

Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices

Suzanne C. Mills^{1,2,*}, Alessandro Grapputo^{1,3}, Esa Koskela¹ and Tapio Mappes¹

¹Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35 YAC, Jyväskylä 40014, Finland

²FRE 2935 CNRS-EPHE, Ecosystèmes Coralliens, Université de Perpignan, 66860 Perpignan Cedex, France

³Department of Biology, University of Padova, Via U. Bassi 58/B, 35121 Padova, Italy

Despite numerous indices proposed to predict the evolution of mating systems, a unified measure of sexual selection has remained elusive. Three previous studies have compared indices of sexual selection under laboratory conditions. Here, we use a genetic study to compare the most widely used measures of sexual selection in natural populations. We explored the mating and reproductive successes of male and female bank voles, *Clethrionomys glareolus*, across manipulated operational sex ratios (OSRs) by genotyping all adult and pup bank voles on 13 islands using six microsatellite loci. We used Bateman's principles (I_s and I and Bateman gradients) and selection coefficients (s' and β') to evaluate, for the first time, the genetic mating system of bank voles and compared these measures with alternative indices of sexual selection (index of monopolization and Morisita's index) across the OSRs. We found that all the sexual selection indices show significant positive intercorrelations for both males and females, suggesting that Bateman's principles are an accurate and a valid measure of the mating system. The Bateman gradient, in particular, provides information over and above that of other sexual selection indices. Male bank voles show a greater potential for sexual selection than females, and Bateman gradients indicate a polygynandrous mating system. Selection coefficients reveal strong selection gradients on male bank vole plasma testosterone level rather than body size.

Keywords: *Clethrionomys glareolus*; mating system; testosterone; opportunity for sexual selection; Morisita index; index of resource monopolization

1. INTRODUCTION

Sexual selection theory was developed to explain the evolution of sexually dimorphic characters (Darwin 1859, 1871) and is widely used to explain the evolution of mating systems (Shuster & Wade 2003). In turn, mating systems also influence sexual selection (Andersson 1994). However, there is no unified agreement in the literature as to the best quantitative measure that provides a direct relationship between sexual selection and the mating system, allows the possibility of cross-taxonomic comparative analyses and can be used in quantitative genetic theory (Arnold & Duvall 1994). Up to this point, the different quantitative measures of a mating system had not been rigorously tested. Recently, Kokko *et al.* (1999) emphasized that a unique 'best' measure is not available and advocated the use of several measures, whereas comparative studies on experimental populations recommended Morisita's index (Fairbairn & Wilby 2001) or Bateman's principles (Wade 1979; Jones *et al.* 2000, 2002, 2004, 2005).

Bateman's first two principles are based on the standardized variance in either the number of mates that sire or bear progeny (mating success) or the total number of offspring sired (reproductive success) and indicate the maximum strength on sexual selection acting in a population (Bateman 1948). Bateman's variances were

formulated quantitatively as the opportunity of sexual selection (I_s) and selection (I), respectively (Crow 1958; Wade 1979; Wade & Arnold 1980). However, two alternative indices that measure mate or resource acquisition were proposed: the index of resource monopolization (Q ; Green 1966; Ruzzante *et al.* 1996), which measures the observed variance as a fraction of the maximum possible variance corrected by the variance when acquisition is equal; and the Morisita index (I_δ ; Morisita 1962) which is similar to Q , but is not expressed as a fraction of the maximum variance. Bateman's third principle, the sexual selection gradient, defined recently as the Bateman gradient (Andersson & Iwasa 1996), is the statistical relationship between mating and reproductive successes approximated by a regression line (Arnold & Duvall 1994). Alternative measures of sexual selection include selection differentials (s') and selection gradients (β') that measure the direct selection on phenotypic characters to reveal the target(s) of sexual selection (Lande & Arnold 1983). These coefficients quantify the intensity of sexual selection and have greater predictive value in relation to evolutionary change; however, they cannot be used for cross-taxonomic comparisons owing to their dependency on specific phenotypic traits.

Fairbairn & Wilby (2001) recently compared the different quantitative measures of sexual selection in laboratory populations of mealworm beetles, *Tenebrio molitor*, at different operational sex ratios (OSRs) and

* Author for correspondence (mills@soozie.co.uk).

densities. Sexual selection on male traits is predicted to covary with the OSR, or the proportion of sexually active males to fertilizable females at any one time (Emlen & Oring 1977; Clutton-Brock & Parker 1992), and is supported with empirical data (e.g. Fleming & Gross 1994; Kvarnemo 1994; Mills & Reynolds 2003). Fairbairn & Wilby (2001) questioned the validity of I_s and I , particularly in populations with female-biased sex ratios and recommended using Morisita's index. However, comparisons of experimental rough-skinned newt, *Taricha granulosa*, populations at two OSRs and sex-role reversed pipefish, *Syngnathus typhle*, populations at three OSRs concluded that Bateman's principles, in particular the Bateman gradient, are the best measures of sexual selection and mating systems (Jones *et al.* 2004, 2005).

In this paper, we empirically test the different quantitative measures of sexual selection by manipulating OSR in natural populations of the bank vole, *Clethrionomys glareolus*. Although bank voles serve as a model species for many different purposes (e.g. Koskela *et al.* 1998; Oksanen *et al.* 2002; Mappes & Koskela 2004), experimental data studying sexual selection in natural populations are lacking. Although two studies have analysed paternity using molecular techniques (Sikorski & Wójcik 1990; Ratkiewicz & Borkowska 2000), only one found evidence of multiple paternity in natural populations (Ratkiewicz & Borkowska 2000). In Central Finland, reproduction takes place from May to mid-September, during which time up to four litters ranging from 2 to 10 pups (5.27 ± 1.32 , mean \pm s.e.) can be born (Koivula *et al.* 2003). Males provide no care for the young or the mother and except for a brief period following mating ('time out', cf. Ahnesjö *et al.* 2001), they are always able to mate once mature. However, females are in oestrus for only 1–2 days following birth and their 21 day period of gestation further shortens the period when they are able to mate ('time in' cf. Ahnesjö *et al.* 2001). As such, even though the primary sex ratio is equal, we predict that the potential for a male-biased OSR in natural bank vole populations is high.

We report the results of an experiment in which we measured male parameters, body mass and plasma testosterone level, and manipulated OSR in natural populations of *C. glareolus* in order to determine its (genetic) mating system and compare the measures of sexual selection using I_s , I , $Q_{\text{matingsuccess(ms)}}$, $Q_{\text{reproductivesuccess(rs)}}$, $I_{\delta\text{-matingsuccess(ms)}}$, $I_{\delta\text{-reproductivesuccess(rs)}}$, Bateman gradient and selection coefficients, s' and β' .

