Contrasting effects of large density changes on relative testes size in fluctuating populations of sympatric vole species

Ines Klemme, Carl D. Soulsbury and Heikki Henttonen

1Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, 40014 Jyväskylä, Finland
2School of Life Sciences, University of Lincoln, Riseholme Park Campus, Lincoln, UK
3Finnish Forest Research Institute, Vantaa Unit, Vantaa, Finland

Across species, there is usually a positive relationship between sperm competition level and male reproductive effort on ejaculates, typically measured using relative testes size (RTS). Within populations, demographic and ecological processes may drastically alter the level of sperm competition and thus, potentially affect the evolution of testes size. Here, we use longitudinal records (across 38 years) from wild sympatric Fennoscandian populations of five species of voles to investigate whether RTS responds to natural fluctuations in population density, i.e. variation in sperm competition risk. We show that for some species RTS increases with density. However, our results also show that this relationship can be reversed in populations with large-scale between-year differences in density. Multiple mechanisms are suggested to explain the negative RTS–density relationship, including testes size response to density-dependent species interactions, an evolutionary response to sperm competition levels that is lagged when density fluctuations are over a certain threshold, or differing investment in pre- and post-copulatory competition at different densities. The results emphasize that our understanding of sperm competition in fluctuating environments is still very limited.

1. Introduction

Females of many species mate with multiple males within a single reproductive cycle, providing the conditions for post-copulatory sexual selection [1]. In particular, when the sperm of two or more males compete for fertilization (sperm competition), selection acts on a number of ejaculate traits that enhance competitive success [2,3]. Such traits have been shown to be sperm size, motility, longevity and morphology (reviewed in [4]). Moreover, investment in the number of sperm is a key predictor of success in sperm competition [5], which typically manifests itself as increased investment in testicular tissue—the site of spermatogenesis. Indeed, there is considerable evidence of increased sperm competition and larger testes across multiple taxa using both behavioural [6–8] and genetic mating systems [9,10].

In nature, there can be substantial variation in the degree of sperm competition, both between populations [11] and temporally within populations [12]. Empirical investigations have shown that increasing levels of sperm competition will favour selection for larger testes in invertebrates [13,14] and greater testicular activity in mammals [15]. However, evidence for natural within-population temporal variation in sperm competition risk and testicular size is limited, within only a single long-term study (9 years of data) of deer mice (Peromyscus maniculatus), showing that testes size correlated positively with density [16]. Large changes in density can affect competition not only for reproduction, but also for other resources such as space and food. These in turn, could potentially feedback and alter the ability of males to invest in reproductive tissue.

†Joint first author.
Thus, knowing whether testes size can respond to rapid and large natural changes in sperm competition is an important evolutionary question.

Here, we investigated whether relative testes size (RTS) responds to natural fluctuations in density of wild promiscuous arvicoline rodents. In these populations, density is extremely variable, undergoing population cycles of 3–5 years [17]. Population density is likely to be directly linked to sperm competition as both inter- and intraspecific home ranges overlap [18,19] and multiple paternity rates increase at high densities [20,21]. Using longitudinal records (across 38 years) of testes size from five species of voles, which have undergone variation in the magnitude of population size and changes in the patterns of cyclicity [22,23], we tested whether RTS was correlated to: (i) current spring densities, i.e. current sperm competition risk; and to (ii) previous year’s spring densities, i.e. lagged response in sperm competition risk. We predicted that differences in the scale of fluctuations would alter the relationship between RTS and density. Our results showed that testes size responds to increases in sperm competition in some species, but is constrained in responding to large population fluctuations in others.

