Effect of nutrition, temperature and photoperiod on the rate of sexual maturation of the field vole (*Microtus agrestis*)

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Summary. Weanling male and female field voles were placed in long or short photoperiods, kept at 18°C or 4°C, and fed (*ad libitum*) diets containing 24%, 16%, 8% and 4% protein, for 6 weeks. Animals in the long photoperiod were more sexually mature than were animals in the short photoperiod. Temperatures had no effect on females, but did affect males: those kept at 4°C had heavier testes and wider seminiferous tubules than those kept at 18°C. There was little difference between the animals on 24%, 16% and 8% protein diets. Animals on 4% protein diets had retarded growth rates and were significantly less sexually mature than those on the other 3 diets, males having smaller testes and seminal vesicles and narrower seminiferous tubules and females having smaller ovaries and uteri.

Introduction

The field vole (*Microtus agrestis*) is a seasonal breeder, reproducing from spring to autumn each year. The main environmental factor regulating this is photoperiod: long photoperiods promote gonadal development and activity, while short photoperiods result in gonadal regression (Baker & Ranson, 1932; Clarke & Kennedy, 1967; Grocock & Clarke, 1974).

There are, however, some aspects of the reproductive activity of the field vole which cannot be explained by photoperiod alone. For example, it is not uncommon to find reproductively active animals in mid-winter, and this winter breeding has sometimes coincided with an unusually good food supply (Myllymäki, 1977; Hansson, 1984). On the other hand, young born in the late summer or early autumn, when there is still more than 12 h light per day, usually do not start to mature sexually until the following spring (Clarke & Forsyth, 1964): it is possible that the particular combination of photoperiod, temperature and nutrition found at that time delays the sexual maturation of these animals.

There are two ways in which diet can influence the reproductive state of animals, (1) through specific chemical compounds such as oestrogens (Moule *et al.*, 1963) or cyclic carbonates (Sanders *et al.*, 1981), or (2) through the overall plane of nutrition, such as the energy or protein content of the food. Low protein diets can cause a reduction in body growth (Anthony & Edozien, 1975) and affect the reproductive state of the animals (Leathem, 1970). Such diets have been shown to result in smaller and less developed testes in male rats (Horn, 1955), mice (Leathem & de Feo, 1952) and bulls (Meachem *et al.*, 1964), and retard the growth of uteri and ovaries in female rats (Srebnik *et al.*, 1978).

The field vole is a herbivore, feeding mainly on grass. The protein content of grass varies from around 8% and 30% throughout the year (Holmes 1980). Such variations, combined with the variations in ambient temperature that accompany these changes, could be additional factors causing the seasonal breeding exhibited by these animals.

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The following experiment was designed to investigate the separate and interactive influences of photoperiod, protein intake and ambient temperature on the rate of sexual maturation of field voles.

Materials and Methods

Animals were from the field vole colony maintained in the Department of Agricultural Science, Oxford, on a photoperiodic regimen of 16 h light:8 h dark (16L:8D). The husbandry of this colony is described by Baker & Clarke (1987). The breeding stock is fed oats and Dixon's diet 41B *ad libitum*, supplemented twice weekly by carrots and hay, with water constantly available. The experimental animals were fed a pelleted diet, described below, with water constantly available.

The experiment was designed as a $2 \times 2 \times 4$ factorial (Bailey, 1981), the three factors being (i) photoperiod, long (16L:8D) or short (6L:18D); (ii) temperature, warm ($18 \pm 2^\circ\text{C}$) or cold ($4 \pm 2^\circ\text{C}$); and (iii) diet protein level, P 24 (24%), P 16 (16%), P 8 (8%) or P 4 (4%), making 16 treatments in all. Groups of 16 male and female weanling voles were matched for age (16 ± 1 days), body weight (8–12 g) and absence of sexual maturity (females with non-perforate vaginas and males with no visible testicular development, a smooth, hairless perineum and a juvenile, small penis). Within each group, or block, of 16 animals, one animal was assigned to each of the treatments. There were 6 such blocks of male voles (6 animals per treatment) and 5 of females (5 animals per treatment).

