

## Seasonal reproduction and delayed sexual maturity in mound-building mice *Mus spicilegus*

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**Abstract.** In the mound-building mouse, *Mus spicilegus*, which is found from Central to Eastern Europe, reproduction is seasonal and limited to spring and summer. In autumn, the mice build voluminous mounds composed of vegetable matter covered with earth, where juvenile animals (autumnal individuals) over-winter in groups without reproducing. Autumnal animals delay reproduction until the next spring when they are 6 months old. The influence and interactions of environmental (short light period and cold temperature (C conditions) compared with long light period and temperate temperature (T conditions)) and social factors (lack of odours from breeding adults (NB conditions) compared with presence of odours from breeding adults (B conditions)) on reproduction and sexual maturation were studied. Forty groups of three autumnal individuals (two males and a female or two females and a male) were placed in four experimental conditions (CB, CNB, TB and TNB), corresponding to interactions between environmental and social factors ( $n = 10$  groups for each condition). Of the 40 groups only one initiated reproduction during the 18 weeks of cohabitation. Subsequently, animals were separated and isolated for 1 month and then paired with unfamiliar partners. Reproduction was monitored for an additional month, and 24 out of 39 females reproduced. In addition, of eight reproducing pairs placed in C conditions and 10 reproducing pairs maintained in T conditions, all but one pair continued reproduction. It was concluded that the delay in reproduction observed in autumnal individuals was the result of the social effects of living in groups as opposed to the environmental conditions of winter.

*Extra keywords:*

### Introduction

Several species of rodents from temperate latitudes limit their reproduction to specific times of the year when environmental conditions permit successful reproduction (reviewed by Bronson and Heideman 1994). Change in daily photoperiod appears to be a good predictor of oncoming favourable periods and many seasonal breeding species use this cue to time breeding accurately (Heideman 2000). Short daylengths inhibit breeding in adults and typically delay sexual maturation until the following spring in animals born at the end of the breeding season.

In the house mouse, *Mus musculus domesticus*, photoperiod does not appear to be a major proximate environmental cue regulating reproduction (Bronson 1979). Commensal populations breed continually, whereas feral populations may modulate their reproductive activity according to environmental conditions. Reproduction is an energy-consuming process, especially for females, and availability of food affects reproductive success in mice (Bronson and Marsteller 1985). The cost of foraging increases with food scarcity and with cold temperature and may inhibit reproduction in

mice (Perrigo and Bronson 1983, 1985; Barnett and Dickson 1989, Manning and Bronson 1990). Other species of European mice studied appear to be opportunistic breeders. *Mus musculus musculus*, the eastern subspecies of the house mouse, migrates seasonally from an outdoor habitat to human buildings, but reproduction is not altered by these migrations and is continuous when conditions remain favourable (Pelikán 1981; Carlsen 1993). The Algerian mouse, *Mus spretus*, a feral species found in south-western Europe as well as in North Africa, is able to reproduce throughout the year depending on climatic conditions and food availability (Cassaing 1984; Cassaing and Croset 1985; Fons and Saint Girons 1993).

The mound-building mouse, *Mus spicilegus*, differs dramatically from these other species of mice by displaying a high level of seasonality in reproduction. In autumn, mice build voluminous mounds composed of vegetable matter (seeds, spikes and other vegetal material) and covered with earth. Underneath this mound, the mice excavate a set of galleries and one or two nest chambers. Individuals in a mound are juvenile animals born in late summer and early autumn

