Spatial relationships of Apodemus Sylvaticus and Apodemus flavicollis in a syntopic habitat in Southwest Bulgaria during breeding season

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Abstract

In Bulgaria Apodemus sylvaticus and Apodemus flavicollis are sympatric and in many regions syntopic, but how individuals occupy the habitat when they co-occur, and how they interact is not enough studied. The purpose of this work was to study the spatial distribution of both species during breeding season in a syntopic habitat in Southwest Bulgaria and character of their interaction to insight into the nature of their cohabitation. It was found that A. flavicollis was dominant and its number was almost double compared to that of A. sylvaticus. In both species in the rocky patches the number of juveniles was close to that of adults, while the tree-shrub patches were dominated by adults. In the interspecific male-male and female-female dyadic encounters high aggressiveness was not demonstrated. Therefore, it could be assumed that sharing of the habitat by two syntopic species may be enhanced by the use of olfactory cues.

Key words: spatial distribution, habitat segregation, interspecific interactions, competition

Introduction

The wood mouse (Apodemus sylvaticus Linnaeus, 1758) and the yellow-necked field mouse (Apodemus flavicollis Melchior, 1834) are sympatric and syntopic in many regions in their European range, including Bulgaria (Obert, Holíšová 1983; Frynta et al., 2006; Popov, 2015). Many studies demonstrate that Apodemus species share generalized muroid morphology and exhibit high phenotypic similarity, but the individual species/populations differ in their preferred habitats and behaviour (Frynta et al., 2006; Popov, 2007; Kuncová, Frynta 2009). Although the spatial distribution is studied by many authors (Montgomery, 1981; Montgomery, Dowie, 1993; Marsh et al., 2001; Vukičević-Radić et al., 2006; Popov 2015), it is still not entirely clear how individual species occupy the habitat when they are syntopic, and how they interact, especially if they are closely related. Aggressive interactions often play an important role in the ecology of closely related wood mice, and affect their coexistence. According to some authors (Hoffemeyer, 1973; Montgomery, 1978, 1980; Čiháková, Frynta, 1996) A. sylvaticus is a subordinate
species in its interactions with *A. flavicollis*, while others (Bovet, 1972) do not establish clear behavioural differentiation between them.

Unlike Central and Northern Europe, in Bulgaria *A. sylvaticus* and *A. flavicollis* do not differ in external morphological features. They are widely distributed and characterized by high ecological plasticity, increased density and mobility (Popov, 2007). Although both species can be distinguished by skull morphometry (Popov, 1993, Chassovnikarova, Markov 2007) or by biochemical analyses (Gemmeke, 1981; Vogel et al. 1991; Filippucci 1992), these techniques require either for the specimens to be killed or the use of heavy field equipment (i.e. special freezer) for tissue sample processing and analysis (Michaux et al., 2001). Because of this until recently it was difficult to carry out relevant behavioural studies with these species in Bulgaria. Prevailing studies are mainly in the field of ecology of communities, based on craniometrical measurements and analyses (Minkova, Popov, 2002; Chassovnikarova, Markov, 2007; Popov 2015). However, with the application of a new method on the basis of PCR amplification of part of the mitochondrial cytochrome b gene by using species-specific primers, allowing these *Apodemus* species to be rapidly and easily distinguished (Michaux et al., 2001) without killing individuals, it is now possible to carry out such studies. On this basis, the purpose of this work is to study the spatial distribution of *A. sylvaticus* and *A. flavicollis* during breeding season in a syntopic habitat in Southwest Bulgaria and the character of their interspecific interaction by dyadic encounters in order to insight into the nature of their cohabitation. Breeding occurs from March to the end of October (Peshev et al., 2004). According to previous studies on the ecology and competitive relationships between both species (Montgomery, 1978, 1980; Čiháková, Frynta, 1996; Peshev et al., 2004; Minkova, Popov, 2002; Popov 2015), the hypothesis was that *A. flavicollis* will show both behavioural dominance over *A. sylvaticus* in dyadic encounters and higher density.

