Effects of litter size on pup defence and weaning success of neighbouring bank vole females

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Abstract: Reproductive success of territorial female mammals depends partly on their capability to defend their young from conspecific intruders. However, how this is related to the characteristics of females and their litter sizes is largely unknown. The defence activity of 25 female bank voles (*Clethrionomys glareolus*) in relation to the number of offspring was studied in a behavioural arena by manipulating litter sizes (–2 pups or +2 pups). Infanticidal male bank voles were used as intruders–predators. Moreover, the weaning success (weaned at least one offspring or none) of 15 pairs of neighbouring females was investigated in a large indoor runway system. In each pair of females, the litter size of one female was reduced (–2 pups) and the litter size of the other enlarged (+2 pups). Defence activity of females increased with the number of offspring and the mother's size. However, weaning success of neighbours was related only to their body mass, and litter-size manipulation did not affect weaning success. Present results indicate that, although bank vole females increase their defence intensity with an increase in the number of pups, the weaning success of neighbouring females may be primarily determined by their size and dominance rank.

Résumé : Le succès de la reproduction chez des femelles territoriales de mammifères est en partie attribuable à leur capacité de défendre leurs petits contre des intrus de la même espèce. Cependant, en quoi cela est relié aux caractéristiques des femelles et à la taille de leurs portées reste à déterminer. L'activité de défense a été étudiée chez 25 femelles du campagnol roussâtre (*Clethrionomys glareolus*) en fonction du nombre de petits dans la progéniture dans une arène comportementale où le nombre de petits dans les portées était manipulé (–2 petits ou +2 petits). Des mâles infanticides du campagnol roussâtre ont été utilisés comme intrus–prédateurs. En outre, le succès du sevrage (au moins un petit sevré dans la portée ou aucun) a été évalué chez 15 paires de femelles voisines dans un vaste réseau intérieur de pistes. Chez chacune des paires de femelles, une femelle a été privée de deux de ses petits (–2 petits), alors que la portée de l'autre femelle était augmentée (+2 petits). L'activité de défense des femelles s'est avérée fonction du nombre de petits dans la portée et de la taille de la femelle. Cependant, le succès du sevrage chez les femelles voisines était relié uniquement à leur masse corporelle et la manipulation du nombre de petits dans la portée n'avait aucun effet sur le succès du sevrage. Nos résultats indiquent que, bien que les femelles du campagnol roussâtre augmentent l'intensité de leur activité de défense à mesure qu'augmente le nombre de petits dans la portée, le succès du sevrage des femelles voisines est probablement déterminé par leur taille et leur rang de dominance.

[Traduit par la Rédaction]

Introduction

Individuals are continually competing for resources such as food, shelter, and mating partners, and the outcome of these contests affects their reproductive success. Accordingly, the reproductive success of females is often influenced by their social status and (or) dominance rank (e.g., Clutton-Brock et al. 1986; Woodroffe and McDonald 1995). For instance, dominant red deer hinds produce about 10% more calves than subordinates (Clutton-Brock et al. 1986). In lemmings, mothers were able to successfully defend their litter only when they could dominate the intruder completely (Mallory and Brooks 1978). Dominance rank in mammals is also often related to their body mass, so that larger individuals have higher social status (Clutton-Brock 1988).

Offspring defence can be viewed as parental investment, since increase in defence of the offspring is likely to reduce the probability that the parent will survive and produce offspring in the future (Barash 1975; Montgomerie and Weatherhead 1988). The parental-investment theory predicts that the benefits of offspring defence should be positively correlated with the number and quality of the offspring (Trivers 1972; Barash 1975). In fact, it has been shown that the intensity of parental defence increases with the number of offspring in birds (Lazarus and Inglis 1986; Curio and Regelmann 1987; Montgomerie and Weatherhead 1988; Wiklund 1990), fish (Carlisle 1985; Knight and Temple 1986; Lavery and Keenleyside 1990), and a few mammals (Maestripieri and Alleva 1990, 1991). However, most studies have concentrated only on measuring the intensity of offspring defence in relation to the characteristics of the brood and have not followed the reproductive success of the parents.

