OIKOS 82: 365–369. Copenhagen 1998

Breeding suppression in voles under predation risk of small mustelids: laboratory or methodological artifact?

Tapio Mappes, Esa Koskela and Hannu Ylönen

Mappes, T., Koskela, E. and Ylönen, H. 1998. Breeding suppression in voles under predation risk of small mustelids: laboratory or methodological artifact? – Oikos 82: 365–369.

Many prey animals have life-history strategies that seem to have evolved to avoid predation from specialist predators. During the past few years, the hypothesis of breeding suppression (BSH) of voles as an adaptation to avoid small mustelid predation has prompted several empirical and theoretical studies. However, the validity of empirical work as well as justification of the conclusions in these studies have been criticised. Here we report results of an experiment in which we studied the breeding suppression of bank voles, *Clethrionomys glareolus*, in four replicated enclosures. We found no effects of simulated least weasel, *Mustela nivalis nivalis*, predation risk on the reproductive output of female voles. In contrast to earlier laboratory studies, the weights of females did not differ between treatment groups after the experiment. We reanalysed results of our earlier laboratory studies in light of these results and criticism of the BSH. At present there is no direct evidence for breeding suppression of voles in field populations. Furthermore, the underlying assumptions of the BSH have not been tested experimentally. Thus the most parsimonious explanation for breeding suppression is that it may be a methodological or laboratory artifact.

T. Mappes, E. Koskela and H. Ylönen, Dept of Biological and Environmental Science, Univ. of Jyväskylä, FIN-40351 Jyväskylä, Finland (tmappes@jylk.jyu.fi).

Recently several authors (Hansson 1995, Lambin et al. 1995, Korpimäki and Krebs 1996) have questioned the validity of studies indicating that some rodents respond adaptively to the odour of mustelid predators by suppressing reproduction (breeding suppression hypothesis; BSH) (Ylönen 1989, Korpimäki et al. 1994, Ylönen and Ronkainen 1994, Koskela and Ylönen 1995, Mappes and Ylönen 1997) or by delaying maturation (Heikkilä et al. 1993, Norrdahl and Korpimäki 1995, Heikkilä 1996). Further, a common theoretical view on the phenomenon is ambiguous (Kokko and Ranta 1996, Kaitala et al. 1997, Ruxton and Lima 1997).

The BSH states that because reproduction under predation risk causes significant survival cost for female voles and/or their offspring, voles should decrease these costs by delaying their breeding to the future when predation risk is lower. Further, the benefits of delayed breeding are hypothesized to be based on synchronous population crashes of voles and mustelid predators (for details see Mappes and Ylönen 1997).

Empirical evidence in support of BSH is based largely on laboratory studies and is plagued by problems with experimental design (e.g. lack of suitable controls) as suggested by Hansson (1995), Lambin et al. (1995) and Korpimäki and Krebs (1996). A second problem is that predictions of BSH (e.g. breeding suppression is an adaptation to minimise predation risk) have been stated before the assumptions have been adequately tested. This has led to lack of rigour in formulating the BSH at individual, population and community levels ((see, e.g., Ylönen 1994).

Here we present results of a field experiment which tend to support the above criticisms. We studied the effects of simulated least weasel predation risk on re-

Accepted 28 December 1997

ISSN 0030-1299

productive output of female bank voles *Clethrionomys glareolus* in a four-fold replicated enclosure experiment. Further, by reanalysing earlier laboratory data on weights of individuals we examined differences in physiological condition of voles between laboratory and field studies. We also discuss the validity of the recent laboratory and field studies and the underlying assumptions of the BSH.

Methods

Field study

In the field experiment the manipulation of predation risk was very similar to the earlier laboratory studies (see references in Figs. 1 and 2). The only main difference was that the voles were free-ranging in the large outdoor enclosures in the present experiment, and so we are able to compare the possible differences in the breeding tactics of voles in the field and the laboratory.

The field study was conducted at Konnevesi, central Finland in eight 0.25-ha enclosures (described in Mappes and Ylönen 1997) in June 1994. We used 40 wild-caught overwintered female bank voles (multiparous, non-pregnant) and eight captive weasels in the study. Bedding (sawdust and hay) with faeces and urine of four least weasels were collected daily and spread in four enclosures to stimulate predator odours, as follows: half of the amount was distributed randomly in the enclosures and the other half was spread in places where voles frequently move (trails and trap stations, not directly on traps). The other four enclosures served as controls; clean bedding was distributed similarly as in experimental enclosures. Approximately 1-m high fences around the enclosures prevented the access of wild mustelids to the enclosures.

