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Effects of diet and origin of animals on growth and reproduction of *Clethrionomys glareolus*

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Populations of bank voles (*Clethrionomys glareolus*) in Northern Sweden have larger body size and larger litters than populations in Southern Sweden. These differences were maintained on a laboratory diet, which suggests that they are genetic, but voles might respond differently to natural diets. In the experiment reported here, we fed voles with different origins (Ammarnäs in the north and Revinge in the south) each of two diets, one mimicking the northern diet and one mimicking the southern diet. Northern animals grew better on the northern diet, suggesting an effect of diet quality, but litter sizes differed with origin of the animals and not with diet. High mortality of the young on both diets indicated that the test diets were not entirely satisfactory. Until a more definitive experiment can be done, our results suggest that differences in body size of the two populations may be linked to nutritional factors, but differences in litter size probably reflect different genotypes.

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1. Introduction

Clethrionomys glareolus from cycling populations at Ammarnäs in Northern Sweden (Swedish Lapland), have larger body sizes and larger litters than *C. glareolus* from noncycling populations in southern Sweden (Gustafsson 1983). This could be a genetic difference caused by selection for higher reproductive rate in the Lapland population or by selection for larger body size which is associated by larger litter size. On the other hand, a direct influence of some environmental factor, e.g. different food availability or quality at the two localities could be responsible. Previous breeding experiments with animals collected from these localities and fed a laboratory diet supported the hypothesis of genetic differences (Gustafsson et al. 1983a), but the difference found when using laboratory diets would probably not have occurred if the animals had been fed natural foods.

Here we report an experiment using food items chosen to mimic natural diets of the populations under study. Our purpose was to test the hypothesis that both nutritional and genetic factors influence the difference in body size and litter size between the two populations. If nutritional differences contribute to better performance in the north, we would expect northern animals to grow and reproduce better on a northern diet than on a southern diet. If northern animals do as well or better on a southern diet, the differences between field populations is not likely to be linked to diet. Southern animals might do better on either a northern or southern diet, depending on their nutritional adaptations and diet quality, but on either diet they should perform worse than the northern animals if differences in litter size and body size are simply genetic.

2. Material and methods

2.1. Animals

Two breeding colonies of animals, one originating from Revinge (Southern Sweden) and one from Ammarnäs (Northern Sweden) were established with founders caught in 1981 and 1982 at the same localities as those described by Gustafsson et al. (1983a). Voles were kept in plastic "shoebox" cages and fed a combination of mouse and rabbit chow *ad libitum*. All animals were kept on a long photoperiod (18L:6D) at approximately 20°C in the animal care facility of the Department of Biology, Division of Zoology, University of Oslo. The reproductive differences between the two present colonies when kept under standard conditions were essentially the same as those reported before (mean litter size of 6 for northern voles and 5 for southern voles).

2.2. Diets

The experimental diets were based upon the analyses of Hansson (1971, 1979) and Larsson & Hansson (1977) and designed to provide 50% herbs, 25% mast or seeds and 25% animal food. Sufficient amounts of seed and animal food could not be collected in natural localities so commercial hazelnuts (*Corylus avellana*) and mealworms (*Tenebrio molitor*) were used.

During July 1982 herbs were collected at Revinge and Ammarnäs, frozen on dry ice and transported to Oslo, where they were stored at −20°C. The following herbaceous species were used after determining that they were common in the habitats of the voles and that they were more palatable to the voles than other common herbs in the respective regions: *Artemisia vulgaris, Impatiens parviflora, Stellaria media, S. nemorum* and *Rumex domesticus* from southern Sweden and *S. nemorum, Lactuca alpina, Melampyrum pratense* and *Potentilla erecta* from northern Sweden.

Herbs were provided in excess (25 g herbs per animal per day) with proportions of different species that reflected their availability (Table 1). Hazelnuts and mealworms were supplied in different amounts depending on age (size) and, in females, reproductive state of the voles (Table 2). This was done to compensate for increasing metabolic demands. Adult animals seldom consumed all the hazelnuts, but most animals consumed all mealworms. Animals always ate substantial amounts of herbs but they seemed to prefer the nuts and mealworms and did not grow well on lesser amounts of these foods.

