Humoral Immune Response of Overwintered Gray Red-Backed Voles (\textit{Myodes rufocanus bedfordiae}) under Cold Stress in Spring

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\section*{Summary}
Effects of low temperature on immune responses of non-overwintered autumn voles (experiment 1) and overwintered spring voles (experiment 2) were examined in gray red-backed voles (\textit{Myodes rufocanus bedfordiae}) under conditions without predators and food limitation. The gray red-backed voles (\textit{M. r. bedfordiae}) were sampled in the study area, a wind shelterbelt (Fure, Hokkaido: 44°18’N, 142°25’-142°28’E) with seasonality and strong direct and delayed density-dependent population fluctuations. The voles in two experiments were captured in the wind shelterbelt and were divided into two experimental groups (room temperature (23°C) and low temperature (5°C)). All voles were immunized with non-pathogenic antigens, sheep red blood cells (SRBCs). Immune responses against SRBCs were measured using a hemagglutination test. Immune responses of non-overwintered autumn voles (wild autumn voles) exposed to low temperature (5°C) decreased in comparison with those of wild autumn voles at room temperature (23°C). However, immune responses of overwintered spring voles (wild spring voles) exposed to low temperature (5°C) were similar to those of wild spring voles at room temperature (23°C). The wild spring voles showed superior immune response to the wild autumn voles at low temperature. These results would suggest that most of the overwintered voles might have inherently superior immunocompetence, tolerance to cold with inherent ability and/or with acclimation, and/or enough energy for mounting an adaptive immune response to survive over winter.

\textbf{Key words:} voles, immune response, survival strategy, over winter

\section*{Introduction}
Arvicoline rodent populations are known to exhibit seasonal fluctuations and to show stronger population decrease during winter. The gray red-backed voles (\textit{Myodes rufocanus bedfordiae}) show dramatic population fluctuations. Statistical and mathematical analyses have indicated that the population fluctuations exhibit direct and delayed density dependence\textsuperscript{11,12,20,21,24,25,26,27}. According to recent analyses of density dependence, the population fluctuations have exhibited seasonality and much stronger density dependence during winter in Hokkaido\textsuperscript{21,24,27,28,29}. Predator, food supply and pathogen are proposed as density-dependent factors of the density-dependent population decline, and climate (e.g. winter length) is proposed as a density-independent factor of the density-dependent population decline\textsuperscript{11,2,8,10}. Animals would show various strategies against vari-
ous surrounding factors (e.g., predator, food supply, pathogen, and winter length). Anti-predatory behavior for predators, decrease of body weight and suppression of growth and reproduction for winter conditions (food limitation and cold stress on short photoperiods), and formation of communal group for cold stress are known as survival strategies. Furthermore, superior immunocompetence against pathogens (e.g., virus and parasite) would be proposed as one of survival abilities.

Winter is energetically demanding, and the time of high thermoregulatory requirements for small mammals and birds generally coincide with the season when food availability decreases. Energy demands for maintenance of body condition and thermoregulation increase in winter. In life-history theory, a common assumption is that trade-offs exist between costs and benefits of various physiological functions. Recently, many researchers have focused on immune function as one of important functions for survival in animals. The immune function is one of the major physiological mechanisms regulating host survival. The stress of coping with energetically demanding conditions may indirectly cause illness and death by compromising immune function. Therefore, it is considered that immunocompetence for antigens (e.g., virus) may become one of survival abilities, and trade-offs exist between metabolism (including thermoregulation, maintenance of body condition, and activity) and immune function in an individual consisting of living cells (e.g., viruses depend on metabolism of living cells in hosts). In fact, Cichon et al. indicated that laboratory mice (Swiss mice) exposed to low temperature (5°C) had an inferior ability to mount a humoral immune response against a standard novel non-pathogenic antigen (sheep red blood cells (SRBCs)) and suggested that a trade-off between thermoregulation and immune function in laboratory mice. Similarly, Kusumoto and Saitoh found that the wild non-overwintered autumn gray red-backed voles (M. r. bedfordiae) exposed to low temperature (5°C) showed inferior immune responses to the wild non-overwintered autumn voles exposed to adequate conditions (23°C). However, it could be considered that immune responses of the wild overwintered spring gray red-backed voles (M. r. bedfordiae) experienced sever cold winter might differ from immune responses of the wild non-overwintered autumn voles under low temperature.