(called hereafter autumnal individuals). With the exception of rare adult females, adult mice are not found inside mounds and disappear soon after the construction of mounds. Autumnal individuals in a mound may belong to a single litter but more commonly are from differently related female parents and their unrelated mates (Garza *et al.* 1997). In early spring, autumnal individuals aged 6 months leave the mounds, disperse and reproduce (Naumov 1940; Pisareva 1948; Murariu 1981; Orsini *et al.* 1983; Duryadi 1993; Milishnikov *et al.* 1998). Under laboratory conditions and a long photoperiod (14 h L : 10 h D), mound-building mice are able to initiate reproduction when they are 2 months old and a breeding pair can reproduce continuously for more than 1 year, with approximately one litter every 28 days (P. Gouat *et al.*, unpublished data). Two types of proximal factors may affect the timing of reproductive maturation: (i) environmental; and (ii) social factors. The geographical range of mound-building mice extends in temperate latitudes from Central to Eastern Europe (Orsini *et al.* 1983; Bonhomme 1992; Sage *et al.* 1993; Sokolov *et al.* 1998) where winters are cold and snowy with short daylengths. Cold ambient temperatures encountered by mound-building mice during winter may challenge the energy balance of adults living outside mounds and, as a result, reproduction may be inhibited. Food availability must not be a limiting factor for autumnal individuals, in so far as there is no reproduction in mounds; mounds constitute a reservoir of food available without any high foraging cost and seeds are still plentiful in the spring (Murariu 1981; Gouat *et al.* 2003). The lack of fresh material and of specific plant compounds may contribute to reproductive failure (Nelson and Shiber 1990; Meek *et al.* 1995). Yet, under laboratory conditions, mice fed standard mice pellets and no additional fresh food reproduce continuously (P. Gouat *et al.*, unpublished observations). A short photoperiod may serve as a synchronizer, as described in many seasonal breeding species (Bronson and Heideman 1994), either to inhibit reproduction during a short photoperiod or to initiate reproduction when daylength increases in the spring (Nelson *et al.* 1990; Prendergast *et al.* 2001). The lack of any cues from adult breeders during the winter may be a social factor affecting sexual maturation of autumnal individuals. In house mice, many laboratory studies have demonstrated the influence of social environment on sexual maturation and adult reproduction. Adult male cues restrain young male sexual development (Vandenbergh 1971; McKinney and Desjardins 1973) and, conversely, stimulate female puberty and oestrous cycles (Whitten 1956, 1966; Vandenbergh *et al.* 1975, 1976; Jemiolo *et al.* 1986; Novotny *et al.* 1995, 1999). Chemosignals of oestrous females and of pregnant and lactating females accelerate puberty in young females and promote oestrus in adults (Drickamer and Hoover 1979; Drickamer 1982a, 1982b; Jemiolo *et al.* 1989). Social and environmental factors are known to affect house mice (Drickamer 1984, Pandey and Pandey 1990). In mound-building mice,

chemosignals from adult breeders and their physiological consequences still remain unknown. Their role in sexual maturation and reproduction require further study. The present study examines the influence of and interactions between environmental and social factors on reproduction and sexual maturation in mound-building mice. According to the seasonality of reproduction in mound-building mice, the present study's main hypothesis is that winter climatic conditions delay sexual maturity in autumnal individuals and inhibit reproduction in adults. A secondary hypothesis is that chemosignals from adult breeders might accelerate puberty, at least in female autumnal individuals.

## Materials and methods

### *Animals*

Mound-building mice were captured inside mounds found in the Gyöngyös region, Hungary (47°44'N and 19°55'E) as juveniles in October 1999. The number of animals per mound varied from six to 23 (mean  $\pm$  SE,  $11.6 \pm 2.4$ ) with a male-biased sex ratio of 1.7. Temperature measured inside the mounds was 17°C for an ambient temperature of 10.5°C. The vegetal material of the mounds forms an efficient protection against the cold and produces heat through fermentation.

In March 2000, animals were paired and started to reproduce under standard laboratory conditions (20.2  $\pm$  0.4°C; 14 h L : 10 h D with the dark phase starting at 12.00 h). Mice were housed in polycarbonate standard cages measuring 26 cm  $\times$  16 cm  $\times$  14 cm, with food (mice pellets, UAR type AO4; Usine d'Alimentation Rationnelle, Epinay sur Orge, France) and water supplied *ad libitum*. Cotton wool was provided in abundance for nesting material. The mice built large spherical nests with one or two circular entrances. Nests formed efficient shelters in which the mice spent a major part of the light period. Activity was then strictly nocturnal, as observed in the field (P. Gouat and K. Katona, unpublished observations).

Animals used in the experiments included reproducing pairs of adults and their young born between August and November 2000 (autumnal individuals). Our study was conducted under authorizations 93-006 and 006399 at the Université Paris Nord (approval from the Prefecture of Seine Saint Denis, prefectorial decree 02-2651).