The four diets were obtained from Special Diets Service Ltd, Witham, Essex, U.K. The 24% protein diet used was the Rat and Mouse No. 3 Breeding Diet (gross energy 15.4 MJ/kg), and the 16% protein diet was the Rat and Mouse No. 1 Modified Maintenance Diet. The 8% and 4% protein diets were made up by SDS Ltd to be compatible, from the point of view of energy content and minor nutrients, with the Rat and Mouse No. 1 Modified Maintenance Diet (gross energy varied from 14 to 16 MJ/kg). All diets were supplied in an expanded pellet form, and were available to the animals *ad libitum*.

Animals were kept in photoperiod cabinets (Crockett & Clarke, 1974), equipped with four 25-W tungsten bulbs, giving a light intensity of 188.3 klux at the front of the cage and 27.2 klux in the nest.

Treatments continued for 6 weeks, and animals were then killed in ether vapour. Ovaries, uteri and testes were weighed fresh and fixed in Bouin's fluid. Carcasses of males were preserved in 10% formalin, and the seminal vesicles were later weighed. Ovaries and testes were embedded in paraffin wax. Serial sections of ovaries and sections of testes, cut at 6 μm , were stained in Ehrlich's haematoxylin and eosin. Using the follicle classification of Pedersen & Peters (1968), total numbers of types 6, 7 and 8 follicles were counted in pairs of ovaries. All atretic follicles (defined as those containing 5 or more pycnotic nuclei in the granulosa) were also counted. Width of seminiferous tubules was estimated by measuring 15 tubules in each male, and using the mean of these figures in the statistical analysis.

Growth rates were calculated from the difference between final and initial body weights divided by time in days.

The significance of effects of treatments was tested by a Factorial Analysis of Variance (Bailey, 1981).

Results

Females

Uterine and ovarian weights. There was a significant photoperiod effect on uterine and ovarian weights ($P < 0.001$ for both) such that these organs were heavier in voles kept in long than in short photoperiods (Table 1). Temperature had no effect on either organ. There was no significant difference between the uteri or ovaries of females on the P 24, P 16 or P 8 diet. There was, however, an effect of the P 4 diet. There was a photoperiod $\times$ diet interactive effect on uterine weights ($P < 0.001$), such that, in the long photoperiod only, females fed the P 4 diet had significantly smaller uteri than those fed the other 3 diets. In the short photoperiod, females fed the P 4 diet were not significantly different from those fed the P 24, P 16 and P 8 diets. Ovarian weights were depressed by the P 4 diet in the long and short photoperiods, and showed a diet effect ($P < 0.001$).

Ovarian follicle numbers. Ovarian follicle numbers showed no clear pattern, the only significant effect being one of photoperiod on type 8 follicles: females in the long photoperiod had more type 8 follicles than did those in the short photoperiod (2.52 ± 0.50 compared with 0.96 ± 0.30 follicles: $P < 0.01$). No corpora lutea were found in any of the ovaries.

Growth rates. There was no photoperiod or temperature effect on growth rates, and no effect of diet amongst the P 24, P 16 and P 8 females (Table 2). P 4 females had significantly lower growth rates than P 24, P 16 or P 8 females in the long and short photoperiods ($P < 0.001$).

Table 1. Uterine and ovarian weights (mg) of females exposed for 6 weeks to long (16L:8D) or short (6L:18D) photoperiods, kept at 18°C or 4°C, and fed diets of 24% (P24), 16% (P16), 8% (P8) or 4% (P4) protein levels

Photoperiod		Temperature and diet							
		18°C				4°C			
		P24	P16	P8	P4	P24	P16	P8	P4
Long	Uterus	19.6 ±0.7	47.5 ±7.6	19.3 ±4.9	5.7 ±0.6	25.3 ±8.8	25.8 ±9.8	26.9 ±9.8	6.0 ±0.4
	Ovary	3.32 ±0.55	3.56 ±0.37	3.36 ±0.39	1.42 ±0.18	3.80 ±0.51	4.52 ±0.26	4.10 ±0.56	2.08 ±0.45
Short	Uterus	7.9 ±1.0	9.5 ±1.8	7.8 ±1.0	5.2 ±0.7	7.2 ±1.0	6.4 ±0.5	10.8 ±4.0	6.8 ±1.5
	Ovary	2.08 ±0.36	2.36 ±0.36	3.12 ±0.18	1.32 ±0.20	2.04 ±0.26	2.40 ±0.22	2.42 ±0.42	1.72 ±0.14

Values are mean ± s.e.m., 5 animals per treatment.