**MATERIAL AND METHODS**

**Field investigations.** The study was conducted in the farthest Southwest region of Bulgaria (41°27’N, 23°15’E). The climate is continental-Mediterranean, with hot summers and mild winters. The average July temperatures are in the range of 22°C-24°C, and the annual average precipitation is about 500 mm. During summer the average precipitation is about 100 mm, and during winter – about 130 mm. Rainfall peaks are in the late fall, and the minimum is in September (www.stringmeteo.com).

The field investigations were carried out on the eastern slope of the elongated hill Kozuh, with a length of 2 km, a width of 500 m and an altitude of about 250 m. The study habitat was a mosaic of tree-shrub and rocky patches. The dominant species in the tree-shrub patches were *Quercus pubescens* Willd., *Carpinus orientalis* Mill., *Paliurus spinachristi* Mill., more rarely *Fraxinus ornus* L., *Juniperus oxycedrus* Sibth. et Sm. Dominant grass species were *Avena fatua* L., *Filago arvensis* L., *Bromus* spp. Here and there in the rocky patches there were trees and bushes, mainly *Q. pubescens* and *J. oxycedrus* as well as
Eryngium campestre L., Hypericum olympicum L., Pteridium aquilinum (L.) Kuhn, Melica ciliata L. Over the study area, altitude ranges between 110-210 m.

The study was conducted by The Capture-Mark-Recapture method in spring – March and April 2011. Each trapping session lasted 5 days and one hundred live traps, Sherman model were used on an area of 1 ha. They were arranged in a grid at a 10-meter distance from each other, so that some of them fell into tree-shrub patches and the other in the rocky part. In March, about 3/4 of the study area was tree-shrub, while in April it was the opposite – about 3/4 was rocky. For each microhabitat, the percentage of vegetation cover and the number of traps in it were described. The distance between grids set in March and April was about 100 m. In March the average daily temperatures were between 8-11°C, and in April – 14-19°C. The relative humidity in March was in the interval 35-85%, and in April – 30-70%.

Each individual was marked by toe clipping when trapped for first time. Further, at each capture the following was recorded: trap location, individual number, sex, body mass to ±1 g and reproductive status (females: pregnant, lactating, or perforated vagina; scrotal testes for males). The age of each animal was determined on the basis of its body mass and reproductive status. The weight criterion in combination with some exterior features about reproductive stage of the mice were used to estimate the age of animals according to other works with wood mice (Cockel, Ruf, 2001) and personal experience from the field. Traps were baited with oat flakes, set in the evening and checked at 6:00-8:00 a.m.

The number of animals captured, and the percentage of the trap points occupied by every species in each microhabitat patch were calculated for each trapping session. Additionally, distribution of captures on the trap-grid was superimposed onto the microhabitat patches in order to assess possible differences between species. The percentage of interspecific overlap (Ov %) in trap use was calculated following Löfgren (1995) – (Ov (%) = (Ns/T) × 100; Ns = number of traps shared by two or more individuals, T = total number of traps used, and used as an indicator of microhabitat selectivity (Vukićević-Radić et al., 2006). The non-parametric Gamma test by ranks was used to obtain the correlation between habitat variables and the quantitative representation of species across the local patches. Difference between species, sexes and age groups were tested using One-sample chi-squared test.

**Species determination.** The toes cut during marking were used for genetic analysis for species identification. Amplification reactions were carried out in 25 µL volumes including 0.5 µL of each 20 pm primer, 1 µL of 10 mm dNTP, 2,5 µL of 10× reaction buffer and 0.1 µL of 5 U/µL Taq Prime DNA polymerase (Genet bio). One µL of DNA was used for a PCR amplification with concentration of 200 ng. All PCRs were performed for 35 cycles (30 s at 94°C, 30 s at 55°C and 1 min 30 s at 72°C) plus 7 min final extension at 72°C. The used primers are as described by Michaux et al. (2001).