Determinants of small-mammal breeding success have lately been studied using litter-size manipulations (e.g., Hare and

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Murie 1992; Mappes et al. 1995; Koskela et al. 1998, 1999; Humphries and Boutin 2000; Oksanen et al. 2001). In these field experiments, a high proportion of females weaned no offspring. Still, the mechanisms causing the total loss of litters are largely unknown, for example, we do not know whether breeding failures are related to litter-size manipulation or to characteristics of neighbouring females.

The bank vole (*Clethrionomys glareolus*) is well suited for behavioural experiments, as it is relatively insensitive to disturbance. The failure of females to discriminate between their own pups and those of strangers permits litter-size manipulations (Mappes et al. 1995). Breeding female bank voles are strictly territorial (e.g., Bujalska 1973; Koskela et al. 1997) and, apparently, possession of a territory is a prerequisite for breeding (Kawata 1987). As is the case for many other mammals, bank voles (both sexes) are infanticidal, and maternal aggression has been hypothesized to protect the offspring from conspecifics (Hausfater and Hrdy 1984; Labov et al. 1985; Wolff 1985; Maestripieri 1992). Thus, pup-defence activity can be studied against strange conspecific individuals.

The purpose of our study was to investigate the factors that determine the reproductive success of neighbouring female bank voles. In particular, using experimental alteration of litter sizes, we investigated whether intensity of pup defence, dominance relationships, and breeding success of females were related to litter size.

Methods

Study animals

The bank voles used were mature individuals of first and second laboratory generations of wild bank voles. Animals were housed individually in breeding cages (38 × 22 × 15 cm), with sawdust and paper or hay as bedding, and maintained under standard conditions (16 h light (L) : 8 h dark (D); 20°C). Voles were provided with water and "mouse chow" (1260 kJ/100 g) ad libitum before and during the experiments.

Defence trials

The defence activity of females in relation to offspring number was studied by manipulating litter sizes within 2 days of the females giving birth. There were two manipulation groups: reduced litters (R, two pups removed) and enlarged litters (E, two pups added). The initial size (body mass and head width) of the mothers did not differ significantly between the manipulation groups (body mass: R, 19.5 ± 1.0 g (mean ± SE) (n = 12); E, 20.5 ± 1.0 g (n = 13); two-sample t test, df = 23, t = –0.75, $p > 0.05$; head: R, 13.4 ± 0.2 mm (mean ± SE); E, 13.6 ± 0.1 mm; two-sample t test, df = 23, t = –0.80, $p > 0.05$). There were also no significant differences in the original litter sizes of females (R, 5.0 ± 0.2 (mean ± SE) (n = 12); E, 5.2 ± 0.3 (n = 13); two-sample t test, df = 23, t = –0.36, $p > 0.05$). Defence trials were conducted when the pups were 3 days old. The trials were carried out in a 1 × 1 m arena, which was covered with transparent Perspex. The smell of the arena was familiar to the female, as sawdust and hay from her cage were spread over the floor. All the pups were protected from the intruder during the trials by placing them, together with their own bedding, in a small wire-mesh cage (15 × 10 × 7 cm) in the centre of the arena. The mother of the pups was released into the arena and, after a 3-min familiarisation period, beginning from when she first noticed the pups, a strange male was also introduced into the arena. We used males as intruders in the trials, as their behaviour in the arenas turned out to be more consistent than female behaviour (patrolling the arena and approaching the female). By observing female–male pairs, we could also avoid the possible confounding effect on the results of a dominance relationship between the two females. The behavioural trials of the female–male pairs were filmed and analysed by the same observer, who was unaware of the manipulation groups to which the females belonged. The defence behaviour of females was defined as the number of attacks against males during the 10-min trials. The variable was $\ln(x + 1)$ transformed before analyses.