Here, immune responses of the wild non-overwintered autumn and the wild overwintered spring voles were investigated under experimental conditions without predators and food limitation in the gray red-backed voles (Myodes rufocanus bedfordiae) captured from area showed seasonality and strong direct and delayed density-dependence (Group2). This study will provide new knowledge for understanding mechanisms of density-dependent population decline during winter in the gray red-backed vole (M. r. bedfordiae).

Materials and Methods

1. Animals and Maintenance

All gray red-backed voles (Myodes rufocanus bedfordiae) used in the experiments
were collected from a wind shelterbelt in Furen, Hokkaido, Japan (44° 18’N, 142° 25’-142° 28’E) by using Sherman-type live traps. Twenty-three non-breeding voles were captured in September 2003 for the Experiment 1\(^\text{13,15}\) and eight non-breeding voles were captured in May 2006 for the Experiment 2. All the captured voles were housed individually in a transparent polymer X (TPX) cage (145 by 290 by 150 mm, SKL-109; Toyoriko, Japan) with 250 ml bedding materials (ALPHA-dri\(^\text{7th}\); Shepherd, USA) around 23°C with natural light, and were allowed free access to water and commercial foods (ZC-2; Funabashi Farms, Chiba, Japan for the Experiment 1, and ZF; Oriental Yeast, Tokyo, Japan for the Experiment 2). The difference of these commercial foods (ZC-2 and ZF) in the Experiment 1 and 2 was caused by production stoppage of ZC-2 because of the meat and bone meal. Furthermore, a handful of oats and/or sunflower seeds were additionally supplied for the first few days before beginning the experiments, because the voles had not become familiar with the commercial foods.

2. Experimental protocol

Two thermal conditions were set to examine the effects of cold stress on immune responses in non-overwintered autumn and overwintered spring gray red-backed voles (\(M. r. bedfordiae\)). The autumn and the spring voles were divided into two groups (low temperature and room temperature), respectively. Low temperature group (LT) was kept at 5°C according to previous study\(^9\). The low temperature (5°C) could be regarded as a non-fat al temperature for this species because of the temperature range from -2.4°C to +1.4°C under snow during winter (about five months) in Furen, Hokkaido. Room temperature group (RT) was kept at 23°C, because the room temperature (23°C) could be regarded as an optimal temperature for gray red-backed voles\(^16\). Both groups were kept in each incubator (FMU-130I; FUKUSIMA, Japan) on a 12L:12D light cycle (lights on at 0700). All voles were housed individually in a transparent polymer X (TPX) cage (145 by 290 by 150 mm, SKL-109; Toyoriko, Japan) with 250ml bedding materials (ALPHA-dri\(^\text{7th}\); Shepherd, USA) and were allowed free access to water and commercial foods (ZC-2; Funabashi Farms, Chiba, Japan for the Experiment 1, and ZF; Oriental Yeast, Tokyo, Japan for the Experiment 2). All cages were cleaned every day, and bedding materials were changed for new ones every day. All voles were allowed free access to commercial foods (ZC-2 in the Experiment 1, 2003; ZF in the Experiment 2, 2006) and water throughout the experiments.

**Experiment 1:**

The Experiment 1 was performed from September to October in 2003\(^\text{13,15}\). As mentioned above, twenty-three non-breeding voles (13 males and 10 females, 23.5g – 34.4g) were captured in September of 2003. The wild-caught autumn voles were divided into low temperature group (LT) and room temperature group (RT). The sex ratio and body weight were adjusted evenly between the two groups (LT and RT) in the Experiment (WALT voles; 7 males, 5 females, WART voles; 6 males, 5 females).