Table 1. Amounts of herbs (g) supplied per animal per day.

Southern diet		Northern diet	
Stellaria media	7	*Stellaria nemorum*	10
S. nemorum	5	*Lactuca alpina*	5
Artemisia vulgaris	5	*Melampyrum pratense*	5
Impatiens parviflora	5	*Potentilla erecta*	5
Rumex domesticus	3		

Table 2. Hazelnuts and mealworms supplies per day to different categories of animals.

Animal category	Hazel-nuts (g)	Mealworms Number	g
Males-females 18-20 days old	1.0	3	0.3
Males-females 21-26 days old	1.2	5	0.5
Males-females 27-35 days old	1.7	5	0.5
Males-nonlactating females, >35 days old	2.2	5	0.5
Females day 1-6 of lactation	2.8	7	0.7
Females day 7-12 of lactation	3.4	9	0.9
Females day 13-18 of lactation	4.0	11	1.1

2.3. Experimental design

Young animals from both colonies remained with their parents, on laboratory diet, until an age of 18 days. At day 18 they were randomly assigned to the experimental northern and southern diets. During a three-day transition (day 18-21) they also had access to laboratory mouse food.

After weaning males were kept isolated until an age of 60 days or older, when they were paired with females. Initially, females were paired with males at an age of 30 days, but this seemed to result in low incidence of pregnancy, so pairing was postponed until 40 days old in the second half of the experiment. Males and females were kept in pairs until three days after the birth of litters; a perforated dividing wall was then inserted in the cage to make separate compartments, one for the male and one for the female together with her young. This was done to enable us to increase the female's diet without any interference from the male. When the young were weaned at 18 days old or when a litter died, the barrier was removed. If a female was not pregnant by an age of 70 days old, a new male was placed together with the female.

Females were kept on experimental diets until 100 days old, or, if lactating at day 100, until weaning of their litter. At the end of the experiment, females were killed and uteri were examined for embryos and placental scars. Placental scars were used for litter sizes on four occasions when no young were found after parturition because they had been eaten shortly after birth.

3. Results and discussion

Body weights of 60 day old males and 40 day old females did not differ significantly either between colonies or between diets (Table 3 and 4), but the northern males grew less than the southern ones from weaning until 60 days. The only clear dietary effect was a significant diet-origin interaction in male body weights (Table 3): northern males were larger on the northern diet but southern males

Table 3. Body weight at days 18 and 60 and growth from day 18 to 60 for northern and southern males on northern and southern diets (mean±*SE* and (*n*)). Effects calculated with ANOVA.

		Origin			
	Diet	South	North	Effect	
Weight					
18 d.	South	8.2±0.4 (7)	10.4±0.7 (5)	Origin	$p<0.01$
	North	8.0±0.5 (7)	10.8±0.7 (5)	Diet	NS
				Interact.	NS
60 d.	South	19.7±1.2 (7)	16.3±0.6 (5)	Origin	NS
	North	18.2±0.9 (7)	19.4±1.2 (5)	Diet	NS
				Interact.	$p<0.05$
Growth					
	South	11.4±1.2 (7)	5.9±0.7 (5)	Origin	$p<0.001$
	North	10.1±0.8 (7)	8.6±1.6 (5)	Diet	NS
				Interact.	NS

Table 4. Body weight at days 18 and 40 and growth from day 18 to 40 for northern and southern females on northern and southern diets (mean±*SE* and (*n*)). Effects calculated with ANOVA.