Species identification of *Mus macedonicus* Petrov et Ruzic was made on the basis of external morphological features, body size, range description, habitat and ecology according to Popov, Sedefchev (2003).

**Laboratory experiments.** All individuals captured in the rocky and tree-shrub patches on the last day of the trapping sessions, as well as additionally captured wood
mice from adjacent areas 1-2 days after completion of the field studies, were transported to the laboratory and tested after about one-week adaptation period. The additionally captured individuals from adjacent areas were also marked, and their gender and reproductive status determined. The toes cut in the marking procedure were used for species identification according to the method described above.

A total of 10 *A. sylvaticus* and 10 *A. flavicollis* were studied in 5 male-male and 5 female-female interspecific dyadic encounters. Since age is an important factor that can modulate relationships between mice and their responses to odours, only adult and sexually active individuals were included in the experiments. Pregnant mice were not used. The captured animals were individually housed in standard laboratory rodent cages. The mice were fed on mixed seed diet supplemented with carrots, apples and provided with water *ad libitum*. The mice were maintained at natural ambient temperatures, 15-18°C and 12-12 h of light/day during the experiments.

The encounters were carried out on 'neutral ground' in a 50x50 cm glass arena provided with fresh sawdust as bedding and hay for shelters. The duration of each encounter was 10 min. Mice were tested in the morning and in the evening. Natural light during the morning and artificial red light during the evening were used for observations. The cage was cleaned before each test. Each animal was used for one encounter only. A camcorder JVC GZ-MG365HE was positioned above the arena in order to record the animals’ reaction.

The behavioural events were categorized as follows: agonistic behaviour-offensive behaviour (threat, attack, fight, chase, offensive-upright and sideways postures), and defensive behaviour (defensive-upright posture, retreat, running away, jumping apart), amicable behaviour (passing above, grooming, clambering on, standing side by side) and introductory behaviour (approaching, following, nose-nose, nose-anal, nose-body). The number of behavioural events demonstrated in dyadic encounters were calculated for each encounter and averaged for male-male and female-female encounters. To compare specific levels of agonistic, amicable and introductory behaviour in interaction between two species, data were quantitatively analysed. The results are represented by the median and the dispersion – by the extremes. The significance of species-specific differences between behavioural patterns, demonstrated by male and female *A. flavicollis* and *A. sylvaticus* was estimated by Mann-Whitney *U*-test at *p* < 0.05. The STATISTICA software (Version 7.1, StatSoft Inc., Tulsa, Oklahoma, USA) was used to analyze the data for both field and laboratory experiments.

**Ethical note.** The research conformed to the international requirements for ethical attitude towards animals. After the end of the laboratory experiments the animals were taken back to their habitats.

**RESULTS**

**Field investigations.** Three species of small mammals were captured during the study period – *A. flavicollis*, *A. sylvaticus* and *M. macedonicus*. *A. flavicollis* was the dominant species (62.0 %), followed by *A. sylvaticus* (34%) and *M. macedonicus* (4%). A total of 31 *A. flavicollis* and 17 *A. sylvaticus* individuals were trapped during March and April 2011.
The number of *A. flavicollis* was almost two times higher than that of *A. sylvaticus* ($\chi^2 = 4.08, p < 0.05$). (Table 1) The ratio of males to females was 1.2:1 for *A. flavicollis* and 1.4:1 for *A. sylvaticus*. Adult individuals were significantly more numerous that juveniles in both *A. flavicollis* ($\chi^2 = 9.32, p < 0.05$) and *A. sylvaticus* ($\chi^2 = 5.55, p < 0.05$).