2. MATERIAL AND METHODS

(a) Animals and collection of samples

Nineteen islands situated on Lake Konnevesi, Finland ($62^{\circ}37'$ N, $26^{\circ}20'$ E) were suitable owing to their size, ranging from 0.32 to 2.48 ha and their accessibility for trapping. The islands were live trapped in May 2002 for a period of 4 days and all the small mammals were removed from the islands.

Thirty-eight adult female and fifty-six adult male bank voles were selected from wild-caught bank voles captured in Konnevesi. All the animals used in the experiment were of proven fertility; each had sired or given birth to at least one litter in the laboratory. Body mass (g) and head width (± 0.1 mm) were measured for all the animals, individually marked, prior to release. Male tissue (2 mm in diameter) was

stored at -70°C to be used in paternity analyses. A 75 μl intra-orbital blood sample was taken (see methods in Oksanen *et al.* 2003) for testosterone analysis from all the males 3 days prior to release.

Females and males were randomly assigned to groups corresponding to the 19 islands. The groups contained two females, with the number of males varying between 2, 3 or 4 to create OSRs of 1, 1.5 and 2, which were released to seven, six and six islands, respectively, with no effect of island size on OSR distribution (one-way ANOVA, $F_{2,19} = 0.451$, $p = 0.72$). Our mean \pm s.e. population density (5.95 ± 0.63 individuals per hectare) corresponds to the natural density during the breeding season for these islands (9.28 ± 2.67 individuals per hectare; Hakkarainen *et al.* in press). The groups were released onto the islands on 27 June 2002. After a period of three weeks, each island was live trapped, twice daily, for 4 days, using Uggländ special multiple-capture live traps baited with oats and potatoes. The trap density was 25 traps per hectare spaced *ca* 20 m from each other. This trapping procedure has proved effective in trapping all the bank voles from small island populations (T. Mappes & E. Koskela 1999, personal communication). All the females were brought to the laboratory to give birth in order to record their litter size. The pups were individually marked and tissue samples stored at -70°C for paternity analysis. All the pups were returned to the islands with their mothers within 4 days of their birth.

(b) Laboratory procedures and microsatellite genotyping

Total genomic DNA was extracted using a solution of 5% chelex resin (Sigma; Pearce *et al.* 1997). Individuals were genotyped at six microsatellite loci, MSCg 04, 07, 09, 15, 18 and 24, respectively (Gockel *et al.* 1997). The amplifications were carried out in a total volume of 10 μl , with the use of 75 mM Tris-HCl, 1.25 mM MgCl₂, 20 mM (NH₄)₂SO₄, 0.01% Tween 20, 5 pmoles of each primer (1/10 of one of the two primers labelled with either IDR-700 or IDR-800 fluorescent dye), 200 μM of each dNTPs, 0.5 unit *Taq* polymerase (Gibco-BRL) and 20–50 ng DNA. Amplification mixes were subjected to a denaturation step at 94°C for 5 min followed by 30 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, followed by an elongation step at 72°C for 5 min. Products of amplifications were run in a LiCor automatic sequencer. Alleles were scored by eye and the size was determined by running the sequence of the plasmid pUC18.

(c) Paternity assignment

Likelihood-based analysis of paternity was conducted with the software CERVUS v. 2 (Marshall *et al.* 1998). The following simulation parameters were used: 10 000 cycles, 100% of candidate parents sampled, 100% of loci typed and a genotyping error rate of 1%. We used the 'one parent known' option in CERVUS to assign paternity. All the males on the same islands (2–4) were included as candidate fathers. We accepted paternity assignment for the candidate with the highest LOD score at confidence level of 95% and with no mismatches (128 of 140 assignments, 91%). Only 12 pups remained unassigned (9%).

(d) Testosterone (T) assay

Plasma T was measured using a radioimmunoassay kit (TESTO-CTK, DiaSorin, Byk-Sangtec Diagnostica GmbH & Co, Germany). Fifty microlitres of the seven standards or 20 μl of the blood plasma samples were added with 500 μl of

¹²⁵I-labelled T to tubes coated in a T antiserum. During the 3 h reaction period at 37°C, labelled T and sample or standard T competed for a fixed number of antibody-binding sites. After aspiration of the mixture, the radioactivity of the tubes was measured in a gamma counter and is inversely related to the amount of unlabelled T in the samples and the standards. Sample T concentration was determined by interpolation from the standard calibration curve and corrected by $((50/20) \times (550/520))$. We screened plasma from *C. glareolus* for parallelism with the kit's standard curve using a series of six dilutions. The dilutions run parallel to the standard curve (homogeneity of slopes for sample versus standard ANCOVA: $F_{1,22}=2.269$, $p=0.148$), thus validating the use of this kit in bank voles. This technique also enabled us to determine that pooled vole plasma samples measured without dilution correspond to 50% of antibody bound. We found that this kit is also highly repeatable for bank voles ($r=0.961$, $n=26$, $p<0.01$).

(e) Selection measures

Our molecular paternity analysis enabled us to determine the bank vole genetic mating system, defined as the distribution of biological parentage in a population (Jones et al. 2005), by estimating the number of genetic mates (mating success) and genetic offspring (reproductive success; Jones et al. 2004).

Bateman's first and second principles were calculated following the methodology of Wade (1979) and Wade & Arnold (1980),

$$I_s \text{ and } I = \frac{\sigma^2}{\bar{X}^2},$$

where \bar{X} and σ^2 are the mean and the variance in either mating or reproductive success for the opportunity for sexual selection (I_s) and selection (I), respectively.

Bateman's third principle: the Bateman gradient, β_{ss} , was determined by the least-squares regression of reproductive success on mating success and is the slope of the regression. Differences between Bateman gradients were examined using the homogeneity of regression slope test in ANCOVA (Field 2005).

Selection coefficients on phenotypic characters were measured using the techniques developed by Lande & Arnold (1983). Phenotypic measurements were log transformed and standardized to have a mean of 0 and a variance of 1 before analysis. Directional selection differentials (s') are calculated as the covariances between fitness and each trait, and the selection gradients (β') are estimated from the linear multiple regression coefficients of fitness on the traits (Lande & Arnold 1983).

Index of resource monopolization (Q) is calculated following the theory of Green (1966) and Ruzzante et al. (1996),

$$Q = \frac{(\sigma^2 - \bar{X})}{(n\bar{X}^2 - \bar{X})},$$

where \bar{X} and σ^2 are the mean and the variance in either mating or reproductive success for Q_{ms} and Q_{rs} , respectively, and n is the number of individuals.