2. Material and methods

(a) Study site and capture methods

Vole trapping was conducted in the Pallasjärvi area (68°30’ N, 24°09’ E) in western Finnish Lapland (for details, see [23,24]). Voles were trapped in three main habitats, i.e. old taiga forests, peatlands and clear cuts. Each main habitat contained 30 permanent trapping quadrats of 15 x 15 m with three snap traps at each corner. In old taiga forests and peatland habitats, trapping quadrats were set in groups of three to seven with a minimum distance between quadrats of 50 m. The distance between the nearest groups was 0.2–2.1 km (mean ± s.e., 1.1 ± 0.2). All quadrats in clear cut habitats were set in one large clear cut area. Trapping was carried out biannually in spring/early summer, at the beginning of the breeding season (late May–mid June) and in autumn, at the end of or after the breeding season (September) between 1970 and 2008. We calculated the number of voles trapped per 100 trap nights as an index of density.

We collected data on five vole species from two genera; the field vole (Microtus agrestis L.), the root/tundra vole (Microtus oeconomus Pall.), the bank vole (Myodes (formerly Clethrionomys) glareolus Schreb.), the grey-sided vole (Myodes rufocanus Sund.) and the red vole (Myodes rutilus Pall.). For all species, there is evidence for a multi-male mating system [25–29].

(b) Testes size

Snap-trapped males were taken to the laboratory, dissected and the length of the right testis was measured with a ruler to 0.5 mm precision. Typically, testes size is estimated via testes mass (e.g. [7,8,10]), but see, e.g. [30] for a study using testes length, but we show that length is also a reliable measure of testes size in all five species studied: in a subsample of our data, length and mass of the right testis (mg) were measured from individual males and found to be correlated (see Results). Further, in deer mice, length of one testis is a good proxy for mass of both testis [31]. Body mass in overwintered voles in all species increases with increasing density in a process known as the Chitty effect [32,33], hence testes size could be increasing through simple allometry [34]. To account for this, we used log testes length as the dependent variable and entered body mass as a covariate into the models (RTS). At this latitude, mature males regress their testes before the onset of winter, but rarely survive the winter. All males born late in the breeding season delay their maturation until the following spring, while males born early in the breeding season mature at the age of three to four weeks and have smaller testes size than overwintered males. The spring trapping took place before the young of the year entered the populations, and in few cases with early breeding, young males were easy to identify on the basis of juvenile and early post-juvenile fur and moulting patterns. Thus, we included only overwintered adult voles trapped during spring, and as rarely testicular abnormalities were found, we included only testes length more than 8 mm [35]. Data on testis size were measured between 1970 and 2008. In some years, vole density was 0 (table 1).

(c) Statistical analyses

All analyses were carried out in R v. 2.14.1 (R development Core Team 2011). Voles have undergone temporal changes in patterns

---

Table 1. The temporal patterns in between-year changes of population density (voles per 100 trap nights) for which all positive and negative changes were combined. (Owing to temporal changes in patterns of cyclicity ca mid-1980s, and species-specific temporal variation in mean autumn density change, we analysed the relationship between density and RTS either across the whole 38 years (My. glareolus, My. rutilus and Mi. agrestis) or separately for time periods with large-scale and small-scale between-year differences (Mi. oeconomus and My. rufocanus). n = total samples of adult males used in the analyses. Years with testes size data = number of years in which testes size was measured.)