When distribution of captures on the trap-grid was superimposed onto the microhabitat patches *A. flavicollis* was concentrated mainly in the tree-shrub patches and its number here was significantly higher compared to rocky patches ($\chi^2 = 4.6, p < 0.05$), Fig. 1, Table 2. Gamma tests indicated significantly positive correlations between degree of vegetation cover of studied patches and number of *A. flavicollis* ($G = 1, Z = 1.86, p = 0.06$). Most *A. sylvaticus* individuals were also captured in the tree-shrub patches, although a statistically significant difference was not established. In the rocky patches the number of individuals from both species was lower and approximately equal. However, in the rocky patches the number of juveniles was close to that of adults, while the tree-shrub patches were dominated by adults, including two pregnant females. Three recaptures of *A. flavicollis* were registered in the tree-shrub patches – 1 male and 2 females, one of which was pregnant. Recaptures of females were at distance of 10-30 m, and of the male – 40 m. Two males of both *A. flavicollis* and *A. sylvaticus* were captured at the same point. Thus, interspecific overlapping is 1.25-4.16 %, Table 2, Fig 1.

**Laboratory experiments.** The behavioural observations showed that the majority of the encounters were with approximately equal number of agonistic (offensive and defensive) events and amicable and introductory behaviour, which indicates a low level of aggressiveness. During dyadic encounters attacks and chases were observed mainly in the 2 male-male encounters. The male *A. flavicollis* presented more offensive and less defensive behaviours than male *A. sylvaticus* (Table 3). Although male *A. flavicollis* seemed to be more aggressive than male *A. sylvaticus* a statistically significant difference was not established. The female *A. sylvaticus* more often performed upright defensive postures in response to the threatening approaches of *A. flavicollis* females, but significant species-specific differences in offensive and defensive behaviours were not established (Table 3).

| **Table 1.** Mice demographic structure in the studied habitat in spring 2011 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Microhabitat    | *A. flavicollis* | *A. sylvaticus* |
|                 | Sex ratio       | Age distribution | Sex ratio       | Age distribution |
|                 | M   | F   | juv | ad | pregnant | M   | F   | juv | ad | pregnant |
| Rocky           | M   | 4   | 2   | 3   | 1     | 1   | 1   | -   | 2   | 1     |
| Tree-shrub      | 13  | 12  | 2   | 1   | 11    | 11  | 2   | 6   | 5   | 1     |
| Total           | 17  | 14  | 5   | 2   | 12    | 12  | 10  | 7   | 3   | 1     |
|                 | 31*  | 7** | 24** |    | 17*   | 4** | 13** |    | 7** | 24** |

* indicates the significant differences between number of captured *A. flavicollis* and *A. sylvaticus* individuals, and ** the significant differences in the number of juveniles and adults in each species, revealed by One sample $\chi^2$ test.
The study region is famous for its great biodiversity, including rich herpetofauna (Beron, 2001). For small mammals, the higher degree of vegetation cover with shrubs and trees provides protection against predators, and sources of food. In this regard the majority of captures in both species in the tree-shrub patches as well as the positive correlation between number and vegetation cover in *A. flavicollis* allow us to suggest that the degree of vegetation cover seems to be associated with perception of overhead predation risk. This selection by *A. flavicollis* and *A. sylvaticus* seems to be an antipredator strategy, displayed by most small mammals (Gray et al., 1998; Torre, Diaz, 2004; Cassaing, 2013).

### DISCUSSION

Table 2. Vegetation cover of studied patches and parameters for trap success during study period

<table>
<thead>
<tr>
<th>Month</th>
<th>Microhabitat</th>
<th>Vegetation cover (%)</th>
<th>Number of individuals captured</th>
<th>% of the trap point occupied</th>
<th>Number of individuals captured</th>
<th>% of the trap point occupied</th>
<th>% of interspecific overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>Rocky</td>
<td>15</td>
<td>4</td>
<td>16</td>
<td>3</td>
<td>12.5</td>
<td>4.16</td>
</tr>
<tr>
<td>2011</td>
<td>Tree-shrub</td>
<td>80</td>
<td>17</td>
<td>22</td>
<td>6</td>
<td>7.9</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>Rocky</td>
<td>10</td>
<td>3</td>
<td>3.75</td>
<td>3</td>
<td>3.75</td>
<td>1.25</td>
</tr>
<tr>
<td>2011</td>
<td>Tree-shrub</td>
<td>75</td>
<td>7</td>
<td>35</td>
<td>5</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