The Morisita index (I_δ) is calculated from Morisita (1962),

$$I_\delta = n \frac{\left[\frac{\sum x^2 - \sum x}{(\sum x)^2 - \sum x} \right]},$$

where x is the individual mating or reproductive success for $I_{\delta-ms}$ and $I_{\delta-rs}$, respectively.

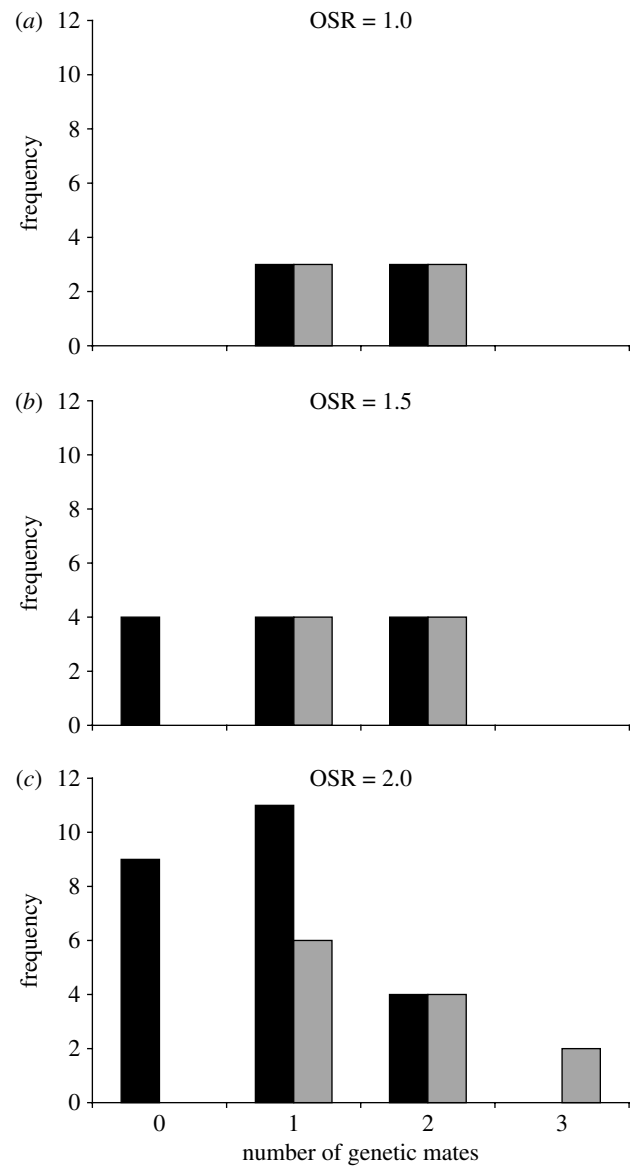


Figure 1. Distributions of genetically documented mating events for male and female bank voles from 13 island populations at OSRs of (a) 1, (b) 1.5 and (c) 2. Black bars, males; Grey bars, females.

(f) Statistics

ANCOVA was carried out with I_s , I , Q_{ms} , Q_{rs} , $I_{\delta-ms}$ and $I_{\delta-rs}$ as dependent variables, OSR as a fixed variable and density (individuals per hectare) as a covariate. Contingency tables were calculated following Siegel & Castellan (1988).

3. RESULTS

Of the 38 females released on 19 islands, six from six islands were not re-caught. As a result, we excluded those islands that lacked the original number of founder individuals from further analyses. All the 26 females on the remaining 13 island populations gave birth and 140 pups were analysed for paternity from 42 males. Of these 26 litters, 13 had a single sire and 13 had multiple sires. All males at an OSR of 1 were successful in mating, whereas 33 and 38% of males failed to sire any pup at the OSRs of 1.5 and 2, respectively (figure 1). Male mating success is only statistically different from that of females at an OSR of 2 (contingency χ^2 -tests, OSR=1: $\chi^2_2=0.07$, $p=0.97$; OSR=1.5: $\chi^2_2=3.33$, $p=0.19$; OSR=2: $\chi^2_3=9.53$, $p=0.023$; figure 1).