**Experiment 2:**

The Experiment 2 was performed from May to June in 2006. As mentioned above,
eight non-breeding voles (6 males and 2 females, 26.1g – 42.8g) were captured in May of 2006. The wild-caught spring voles were divided into low temperature group (LT) and room temperature group (RT). The sex ratio and body weight were adjusted evenly between the two groups (LT and RT) in the Experiment (WSLT voles; 4 males, 1 females, WSRT voles; 2 males, 1 females).

3. Body weight, Food intake, Riney’s Kidney Fat Index, and Weights of internal and lymphatic organs

All the gray red-backed voles (M. r. bedfordiae) were weighed daily with accuracy to 0.1 g by an electronic balance. Kidney and heart were weighed with accuracy to 0.01 g. The lymphatic organs (thymus and spleen) were weighed with accuracy to 0.001 g. Riney’s Kidney Fat Index (RKFI) was measured to compare fat reserves between the two groups (LT and RT) in the Experiment 1 and 2. Food remains were collected daily and were measured after drying at 105°C for 24 h in the Experiment 1 and 2. Food intakes were calculated for 8th-9th days and for 14th-15th days of the experiments. Those days correspond to the day just before immunization and the final day of the experiments, respectively. Those days may represent adequate body conditions of voles before and after immunization.

4. Immunization

The standard non-pathogenic antigen-sterile sheep red blood cells (Inter-Cell Technologies, Florida, America) with 20 μg•g⁻¹ body weight were injected into the peritoneal cavity of all gray red-backed voles (M. r. bedfordiae) on the 10th day of the experiments. The sheep red blood cells (SRBCs) were adjusted to 6 × 10⁶ cells mm⁻³ in phosphate-buffered saline. On the 6th day after immunization, the voles were killed by cervical dislocation. Blood was collected using an injector (1 ml or 2 ml) from a heart of the vole. The blood was incubated at 37°C for 2 h, and kept at room temperature for 1 h. After the blood was centrifuged at 2,500 rpm for 15 min, the serum was collected. The test serum was heat-inactivated at 56°C for 30 min. Antibody (IgM) production against SRBCs was assessed by a hemagglutination test, as described below. The simplest form of this test involves the agglutination of erythrocytes (as antigens) by increasing dilutions of anti-erythrocyte sera.

5. Hemagglutination test

A hemagglutination was performed with 96 well plates to measure antibody production (IgM) against SRBCs. First, 20 μl of phosphate-buffered saline (PBS) was added to all the test wells. Second, each test serum was made with a 1:2 dilution, and a 1:2 dilution was repeated to the end well in each test serum line. Third, 20 μl of 1% SRBCs suspension was added to all the test wells. The number of titers showing positive hemagglutination represents antibody production⁹. Titers refer to log₂ antibody concentrations.
6. Statistical analyses

JMP (SAS Institute Inc., Cary, North Carolina) was used for all analyses. In most analyses, T-test and Paired T-test were used to compare between the two when there were no correlations with body weight. ANOVA was used to compare the results among the experimental groups when there was no effect of body weight, and the ANCOVA was used for the data correlated with body weight. If the differences were significant among the experimental groups, they were analyzed using Tukey-Kramer HSD.