![Spatial distribution of *A. flavicollis* and *A. sylvaticus* in studied mosaic habitat](image_url)

**Fig. 1.** Spatial distribution of *A. flavicollis* and *A. sylvaticus* in studied mosaic habitat (white circles – adult female *A. flavicollis*, white circles intersected – pregnant females *A. flavicollis*, white squares – adult male *A. flavicollis*, black ellipses – adult female *A. sylvaticus*, black rhombs – adult male *A. sylvaticus*; the same symbols in bold and in gray – young individuals; circled points indicate interspecific overlapping), photo Google Earth.
The mouse species, present on this habitat was *M. macedonicus*, which is typical for Southwest Bulgaria (Popov, 2007, 2015). Only two specimens were captured in the lowest part of the hill, which can be explained with the nearby agroecosystems, its typical habitat.

Considering that aggressive residents limit settlement of migrants and survival of juveniles (Flowerdew, 1984; Gurnell, 1978) it could be assumed that in a mosaic landscape such as the present one, breeding sites will be scarce, which could explain the low number of juveniles registered and their presence in the less favourable rocky patches. This assumption was supported by recaptures of adult *A. flavicollis* in trap-points near the first ones in the tree-shrub patches, as well as the presence of pregnant females there. According to Wolff (1993) *Apodemus* females are territorial when breeding. In *A. sylvaticus*, the property of a nest site of good quality is also very important for the female’s fitness (Musolf, 2002).

One of the most important topics in ecology is the issue of species competition and coexistence (Polechová, Storch, 2008). Andrzejewski et al. (1978) found that in syntopic habitats with *A. sylvaticus*, *A. agrarius* reached high density, while *A. sylvaticus* remained poorly represented. In the absence of *A. agrarius*, *A. sylvaticus* occupied its niche (Yalden, 1980; Dickman, Doncaster, 1986). Since *A. flavicollis* was twice more numerous than *A. sylvaticus*, it could be assumed there are either slight differences in habitat selection between the two murid species or that the abundance of *A. sylvaticus* is affected by the presence of *A. flavicollis*. According to Kuncová, Frynta (2009) in Central Europe, the two species exhibit contrasting ecological strategies – *A. flavicollis* is forest-dweller, and *A. sylvaticus* exhibits less specialised requirements, reaching its maximal abundance in ecotones including forest margins, bushes, set aside fields, parks, etc. Using cranial measurements in a study on small mammal assemblages from North to South along the Bulgarian Black Sea coast Popov (2000) established that *A. flavicollis* was better represented in the southern area where the forests were the prevailing vegetation.

**Table 3.** Median and extreme values (min and max) of behaviour during dyadic encounters and interspecific significant differences revealed by Mann-Whitney *U*-test. The extreme values are given in brackets.