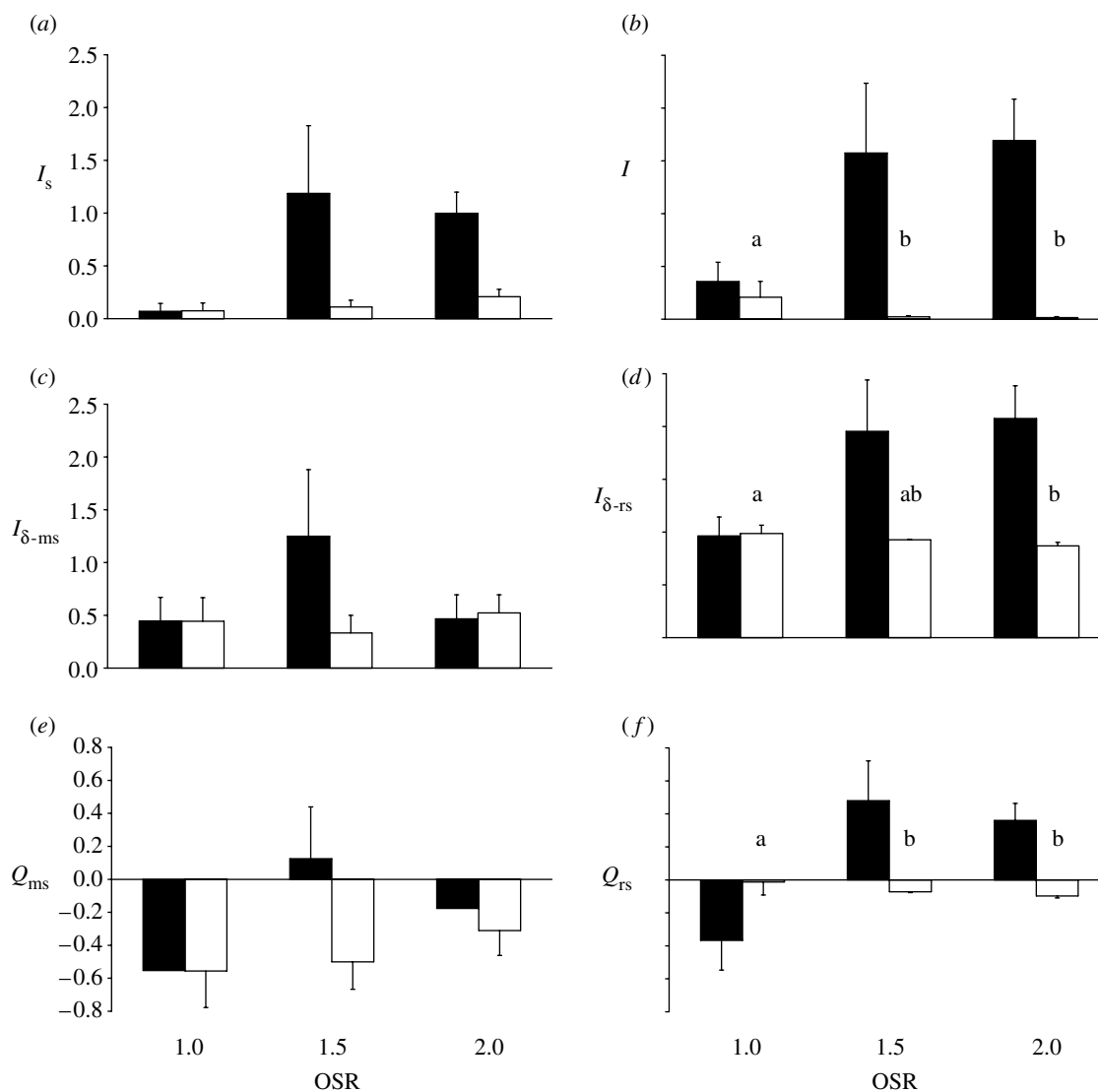


Figure 2. Sexual selection measures for bank voles from 13 island populations at OSRs of 1, 1.5 and 2: (a,b) I_s and I , the opportunity for sexual selection and selection; (c,d) $I_{\delta\text{-ms}}$ and $I_{\delta\text{-rs}}$, the Morisita indices for mating and reproductive success and (e,f) Q_{ms} and Q_{rs} , the indices of monopolization for mating and reproductive success. Males are represented by black bars and females by white bars. Significant differences are shown by different letters; means shown as ab are not significantly different from either a or b.

(a) Effect of operational sex ratio on sexual selection based on mating success: I_s , $I_{\delta\text{-ms}}$ and Q_{ms}

Estimates of the opportunity for sexual selection (I_s), the Morisita index ($I_{\delta\text{-ms}}$) and the index of resource monopolization (Q_{ms}) are shown in figure 2a,c,e. For males, opportunities for sexual selection, I_s , $I_{\delta\text{-ms}}$ and Q_{ms} , closely resemble each other, but although they appear higher at an OSR of 1.5, there was no significant effect of OSR on any estimate (ANCOVA, I_s : $F_{2,12}=0.085$, $p=0.920$; $I_{\delta\text{-ms}}$: $F_{2,12}=0.007$, $p=0.993$; Q_{ms} : $F_{2,12}=1.040$, $p=0.402$).

In females, there was neither an obvious visual trend (figure 2a,c,e) nor a significant effect of OSR on the estimates of sexual selection (ANCOVA, I_s : $F_{2,12}=0.600$, $p=0.575$; $I_{\delta\text{-ms}}$: $F_{2,12}=1.129$, $p=0.376$; Q_{ms} : $F_{2,12}=2.291$, $p=0.172$).

(b) Effect of operational sex ratio on selection based on reproductive success: I , Q_{rs} and $I_{\delta\text{-rs}}$

The estimates of the opportunity for selection (I), the Morisita index ($I_{\delta\text{-rs}}$) and the index of resource

monopolization (Q_{rs}) are shown in figure 2b,d,f. For males, opportunities for sexual selection, I , $I_{\delta\text{-rs}}$ and Q_{rs} , again closely resemble each other and appear higher at OSRs of 1.5 and 2, but there was no significant effect of OSR on any estimate (ANCOVA, I : $F_{2,12}=0.180$, $p=0.839$; $I_{\delta\text{-rs}}$: $F_{2,12}=0.365$, $p=0.707$; Q_{rs} : $F_{2,12}=0.735$, $p=0.513$).

However, in females, we did find a significant effect of OSR. For the estimates of I and Q_{rs} , we found a significant interaction between OSR and density (ANCOVA, OSR \times density, I : $F_{2,12}=6.920$, $p=0.022$; Q_{rs} : $F_{2,12}=8.599$, $p=0.013$). For both estimates, Bonferroni-corrected *post hoc* tests revealed that sexual selection in females was the greatest at an OSR of 1 compared with the OSRs of both 1.5 and 2 ($p<0.01$; figure 2b,f). For $I_{\delta\text{-rs}}$, we found a non-significant interaction trend between OSR and density (ANCOVA: $F_{2,12}=4.151$, $p=0.065$). Bonferroni-corrected *post hoc* tests revealed that sexual selection in females was greater at an OSR of 1 compared with 2 ($p=0.025$; figure 2d). Although significant effects of OSR on female selection were found, these results should be interpreted cautiously, as variances were calculated using only two females per island.

Table 1. Pearson's correlation coefficients (two-tailed) between the different indices of sexual selection based on mating and reproductive successes. (I_s and I , the opportunity for sexual selection and selection; Q_{ms} and Q_{rs} , the index of monopolization and $I_{\delta-ms}$ and $I_{\delta-rs}$, the Morisita index. The sample size is 13 for all comparisons. * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.)

		mating success		reproductive success	
		Q_{ms}	$I_{\delta-ms}$	Q_{rs}	$I_{\delta-rs}$
female	I_s	0.680*	0.547*		
	Q_{ms}		0.692**		
				I	0.960***
male	I_s	0.840***	0.744**		0.774**
	Q_{ms}		0.916***		0.798**
				I	0.941***
				Q_{rs}	0.948***

We calculated two-tailed Pearson's correlation coefficients between I_s , Q_{ms} and $I_{\delta-ms}$, and between I , Q_{rs} and $I_{\delta-rs}$ for males and females separately. All of the measures are significantly positively intercorrelated (table 1).

(c) Effect of operational sex ratio on the Bateman gradient

Bateman gradients showed that the reproductive success of males increased significantly with the number of females with which a male sired young (figure 3). However, for females, reproductive success does not increase significantly with the number of males that sired their young and the females' slopes do not differ significantly from 0 (figure 3). There are no significant differences between the gradients of either the male or the female across the OSRs (ANCOVA, all $p > 0.3$); however, male gradients are significantly steeper than the female gradients at OSRs of 1.5 and 2 (homogeneity of slopes using ANCOVA, sex \times mating success: OSR = 1.5: $F_{1,19} = 34.23$, $p = 0.017$, OSR = 2: $F_{1,35} = 34.39$, $p < 0.001$, OSR = 1: $F_{1,11} = 10.08$, $p = 0.108$).