<table>
<thead>
<tr>
<th>species</th>
<th>years</th>
<th>mean ± s.e. autumn density change</th>
<th>years with testis size data</th>
</tr>
</thead>
<tbody>
<tr>
<td>My. glareolus (n = 1065)</td>
<td>1970–1985</td>
<td>8.70 ± 1.95</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>1986–2008</td>
<td>7.60 ± 1.37</td>
<td>19</td>
</tr>
<tr>
<td>My. rutilus (n = 267)</td>
<td>1970–1985</td>
<td>3.75 ± 0.99</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1986–2008</td>
<td>2.34 ± 0.99</td>
<td>15</td>
</tr>
<tr>
<td>Mi. agrestis (n = 200)</td>
<td>1970–1979</td>
<td>6.11 ± 1.88</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1980–2008</td>
<td>2.16 ± 0.18</td>
<td>19</td>
</tr>
<tr>
<td>Mi. oeconomus (n = 157)</td>
<td>1970–1989</td>
<td>2.64 ± 0.65</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1990–2008</td>
<td>0.56 ± 0.26</td>
<td>12</td>
</tr>
<tr>
<td>My. rufocanus (n = 507)</td>
<td>1970–1985</td>
<td>3.04 ± 1.00</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1986–2008</td>
<td>0.51 ± 0.12</td>
<td>15</td>
</tr>
</tbody>
</table>
of cyclicity. The characteristic 4 year vole cycles in the Pallasjärvi area prevailed during 1970—ca 1985, but underwent drastic changes in the mid-1980s [23,24]. The population dynamics of some species turned much more stable (multiannual cyclicity lost), and some species became generally much less common (figure 1) [23]. However, because we were interested in the amplitude of population changes regardless of cyclicity patterns, we quantified the variation in population density based on the magnitude of population change as the mean ± s.e. change between years (table 1). Three species retained strong large-scale between-year differences in density throughout the time series (table 1 and figure 1) and thus, the relationship between density and RTS was analysed across the whole 38 years. Because the other two species had distinct periods with both large-scale and small-scale between-year differences (table 1 and figure 1), the relationship between RTS and density was analysed separately for both time periods. For each species, we considered the effects of (i) current spring density and (ii) previous spring density. Current spring densities were significantly correlated with previous autumn densities (My. glareolus: $r = 0.56$, $p = 0.002$; My. rutilus: $r = 0.77$, $p < 0.001$; Mi. oeconomus: $r = 0.86$, $p < 0.001$; Mi. agrestis: $r = 0.70$, $p < 0.001$; My. rufocanus: $r = 0.80$, $p < 0.001$), but not with previous spring density (My. glareolus: $r = 0.19$, $p = 0.260$; My. rutilus: $r = 0.04$, $p = 0.818$; Mi. oeconomus: $r = 0.25$, $p = 0.123$; Mi. agrestis: $r = 0.14$, $p = 0.402$; My. rufocanus: $r = 0.26$, $p = 0.111$).

We first carried out a cross-species comparison of allometric relationships using standardized major axis regression (SMA). We tested the relationship between log testes mass and log body mass, log testes length and log body mass, and log testes mass and log testes length. For each model, we tested for species-specific differences in slopes and intercepts with post hoc pairwise analyses where significant. SMA was carried out using the sma function from the R package smatr [36].

For each species, we built linear models with log testes length as a dependent variable, log body mass as a covariate and density as a fixed factor. For each density model (current spring density and previous spring density), we compared the model with and without the interaction using likelihood ratio tests; we report results for the model without interaction if the interaction model is not a significant improvement. Since period and density changes are non-independent, for the two species with marked reductions in density changes (Mi. oeconomus and My. rufocanus), we considered the two periods separately. For all models, we calculated effect sizes and non-central 95% confidence intervals [37].

3. Results

(a) Density change and population cycles

Even though all vole species showed a decline in multiannual cyclicity (figure 1), My. glareolus (figure 1a), My. rutilus (figure 1b) and Mi. agrestis (figure 1c) retained large-scale between-year differences in density across the dataset (table 1). By contrast, Mi. oeconomus and My. rufocanus showed two distinct periods with much lower variability in densities in the second period (figure 1d,e and table 1).

(b) Interspecific differences in allometric relationships

There were species-specific differences in the slopes of relationship between testes mass and body mass (likelihood ratio test (LRT) = 27.08, $p < 0.001$) and in intercepts (Wald $\chi^2 = 1173$, $p < 0.001$). Post hoc pairwise analyses showed that slopes of lines for Myodes spp. appeared to be steeper than Microtus spp., although there were also within-genus differences (electronic supplementary material, figure S1). Similarly, the relationship between testes length and body mass differed significantly between species (slope: LRT = 60.06, $p < 0.001$; intercepts: Wald $\chi^2 = 5414$, $p < 0.001$), with post hoc pairwise

Figure 1. Line charts showing spring and autumn densities (voles per 100 trap nights) from 1970 to 2008 in (a) My. glareolus, (b) My. rutilus, (c) Mi. agrestis, (d) Mi. oeconomus and (e) My. rufocanus.
analyses showing no differences between species within either the *Myodes* genus or the *Microtus* genus, but significant difference between genera (figure 2).