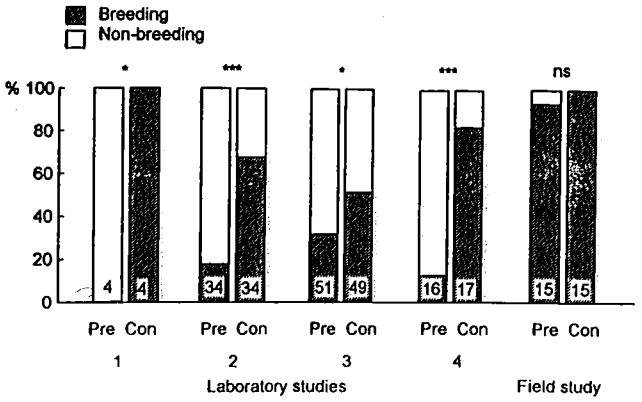

Fig. 1. Proportion of breeding and non-breeding females under simulated predation risk (Pre) and among control groups (Con) in the laboratory studies ((1) Ylönen 1989/Ylönen et al. 1992, (2) Ylönen and Ronkainen 1994, (3) Mappes and Ylönen 1997, (4) Koskela and Ylönen 1995) and in the present field study. Studies are in chronological order. Asterisks indicate significant differences ($*P < 0.05$, $***P < 0.001$).

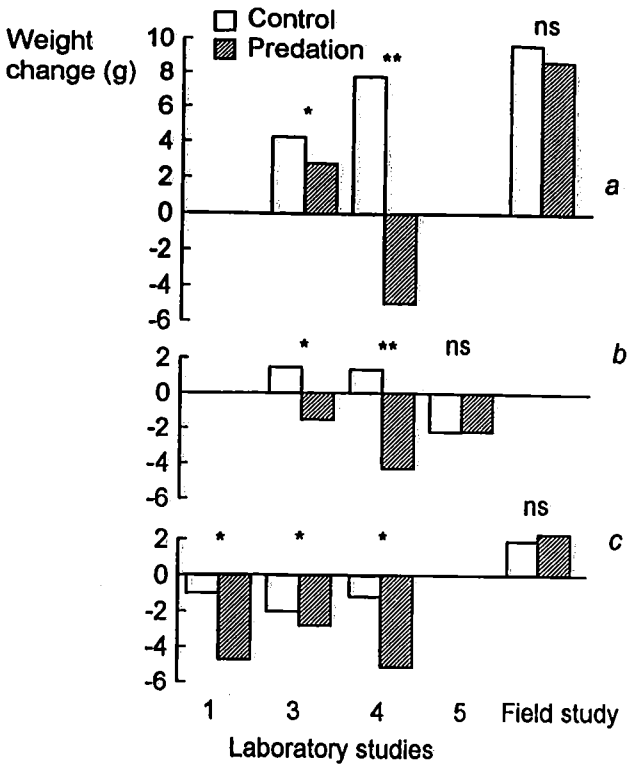

Fig. 2. Mean change in weight among breeding females (a), non-breeding females (b) and males (c). After the experiments, control animals were heavier than experimental animals in three studies (length of the experiment in parenthesis): (1) Ylönen 1989/Ylönen et al. 1992 (15 d), (3) Mappes and Ylönen 1997 (21 d), (4) Koskela and Ylönen 1995 (14 d). In the study (5) Koskela et al. 1996 (20 d) where an appropriate control was used, the final weight of females did not differ between the treatments, but weights decreased significantly in both treatments during the experiment. In the field study neither the weights of females or males differed significantly between treatments at the end of the study. Asterisks indicate significant differences ($*P < 0.05$, $**P < 0.01$). Details of statistics are in Table 2.

Five females were placed in each enclosure (mean weight $\pm$ s.e. $= 23.3 \pm 0.6$ g, no differences in weights between the treatments or enclosures, one-way ANOVA, in both cases $P > 0.4$). Spreading of predator and control odour started after ten days and continued daily until the end of the field portion of study (day 37). On day 20, three mature males were released in each enclosure. Before possible parturitions, females were removed from enclosures and housed in standard breeding cages in the laboratory to the end of study (day 46). All births occurred in the lab within eight days (mean = 4.1). Litter size was determined at parturition and pups were weighed and sexed at the age of one day.