(d) Effect of operational sex ratio on selection coefficients, s' and β'

At an OSR of 1.5, both selection coefficients (s' and β') with respect to male reproductive success showed that sexual selection acted significantly on a male's plasma testosterone (T; table 2). Similar, but non-significant, trends were found with respect to genetic mating success at an OSR of 1.5, revealing that sexual selection also acts on T (table 2).

A simple linear regression on unstandardized data between T and reproductive success (the total number of pups sired) revealed a significantly positive relationship (simple linear regression for all OSRs pooled together: $F_{1,37} = 11.95$, $p = 0.001$, $R^2 = 0.25$). Unfortunately, with the present sample size, we failed to find a significant effect of OSR on the regression equations (homogeneity of slopes using ANCOVA, OSR \times T: $F_{1,37} = 0.529$, $p = 0.472$; figure 4). A significant linear regression was also found between T and the total number of genetic mates (simple linear regression for all the OSRs pooled together: $F_{1,37} = 6.61$, $p = 0.014$, $R^2 = 0.16$); however, again, no significant

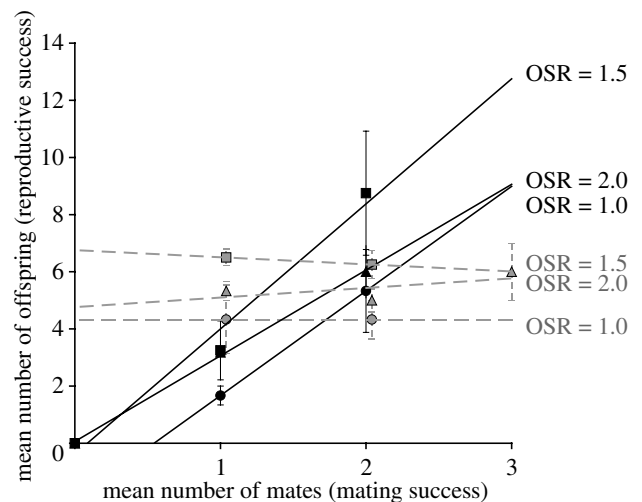


Figure 3. A plot of reproductive success versus genetic mating success for bank voles, showing the Bateman gradient for males (black points and solid lines) and females (grey points and dashed lines) at different OSRs. Filled circle, OSR = 1: $y = 3.667x - 2$; $R^2 = 0.60$, $N = 6$, $p = 0.07$, $\beta_{ss} = 3.67$. Filled square, OSR = 1.5: $y = 4.375x - 0.375$; $R^2 = 0.68$, $N = 12$, $p = 0.001$, $\beta_{ss} = 4.38$. Filled triangle, OSR = 2: $y = 3.035x + 0.056$; $R^2 = 0.67$, $N = 24$, $p < 0.001$, $\beta_{ss} = 3.04$. Shaded circle OSR = 1: $y = 4.33$; $R^2 = 0$, $N = 6$, $p = 1$, $\beta_{ss} = 0$. Shaded square, OSR = 1.5: $y = -0.25x + 6.75$; $R^2 = 0.032$, $N = 8$, $p = 0.67$, $\beta_{ss} = -0.25$. Shaded triangle, OSR = 2: $y = 0.2x + 5$; $R^2 = 0.03$, $N = 12$, $p = 0.586$, $\beta_{ss} = 0.2$.

differences were found between the regression equations at different OSRs (homogeneity of slopes using ANCOVA, OSR \times T: $F_{1,37} = 1.63$, $p = 0.21$).

4. DISCUSSION

Only three previous studies have experimentally compared different measures of sexual selection with OSR, yet in both experiments, laboratory or artificial populations were used (Fairbairn & Wilby 2001; Jones et al. 2004, 2005). Our results provide an empirical test of the widely used measures of selection using experimental populations of the bank vole, *C. glareolus*, in natural habitats at three manipulated OSRs.

(a) Comparison between sexual selection and selection indices: I_s , I , Q_{ms} , Q_{rs} , $I_{\delta-ms}$ and $I_{\delta-rs}$

All indices agreed that selection on females, based on I , Q_{rs} and $I_{\delta-rs}$, was the highest at an OSR of 1 (figure 2b,d,f) and that there was no evidence for an effect of OSR on sexual selection in males or females. We also show significant positive correlations between all indices of selection (table 1) and as such, we find that sexual selection and selection estimates covary with OSR in a similar manner.

However, Ruzzante et al. (1996) and Blanckenhorn et al. (1998) showed that when either the number of resources per competitor or the number of mates differs between populations, Q is a better measure of sexual selection than I . Even though the relative number of mates differed between our island populations, we found that all selection estimates based on Q and I show positive intercorrelations. Populations with female-biased sex ratios is another situation in which the validity of I has been questioned and in this case, the Morisita index is preferred (Fairbairn & Wilby 2001). However, as natural

Table 2. Estimates of the coefficients of sexual selection, selection differentials (s') and selection gradients (β') on body mass and testosterone levels of males at different operational sex ratios. (In both the analyses, relative fitness and standardized traits values are used. Significantly positive correlations are given in bold, trends (non-significant) in italics and non-significant results in plain text.)

OSR	character	n	fitness = genetic mating success				fitness = reproductive success			
			s'	<i>p</i>	β'	<i>p</i>	s'	<i>p</i>	β'	<i>p</i>
1	body mass	6	-0.11	0.763	-0.13	0.432	-0.13	0.819	-0.23	0.460
	testosterone	6	0.38	0.123	0.28	0.131	0.59	0.099	0.59	0.115
1.5	body mass	10	-0.09	0.743	-0.15	0.578	0.29	0.515	0.03	0.922
	testosterone	10	<i>0.59</i>	<i>0.073</i>	<i>0.54</i>	<i>0.079</i>	1.50	0.004	0.97	0.011
2	body mass	22	0.10	0.524	0.06	0.767	0.16	0.382	0.16	0.546
	testosterone	22	-0.04	0.760	-0.06	0.767	0.05	0.785	0.07	0.786

bank vole populations are male biased, our experiment did not include islands with female-biased sex ratios; therefore, we are unable to corroborate the results of Fairbairn & Wilby (2001). In terms of our study system and the OSR manipulations we carried out, we agree with Jones *et al.* (2004, 2005) that the use of I_s and I as measures of sexual selection is justified.