By contrast, the relationship between testes mass and testes length was isometric between species (slope: LRT = 0.97, \( p = 0.91 \)), but there were species-differences in intercepts (Wald \( \chi^2 = 46.98, p < 0.001 \)), mainly driven by species-differences in body mass. For all species, testes mass was significantly related to testes length (\( My. \ glareolus: n = 116, R^2 = 0.22, p < 0.001 \); \( My. \ rufocanus: n = 267, R^2 = 0.41, p < 0.001 \); \( My. \ rutilus: n = 35, R^2 = 0.21, p = 0.005 \); \( Mi. \ agrestis: n = 26, R^2 = 0.71, p < 0.001 \); \( Mi. \ oeconomus: n = 31, R^2 = 0.65, p < 0.001 \); electronic supplementary material, figure S2).

### (c) Density, relative testes size and large amplitudes of population change

For *My. glareolus*, the best model included the interaction between body mass and density, for both current and past density models (electronic supplementary material, table S1). The interaction was not the best model for all other species. For three species (*My. glareolus, Mi. agrestis* and *Mi. oeconomus*), there was a significant negative relationship between RTS and current spring density (table 2 and figure 3A, C, D). By contrast, both *My. rutilus* and *My. rufocanus* had a positive relationship between RTS and current density (figure 3B, E).

Relationships between RTS and past density were similar; there was a significant negative relationship between RTS and past density in *My. glareolus* and *Mi. agrestis*, but not *Mi. oeconomus* (table 2). By contrast, *My. rutilus* showed a significant negative relationship with previous spring density (table 2). There remained a significant positive relationship between RTS and previous spring density in *My. rufocanus* (table 2), but the strength of the relationship was greater than for current spring density.

### (d) Density, relative testes size and small amplitudes of population change

During the small population variability phase, *Mi. oeconomus* showed a significant positive relationship between RTS and current spring density, but a significant negative relationship with past spring density (table 2). For *My. rufocanus*, there was a weak positive trend with current and past density (table 2).

### 4. Discussion

A positive relationship between sperm competition level and gonadal investment has often been demonstrated across taxa and populations [6–11,13,14,39], but within-population studies are rare. A single study on a population showed a positive relationship with testes size in deer mice [16]. However, our long-term multi-species data on fluctuating populations suggest that the relationship between density and RTS is somewhat more complex. During periods with small-scale differences in density, there was a positive relationship between RTS and current spring density in 2 out of 2 species. By contrast, during periods with large-scale differences in density only 2 out of 5 species showed a positive relationship of current density and RTS, while the other three species showed a negative relationship. Relationships between RTS and past density were similar, though a positive relationship was found in only 1 out of 5 species during periods with large population fluctuations and 1 out of 2 species during small population fluctuations. Thus, our data partly support theoretical models that predict increased investment in testicular tissue in response to increases in sperm competition [40], but also show that this relationship can be reversed when populations fluctuate strongly in density.

We found genera-specific relationships between testes size and body mass that are indicative of higher RTS in *Myodes* compared with *Microtus* species. Owing to differences in the spatio-social organization between genera, *Myodes* voles may face higher levels of sperm competition. Males of *Mi. agrestis* and *Mi. oeconomus* defend exclusive intrasexual territories that overlap with several smaller female home ranges [41,42], leading to female-biased operational sex ratios [41,43]. By contrast, male home ranges of *Myodes* voles overlap extensively with both several conspecific males and intrasexually territorial females [44]. Thus, although all studied species are polyandrous, males may be better able to monopolize females in *Microtus* spp. compared with males in *Myodes* spp. In addition, sperm competition levels may increase with density at a faster rate in *Myodes* voles, because intrasexual range overlap increases with density [45].