Result and discussion

In the Experiment 1\(^{15,19}\), body weights of the gray red-backed voles (*Myodes rufocanus bedfordiae*) did not differ between the two groups (wild autumn voles exposed to low temperature (WALT) and wild autumn voles kept at room temperature (WART)) at the beginning of the experiment (mean ± SE [g]: 29.8 ± 0.90 for WALT, 30.0 ± 0.94 for WART, T-test; \( t = 0.17, P = 0.864 \) for the autumn voles (WALT and WART). Body weights of WART significantly increased during the experimental period: from 30.0 ± 0.94 (mean ± SE [g]) to 33.0 ± 1.11 for the first 10 days (Paired T-test; \( t = 7.06, P < 0.001 \)); from 33.0 ± 1.11 to 34.0 ± 1.20 for 6 days after immunization (Paired T-test; \( t = 5.01, P < 0.001 \)). Body weights of WALT also increased from 29.8 ± 0.90 to 32.5 ± 1.18 for the first 10 days (Paired T-test; \( t = 4.94, P < 0.001 \)), but did not change thereafter (32.7 ± 1.23 at the end of the experiment, Paired T-test; \( t = 1.01, P = 0.336 \)). The difference in body weights between WALT and WART was not statistically significant at the end of the experiment (T-test; \( t = 0.73, P = 0.474 \)). Food intake of the wild autumn voles exposed to low temperature (WALT) was significantly higher than that of the wild autumn voles kept at room temperature (WART) in the both periods of 8th-9th days and 14th-15th days (Fig. 1; 8th-9th days [before immunization], mean ± SE [g]: 6.43 ± 0.21 for WALT, 9.80 ± 0.29 for WART, T-test, \( t = 8.99, P < 0.001 \); 14th-15th days [after immunization], 6.01 ± 0.25 for WART, 9.16 ± 0.26 for WALT, T-test, \( t = 8.68, P < 0.001 \)). Riney’s Kidney Fat Index (RKFI) of WALT was significantly lower than that of WART in the wild autumn voles (Table 1). Kidney and heart of WALT were significantly heavier than those of WART, respectively (Table 1). Spleen and thymus of WALT had similar weights to those of WART, respectively (Table 1). The results of food intake and internal organs would suggest that energy demands for maintenance of body condition and thermoregulation increase at low temperature\(^{11,16,17,31}\). Thus, the metabolic cost of the wild autumn voles exposed to low temperature (WALT) would become higher than that of the wild autumn voles kept at room temperature (WART). Furthermore, the wild autumn gray red-backed voles exposed to low temperature (WALT; \( n = 12 \)) showed inferior immune responses in comparison with the wild autumn voles kept at room temperature on a 12L:12D light cycle (WART; \( n = 11 \), Fig. 2; see also previous studies\(^{13,19}\)). There were significant differences of titers between the two groups, although there was not an effect of body weight (ANCOVA; \( F_{1.20} = 3.972, P = 0.035 \) for titers; \( F = 6.973, df = 1, P = 0.016 \) for group; \( F = 0.311, df = 1, P = 0.583 \) for body weight, ANOVA; \( F_{1.21} = 7.891, \)
Fig. 1. Food intakes before immunization (8th-9th days) and after immunization (14th-15th days) in gray red-backed voles (Myodes rufocanus bedfordiae) in laboratory experiments. White bar: wild autumn voles under 5°C [WALT]; black bar: wild autumn voles under 23°C [WART]; striped white bar: wild spring voles under 5°C [WSLT]; striped black bar: wild spring voles under 23°C [WSRT].

Table 1. Riney’s KFI and weights of internal and lymphatic organs of wild autumn [WA] and spring [WS] voles for two experimental groups (room temperature [RT] group and low temperature [LT] group). Riney’s KFI and weights of internal and lymphatic organs were compared between experimental groups by ANCOVA using body weights as covariance. F-value and probability (P) indicate effects of experimental group.

<table>
<thead>
<tr>
<th>Group</th>
<th>ANCOVA</th>
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<tbody>
<tr>
<td></td>
<td>RT [Mean ± SE]</td>
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<tr>
<td>Wild autumn voles</td>
<td></td>
</tr>
<tr>
<td>RKFI [%]</td>
<td>32.8±3.79</td>
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<tr>
<td>Kidney [g]</td>
<td>0.17±0.01</td>
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<tr>
<td>Heart [g]</td>
<td>0.15±0.01</td>
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<tr>
<td>Spleen [mg]</td>
<td>56±6.1</td>
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<tr>
<td>Thymus [mg]</td>
<td>15±2.4</td>
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<tr>
<td>Wild spring voles</td>
<td></td>
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<tr>
<td>RKFI [%]</td>
<td>18.4±5.54</td>
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<tr>
<td>Kidney [g]</td>
<td>0.18±0.00</td>
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<tr>
<td>Heart [g]</td>
<td>0.18±0.00</td>
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<tr>
<td>Spleen [mg]</td>
<td>106.0±16.3</td>
</tr>
<tr>
<td>Thymus [mg]</td>
<td>0.00±0.00</td>
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</tbody>
</table>