### *Reproducing pairs of adults*

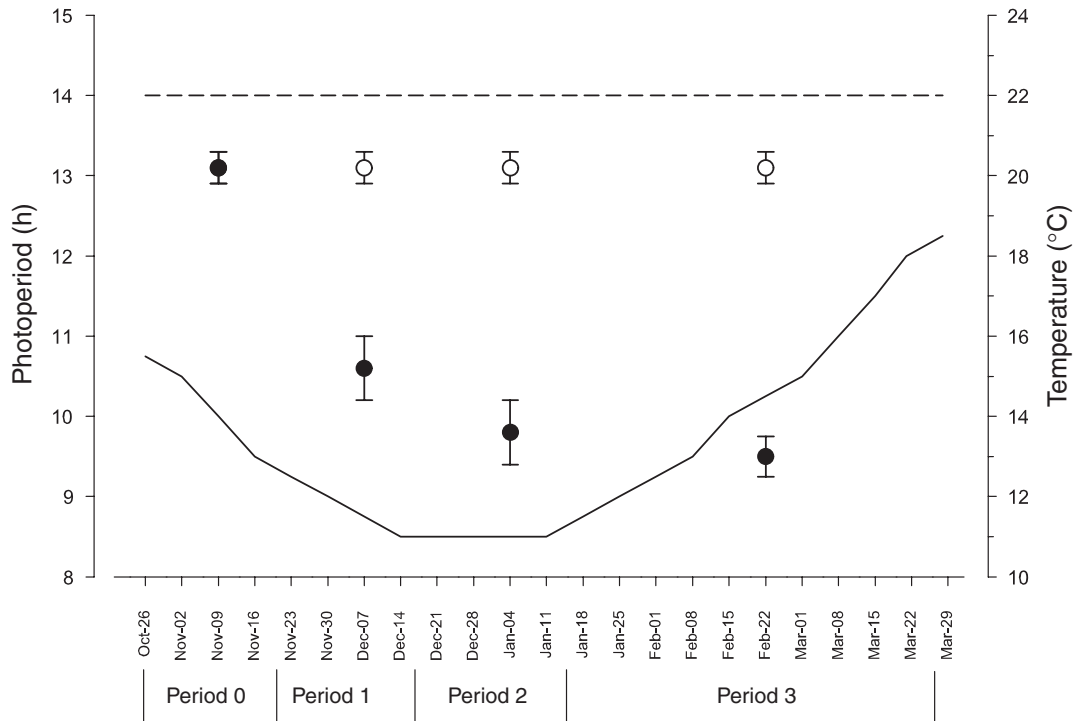
In order to determine whether winter climatic conditions inhibit reproduction, reproducing pairs of adults (two to five litters produced before the experimental period) were placed either in temperate conditions (14 h L : 10 h D, 21°C; 10 pairs) or in cold conditions (eight pairs) similar to the juvenile groups (discussed later). Reproduction was assessed by monitoring weekly the presence of any newborn animals with each pair.

### *Autumnal individuals*

#### *Breeding conditions*

After weaning (mean age  $\pm$  SEM, 31  $\pm$  0.8 days), each litter was divided into groups of three individuals (trio) composed of either one male and two females or two males and one female. One to three trios were set per litter. Trios from the same litter were allocated to different experimental groups.

Twenty trios were raised under standard laboratory conditions of temperature and photoperiod (temperate groups, T). In order to test the influence of cues from a reproducing pair, ten of these trios were given, twice a week, approximately 3 g of sawdust soiled by a reproducing pair (breeders) of mound-building mice (temperate conditions and breeder odours group, TB). The ten remaining trios received the same amount



**Fig. 1.** Temperature and photoperiod in the two experimental conditions. In the temperate condition, photoperiod (dotted line) and temperature (white circles) were maintained constant, whereas in the cold condition, photoperiod (straight line) roughly followed the annual cycle observed in Hungary and temperature (black circles) decreased during the experimental period. Mean temperatures  $\pm$  SEM are given for each period. Period 0 encompassed the 4 weeks when groups of three individuals were set up.

of clean sawdust twice per week (temperate conditions and no breeder odours group, TNB). The donor reproducing pairs were unrelated and unfamiliar to the trios. To prevent any odour transferring from the TB to the TNB group, the TB group was placed near an air outlet, whereas the TNB group, which was placed at a distance of 1.6 m, was placed above the air inlet. No adults were present in the experimental room.