By contrast, the observed differences in the relationship of testes size and density were not genera specific, and there are at least three possible explanations as to why the species responded differentially. First, testis size may not only be affected by sperm competition level, but also by a density-dependent rise in competition for other resources, such as space and food. Our site of study is characterized by an exceptionally high number of co-occurring arvicoline species (\( n = 8 \)) that face competitive interactions [46,47]. During peak phases of synchronous population density oscillations, the habitats of these species increasingly overlap causing not only an increase in intraspecific, but also in interspecific interactions [48]. Earlier studies on *Microtus* and *Myodes* voles have shown that stress hormone levels increase with density [49–51], probably owing to a rise in intrinsic social stress. This may feedback negatively on reproductive physiology and thus, may affect testes growth. Given that
Table 2. $\beta \pm$ s.e., $t$- and $p$-values for linear models for the relationship between testes length and body mass and current or past density. (Results are shown for periods with large and small density changes separately. Effect sizes ($r$) and 95% confidence intervals (CI) are shown. Conventions for effect sizes: small effect $r = 0.10$, medium effect $r = 0.30$, large effect $r = 0.50$ [38]. For full 38 year dataset: Mi. oeconomus (body mass: estimate $= 0.17 \pm 0.02$, $t = 6.88, p < 0.001$; current density $= -0.05 \pm 0.00, t = -10.95, p < 0.001$) and My. rufocanus (body mass: estimate $= 0.14 \pm 0.0, t = 5.75, p < 0.001$; current density: estimate $= 0.00 \pm 0.00, t = 2.31, p = 0.02$.)