Weight					
18 d.	South	8.3±0.5 (12)	10.9±0.6 (9)	Origin	$p<0.01$
	North	8.8±0.5 (11)	10.5±0.6 (9)	Diet	NS
				Interact.	NS
40 d.	South	15.5±1.1 (12)	16.8±0.7 (9)	Origin	NS
	North	15.9±1.2 (11)	18.2±0.9 (9)	Diet	NS
				Interact.	NS
Growth					
	South	7.0±0.8 (12)	5.9±1.1 (9)	Origin	NS
	North	7.3±1.0 (11)	7.7±1.1 (9)	Diet	NS
				Interact.	NS

Table 5. Litter size in northern and southern Swedish females on northern and southern diets (mean ±*SE* and (*n*)). Effects calculated with ANOVA.

	Origin			
Diet	South	North	Effect	
South	4.5±0.3 (8)	7.1±0.4 (9)	Origin	$p<0.01$
North	3.6±0.5 (8)	6.0±0.7 (11)	Diet	NS
			Interact.	NS

were not. The trends in growth rate of males and in body weight and growth of the females were the same, but the differences were not significant.

The higher growth rate of southern males was surprising because both the genetic and nutritional hypotheses predict better growth by northern animals. A confounding factor may be that growth rate declines at sexual maturation and that northern animals are variable in the age at which they reach sexual maturity, while South Swedish animals are much more homogeneous (Gustafsson et al. 1983b). Furthermore, northern young grow faster from birth to weaning (Gustafsson et al. 1983a), and, as can be seen in Tables 3 and 4, the northern animals were slightly larger than the southern ones at the start of the experiment (10.7 g vs. 8.4 g, respectively). Thus growth rates after weaning are not entirely consistent with weight at maturity. Nevertheless, the fact that body size differences found in the field were not expressed during this experiment suggests that some kind of genetic-nutritional interaction occurred.

As previously reported for animals fed laboratory mouse food (Gustafsson et al. 1983a), northern animals on "natural" diets had much larger litters than southern ones. No significant difference could be ascribed to diet (Table 5). The fact that this difference occurred even when body weights and diets of northern and southern animals were identical is consistent with the hypothesis that direct selection for large litter size has occurred in northern populations.

Differences in food quality could be expected to affect density, growth, and reproductive performance in different habitats. *Microtus ochrogaster* in central Illinois showed better growth, reproduction and survival and therefore more dramatic population cycles, in habitats with higher quality food (Cole & Batzli 1979). Animals in a habitat with poorer quality food responded to provision of high quality food with increased litter size, more winter breeding, and increased density (Cole & Batzli 1978). However, these differences between habitats within the same area may be somewhat different from the situation seen in *Clethrionomys glareolus* and *Microtus agrestis* in Scandinavia, where there is a substantial geographic separation between the noncyclic populations in Denmark and southern Sweden and cyclic ones in northern Scandinavia (Hansson 1979). The present experiments as well as previous comparisons using laboratory mouse food (Gustafsson et al. 1983a) suggest that the difference in reproductive performances seen between northern and southern Swedish animals is genetic; even though the ultimate factor responsible for this pattern may be different quality of available food.

On the other hand, our experimental sample sizes were small, we needed to adjust our protocols during the experiment, southern males grew better than northern males (contrary to expectations), and the trend in growth for northern animals was for better performance on northern diets (as predicted if differences between populations were linked to food quality). Furthermore, the mortality of young was high on both experimental diets, which may indicate some kind of deficiency in the test diets. *Clethrionomys glareolus* uses a wide range of food (Hansson 1971), but for practical reasons only the herb component could be collected in nature. Also, for practical reasons only a few of the most readily available herbs could be used. Bank voles probably cannot survive on a diet of only herbs (Hansson 1971), so a supplement was required. Hazelnuts and mealworms were supplied because they were readily available and because the quality of seed and insect components of the diet probably differ less than does the quality of herbs in northern and southern Sweden. Nevertheless the composition of the diet was not entirely natural, and a direct effect of nutrition on reproduction cannot be excluded.

A more definitive experiment on the influence of forage quality on population parameters in northern and southern Sweden should therefore be done. Until such results are available, those reported in this note suggest that differences in body size may be linked to nutritional factors, but differences in litter size probably reflect differences in genotypes.

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