Breeding-success experiment

The experiment was conducted in vertical runway complexes, where each complex (190 × 60 × 15 cm) had 26 floors with one or two door holes on each floor. The total length of each runway system was about 15.5 m from the top to the ground floor. The front of the runway system was made of transparent Perspex, which permitted direct observations of all individuals. The voles in the system were maintained under summer conditions (16 h L : 8 h D; 18°C). Each female was provided with hay as nest material.

Thirty females in late pregnancy were used in the experiment and randomly divided into pairs (n = 15 pairs). The female pairs were released at the same time in each system. At first, the two females were separated by a closed passage, which allowed no interaction between them. After births, females and pups were removed for measurement (postpartum body mass, litter size, body mass of the pups) and litter-size manipulation. The females in the study were breeding synchronously (all females had mated within 1 day) and gave birth within 3–6 days after their introduction into the runway system. In each female pair, there were two manipulation groups: reduced litters (R), with two pups removed, and enlarged litters (E), with two pups added. To enlarge litters, two extra pups were always taken from a female in another female–female pair. The number of offspring before litter manipulation did not differ significantly between the two treatment groups (E, 4.0 ± 0.3 (mean ± SE); R, 4.9 ± 0.4; two-sample t test, df = 28, t = –1.90, $p > 0.05$). Further, there was no significant difference in the post-partum body mass of females between the treatments (E, 25.2 ± 0.8 g (mean ± SE); R, 24.7 ± 0.6 g; two-sample t test: df = 28, t = 0.52, $p > 0.05$). The initial litter size varied from 2 to 8 pups. The post-partum body mass of the females did not correlate significantly with litter size (Pearson's correlation coefficient, r = 0.048, p = 0.80).

After the litter-size manipulations, females with their pups were returned to the runway complex and allowed to adjust for 2–4 days before the passage between the females was opened. Behavioural interactions (occurrence of infanticidal behaviour, aggression) and survival of the pups were monitored for 10–20 min, five times (08:00, 11:00, 15:00, 18:00, 22:00) per day for 3 weeks. The dominance relationship between the females was defined when the one female visited the other female's nest and the latter abandoned her nest and (or) when the subordinate female hid (stayed inactive or actively moved away) from the other female.

Table 1. Analysis of deviance in logistic regression for the probability that a mother will wean at least one offspring (weaning success).

	Deviance	G	df	Tested for:	p
Intercept only	40.38	6.04	2	full model	0.049
Full model – body mass	40.38	0	1	treatment	1.00
Full model – treatment	34.35	6.03	1	body mass	0.014
Full model	34.34				

Note: Full model includes the intercept, treatment, and body mass variables.

Fig. 1. Weaning success of a female increases with an increase in her body mass, whereas litter-size manipulation has no effect on the probability of weaning offspring; solid line, litters with –2 pups; broken line, litters with +2 pups. For statistics see Table 1.