<table>
<thead>
<tr>
<th>population phase</th>
<th>species</th>
<th>variable</th>
<th>$\beta$</th>
<th>s.e.</th>
<th>$t$</th>
<th>$p$-value</th>
<th>$r$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>large density changes</td>
<td><strong>My. glareolus</strong></td>
<td>body mass</td>
<td>0.51</td>
<td>0.05</td>
<td>12.10</td>
<td>&lt;0.001</td>
<td>0.35</td>
<td>(0.30/0.40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current density</td>
<td>0.12</td>
<td>0.02</td>
<td>5.46</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>(0.11/0.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>body mass × current density</td>
<td>-0.04</td>
<td>0.01</td>
<td>-5.54</td>
<td>&lt;0.001</td>
<td>-0.17</td>
<td>(-0.22/-0.11)</td>
</tr>
<tr>
<td></td>
<td><strong>My. glareolus</strong></td>
<td>body mass</td>
<td>0.37</td>
<td>0.03</td>
<td>11.82</td>
<td>&lt;0.001</td>
<td>0.35</td>
<td>(0.29/0.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>past density</td>
<td>0.05</td>
<td>0.02</td>
<td>2.54</td>
<td>0.011</td>
<td>0.08</td>
<td>(0.02/0.14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>body mass × past density</td>
<td>-0.02</td>
<td>0.01</td>
<td>-2.68</td>
<td>0.007</td>
<td>-0.08</td>
<td>(-0.14/-0.02)</td>
</tr>
<tr>
<td></td>
<td><strong>My. rubinus</strong></td>
<td>body mass</td>
<td>0.10</td>
<td>0.04</td>
<td>2.83</td>
<td>&lt;0.001</td>
<td>0.17</td>
<td>(0.05/0.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current density</td>
<td>0.01</td>
<td>0.00</td>
<td>5.31</td>
<td>&lt;0.001</td>
<td>0.31</td>
<td>(0.20/0.41)</td>
</tr>
<tr>
<td></td>
<td><strong>My. rubinus</strong></td>
<td>body mass</td>
<td>-0.15</td>
<td>0.04</td>
<td>-4.32</td>
<td>&lt;0.001</td>
<td>0.26</td>
<td>(0.14/0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>past density</td>
<td>-0.01</td>
<td>0.00</td>
<td>-2.85</td>
<td>0.004</td>
<td>-0.17</td>
<td>(-0.28/-0.05)</td>
</tr>
<tr>
<td></td>
<td><strong>Mi. agrestis</strong></td>
<td>body mass</td>
<td>0.26</td>
<td>0.03</td>
<td>8.47</td>
<td>&lt;0.001</td>
<td>0.52</td>
<td>(0.41/0.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current density</td>
<td>-0.01</td>
<td>0.00</td>
<td>-3.76</td>
<td>&lt;0.001</td>
<td>-0.26</td>
<td>(-0.38/-0.12)</td>
</tr>
<tr>
<td></td>
<td><strong>Mi. agrestis</strong></td>
<td>body mass</td>
<td>0.23</td>
<td>0.03</td>
<td>7.74</td>
<td>&lt;0.001</td>
<td>0.48</td>
<td>(0.37/0.57)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>past density</td>
<td>-0.01</td>
<td>0.00</td>
<td>-2.64</td>
<td>&lt;0.001</td>
<td>-0.18</td>
<td>(-0.31/-0.05)</td>
</tr>
<tr>
<td></td>
<td><strong>Mi. oeconomus</strong></td>
<td>body mass</td>
<td>0.15</td>
<td>0.03</td>
<td>4.97</td>
<td>&lt;0.001</td>
<td>0.48</td>
<td>(0.30/0.61)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current density</td>
<td>-0.02</td>
<td>0.01</td>
<td>-3.39</td>
<td>&lt;0.001</td>
<td>-0.35</td>
<td>(-0.51/-0.15)</td>
</tr>
<tr>
<td></td>
<td><strong>Mi. oeconomus</strong></td>
<td>body mass</td>
<td>0.12</td>
<td>0.03</td>
<td>3.53</td>
<td>&lt;0.001</td>
<td>0.36</td>
<td>(0.16/0.52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>past density</td>
<td>-0.01</td>
<td>0.01</td>
<td>-0.83</td>
<td>0.408</td>
<td>-0.09</td>
<td>(-0.29/0.13)</td>
</tr>
<tr>
<td></td>
<td><strong>My. rufocanus</strong></td>
<td>body mass</td>
<td>0.23</td>
<td>0.03</td>
<td>9.13</td>
<td>&lt;0.001</td>
<td>0.40</td>
<td>(0.32/0.47)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current density</td>
<td>0.01</td>
<td>0.00</td>
<td>5.35</td>
<td>&lt;0.001</td>
<td>0.25</td>
<td>(0.16/0.33)</td>
</tr>
<tr>
<td></td>
<td><strong>My. rufocanus</strong></td>
<td>body mass</td>
<td>0.30</td>
<td>0.02</td>
<td>12.24</td>
<td>&lt;0.001</td>
<td>0.50</td>
<td>(0.43/0.56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>past density</td>
<td>0.03</td>
<td>0.00</td>
<td>11.00</td>
<td>&lt;0.001</td>
<td>0.46</td>
<td>(0.39/0.53)</td>
</tr>
</tbody>
</table>

(Continued.)
Table 2. (Continued)