The samples sizes are shown as below: WALT (n=11), WALT (n=12), WSRT (n=3), and WSLT (n=5).

P = 0.011, Mean (SE): 1.56 (0.37) for WALT and 3.45 (0.58) for WART). The decreased humoral immune responses of the gray red-backed voles exposed to low temperature (WALT) were consistent with the research reported by Cichor et al. which the cold-stressed mice showed inferior humoral immune responses against a novel non-pathogenic antigen (sheep red blood cells) on a 12L:12D light cycle. In life-history theory,
it is assumed that trade-offs exist between costs and benefits of various physiological functions\(^{23,28,30,33}\). Cichoń et al.\(^{9}\) suggested the existence of a trade-off between thermoregulation and immune function in the laboratory mice (Swiss mice). Similarly, it could be considered that there is a trade-off between thermoregulation and immune function in the gray red-backed voles (*M. r. bedfordiae*).

In the Experiment 2, body weights of the gray red-backed voles (*M. r. bedfordiae*) did not differ between the two groups (wild spring voles exposed to low temperature (WSLT) and wild spring voles kept at room temperature (WSRT) at the beginning of the experiment (mean ± SE [g]; 37.0 ± 2.41 for WSLT, 35.1 ± 3.20 for WSRT, T-test; \(t = 0.484, P = 0.646\)). It was observed that body weights of WSLT and WSRT increased (Changes in body weights from 37.0 ± 2.41 to 39.7 ± 1.15 for WSLT, from 35.1 ± 3.20 to 37.2 ± 1.91 for WSRT, respectively) for the first 10 days, however, significant differences were not detected (Paired T-test; \(t = 1.24, P = 0.282\) for WSLT, \(t = 1.54, P = 0.263\) for WSRT). After then, changes in body weights were not significantly observed (Changes in body weights from 39.7 ± 1.15 to 39.2 ± 1.62 for WSLT, from 37.2 ± 1.91 to 37.4 ± 2.72 for WSRT, Paired T-test; \(t = 0.52, P = 0.632\) for WSLT, \(t = 0.30, P = 0.794\) for WSRT). At the end of the experiment, there was no significant difference in body weight between WSLT and WSRT (T-test; \(t = 0.602, P = 0.569\)). Food intake of WSLT exposed to cold stress was higher than that of WSRT kept at room temperature in the experiment (Fig. 1; 8th-9th days [before immunization], 9.35 ± 0.11 for WSRT, 13.16 ± 0.70 for WSLT, T-test, \(t = 4.06, P = 0.007\); 14th-15th days [after immunization], 9.38 ± 0.53 for WSRT, 13.12 ± 0.57 for WSLT, T-test, \(t = 4.41, P = 0.005\)). RKFI of WSLT was lower than that of WSRT in wild spring voles, although there was not a significant difference (Table 1). Kidney and heart of WSLT were significantly heavier than those of WSRT, respectively (Table 1). Spleen and thymus of WSLT had similar weights to ones of WSRT, respectively (Table 1). In the same as the wild non-overwintered autumn voles, their energy demands of the wild overwintered spring voles exposed to low temperature (WSLT) would become higher than that of the wild overwintered spring voles kept at room temperature (WSRT) for maintenance of body condition and thermoregulation at low temper-