Populations were monitored three times by live-trapping (days 6–10, 24–28 and 33–37). During each trapping period traps were checked 10 times, twice a day for five days. Disappeared individuals (not caught during four subsequent trap checks) were replaced with new similar individuals. During the study 13 individuals

Table 1. Characteristics of breeding under simulated predation risk and in the control group in the field study (mean ± sd). Analysis of variance used where the enclosures were nested within treatments. Sex ratio variable was arcsine square-root transformed before analysis.

	Control	Predation	F	d.f.	P
Breeding delay (d)	20.7 ± 1.8	21.5 ± 1.9	1.02	1,6	0.352
Litter size	5.9 ± 1.1	5.9 ± 1.3	0.23	1,6	0.651
Mean weight of pup in litter (g)	2.2 ± 0.3	2.1 ± 0.5	0.85	1,6	0.393
Litter sex ratio (no. of males/litter size)	0.57 ± 0.28	0.48 ± 0.25	2.91	1,6	0.139

were lost (five females/one male from the control and five females/two males from the experimental group). Only the individuals that were present throughout the study were used in the analyses (30 females and 21 males).

Laboratory studies

We compared the results of earlier laboratory studies (see references in Figs. 1 and 2) to the results from the field experiment. The main procedure in laboratory studies was as follows (see the papers for details). Male-female pairs of voles were placed in breeding cages for three weeks and exposed to predation risk by placing a real predator in an adjacent cage or spraying odours of mustelids on surroundings of experimental cages when control cages were sprayed with distilled water. Appropriate control (domestic rabbit *Oryctolagus cuniculus*) was used only in one experiment to control for possible neophobia (Fig. 2). In all experiments differences in weights before and after predator exposure were analysed separately for both sexes (Table 2). In two experiments the amount of food consumed was also studied (Koskela and Ylönen 1995, Koskela et al. 1996).

Results

Field study

The number of females breeding in the experimental enclosures (14 out of 15) did not differ significantly from the number breeding in the control enclosures (15 out of 15; Fisher's exact test, $P = 1.00$; Fig. 1). The time elapsed between the day when males were released into the enclosures and parturitions (breeding delay) was not significantly different between the treatments (Table 1). No significant differences were found for litter size, sex ratio and mean weight of pups between the females in two treatments (Table 1). Neither the weights of females nor males differed significantly between treatments at the end of the study (Fig. 2).

Laboratory studies

Breeding was suppressed significantly by female voles in all four laboratory studies (Fig. 1). After the experiments, control animals were heavier than experimental animals in three studies (Fig. 2 and Table 2). In the study where an appropriate control was used, the final weights of females did not differ between treatment groups (Fig. 2). However, during that experiment the weight of females decreased significantly among both treatment groups (Fig. 2).

Under simulated predation risk voles ate less than the control voles (Koskela and Ylönen 1995), but similar effects were not found when an appropriate control was used (Koskela et al. 1996).

Discussion

Earlier laboratory studies indicate that small rodent species, the bank vole and the field vole *Microtus agrestis*, suppress their breeding when exposed to odours of small mustelid predators. In our field experiment we did not find a similar response nor could we find any differences in reproductive output or weights of females and males in two treatments. We consider our results surprising because we simulated the breeding environment under high predation risk (daily faeces and urine of one least weasel/0.25 ha enclosure/day); nine times higher than the density of weasels observed in nature (Oksanen and Oksanen 1992) and still breeding suppression was not observed. While these results do not support our earlier laboratory experiments they agree with a recent field experiment conducted with the gray-tailed vole *Microtus canicaudus* in North America (Wolff and Davis-Born 1997) where voles did not suppress reproduction under simulated mustelid predation risk (mink *Mustela vison* scent).

In the laboratory studies of breeding suppression (Table 2) and delayed maturation (Heikkilä 1996) it is not possible to separate the role of laboratory conditions and methodological flaws to the results. In the only study where an appropriate control was used (Koskela et al. 1996) there were no differences in feed-

Table 2. Details of ANCOVA analyses of differences in final weights between treatments. In the analyses final weight was the dependent variable and treatment, pregnancy (females) and initial weight (covariate) were independent variables. In the field study the enclosures were nested within treatments.