<table>
<thead>
<tr>
<th>population phase</th>
<th>species</th>
<th>variable</th>
<th>$\beta$</th>
<th>s.e.</th>
<th>$t$</th>
<th>p-value</th>
<th>$r$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>small density changes</td>
<td>$Mi. oeconomus$</td>
<td>body mass</td>
<td>0.16</td>
<td>0.03</td>
<td>4.77</td>
<td>$&lt;0.001$</td>
<td>0.51</td>
<td>(0.30/0.64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>current density</td>
<td>0.18</td>
<td>0.08</td>
<td>2.11</td>
<td>0.038</td>
<td>0.25</td>
<td>(0.01/0.444)</td>
</tr>
<tr>
<td>$Mi. oeconomus$</td>
<td>body mass</td>
<td>0.13</td>
<td>0.03</td>
<td>3.96</td>
<td>$&lt;0.001$</td>
<td>0.43</td>
<td>(0.22/0.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>past density</td>
<td>-0.21</td>
<td>0.06</td>
<td>-3.82</td>
<td>$&lt;0.001$</td>
<td>-0.42</td>
<td>(-0.58/-0.21)</td>
<td></td>
</tr>
<tr>
<td>$My. rufocanus$</td>
<td>body mass</td>
<td>0.00</td>
<td>0.06</td>
<td>0.07</td>
<td>0.948</td>
<td>0.12</td>
<td>(-0.12/0.33)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>current density</td>
<td>0.05</td>
<td>0.03</td>
<td>1.70</td>
<td>0.094</td>
<td>0.20</td>
<td>(-0.04/0.41)</td>
<td></td>
</tr>
<tr>
<td>$My. rufocanus$</td>
<td>body mass</td>
<td>0.03</td>
<td>0.06</td>
<td>0.46</td>
<td>0.648</td>
<td>0.08</td>
<td>(-0.16/0.30)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>past density</td>
<td>0.02</td>
<td>0.01</td>
<td>1.47</td>
<td>0.145</td>
<td>0.18</td>
<td>(-0.06/0.39)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Surface plot showing the relationship between log testes length (mm), log body mass (g) and current density (voles per 100 trap nights) in (a) $My. glareolus$, (b) $My. rutilus$, (c) $Mi. agrestis$, (d) $Mi. oeconomus$ and (e) $My. rufocanus$. (Online version in colour.)
breeding males of both *Microtus* species studied here are strictly territorial, stress levels are particularly expected to rise with density when competition for territories increases. Indeed, a study on two *Microtus* species showed that aggressive behaviour of voles changed during the population cycles, with males from peak populations being most aggressive [52]. In addition to space deprivation, high-density vole populations can also face food deprivation beyond levels that cause malnutrition (e.g. [53,54]). This in turn induces poor physiological condition of individuals [55] and thus may significantly affect investment in reproductive tissues. For example, studies on wild rabbit (*Oryctolagus cuniculus*) populations that differ in food quality showed significant differences in testes development [56]. Furthermore, in insects food deprivation has been shown to negatively affect testis mass [57] and sperm production [58,59]. Among all *Myodes* species studied here, *My. glareolus* showed the highest abundances (figure 1) as well as greater RTS. Thus, intraspecific competition is expected to be most intense, potentially explaining the negative relationship between RTS and density in *My. glareolus*.

A second explanation could be that there is differing investment in pre- and post-copulatory competition at different densities [60]. For example, in meadow voles (*Microtus pennsylvanicus*) mate guarding may be more effective at acquiring reproductive success at high densities, placing emphasis on greater male body mass rather than spermatogenic tissue [61]. In *Mi. agrestis* too, the ability to defend a territory depends solely on the ability to fight and is thus probably related to body size [44]. Male *My. glareolus*, on the other hand, regulate dominance hierarchies mostly through social odours [44], and males with greater body mass are more successful when sperm competition levels are low (female-biased operational sex ratio) compared with situations with strong sperm competition (male-biased operational sex ratio) [62]. Hence, investment in pre- and post-copulatory tactics may vary with sperm competition risk and possibly between species. However, given that we found no genera-specific difference in the relationship of RTS and density that would relate to the different spatio-social organization, this explanation seems less likely.