Table 2. Growth rates (g/day) of females (5 animals per treatment) and males (6 animals per treatment) exposed for 6 weeks to long (16L:8D) or short (6L:18D) photoperiods, kept at 18°C or 4°C, and fed diets of 24% (P24), 16% (P16), 8% (P8) or 4% (P4) protein levels

		Temperature and diet							
		18°C				4°C			
Photoperiod	Sex	P24	P16	P8	P4	P24	P16	P8	P4
Long	Females	0.34 ±0.03	0.33 ±0.06	0.34 ±0.05	0.09 ±0.04	0.37 ±0.03	0.36 ±0.02	0.36 ±0.03	0.13 ±0.01
	Males	0.43 ±0.05	0.38 ±0.05	0.34 ±0.03	0.08 ±0.04	0.48 ±0.04	0.42 ±0.03	0.42 ±0.03	0.19 ±0.04
Short	Females	0.31 ±0.04	0.32 ±0.03	0.34 ±0.05	0.13 ±0.03	0.36 ±0.04	0.39 ±0.04	0.33 ±0.04	0.18 ±0.02
	Males	0.42 ±0.05	0.39 ±0.05	0.38 ±0.03	0.08 ±0.02	0.34 ±0.04	0.28 ±0.03	0.35 ±0.03	0.15 ±0.01

Values are mean ± s.e.m.

Males

Testicular and seminal vesicle weights. Males in the long photoperiod had heavier testes and seminal vesicles than those in the short photoperiod ($P < 0.001$ for both) (Table 3), although several of the males in the short photoperiod did have testicular weights ranging from 130 to 300 mg. There was no significant difference in testicular or seminal vesicle weights between males on diets P 24, P 16 and P 8. There was a photoperiod × diet interaction on both organs ($P < 0.001$ and $P < 0.01$ respectively) such that, in the long photoperiod only, males fed the 4% protein diet had significantly smaller testes and seminal vesicles than those fed the other 3 diets. In the short photoperiod, males fed the 4% protein diet were not significantly different from those fed the 24%, 16% and 8% diets. Temperature had a significant effect on testicular weights: males kept at 4°C had heavier testes than males kept at 18°C ($P < 0.01$).

Table 3. Paired testicular and seminal vesicle weights (mg) and seminiferous tubule widths (μm) of males exposed for 6 weeks to long (16L:8D) or short (6L:18D) photoperiods, kept at 18°C or 4°C, and fed diets of 24% (P24), 16% (P16), 8% (P8) or 4% (P4) protein levels

Photoperiod		Temperature and diet							
		18°C				4°C			
		P24	P16	P8	P4	P24	P16	P8	P4
Long	Testes	338 ± 31	340 ± 36	281 ± 29	85 ± 26	399 ± 47	321 ± 36	361 ± 17	198 ± 48
	Seminal vesicles	129.9 ± 40.1	112.4 ± 38.9	80.2 ± 34.7	13.6 ± 11.7	122.6 ± 33.6	113.8 ± 23.2	93.1 ± 33.8	16.7 ± 6.9
	Tubules	185.7 ± 7.4	187.5 ± 5.0	138.8 ± 9.7	104.0 ± 12.3	200.5 ± 7.1	185.7 ± 6.5	164.7 ± 7.5	148.0 ± 17.6
Short	Testes	37 ± 9	40 ± 8	36 ± 4	23 ± 12	63 ± 20	35 ± 9	97 ± 46	25 ± 6
	Seminal vesicles	2.6 ± 0.2	3.1 ± 0.7	2.7 ± 0.6	1.8 ± 0.2	3.4 ± 0.7	2.2 ± 0.2	8.6 ± 5.8	1.8 ± 0.4
	Tubules	87.7 ± 8.9	90.2 ± 4.1	77.8 ± 6.6	62.2 ± 9.1	91.5 ± 13.6	86.2 ± 6.6	91.7 ± 16.0	73.2 ± 6.7

Values are mean $\pm$ s.e.m., 6 animals per treatment.