To test the effect of climatic conditions, twenty trios were transferred from standard laboratory conditions to a climatic chamber in which the temperature and photoperiod reproduced winter conditions (Fig. 1) in the Gyöngyös region (cold groups, C). Four weeks were necessary to form all the trios (from October 26 to November 11) during which the light period was decreased from 10 $\frac{3}{4}$  h to 9 $\frac{1}{2}$  h but temperature was left constant at 20.2  $\pm$  0.4°C. When all the trios were formed, temperature was lowered to 15.2  $\pm$  0.8°C (Period 1) and the light period was decreased from 9 $\frac{1}{4}$  h (November 23) to 8 $\frac{1}{2}$  h (December 14). From December 21 to January 11 (Period 2), the light period was kept constant and at a minimum (8 $\frac{1}{2}$  h) and temperature was maintained at 13.6  $\pm$  0.8°C. In the final period (Period 3), from January 18 to March 28, the light period was increased from 8 $\frac{3}{4}$  h to 12 $\frac{1}{4}$  h and the mean temperature was 13.0  $\pm$  0.5°C. The dark phase always started at 12.00 hours. Temperature was maintained mild according to the temperatures measured inside the mounds. To compare the cold group with the temperate groups, the cold group was divided into two subgroups of 10 trios each: trios from the cold group with breeder odours (CB) received approximately 3 g of sawdust soiled by a reproducing pair, twice a week; trios from the cold group with no breeder odours (CNB) received the same amount of clean sawdust twice a week.

Table 1 indicates the composition and characteristics of each group. In order to allow the temperature to be decreased, the experiments involving the cold groups were completed as a priority and, as a result,

**Table 1. Composition and characteristics of each experimental group**

No. trios of each category (two females and one male FFM or inverse FMM) are given. Ages at the beginning and at the end of the experiment are expressed in days (mean  $\pm$  SEM)

	CB	CNB	TB	TNB
Age begin	31.9 $\pm$ 1.4	31.7 $\pm$ 1.4	31.1 $\pm$ 1.7	29.2 $\pm$ 1.6
Age end	182.8 $\pm$ 2.1	182.6 $\pm$ 2.1	170.8 $\pm$ 4.6	168.9 $\pm$ 3.9
FFM	5	4	4	6*
FMM	5	6	6	4

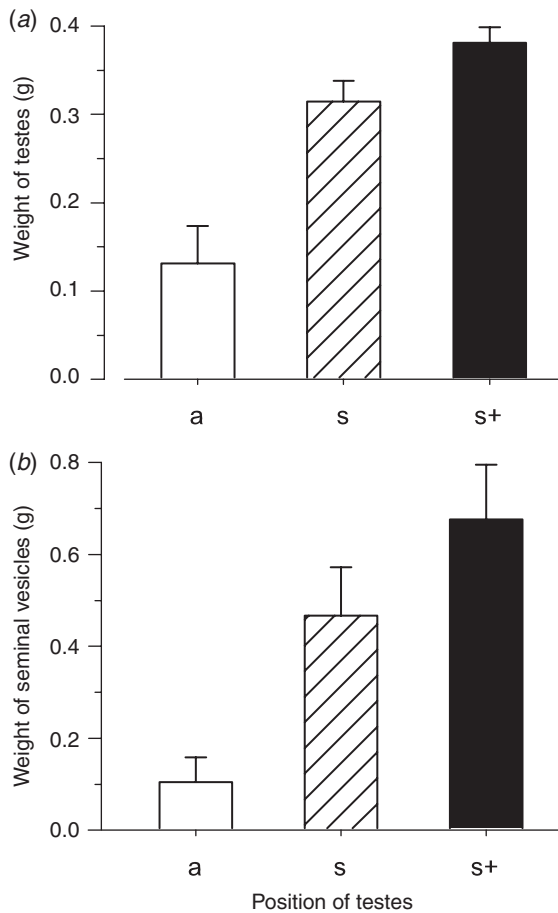
\*In one trio, a female died in Week 9 of cohabitation, and observations were held on the remaining pair.

CB, Cold conditions and breeder odours; CNB, cold conditions and no breeder odours; TB, temperate conditions and breeder odours; TNB, temperate conditions and no breeder odours.

these animals remained longer in the experimental conditions and were older by the end of the experiments compared with animals from the temperate groups.