Studies in chronological order		F	d.f.	P
Laboratory studies				
Ylönen 1989/ Ylönen et al. 1992[a]	Males	8.25	1,5	0.035
Mappes and Ylönen 1997	Females	4.15	1,45	0.047
	Males	4.41	1,48	0.041
Koskela and Ylönen 1995	Females	9.71	1,28	0.004
	Males	5.06	1,30	0.032
Koskela et al. 1996[b]	Females	0.01	1,19	0.941
Field study	Females	2.26	1,5	0.193
	Males	0.02	1,5	0.889

[a] Changes in weights of females were impossible to distinguish from the pregnancy effect because all females were pregnant in the control group; [b] Weights of females decreased significantly in both treatments during the experiment; Repeated measures MANOVA, Time: $F_{(1,20)} = 42.2$, $P = 0.000$, Treatment: $F_{(1,20)} = 0.03$, $P = 0.871$, Interaction $F_{(1,20)} = 0.00$, $P = 0.961$.

ing or final weights of voles between the treatments while weights decreased significantly in both groups (Fig. 2). This is in contrast to other laboratory studies and may indicate a general sensitivity or neophobia of voles to any strange odour (Hansson 1995, Lambin et al. 1995). In our laboratory studies voles have decreased both their activity (Ronkainen and Ylönen 1994) and feeding (Koskela and Ylönen 1995) under simulated predation risk. As reduced feeding may decrease female energy reserves and fecundity (Bronson 1989), the proximate cause of breeding suppression in the laboratory may be reduced foraging in response to laboratory conditions or strange odours.

Some field experiments have demonstrated an inverse relationship between proportion of breeding females and densities of small mustelid predators (Korpimäki et al. 1994, Klemola et al. 1997). This relationship could be explained by selective predation on breeding females, thus resulting in higher proportion of males and non-breeding females in the population (Klemola et al. 1997, Norrdahl and Korpimäki 1998), rather than breeding suppression. In these studies individual females were not monitored repeatedly so it is not known how they perform under varying predator densities. Therefore, at present there exists no direct evidence for breeding suppression of voles in nature.

In earlier papers (Ylönen 1994, Mappes and Ylönen 1997) we hypothesized that under high risk of mustelid predation female voles could benefit by delaying breeding from late in the breeding season to the next one in the following year, because mustelid populations usually crash during the winter. However, several assumptions of this hypothesis require verification. For instance: (1) reproduction must have survival costs for female voles under mustelid predation, (2) the tactic of breeding suppression should decrease these survival costs, (3) the probability of survival to the next breeding attempt (e.g. next breeding season) must be high enough to compensate for the lost opportunity, and (4) with high probability predation risk will decline substantially before the future breeding attempts. Without careful verification of the assumptions of BSH and their causalities one can fall into inaccurate argumentation: Because breeding is hypothesized to be costly under predation risk and voles seem to suppress breeding under predation risk, the tactic of breeding suppression is adaptation to predator induced reproductive costs (e.g. Norrdahl 1993, Korpimäki et al. 1994, Ylönen 1994, Koskela and Ylönen 1995, Heikkilä 1996, Mappes and Ylönen 1997).

At present there exists only some evidence for the first assumption of BSH. Cushing (1985) demonstrated that under laboratory conditions, deer mice (*Peromyscus maniculatus*) in oestrus were more vulnerable to weasel predation than non-oestrous females. However, complete suppression of breeding does not necessarily decrease predation risk if females do not also suppress their oestrous cycles. Koskela et al. (1996) found that female bank voles still had oestrous cycles under predation risk although females tended to decrease the time in oestrus. Whether a longer oestrous cycle is a better antipredatory tactic than to be pregnant and not in oestrous at all is equivocal.

We conclude that our present experiment does not support the BSH. Reduced foraging may be the proximate cause of breeding suppression in artificial laboratory conditions. However, it may be premature to reject the BSH before conducting long-term field experiments with real predators at natural densities (e.g. Klemola et al. 1997). Furthermore, the proposition that breeding suppression is related to cyclic predator-prey fluctuations needs clarification by experiments conducted both in cyclic and non-cyclic populations.

Acknowledgements – We thank R. V. Alatalo, V. Kaitala, E. Korpimäki, X. Lambin, J. Mappes, J. Pusenius and J. O. Wolff for constructive criticisms. Konnevesi Research Station provided working facilities. The study was supported by the Academy of Finland, Emil Aaltonen Foundation and Univ. of Jyväskylä.

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