Seminiferous tubule width. As with testicular weights, the seminiferous tubules showed a photoperiod effect ($P < 0.001$), with males in the long photoperiod having wider tubules than those in the short photoperiod, and a temperature effect ($P < 0.01$), with males kept at 4°C having wider tubules than those kept at 18°C (Table 3). In the long photoperiod, P 24, P 16 and P 8 males had wider tubules than P 4 males ($P < 0.001$). However, a difference also appeared between the P 24, P 16 and P 8 males: the tubules of P 8 males were narrower than those of P 24 and P 16 males ($P < 0.001$). As with the testicular and seminal vesicle weights, there was no effect of diet in the short photoperiod, and therefore the diet effect was shown as a photoperiod $\times$ diet interaction.

Growth rates. There was a photoperiod effect on growth rates only amongst the animals living at 4°C (Table 2): males in the long photoperiod had a higher growth rate than those in the short photoperiod (photoperiod $\times$ temperature interaction: $P < 0.05$). There was no diet effect amongst the P 24, P 16 and P 8 males, but there was an effect of diet at the P 4 level, in the long and short photoperiods, such that P 4 males had lower growth rates than P 24, P 16 and P 8 males ($P < 0.001$).

Discussion

The well established effect of photoperiod upon the sexual development of field voles has again been demonstrated here for various measures of ovarian and testicular activity.

In some laboratory populations of deer mice and white-footed mice, sexual development in a fairly large proportion (20–40%) of males is not inhibited by short photoperiods (Johnston & Zucker, 1980; Beasley *et al.*, 1981; Desjardins & Lopez, 1983). This was also found here, and has been described in the past for the field vole, although the proportion of animals not inhibited appears to be smaller (Clarke, 1977; Spears & Clarke, 1987).

Temperature had no effect on the sexual maturation or the growth rates of the females. Temperatures did, however, have an effect on males: those kept at 4°C had significantly larger testes and

significantly wider seminiferous tubules than did those kept at 18°C. There was no interaction between diet and temperature on the sexual maturation of males or females.

Protein levels in grass vary from around 30% in early spring, when the grass is starting to grow, to its lowest of about 8% in late summer, after flowering (Dent & Aldrich, 1968; Green *et al.*, 1971; Holmes, 1980). In this experiment, there was surprisingly little difference between the animals fed diets containing 24%, 16% or 8% protein. Indeed, the only indication of any diet effect amongst these groups was that the P 24 and P 16 males had wider seminiferous tubules than the P 8 males. In general, therefore, the 8% protein diet, corresponding to late summer/early autumn levels of protein (a time of year when weanlings do not mature sexually in spite of the long photoperiod), had little effect on the sexual maturity of the animals. Although earlier studies considered that most *Microtus* species needed a diet containing about 13% protein, more recently minimum protein requirements have been set at 8% (Batzli, 1985). It is possible that the P8 animals compensated for the low protein content of the diet by eating more food. Unfortunately, it was not possible to measure food intake, due to the large and variable amount of spillage by the animals. However, with the exception of some arctic species, food supply is rarely thought to be limiting to microtine rodents (Southern, 1979), and therefore animals in the wild would be able to increase food intake in response to low food quality. Laboratory rats fed low (1 or 2%) protein diets have been found to have a food intake lower than that of animals fed 20% protein diets, with food consumption per gram body weight remaining the same (Crompton *et al.*, 1981).

When protein levels were reduced to 4% (roughly the level of protein in straw), a strong dietary effect was found: both males and females fed the P 4 diet were less sexually mature and had slower growth rates than did the P 24, P 16 and P 8 animals. Uterine, testicular and seminal vesicle weights were reduced by the P 4 diet only amongst long photoperiod animals, so that there was a photoperiod $\times$ diet interactive effect. Female ovarian weights and growth rates and male growth rates were reduced by the P 4 diet in the long and short photoperiod, and did not show a photoperiod $\times$ diet interactive effect. It seems likely, therefore, that where there was no diet effect in the short photoperiod, this was because uterine, testicular and seminal vesicle weights were already suppressed to a basal level by the short photoperiod. Indeed, the organ weights of the short photoperiod animals on all diets and at both temperatures compare with those of animals in the wild in winter (Clarke & Forsyth, 1964).