#### Evaluation of sexual maturity

Once a week, in the 3 h preceding the end of the light phase, each animal was weighed and checked for wounds. Vaginal aperture and gestation in females were checked while females were hand-held. To evaluate the size and position of testes in males, the male mouse was placed in a transparent box and its testis position assessed independently by



**Fig. 2.** Weight (mean  $\pm$  SEM) of: (a) testes; and (b) seminal vesicles in males according to the position of their testes (a: abdominal,  $n = 8$ ; s: scrotal,  $n = 10$ ; s<sup>+</sup>: scrotal plus,  $n = 12$ ).

two experimenters. Testes were classified as: (i) abdominal; (ii) scrotal (testes made the scrotum appear as a unique mass); or (iii) scrotal plus (two clearly oval masses were observable). To link our evaluation of testis position with the development of reproductive organs, 20 experimental males (four to six individuals per experimental group) and 10 additional subadult males were killed by asphyxiation at the end of the experimental period, on March 28. The position of the testes was evaluated before death. The testes and seminal vesicles were dissected, cleaned of excess tissue and weighed to the nearest 0.1 mg. Bodyweight was recorded to the nearest 0.1 g. The aforementioned classification of scrotal position of the testes was linked to testis weight (Fig. 2a) and to seminal vesicle weight (Fig. 2b). In general, the evaluation of testis position affected both testis weight and seminal vesicle weight (ANOVA with general scores, exact procedure,  $P = 0.0001$  and  $P = 0.0029$ , respectively). The three groups that were classified according to testis position differed significantly from each other according to testis weight (permutation tests, s<sup>+</sup>/s:  $P = 0.032$ , s<sup>+</sup>/a:  $P = 0.0001$ , s/a:  $P = 0.003$ ). The weight of seminal vesicles differed significantly between the abdominal group and the two scrotal groups only (a/s<sup>+</sup>:  $P = 0.0005$ , a/s:  $P = 0.009$ ).

#### *Test of reproductive ability*

At the end of the experimental period, all the females and the remaining males were placed in individual cages under temperate conditions for 4 weeks to check for gestation. At the end of this period, 39 females

were paired with a male from the same experimental group. Animals of a pair were unfamiliar and unrelated. Reproduction was monitored for an additional month.

#### *Statistical analyses*

Data are given as mean  $\pm$  SEM. The proportions of reproductive adult pairs or juvenile trios in different groups were compared using the  $\chi^2$ -test and the exact procedure.

Comparisons between the TB and TNB groups (breeder odour effect) were made using ANOVA with repeated measures for changes in body mass, or by a permutation test and exact procedure for the sexual maturity data.

The effect of climatic conditions was analysed using ANCOVA with repeated measures for changes in body mass. Breeder/non-breeder odour treatment was used as the covariant. Sexual maturity data were analysed using permutation tests with stratification according to the breeder/non-breeder odour treatment.

The proportion of males with a given testis position during each of the three periods was analysed using ANOVA of Friedman (exact procedure) followed by permutation tests in order to compare the successive periods.

Exact procedures were realized using StatXact 3.1 (Cytel Software Corporation), ANOVAs and ANCOVAs were performed using Statistica 5.1 (Statsoft Incorporated, Tulsa, CA, USA).

## **Results**

### *Reproducing pairs of adults*

All of the ten pairs placed in temperate conditions continued to reproduce. Of the eight reproductive pairs placed in cold conditions, seven continued to reproduce. There was no significant difference between the two groups ( $\chi^2 = 1.32$ , d.f. = 1,  $P = 0.44$ ).

### *Autumnal individuals*

#### *Sexual maturity and reproduction*

No mice were wounded during the cohabitation period and individuals in a trio always used a common nest in which they rested during the light period.

Among all of the treatments, reproduction occurred in only one female of a trio of the CB group, which was composed of two females and one male. According to the date of delivery, copulation had occurred in the last week of the experiment at 6 months of age. Despite the lack of difference in reproductive success, groups differed in several developmental parameters.

Most males reached the scrotal plus position at least once during the cohabitation period, and the groups did not differ significantly (Table 2). Males of T groups tended to reach their first scrotal plus position at a younger age ( $52.8 \pm 3.8$  days) than males of C groups ( $68.4 \pm 8.4$  days), although the difference was not significant. Males of T groups displayed proportionally more testes in a scrotal plus position and fewer in abdominal positions than males of C groups. There was no difference in the proportion of testes in a scrotal position between these two groups.