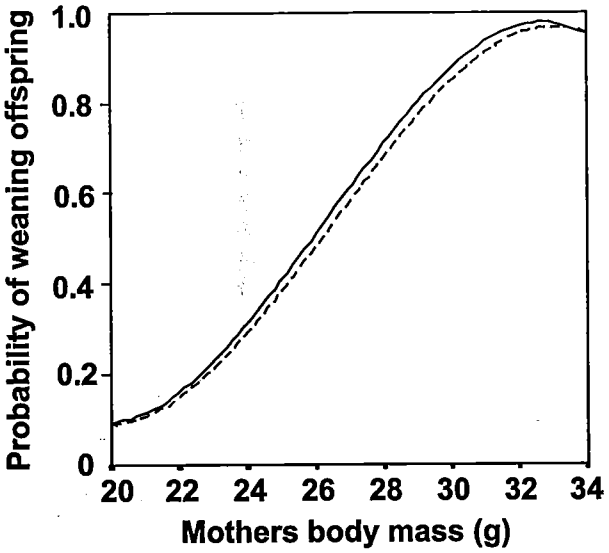
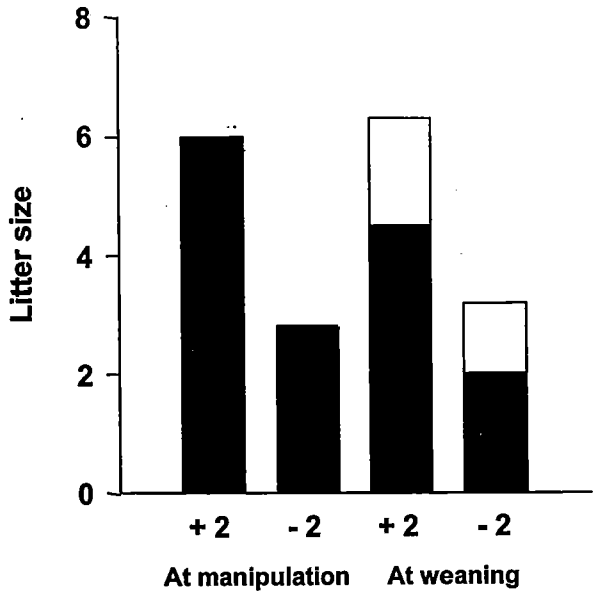


Fig. 2. Mean number of offspring at manipulation and at weaning in the two treatment groups. The number of weaned offspring is divided into two categories. Solid bars show the number of females' own offspring at weaning (n = 12), while the open bars show the mean number of offspring that females adopted from neighbouring females (n = 7).



Results

Offspring-defence trials

Litter-size manipulation affected the intensity of females' defence activity. Females with reduced litters did not attack the intruder males as frequently as did females with enlarged litters (number of attacks in 10 min: R, 11.3 ± 4.5 (mean ± SE) (n = 12); E, 20.5 ± 4.4 (n = 13); two-sample t test, df = 23, t = –2.08, p = 0.049). The effect of the mother's size upon maternal defence was studied using Kendall's partial correlation (treatment as a controlling variable). Mothers with larger body size attacked the intruders more frequently than smaller mothers (head width: r_k = 0.29, n = 25, p = 0.042; body mass: r_k = 0.24, n = 25, p = 0.090).

Breeding-success experiment

In 12 of 15 pairs, only one female in each pair succeeded in weaning at least one offspring (weaning success). In the remaining three pairs, none of the females weaned young. The factors affecting weaning success were studied using logistic regression, with weaning success (weaned at least one offspring or none) as a dependent variable and litter-size manipulation (categorical variable) and mother's size (post-partum body mass) as explanatory variables (for a description of the analytical technique see Hardy and Field 1998). The analyses of deviance showed that litter-size manipulation had no effect on the weaning success of females (Table 1). However, the probability of weaning offspring depended upon female body mass, so that the females who successfully weaned offspring were significantly heavier than unsuccessful females (Fig. 1). The importance of large size is particularly evident when the analysis is carried out for neighbouring females: in pairs of females, those that weaned offspring successfully were significantly heavier than those that were unsuccessful (successful, 26.3 ± 0.9 g (mean ± SE); unsuccessful, 23.9 ± 0.6 g; paired t test, df = 11, t = 4.2, p < 0.001). The benefit of large size is further supported by a significant positive correlation between the number of weaned pups and mother body mass (Pearson's correlation coefficient, r = 0.43, n = 30, p = 0.018). Unsuccessful females lost their young, on average, 2.6 ± 1.3 days after birth.