<table>
<thead>
<tr>
<th>Behavioural patterns</th>
<th>Male-male dyadic encounters</th>
<th>Female-female dyadic encounters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. sylvaticus</em></td>
<td><em>A. flavicollis</em></td>
</tr>
<tr>
<td>Offensive behaviour</td>
<td>2.0 (0-4.0)</td>
<td>4.0 (1.0-5.0)</td>
</tr>
<tr>
<td><em>U</em></td>
<td>Not significant, <em>U</em> = 6.0, <em>p</em> &gt; 0.05</td>
<td>Not significant, <em>U</em> = 7.0, <em>p</em> &gt; 0.05</td>
</tr>
<tr>
<td>Defensive behaviour</td>
<td>9.0 (6.0-17.0)</td>
<td>7.0 (5.0-9.0)</td>
</tr>
<tr>
<td><em>U</em></td>
<td>Not significant, <em>U</em> = 6.0, <em>p</em> &gt; 0.05</td>
<td>Not significant, <em>U</em> = 9.0, <em>p</em> &gt; 0.05</td>
</tr>
<tr>
<td>Amicable behaviour</td>
<td>2.0 (1.0-3.0)</td>
<td>2.0 (1.0-2.0)</td>
</tr>
<tr>
<td><em>U</em></td>
<td>Not significant, <em>U</em> = 9.5, <em>p</em> &gt; 0.05</td>
<td>Not significant, <em>U</em> = 10.5, <em>p</em> &gt; 0.05</td>
</tr>
<tr>
<td>Introductory behaviour</td>
<td>13.0 (9.0-18.0)</td>
<td>11.0 (9.0-15.0)</td>
</tr>
<tr>
<td><em>U</em></td>
<td>Not significant, <em>U</em> = 12.0, <em>p</em> &gt; 0.05</td>
<td>Not significant, <em>U</em> = 9.5, <em>p</em> &gt; 0.05</td>
</tr>
</tbody>
</table>
type. In Central Western Bulgaria the majority of adult wood mice also belong to \textit{A. flavicollis} (Minkova, Popov, 2002; Peshev et al., 2004) as well as in the Southwest part of the country (Popov, 2015), where the patterns of spatial distribution of the two species coincide with the findings in this work. Hence, it be could suggested that the studied habitat seems more favourable to \textit{A. flavicollis} than to \textit{A. sylvaticus}. At the same time, in the present study during dyadic encounters animals did not show high aggressiveness. The majority of the encounters were with approximately equal number of agonistic (offensive and defensive) events and amicable and introductory behaviour, suggesting indifference to the presence of the other species. This response is in contradiction with the presence of intraspecific aggressiveness found in both \textit{A. flavicollis} and \textit{A. sylvaticus} during the breeding period (Hoffmeyer, 1973; Montgomery, 1978; Gurnel 1978; Frynta et al., 1995; Čihákova, Frynta, 1996). In a previous study on interspecific social interactions between \textit{A. agrarius} and \textit{A. flavicollis} in a syntopic habitat Simeonovska-Nikolova (2007) found that mice of these two species showed avoidance to heterospecific odours from the same and the opposite sex in breeding season. The author suggested that the avoidance response to heterospecific odours could serve as a spacing mechanism to avoid aggressive encounters between two species. Based on these finding, it could be assumed that the level of interspecific competition in the studied populations of \textit{A. flavicollis} and \textit{A. sylvaticus} is not high or mice responded indifferently to individuals from the other species, because they had habituated to them in the habitats. Therefore, the sharing of the habitat by \textit{A. flavicollis} and \textit{A. sylvaticus} may be enhanced by the use of olfactory cues, which serve as a spacing mechanism for avoiding aggressive encounters, also established in cohabitation of various other rodents (Cassaing et al., 2013).

\textbf{CONCLUSION}

The present study expands knowledge about the distribution and habitat requirements of \textit{A. flavicollis} and \textit{A. sylvaticus} in their European range. \textit{A. flavicollis} was dominant and its number was almost double that of \textit{A. sylvaticus} in the studied habitat. This result corresponds with the findings of other authors (Popov, 2000, 2015; Minkova, Popov, 2002) for the distribution of both species in Central Western and Southwest Bulgaria, but based on morphometric analyses. The established spatial distribution suggests a habitat preference, similar to those of their conspecifics from Central Europe – \textit{A. flavicollis} prefers forest areas, while \textit{A. sylvaticus} – ecotones, including forest margins, bushes (Marsh, Harris, 2000, Frynta et al., 1994, Kuncová, Frynta, 2009). Because of the low interspecific overlapping and the relatively low aggressiveness, demonstrated by mice in the interspecific male-male and female-female dyadic encounters, it could be assumed that sharing of the habitat by both syntopic species may be enhanced by the use of olfactory cues, which could serve as a spacing mechanism to avoid aggressive encounters between them. Further studies in other types of habitats, seasons and ecological conditions are required to better understand the microhabitat segregation and interspecific relationships between \textit{A. flavicollis} and \textit{A. sylvaticus}. 
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