Influence of photoperiod, nutrition and water availability on reproduction of male California voles (*Microtus californicus*)

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Summary. Male California voles were maintained in long (14L:10D) or short photoperiods (10L:14D) for 10 weeks and fed a standard diet of rabbit chow and water *ad libitum*. One additional group in each photoperiod received the standard diet plus supplements of spinach 3 times weekly. A fifth group was housed in 14L:10D and fed the standard diet, but for 10 weeks water availability was restricted to several hours each morning. Testes and seminal vesicles were heaviest in long-day voles fed spinach supplements and lightest in short-day voles fed only the standard diet; the latter animals manifested reduced testicular spermatogenesis. Testicular weights were also depressed in voles with restricted access to water. It is suggested that photoperiods that simulate those of winter induce regression of the reproductive organs of male California voles but the availability of green vegetation counteracts the inhibitory effects of short daylengths.

Introduction

Photoperiod is a proximate factor used by many temperate zone mammals to restrict breeding to one time of the year (Sadleir, 1969). All *Microtus* species so far examined are 'long-day' breeders, i.e. they are reproductively competent when daylengths exceed some minimum value (usually >12 h light/day) (Lecyk, 1962; Negus & Berger, 1972; Imel & Amann, 1979; Grocock, 1981; Dark, Zucker & Wade, 1983). Some mammals, particularly ungulates, are short-day breeders (reviewed by Sadleir, 1969) in that their reproductive system is stimulated or maintained by short daylengths (usually <12 h light/day) (Lincoln & Kay, 1979; Karsch, Goodman & Legan, 1980; Lincoln & Short, 1980; Legan & Winans, 1981).

The extent to which photoperiod is a useful proximate factor depends on the year to year predictability of favourable conditions (Negus & Berger, 1972). The onset of breeding in montane voles (*Microtus montanus*) inhabiting meadow regions, where the time of snow melt-off varies annually, is influenced by photoperiod; however, ingestion of young growing plants can initiate reproductive activity (Pinter & Negus, 1965; Berger, Negus, Sanders & Gardner, 1981; Sanders, Gardner, Berger & Negus, 1981).

California voles, *M. californicus*, inhabit grasslands from southwestern Oregon through California and south to northern Baja California (Church, 1966). The breeding season of the California vole in the San Francisco Bay area is from November to May (Greenwald, 1956; Batzli & Pitelka, 1971; Lidicker, 1973, 1976). Winter breeding coincides with the seasonal rains and subsequent availability of new plant food (Lidicker, 1973). California voles have not evolved specialized mechanisms for minimizing body fluid loss during droughts (Church, 1966); in the

summer, when free-standing water is unavailable, body weights are low and the voles are infertile (Lidicker, 1973). The regulation of the breeding cycle of California voles requires clarification. Reproduction coincident with the short daylengths of winter is consistent with several hypotheses. (1) These voles may be short-day breeders, i.e. they are stimulated to reproductive activity by short daylengths and perhaps also inhibited by long photoperiods. There are no previous reports of rodent species that are short-day breeders. (2) The California vole may be non-photoperiodic, i.e. reproduction is regulated by other factors and length of the daily photoperiod is irrelevant. (3) These voles may be typical long-day breeders whose reproductive apparatus regresses in short daylengths; however, the inhibitory effects of photoperiod are counteracted by ingestion of green vegetation. The present study addressed the influence of photoperiod, green plant food and water availability on the maintenance of the reproductive apparatus.

Materials and Methods

Sexually mature male voles (>50 days of age; $N = 72$) were randomly assigned to groups balanced for age, body weight and degree of scrotal development; animals were individually housed and maintained in cabinets for 10 weeks. One cabinet was illuminated for 14 h/day (14L:10D; lights on 07:00 h Pacific Standard Time); the other cabinet was illuminated for 10 h/day (10L:14D; lights on 06:00 h PST). Animals were first generation offspring of voles trapped at the Russel Reserve Field Station, Lafayette, California. The standard food (Purina Rabbit Chow) and tap water were available *ad libitum* for one group of 14 voles in each photoperiod (Groups L1 and S1). Separate groups (L2 and S2) in each photoperiod were fed the standard diet plus supplements of fresh spinach (*Spinacia oleracea*) (3–5 g/vole) three times per week (10 voles in 14L:10D; 12 voles in 10L:14D). A fifth group ($N = 16$) maintained in 14L:10D was fed the standard diet *ad libitum*, but water availability was restricted to 4 h each morning (09:00–13:00 h) for 5 weeks and then to 2 h (09:00–11:00 h) for 5 additional weeks (Group L3). Water intake was measured several times during the experiment.

At the end of 10 weeks voles were given a lethal dose of pentobarbitone sodium. Paired testes weights, seminal vesicle weights, body weights and the length and width of the left testis were recorded. Testis volume was calculated using the formula for a prolate spheroid (i.e. $\frac{4}{3}\pi \{0.5 \text{ length}\} \{0.5 \text{ width}\}^2$ or $0.523 \{ \text{length} \} \{ \text{width} \}^2$; Lidicker, 1973).

Testes were stored in buffered formalin, dehydrated, embedded in paraffin wax, sectioned at 5 μm and sections stained with haematoxylin and eosin. Slides of testicular tissue were examined microscopically and rated for spermatogenesis (Spermatogenic Index) using the rating system developed for field voles, *M. agrestis*, by Grocock & Clarke (1974). In this classification a value of 5 = large seminiferous tubules with complete spermatogenesis; 4 = complete spermatogenesis with spermatozoa and elongated spermatids decreased in number; 3 = the number of spermatozoa are further reduced but elongated spermatids are present; 2 = elongated spermatids are absent; 1 = only Sertoli cells, spermatogonia and primary spermatocytes are present; and 0 = only Sertoli cells and spermatogonia are observed.