Sexual selection on male traits is predicted to be the strongest when there is intense competition for females, such as when the OSR is biased towards males (Emlen & Oring 1977). Although figure 2*b,d,f* suggests that sexual selection in males is higher at the male-biased OSRs of 1.5 and 2, we found no significant differences in sexual selection estimates between OSRs in male bank voles. A primary sex ratio of 1 : 1 may already provide a sufficiently male-biased OSR, thus increasing the number of males has only a small effect on the intensity of sexual selection. In terms of females, even though the population sizes used are biologically relevant (Hakkarainen *et al.* in press), only two females were used per island; therefore, we will not draw any detailed conclusions concerning female bank vole genetic mating system in this paper. Nevertheless, at an OSR of 1, female selection estimates are high (figure 2*b,d,f*) yet they cannot be attributed to benefits of multiple mating, as number of mates has no effect on litter size (figure 3) and there is no evidence for sexual selection (Arnold & Duvall 1994). Therefore, another aspect of fitness is increasing the variance in female reproductive success and one such trait may be fecundity selection.

(b) Bateman gradient

The slope of the Bateman gradient reveals the potential for sexual selection. In bank voles, the regression of fecundity on mating success is always steeper for males than females, thus males have a greater potential for sexual selection (figure 3). Selection estimates from the quantitative indices, I_s , I , Q_{ms} , Q_{rs} , $I_{\delta-ms}$ and $I_{\delta-rs}$, also showed higher sexual selection in males (figure 2). However, Bateman gradients further reveal that in females, fecundity reaches an asymptote after one mating. As females receive no provisioning from males, we can conclude that females experience no gain in fecundity once they secure a single mate. Thus, sperm from one male is sufficient to fertilize all their litter.

Our results lead us to expect a polygynandrous mating system in bank voles (Searcy & Yasukawa 1995). An analysis of all 26 litters reveals that 50% were sired by multiple

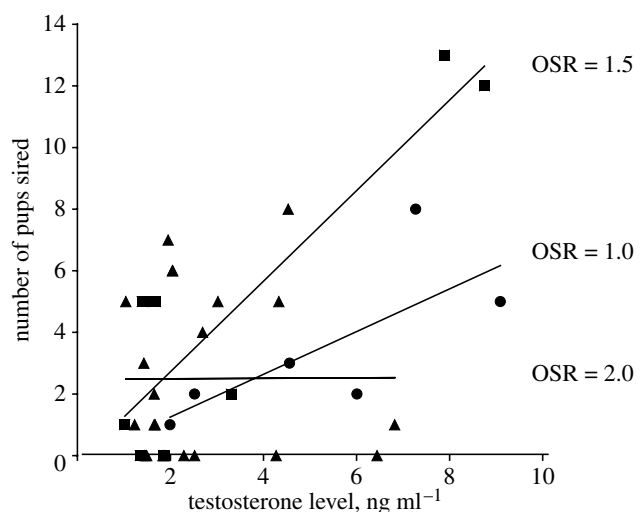


Figure 4. The plasma testosterone level (unstandardized and untransformed) of male bank voles and their reproductive success (number of pups sired) at different OSRs. Filled circle, OSR = 1: $y = 0.693x - 0.134$; $R^2 = 0.54$, $N = 6$, $p = 0.094$. Filled square, OSR = 1.5: $y = 1.468x - 0.212$; $R^2 = 0.75$, $N = 10$, $p = 0.001$. Filled triangle, OSR = 2: $y = 0.006x + 2.483$; $R^2 = 0.004$, $N = 22$, $p = 0.987$.

fathers, a value higher than 0 and 35.5% previously reported (Sikorski & Wójcik 1990; Ratkiewicz & Borkowska 2000), and as many as three fathers sired litters at an OSR of 2 (16.7% of 12 litters). Owing to overlapping ejaculates, male-male competition may therefore be as intense at the ejaculate, as at the population level. Post-mating selection is clearly an important factor influencing male reproductive success in bank voles. Numerous mechanisms have been proposed to explain multi-male mating including: direct non-genetic benefits (Dewsbury 1979; Klemme *et al.* in press); genetic benefits (Jennions & Petrie 2000) including bet hedging in the presence of density cycles, genotype by environment interaction and ontogenetic conflict (Mills *et al.* in press); prevention of inbreeding (Zeh & Zeh 1997); and confusion of paternity to deter infanticide (Wolff & Macdonald 2004).

(c) Selection coefficients, s' and β'

A steep Bateman gradient results in persistent directional selection on mating success and any trait correlated with mating success (such as a secondary sexual character) will be under strong selection (Jones *et al.* 2002). Sexual

dimorphism is common in mammals, with males the larger sex, owing to their advantage in gaining access to receptive females (e.g. McElligott *et al.* 2001). However, in the bank vole, selection coefficients reveal that sexual selection is acting on plasma testosterone level (T) rather than body size (table 2) and T is not correlated with either body mass (Pearson's correlation: $r(40) = 0.09$, $p = 0.564$) or head width ($r(40) = 0.22$, $p = 0.182$). We find evidence for strong selection on T ($\beta' = 0.97$; table 2), which is likely to affect male mating success through its potential influence on both intra- and inter-sexual selection. In terms of intra-sexual selection, male bank voles implanted with exogenous T had larger home range sizes and sired more offspring than saline implanted males (Mills *et al.* in preparation b). T also plays an important role in spermatogenesis (mice and rats: Singh & Handelsman 1996; Spaliviero *et al.* 2004; Sriraman *et al.* 2004); therefore, it may also be acting on internal male reproductive traits such as sperm characteristics. The role of T in inter-sexual selection is evident through female preferences for dominant males based on cues in their urine (Horne & Ylönen 1996; Kruczek 1997), and the preputial gland, the main source of male sexual attractants, is T-dependent (Radwan *et al.* 2006).

The strong selection gradient on T (table 2) coupled with significant heritability of T (Mills *et al.* in preparation b) is predicted to drive high T levels to fixation (Charlesworth 1987). However, the presence of both considerable environmental sources of variation (e.g. population density cycles; Hanski *et al.* 1993) and genotype by environment interactions (Mills *et al.* in press) may maintain additive genetic variation for T in bank voles. Furthermore, genetic variation may also be maintained by the tradeoff between T and immune function, as T is predicted to have an immunosuppressive effect due to the negative feedback within and between the endocrine and immune systems (Folstad & Karter 1992). As both the endocrine and immune systems have high metabolic demands immunosuppression may represent adaptive resource allocation, where resources are redirected away from an immune response towards sexual behaviour (Wedekind & Folstad 1994). This tradeoff in bank voles has concomitant effects on both survival and reproductive success (Mills *et al.* in preparation a).