**Table 2. Comparisons between males of the four experimental groups**

The proportion (mean %  $\pm$  SEM) of observations per male with testis in each of the three positions (% A, abdominal; % S, scrotal; % S<sup>+</sup>, scrotal plus), the latency of the first scrotal plus position (Latency; mean age in days  $\pm$  SEM) and the number of males reaching this status (Nb S<sup>+</sup>) are given

	% A	% S	% S <sup>+</sup>	Latency	Nb S <sup>+</sup>	<i>n</i>
CB	45.2 $\pm$ 4.7	43.7 $\pm$ 3.8	11.1 $\pm$ 1.6	70.4 $\pm$ 12.2	15	15
CNB	39.2 $\pm$ 4.0	49.5 $\pm$ 3.1	11.2 $\pm$ 1.9	56.4 $\pm$ 9.7	14	16
TB	10.9 $\pm$ 4.9	40.9 $\pm$ 5.3	48.2 $\pm$ 7.4	47.1 $\pm$ 2.6	14	15
TNB	15.8 $\pm$ 4.6	55.2 $\pm$ 4.3	29 $\pm$ 5.1	59.3 $\pm$ 7.5	12	14
Climate	$P < 0.001$	$P = 0.73$	$P < 0.001$	$P = 0.25$	$\chi^2 = 1.147, P = 0.35$	
Parents	$P = 0.48$	$P = 0.050$	$P = 0.045$	$P = 0.14$	$\chi^2 = 2.004, P = 0.48$	

*n*, No. males in each group; CB, cold conditions and breeder odours; CNB, cold conditions and no breeder odours; TB, temperate conditions and breeder odours; TNB, temperate conditions and no breeder odours.

Climate: *P* values of the comparison between cold groups and temperate groups.

Parents: *P* values of the comparison between TB and TNB groups.

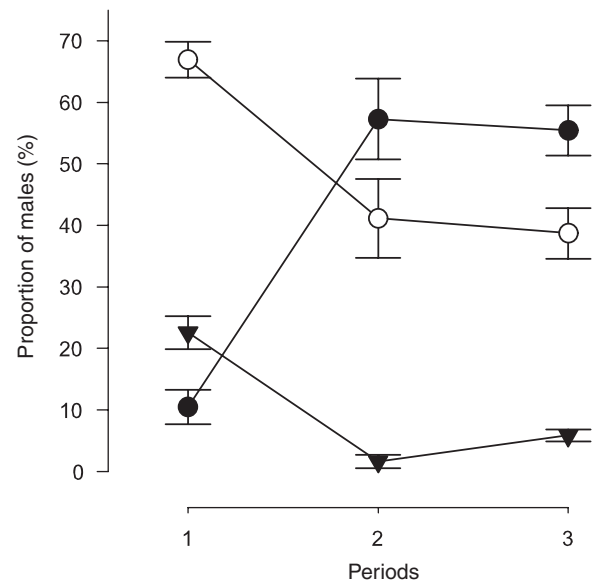
In the C groups (Fig. 3), the proportion of males with testes in the abdominal position varied among the three periods (Friedman test:  $\chi^2 = 39.88$ , d.f. = 60,  $P < 0.001$ ). This proportion was at a minimum in Period 1, increased in Period 2 ( $P < 0.001$ ) and remained at a similar level in Period 3 (comparison between Periods 2 and 3,  $P = 0.8$ ). The proportion of males with testes in a scrotal position decreased from Period 1 to Period 3 (Friedman test:  $\chi^2 = 16.83$ , d.f. = 60,  $P < 0.001$ ), but the difference was significant only between Periods 1 and 2 ( $P < 0.001$ ). The proportion of males with testes in a scrotal plus position varied significantly between the three periods (Friedman test:  $\chi^2 = 34.86$ , d.f. = 60,  $P < 0.001$ ); it was maximum in Period 1, then decreased significantly in Period 2 ( $P < 0.001$ ) and finally increased slightly but significantly in Period 3 ( $P = 0.004$ ).