Water intake data were analysed by Student's *t* test. All other data were assessed by a 2-way analysis of variance.

Results

Photoperiod and diet

Photoperiod and dietary supplements of spinach each affected testicular weight ($P < 0.05$, respectively) (Text-fig. 1). Testes were heaviest in Group L2 voles and lightest in voles in Group S1 ($P < 0.001$). Voles in Groups L1 and S2 had testes of intermediate weight that did not differ

Text-fig. 1. Paired testicular and seminal vesicle weights (mean $\pm$ s.e.m., $N = 14$) in voles after 10 weeks of exposure to experimental treatment.

significantly from those of other groups. Seminal vesicle weights followed a similar trend; however, the effects of photoperiod and of spinach supplements were not significant ($P > 0.10$ in each case; Text-fig. 1). Neither photoperiod nor spinach supplements affected the Spermatogenic Index. In Group S1, 4 of 14 voles (29%) had fully regressed testes (SI ≤ 3 and testes weights < 100 mg). None of the animals in the other groups had completely regressed testes.

Water availability

Water intake was substantially decreased ($P < 0.001$) in Group L3 voles (11.2 ± 0.5 ml) when they had restricted access to daily fluid compared with the value before restriction (26.5 ± 2.7 ml). Body weight gain of Group L3 voles (0.4 ± 1.4 g) was lower than that of control animals (Group L1; 10.0 ± 1.9 g; $P < 0.001$) during the 10-week testing interval.

Testes weights of Group L3 voles were lower than those of unrestricted voles in Group L1 (137.2 ± 0.1 and 182.8 ± 21.2 mg; $P < 0.05$). Neither the weight of the seminal vesicles nor the Spermatogenic Index differed significantly between these groups (60.9 ± 0.01 and 61.1 ± 0.01 mg; $P > 0.05$). Most animals had a Spermatogenic Index of 4. Testicular volumes (mm^3) were 102 ± 11 in Group L1, 112 ± 5 in Group L2, 72 ± 5 in Group L3, 79 ± 7 in Group S1 and 101 ± 9 in Group S2; values for Groups L1 and L3 were significantly different ($P < 0.05$).

Discussion

California voles exposed to short photoperiods had depressed gonadal weights in comparison to animals housed in long days and fed fresh greens. Limiting access to water to 2 h per day decreased daily fluid consumption by approximately 50% and also depressed gonadal weights; reduced water intake is one of the sequelae of summer drought and may account for the summer hiatus in reproduction of California voles.

Short photoperiods exert similar effects on the reproductive apparatus of California voles as on other species of the genus *Microtus*; in all species studied to date, short daylengths decrease the size of gonadal and sexual accessory tissues (Lecyk, 1962; Pinter & Negus, 1965; Grocock & Clarke, 1974). However, responsiveness to short photoperiods was relatively modest in California voles as compared to other vole species (Desjardins, 1981; Dark *et al.*, 1983). In meadow voles (*M. pennsylvanicus*), for example, exposure to short photoperiods reduced testicular weight in virtually all animals (Dark *et al.*, 1983), whereas a relatively small proportion (29%) of California voles fed

the standard diet had completely regressed gonads. Furthermore, absolute degree of gonadal regression induced by short photoperiod was much greater among meadow voles (1404 to 196 mg) (Dark *et al.*, 1983) than in California voles (182 to 75 mg). The California vole is similar to the montane vole (*M. montanus*) in that ingestion of green vegetation protects the reproductive apparatus from the inhibitory effects of short photoperiods (see Berger *et al.*, 1981).

In the San Francisco Bay area California voles breed primarily during the winter months (November to May); breeding is coincident with short photoperiods, the onset of the rainy season, and new growth of annual vegetation. Reproductive arrest is coincident with long photoperiods during spring and summer, annual summer drought, and the presence of dry mature vegetation. Our studies suggest that photoperiod is not the principal proximate stimulus for the seasonal reproductive cycle. Onset of breeding by California voles is most probably initiated by ingestion of new green vegetation during the winter rainy season. Voles fed with fresh spinach showed maintenance of their reproductive apparatus regardless of photoperiod. The disappearance of green vegetation in the spring may not be sufficient to induce reproductive quiescence since voles fed the unsupplemented standard diet and maintained in long photoperiods (Group L1) had large reproductive organs. Limited access to water under otherwise similar conditions reduced gonadal weight, thereby suggesting that termination of reproductive activity in the late spring is caused by insufficient water. Indeed, voles in outdoor enclosures during the summer months showed increased breeding activity when supplied with supplementary water (Lidicker, 1976). The fertility of California voles in the field is correlated with testis dimensions (Lidicker, 1973); testis volumes $\geq 100 \text{ mm}^3$ indicate fertile males while those of $< 100 \text{ mm}^3$ relate to infertile individuals. If testicular volume is a valid indicator of fertility in laboratory populations, then short photoperiods and restricted water intake cause reduced fecundity and green vegetation can restore normal fertility.

Purina Rabbit Chow was used as the standard diet to facilitate comparisons to similar studies with montane voles (e.g. Berger *et al.*, 1981). The high content of green alfalfa in this diet (Batzli & Cole, 1979) may stimulate the voles' reproductive apparatus and could mask the regressive effects of short days. It remains to be determined whether a greater proportion of California voles maintained on a diet devoid of alfalfa would be responsive to short photoperiods. Similarly, only 25% of water-restricted voles showed a complete absence of spermatozoa. It is questionable whether the decreases in weight of the reproductive apparatus observed would translate into diminished production of offspring. However, the impact of reduced fluid availability on reproductive status also may have been ameliorated by the high alfalfa content in the diet.

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