent strong interspecific competition causing the increased arboreal activity of *A. argenteus*. The arboreal habitat shift of *A. argetenus* was considered most likely to have resulted from a behavioral response to temporal and spatial changes in food availability, although exploitative competition seemed to act between the two species when a rich food patch was provided on the ground. Some researchers have reported similar phenomena among arboreal mice. McShea and Franq (1984) demonstrated that *Peromyscus leucopus*, a semi-arboreal species occurring in North America, were captured in great numbers on the ground when the amount of fallen acorns was abundant, and in great numbers in the trees when lepidopteran larvae appeared. Similarly, Shida (1989) also reported that the arboreal activity of *A. argenteus* increased when numbers of insects observed on tree trunks were more abundant. Ida et al. (2004), using a canopy observation tower, confirmed that *A. argenteus* climbed as much as 18 m above the ground during the period from summer to autumn in order to access tree seeds.

Several studies on the habitat use of *A. argenteus* and *A. speciosus* have suggested that horizontal and vertical microhabitat segregation play a role in reducing competition between the two species and as a result facilitate their coexistence (Abe 1986; Shioya et al. 1990). However, Sekijima and Sone (1994) demonstrated by reciprocal removal experiments that, though interspecific competition occurred between *A. argenteus* and *A. speciosus*, intraspecific competition had a greater effect than interspecific competition on the abundance and horizontal habitat use of the two species. Interspecific competition may not have a great effect on either vertical or horizontal habitat segregation in these two species.

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