In light of our results, which highlight that the different indices provide comparable sexual selection estimates, we agree with Jones *et al.* (2004, 2005) and that Bateman's principles are a valid measure for the characterization of mating systems. The Bateman gradient, in particular, provides not only a sexual selection estimate that can be used for cross-taxonomic comparisons, but also a more thorough description of male and female mating systems.

We acknowledge Maria Tuittu for carrying out genetic analyses, Antti Poikonen and Raimo Saunanen for island fieldwork and Tanja Poikonen for laboratory work. We thank Adam Jones for providing helpful advice and Leena Lindström and Dominique Mazzi for their comments on the manuscript. The study was financially supported by the Centre of Excellence for Evolutionary Ecology at the University of Jyväskylä and the Academy of Finland (grant no. 103508 and 108566 to S.C.M.; 63789, 202166 and 206091 to T.M.; 100143, 78777 and 103148 to E.K.)

REFERENCES

- Ahnesjö, I., Kvarnemo, C. & Merilaita, S. 2001 Using potential reproductive rates to predict mating competition among individuals qualified to mate. *Behav. Ecol.* **12**, 397–401. (doi:10.1093/beheco/12.4.397)
- Andersson, M. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
- Andersson, M. & Iwasa, Y. 1996 Sexual selection. *Trends Ecol. Evol.* **11**, 53–58. (doi:10.1016/0169-5347(96)81042-1)
- Arnold, S. J. & Duvall, D. 1994 Animal mating systems: a synthesis based on selection theory. *Am. Nat.* **143**, 317–348. (doi:10.1086/285606)
- Bateman, A. J. 1948 Intrasexual selection in *Drosophila*. *Heredity* **2**, 349–368.
- Blanckenhorn, W. U., Grant, J. W. A. & Fairbairn, D. J. 1998 Monopolization in a resource queue: water striders competing for food and mates. *Behav. Ecol. Sociobiol.* **42**, 63–70. (doi:10.1007/s002650050412)
- Charlesworth, B. 1987 The heritability of fitness. In *Sexual selection: testing the alternatives* (ed. J. W. Bradbury & M. B. Andersson), pp. 21–40. New York, NY: Wiley.
- Clutton-Brock, T. H. & Parker, G. A. 1992 Potential reproductive rates and the operation of sexual selection. *Quart. Rev. Biol.* **67**, 437–456. (doi:10.1086/417793)
- Crow, J. F. 1958 Some possibilities for measuring selection intensities in man. *Hum. Biol.* **30**, 1–13.
- Darwin, C. 1859 *On the origin of species by means of natural selection*. London, UK: Murray.
- Darwin, C. 1871 *The descent of man, and selection in relation to sex*. London, UK: Murray.
- Dewsbury, D. A. 1979 Copulatory behaviour of deer mice (*Peromyscus maniculatus*): III effects on pregnancy initiation. *J. Comp. Physiol. Psychol.* **93**, 178–188. (doi:10.1037/h0077575)
- Emlen, S. T. & Oring, L. W. 1977 Ecology, sexual selection, and the evolution of mating systems. *Science* **197**, 215–223.
- Fairbairn, D. J. & Wilby, A. E. 2001 Inequality of opportunity: measuring the potential for sexual selection. *Ecol. Ecol. Res.* **3**, 667–686.
- Field, A. 2005 *Discovering statistics using SPSS*. Oxford, UK: The Alden Press.
- Fleming, I. A. & Gross, M. R. 1994 Breeding competition in a Pacific salmon (Coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. *Evolution* **48**, 637–657. (doi:10.2307/2410475)
- Folstad, I. & Karter, A. J. 1992 Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**, 603–622. (doi:10.1086/285346)
- Gockel, J., Harr, B., Schlötterer, C., Arnold, W., Gerlach, G. & Tautz, D. 1997 Isolation and characterization of microsatellite loci from *Apodemus flavicollis* (Rodentia, Muridae) and *Clethrionomys glareolus* (Rodentia, Cricetidae). *Mol. Ecol.* **6**, 597–599. (doi:10.1046/j.1365-294X.1997.00222.x)
- Green, R. H. 1966 Measurement of non-randomness in spatial distributions. *Res. Popul. Ecol.* **8**, 1–7.
- Hakkarainen, H., Koskela, E., Mappes, T., Soveri, T., Huhta & E., Suorsa, P. In press. Eimeria-parasites are associated with life history traits in island and mainland populations of the bank vole. *Parasitology*.
- Hanski, I., Turchin, P., Korpimäki, E. & Henttonen, H. 1993 Population oscillations of boreal rodents: regulation by mustelid predators leads to chaos. *Nature* **364**, 232–235. (doi:10.1038/364232a0)
- Horne, T. J. & Ylönen, H. 1996 Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behav. Ecol. Sociobiol.* **38**, 401–405. (doi:10.1007/s002650050257)
- Jennions, M. D. & Petrie, M. 2000 Why do females mate multiply? *Biol. Rev. Camb. Philos. Soc.* **72**, 21–64. (doi:10.1017/S0006323199005423)