A third explanation for our results could be the difference in an evolutionary response owing to sexual selection or from a plastic response to prevailing levels of sperm competition. In a highly variable social environment, such as fluctuating populations, we would expect to see selection on phenotypic plasticity in testes size, so that males would be able to respond quickly to current strengths of sperm competition [63]. Indeed, several studies on invertebrates have shown that individuals raised under high densities during sexual development grow larger testes than individuals raised under low densities [64–66]. Here, the positive relationship of RTS and current density may also suggest phenotypic plasticity in testes size; however, it is not clear why it evolved only in 2 out of 5 species. In general, phenotypic plasticity may involve substantial energetic and genetic costs [67], potentially decreasing its adaptive value. Thus, the negative relationship between RTS and density in 3 out of 5 species may have an underlying genetic basis. Experimental evolutionary studies on invertebrates have shown significant testis size divergence between lines with (polygamy) and without (monogamy) sperm competition [13,14,68]. However, it is possible that large changes in density are too rapid for testes size to co-evolve with sperm competition risk. Here, testes size does increase as a result of an allometric increase in body size (Chitty effect; [32,33]). But the change in body size may occur at a faster rate than in testes, leading to a lagged evolutionary response in RTS. Consequently, under continuously strong density fluctuations, RTS may be low when densities are high and high when densities are low. Therefore, population fluctuations may cause male voles to invest heavily under low competition and little under high competition. While the first implies fitness costs from unnecessary energetic expenditure, the latter causes fitness costs owing to poor competitive ability. However, other components of the ejaculate may be able to compensate the suboptimal testes size. Studies on rodents indicated developmental plasticity in sperm production rate and the size of major accessory reproductive glands, instead of testis size [69–71]. Furthermore, one experimental evolution study on house mice (*Mus domesticus*) indicated that testes size may not respond to increasing levels of sperm competition at least initially (generation 8), instead sperm production may evolve faster [15]. This may be realized by an increase of sperm producing tissue within the testis as observed in birds [72]. Additionally, ejaculate quality may be increased, but in rodents such sperm competition metrics are typically strongly related to RTS [73–76].

In summary, our long-term data on natural populations of five sympatric vole species show that demographic factors affect the evolution of testes size. Our data partly support the assumption that sperm competition selects for increased investment in testicular tissue, but also show that strong fluctuations in population density can reverse this relationship. This may be owing to an increased competition at high densities, differing investment in pre- and post-copulatory competition at different densities, or an evolutionary response to sperm competition levels that is lagged when density fluctuations are over a certain threshold. Overall, testes size can be a good measure of sperm competition levels, particularly for cross-species comparisons [10], but our results indicate that caution must be taken in simple inference about intraspecific variation, particularly in systems where sperm competition risk or intensity can be highly variable or different male mating strategies may change with density.

According to the Finnish Act on the Use of Animals for Experimental Purposes (62/2006) and a further decision by the Finnish Animal Experiment Board (16 May 2007), the animal capture technique we used, i.e. traps that instantly kill the animal, is not considered an animal experiment and therefore requires no animal ethics licence from the Finnish Animal Experiment Board. Permits (23/5713/2001, 4/5713/2007 and 7/5713/2013) for capturing protected species (in our study *My. rutilus* and *My. rufocanus*) were granted by the Finnish Ministry of the Environment. None of the captured species are included in the Red List of Finnish Species.

**Acknowledgements.** We are grateful to Tommaso Pizzari and two anonymous reviewers for extremely helpful and insightful comments. We are also grateful to the members of the Sexual Selection Discussion Group at the University of Jyväskylä, Paul Eady, Renée Firman and Leigh Simmons for comments on earlier drafts of this manuscript.

**Data accessibility.** We provide all data via Dryad repository (doi:10.5061/dryad.p8791).

**Funding statement.** The Pallassjärvi studies have been supported by various grants from the University of Helsinki, the Finnish Academy, the Finnish Forest Research Institute and the EU.
References


51. Eccard JA, Jokinen I, Ylönén H. 2011 Loss of

52. Krebs CJ. 1970 Microtus

53. Huitu O, Koivula M, Korpimaki E, Klemola T, S, Palme R, Cosson JF. 2008 Stress and demographic

54. Laine K, Henttonen H. 1983 The role of plant


56. Stockley P, Seal NJ. 2001 Plasticity in reproductive

57. Droney DC. 1998 The influence of the nutritional


59. Proctor HC. 1992 Effect of food-deprivation on mate