Quantitative and qualitative changes in the diet, without regard to the chemical composition of the food, has been shown to alter the gonadal activity of several species of rodents. Grocock (1972), working with male field voles kept at 18°C, found that reducing the diet of whole oats and carrots by 50% caused a significant decline in testicular weight in long and short photoperiods. Testicular regression also occurs in deer mice (*Peromyscus maniculatus*) given a restricted diet (Desjardins & Lopez, 1983; Blank & Desjardins, 1984). An interactive effect of photoperiod, diet and temperature or of photoperiod and diet on testicular weights has been observed in male Australian bush rats (*Rattus fuscipes*) and golden hamsters (*Mesocricetus auratus*) (Irby *et al.*, 1984; Johnson & Hoffman, 1985). On the other hand, gonadal activity of house mice seems to be little affected by low protein diets, or by quite considerable restriction of diet, particularly amongst males (Vandenbergh *et al.*, 1972; Hamilton & Bronson, 1985).

Under laboratory conditions, supplementing the pelleted food of two species of *Microtus* with lettuce or fresh spinach caused increased sexual development compared with animals fed pellets alone (Berger & Negus, 1974; Nelson *et al.*, 1983). The P 24 pellets used in this experiment are also probably not, on their own, an optimal diet. A comparison of the animals in this experiment with voles of the same age from a previous experiment, fed whole oats, pellets and carrots, shows that the P 24 animals described here had smaller testes and uteri, and lower growth rates, in long and short photoperiods (Spears & Clarke, 1986).

Supplemental feeding also affects the reproductive activity of wild populations of *Microtus* (Negus & Berger, 1977; Cole & Batzli, 1978; Taitt & Krebs, 1981), although such feeding appears

not to affect the 3–4-year population cycles exhibited by many species of *Microtus* (Cole & Batzli, 1978; Abdellatif *et al.*, 1982).

Growth rates of females were unaffected by photoperiod, but males kept at 4°C had reduced growth rates in short photoperiods. Consequently, there was a photoperiod × temperature interactive effect on male growth rates. Decreased growth rates of voles in short photoperiod have already been reported (Dark & Zucker, 1984; Spears & Clarke, 1986), although such an effect does not always occur (Wunder, 1984; Spears & Clarke, 1987).

The temperature effect found here, such that males kept at 4°C had heavier testes than those kept at 18°C, was unexpected. Earlier experiments with field voles found no significant effect of temperature on males or females (Clarke & Kennedy, 1967). In addition, it is not clear why low temperatures (of around 4°C) should stimulate sexual maturation. Low temperatures suppress reproductive productivity in house mice (Pryor & Bronson, 1981), delaying the onset of breeding and increasing the inter-birth interval (Barnett, 1973), result in reduced testicular weights in rats and golden hamsters (Heroux & Campbell, 1959; Hoffman *et al.*, 1965), and, when applied in combination with short photoperiods, cause testicular regression in deer mice (Desjardins & Lopez, 1983). However, the increase in testicular weights by low temperatures has been described before, in the red-backed vole, the desert pocket mouse and in one strain of deer mouse (Roth, 1974; Kenagy & Bartholemew, 1981; Bronson & Pryor, 1983).

It is known that field voles from laboratory stock are not exactly representative of the F1 generation of wild voles in their sexual development (Spears & Clarke, 1987). Any application of the present results to wild vole populations will, therefore, need a degree of caution. It does, however, seem likely that neither the onset nor the cessation of breeding is regulated via the protein content of the diet in field voles in the wild. Protein had to be reduced to a level below that found in winter grass before it had an effect on the reproductive state of the laboratory voles, and no interaction between diet and temperature was found. Any role of nutrition in regulating the breeding season may, therefore, need to be looked for amongst other components of the diet such as energy levels or specific 'signals'.

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