After the litter-size manipulations, the number of offspring differed significantly between the two litter-manipulation groups (E, 6.0 ± 0.3 (mean ± SE); R, 2.9 ± 0.4; two-sample t test, df = 28, t = 6.56, p < 0.001). Females with enlarged litters weaned, on average, three more pups than did females with reduced litters (two-sample t test, df = 10, t = 2.71, p =

0.022; Fig. 2). Seven of the 12 successful females adopted (stole) pups (E, five of six females; R, two of six females; Fisher's exact two-tailed test, $p = 0.242$) from the neighbouring female in the same system. When the number of adopted pups was not included in the analysis, the number of weaned offspring was still significantly different between the two litter-manipulation groups (two-sample t test, df = 10, $t = 2.67$, $p = 0.023$; Fig. 2). The offspring body mass at birth did not differ between the litter-manipulation groups (E, 2.2 ± 0.1 g (mean ± SE); R, 2.0 ± 0.1 g; two-sample t test, df = 28, $t = 1.42$, $p > 0.05$). Neither did the body mass at weaning (day 17) differ significantly between the treatment groups (E, 7.6 ± 0.4 g (mean ± SE); R, 8.5 ± 0.3 g; t test, df = 10, $t = -1.65$, $p > 0.05$).

Discussion

The present results provide experimental evidence that bank vole females adjust their pup-defence intensity according to the size of their litter. These findings are in accordance with the parental-investment theory, which predicts a positive relationship between intensity of offspring defence and the number and (or) quality of offspring (Trivers 1972; Barash 1975). However, only a few studies indicate that aggression actually protects infants from being killed by intruders (Wolff 1985; Wilson et al. 1993). Defence intensity was also dependent on the mother's size, so that larger females defended their pups more vigorously than smaller ones. This illustrates the direct benefit of large size in contests affecting reproductive success.

We further studied the determinants of reproductive success in a situation where two neighbouring females were breeding in synchrony. In particular, we investigated whether the weaning success of females (weaned at least one offspring or none) was related to the number of offspring. This hypothesis was not supported, as weaning success was not related to litter-size manipulations in bank vole females. Instead, it seems that weaning success was particularly dependent on the relative body mass between the neighbouring females. Our results are in agreement with Agrell et al. (1995), who showed that the relative size of the female voles within the population explained their probability to reproduce. Furthermore, the size-dependent dominance hierarchy between the females in the runway system seemed to be settled within a few days after pairing. By that time, the larger female had destroyed the nest of the smaller female, which abandoned the nest and the offspring. No signs of bites or other physical injuries were observed on dead offspring. This could support the idea that, in populations of synchronously breeding females, the opportunity or potential for infanticide is lower (Elwood et al. 1990; Lambin 1993). However, it should be noted that, even when no infanticide was observed in the present study, larger females still caused the death of litters of smaller females.

Larger bank vole females had higher weaning success, which is in accordance with earlier studies (Mallory and Brooks 1978; Clutton-Brock et al. 1986; Woodroffe and McDonald 1995). For instance, in female ground squirrels, the largest and most vigorous females were most successful (through aggressive behaviour) in keeping unrelated intruders from their nest (Sherman 1981). This might also be the case in the bank vole, as the mother's size correlated positively with her intensity of defence.

As is always the case with laboratory studies, care must be taken when generalising the results to naturally occurring populations. For instance, in the current experiment, seven females adopted pups of neighbouring females. Adoption of non-kin offspring might not occur in the wild, since nest sites of bank voles are located in exclusive territories (defended area) in which all the pups are normally their own. As selection would also act against adopting non-kin offspring, it seems evident that, in the social system of a bank vole, such behaviour would occur infrequently in the wild and, in the present experiment, was possibly caused by the experimental setup. Nevertheless, the study indicates that intense aggression between females reproducing in neighbouring areas may have the same effects as infanticide; by chasing away neighbouring and (or) intruding females, dominant females cause the death of unrelated litters and thereby reduce the local competition for their own young.

To conclude, territorial bank vole females seem to increase their defence intensity with the number of pups in the litter. However, the success of a breeding attempt may primarily be determined by characteristics of neighbouring females, with size being of particular importance.

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