![Fig. 2. Titers of immune responses against sheep red blood cells (SRBCs) in gray red-backed voles (*Myodes rufocanus bedfordiae*) in laboratory experiments. White bar: wild autumn voles exposed to 5°C [WALT]; black bar: wild autumn voles kept at 23°C [WART]; striped white bar: wild spring voles exposed to 5°C [WSLT]; striped black bar: wild spring voles at 23°C [WSRT]. Differences between experimental groups in September are significant (after Tukey-Kramer HSD test) if characters above bars are different.](image-url)
temperature\textsuperscript{11,16,17,33}, considering the increased food intake and the hypertrophied internal organs in WSLT. However, the spring voles exposed to low temperature (WSLT; \(n = 5\)) showed much the same immune response as the spring voles kept at room temperature (WSRT; \(n = 3\); Fig. 2). The mean titers (SE) of WSLT and WSRT were 6.20 (0.73) and 5.00 (0.00), respectively (ANCOVA, \(F_{x,5} = 0.796, P = 0.501\) for titers; \(F = 1.539, df = 1, P = 0.270\) for group; \(F = 0.274, df = 1, P = 0.623\) for body weight, ANOVA; \(F_{1,6} = 1.500, P = 0.267\)).

In comparison among the four groups (WALT, WART, WSLT, and WSRT), the body weights significantly differed among the four groups. The body weight of the wild spring voles (WSLT) was significantly heavier than those of the wild autumn voles (WALT and/or WART) (ANOVA; \(F_{x,27} = 5.90, P = 0.003\) for four groups at the beginning of the experiments, Tukey-Kramer HSD; \(P = 0.006\) between WSLT and WALT, \(P = 0.009\) between WSLT and WART, ANOVA; \(F_{x,27} = 5.70, P = 0.004\) at the immunization, Tukey-Kramer HSD; \(P = 0.005\) between WSLT and WALT, \(P = 0.010\) between WSLT and WART, ANOVA; \(F_{x,27} = 3.47, P = 0.030\) at the end of the experiments, Tukey-Kramer HSD; \(P = 0.030\) between WSLT and WALT). The food intakes significantly differed among the four groups, that is, food intake of WSLT was the most, and that of WART was the least, and those of WALT and WSRT showed much the same value (Fig. 1; ANCOVA; \(F_{x,27} = 74.30, P < 0.001\) for 8th-9th days, \(F_{x,27} = 109.2, P < 0.001\) for 14th-15th days). In RKKFI, there were significant differences among the four groups (ANCOVA; \(F_{x,36} = 5.427, P = 0.003; F = 7.171, df = 3, P = 0.001\) for group; \(F = 2.357, df = 1, P = 0.137\) for body weight). The Riney's Kidney Fat Index of WART was higher than those of WSLT and WALT (Table 1, ANOVA; \(F_{x,27} = 6.142, P = 0.003\), Tukey-Kramer HSD; \(P = 0.006\) between WART and WSLT, \(P = 0.008\) between WART and WALT). The weights of kidneys significantly differed among the four groups (ANOVA; \(F_{x,36} = 14.74, P < 0.001; F = 11.04, df = 3, P < 0.001\) for group; \(F = 14.83, df = 1, P < 0.001\) for body weight). The kidneys of WALT and WSLT were significantly heavier than those of WART and WSRT (Table 1). There were significant differences among the four groups in their hearts (ANOVA; \(F_{x,27} = 10.32, P < 0.001; F = 11.84, df = 3, P < 0.001\) for group; \(F = 3.322, df = 1, P = 0.081\) for body weight; \(F = 3.444, df = 3, P = 0.033\) for group \(\times\) body weight). The hearts of WALT and WSLT were significantly heavier than that of WART (Table 1). The weights of spleen differed among the four groups. The spleen of WSLT was heaviest among the four groups (ANOVA; \(F_{x,27} = 4.159, P = 0.015\); Tukey-Kramer HSD; \(P = 0.021\) between WSLT and WART, \(P = 0.030\) between WSLT and WALT, ANCOVA; \(F_{x,36} = 3.300, P = 0.026; F = 2.258, df = 3, P = 0.105\) for group; \(F = 0.800, df = 1, P = 0.379\) for body weight). There were significant differences among the four groups, the thymus of WART was heaviest, and the thymuses of WSRT and WSLT were small (Table 1; ANCOVA; \(F_{x,36} = 4.586, P = 0.006; F = 6.080, df = 3, P = 0.003\) for group; \(F = 5.508, df = 1, P = 0.027\) for body weight). Immune response of the wild overwintered spring voles exposed to low temperature (WSLT) was significantly higher than those of the wild non-overwintered autumn voles (WALT and WART) kept at low or room temperature (Fig. 2; ANCOVA; \(F_{x,36} = 8.702, P < 0.001\) for titers; \(F = 8.104, df = 3, P < 0.001\) for
group; \( F = 0.087, \text{df} = 1, P = 0.771 \) for body weight. ANOVA; \( F_{1,27} = 11.98, P < 0.001 \), Tukey-Kramer HSD; \( P < 0.001 \) between WSLT and WALT, \( P = 0.010 \) between WSRT and WALT, \( P = 0.015 \) between WSLT and WART, \( P = 0.034 \) between WART and WALT. As mentioned above, the wild non-overwintered autumn gray red-backed voles (\( M. \ r. \ bedfordiae \)) exposed to low temperature (5°C) showed inferior immune response compared to the wild non-overwintered autumn voles at room temperature (23°C) on a 12L:12D light cycle (Fig. 2; see also previous studies\(^{12,15}\)) and their internal organs were hypertrophied at low temperature (5°C). However, interestingly, the wild overwintered spring gray red-backed voles (\( M. \ r. \ bedfordiae \)), i.e. the spring voles experienced severe cold winter, exposed to low temperature (5°C) on a 12L:12D light cycle showed significantly superior immune response in comparison with the wild non-overwintered autumn voles at low temperature (5°C) on a 12L:12D light cycle and the wild non-overwintered autumn voles at room temperature (23°C) on a 12L:12D light cycle, although the internal organs of the wild overwintered spring voles exposed to low temperature (5°C) were hypertrophied compared to those of the wild overwintered spring voles kept at room temperature (23°C). These results would suggest that most of the survived voles over winter should have high survival abilities. Therefore, there is a possibility that most of the wild spring voles survived over winter would have inherently superior immunocompetence (e.g., MHC type\(^3\)), tolerance of low temperature with inherent ability and/or with acclimation, and/or enough energy for mounting an adaptive immune response. In the future, it would be necessary to verify these suggestions.