- Jones, A. G., Rosenqvist, G., Berglund, A., Arnold, S. J. & Avise, J. C. 2000 The Bateman gradient and the cause of sexual selection in a sex-role-reversed pipefish. *Proc. R. Soc. B* **267**, 677–680. (doi:10.1098/rspb.2000.1055)
- Jones, A. G., Arguello, J. R. & Arnold, S. J. 2002 Validation of Bateman's principles: a genetic study of sexual selection and mating patterns in the rough-skinned newt. *Proc. R. Soc. B* **269**, 2533–2539. (doi:10.1098/rspb.2002.2177)
- Jones, A. G., Arguello, J. R. & Arnold, S. J. 2004 Molecular parentage analysis in experimental newt populations: the response of mating system measures to variation in the operational sex ratio. *Am. Nat.* **164**, 444–456. (doi:10.1086/423826)
- Jones, A. G., Rosenqvist, G., Berglund, A. & Avise, J. C. 2005 The measurement of sexual selection using Bateman's principles: an experimental test in the sex-role-reversed pipefish *Syngnathus typhle*. *Integr. Comp. Biol.* **45**, 874–884. (doi:10.1093/icb/45.5.874)
- Klemme, I., Ylönen, H. & Ecard, J. A. In press. Why do female bank voles (*Clethrionomys glareolus*) mate multiply? *Anim. Behav.*
- Koivula, M., Koskela, E., Mappes, T. & Oksanen, T. A. 2003 Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology* **84**, 398–405.
- Kokko, H., Mackenzie, A., Reynolds, J. D., Lindström, J. & Sutherland, W. J. 1999 Measures of inequality are not equal. *Am. Nat.* **72**, 358–381. (doi:10.1086/303235)
- Koskela, E., Jonsson, P., Hartikainen, T. & Mappes, T. 1998 Limitation of reproductive success by food availability and litter size in the bank vole, *Clethrionomys glareolus*. *Proc. R. Soc. B* **265**, 1129–1134. (doi:10.1098/rspb.1998.0408)
- Kruczek, M. 1997 Male rank and female choice in the bank vole, *Clethrionomys glareolus*. *Behav. Process.* **40**, 171–176. (doi:10.1016/S0376-6357(97)00785-7)
- Kvarnemo, C. 1994 Temperature differentially affects male and female reproductive rates in the sand goby—consequences for operational sex-ratio. *Proc. R. Soc. B* **256**, 151–156.
- Lande, H. & Arnold, S. J. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226. (doi:10.2307/2408842)
- Mappes, T. & Koskela, E. 2004 Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution* **58**, 645–650. (doi:10.1554/02-104)
- Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x)
- McElligott, A. G., Gammell, M. P., Harty, H. C., Paini, D. R., Murphy, D. T., Walsh, J. T. & Hayden, T. J. 2001 Sexual size dimorphism in fallow deer (*Dama dama*): do larger, heavier males gain greater mating success? *Behav. Ecol. Sociobiol.* **49**, 475–484. (doi:10.1007/s002650000293)
- Mills, S. C. & Reynolds, J. D. 2003 Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behav. Ecol. Sociobiol.* **54**, 98–104.
- Mills, S. C., Alatalo, R. V., Koskela, E., Mappes, J., Mappes, T. & Oksanen, T. A. In press. Signal reliability compromised by genotype by environment interaction. *Evolution*.
- Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T. & Poikonen, T. In preparation *a*. The immunity versus testosterone trade off is the proximal effector of the survival versus mating success trade off in bank voles.
- Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T. & Poikonen, T. In preparation *b*. Is dominance an honest signal of male viability for female bank voles?
- Morisita, M. 1962 *I*—index, a measure of dispersion of individuals. *Res. Popul. Ecol.* **4**, 1–7.
- Oksanen, T. A., Koskela, E. & Mappes, T. 2002 Hormonal manipulation of offspring number: maternal effort and reproductive costs. *Evolution* **56**, 1530–1537. (doi:10.1554/0014-3820(2002)056[1530:HMOONM]2.0.CO;2)
- Oksanen, T. A., Jokinen, I., Koskela, E., Mappes, T. & Vilpas, H. 2003 Manipulation of offspring number and size: benefits of large body size at birth depend upon the rearing environment. *J. Anim. Ecol.* **72**, 321–330.
- Pearce, J. M., Fields, R. L. & Scribner, K. T. 1997 Nest materials as a source of genetic data for avian ecological studies. *J. Field Ornithol.* **68**, 471–481.
- Radwan, J. *et al.* 2006 Metabolic costs of sexual advertisement in the bank vole, *Clethrionomys glareolus*. *Evol. Ecol. Res.* **8**, 859–869.
- Ratkiewicz, M. & Borkowska, A. 2000 Multiple paternity in the bank vole (*Clethrionomys glareolus*): field and experimental data. *Mamm. Biol.* **65**, 6–14.
- Ruzzante, D. E., Hamilton, D. C., Kramer, D. L. & Grant, J. W. A. 1996 Scaling of the variance and quantification of resource monopolization. *Behav. Ecol.* **7**, 199–207.
- Searcy, W. A. & Yasukawa, K. 1995 *Polygyny and sexual selection in red-winged blackbirds*. Princeton, NJ: Princeton University Press.
- Shuster, S. M. & Wade, M. J. 2003 *Mating systems and strategies*. Princeton, NJ: Princeton University Press.
- Siegel, S. & Castellan, N. J. 1988 *Nonparametric statistics for the behavioral sciences*. Singapore, Singapore: McGraw-Hill Book Co.
- Sikorski, M. D. & Wójcik, A. M. 1990 Mating system and reproductive success in a free-living population of the bank vole, *Clethrionomys glareolus*. In *Social systems and population cycles in voles* (ed. R. H. Tamarin, R. S. Ostfeld, S. R. Pugh & G. Bujalska), pp. 193–200. Basel, MA: Birkhäuser Verlag.
- Singh, J. & Handelsman, D. J. 1996 Neonatal administration of FSH increases Sertoli cell numbers and spermatogenesis in gonadotrophin-deficient (*hpg*) mice. *J. Endocrinol.* **151**, 37–48. (doi:10.1677/joe.0.1510037)
- Spaliviero, J. A., Jimenez, M., Allan, C. M. & Handelsman, D. J. 2004 Luteinizing hormone receptor–mediator effects on initiation of spermatogenesis in gonadotrophin-deficient (*hpg*) mice are replicated by testosterone. *Biol. Reprod.* **70**, 32–38. (doi:10.1095/biolreprod.103.019398)
- Sriraman, V., Sairam, M. R. & Jagannadha, R. A. 2004 Evaluation of relative role of LH and FSH in restoration of spermatogenesis using ethanedimethylsulphonate-treated adult rats. *Reprod. Biomed. Online* **8**, 167–174.
- Wade, M. J. 1979 Sexual selection and variance in reproductive success. *Am. Nat.* **114**, 742–747. (doi:10.1086/283520)
- Wade, M. J. & Arnold, S. J. 1980 The intensity of sexual selection in relation to male sexual behaviour, female choice and sperm precedence. *Anim. Behav.* **28**, 446–461. (doi:10.1016/S0003-3472(80)80052-2)
- Wedekind, C. & Folstad, I. 1994 Adaptive or nonadaptive immunosuppression by sex hormones. *Am. Nat.* **143**, 936–938. (doi:10.1086/285641)
- Wolff, J. O. & Macdonald, D. W. 2004 Promiscuous females protect their offspring. *Trends Ecol. Evol.* **19**, 127–134. (doi:10.1016/j.tree.2003.12.009)
- Zeh, J. A. & Zeh, D. W. 1997 The evolution of polyandry II: post-copulatory defences against genetic incompatibility. *Proc. R. Soc. B* **264**, 69–75. (doi:10.1098/rspb.1997.0010)