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References


エゾヤチネズミ（*Myodes rufocanus bedfordiae*）における
越冬した春の個体の体液性免疫反応

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（動物行動生態学教室）
平成26年11月10日 受理

摘　要

エゾヤチネズミ（*Myodes rufocanus bedfordiae*）において、補食および餌制限のない条件下で、越冬していない当年秋の非繁殖個体（実験 1）および越冬した春の個体（実験 2）の免疫反応における低温の効果を調べた。エゾヤチネズミは、北海道風連町に位置する防風林（44°N, 142°25'-142°28'E）で捕獲された。捕獲されたエゾヤチネズミは、2つの実験区（室温区（23℃）および低温区（5℃））に分けられた。実験に使用された全てのエゾヤチネズミは、病原性のない羊赤血球（SRBCs）で免疫された。SRBCs に対する免疫反応を赤血球凝集反応法を用いて測定した。低温にさらされた越冬していない当年秋の非繁殖個体の免疫反応は、室温に維持された越冬していない当年秋の非繁殖個体よりも低かった。しかしながら、低温にさらされた越冬した春の非繁殖個体の免疫反応は、室温に維持された越冬した春の非繁殖個体と似た免疫反応を示し、低温にさらされた越冬していない当年秋の非繁殖個体よりも高い免疫反応を示した。