In the T groups, males receiving breeder adult odours displayed proportionally fewer testes in a scrotal position and more in scrotal plus positions than males of the TNB group, and they tended to reach their first scrotal plus position at a younger age, although the difference was not significant (Table 2).

In contrast to males, signs of sexual maturity onset were scarce in females. Vaginal aperture was observed in less than 30% of females (16/59). The proportion of females with an observed vaginal aperture was significantly higher in T groups than in C groups, and the proportion of vaginal aperture per female tended to be more important in T groups than in C groups, the difference almost reaching significance (Table 3). There was no difference between the two climatic conditions in terms of age at first aperture (T groups:  $133.4 \pm 10.5$  days; C groups:  $137.5 \pm 24.5$  days). Breeding adult odours had no observable influence on these parameters (Table 3).

#### Body mass

The body mass of males (Fig. 4a) increased with age (ANOVA, age effect:  $F_{16,928} = 31.89$ ,  $P < 0.001$ ). Variation



**Fig. 3.** Proportion of cold group males (mean %  $\pm$  SEM) with each of the three types of testis position (abdominal: black circles; scrotal: white circles; scrotal plus: black triangles) in the three different periods: Period 1, decreasing light period and temperature; Period 2, minimum light period and low temperature; and Period 3, increasing light period and low temperature.

in body mass differed significantly between C group males and T group males (ANCOVA, group  $\times$  age effect:  $F_{16,928} = 6.94$ ,  $P < 0.001$ ). Temperate group males became significantly heavier than males of C groups from 17 weeks of age (post-hoc comparisons,  $P < 0.01$ ). Variation in body mass differed significantly between TB and TNB males (ANOVA, group  $\times$  age effect:  $F_{16,432} = 4.01$ ,  $P < 0.001$ ). Males of the TB group were significantly heavier than males of the TNB group from Week 7 to Week 10, and from Week 13 to Week 15 (post-hoc comparisons,  $P < 0.05$ ). Body mass became similar from 17 weeks of age.

**Table 3. Comparisons between females of the four experimental groups**

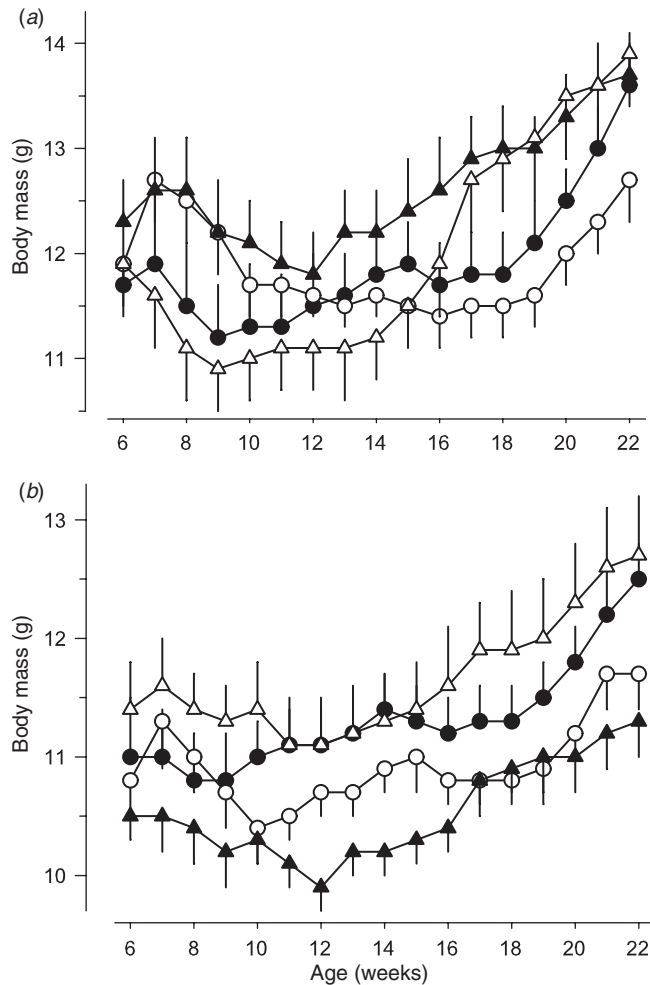
The proportion of observations per female with open vagina (% O; mean %  $\pm$  SE), the latency of the first vaginal aperture (Latency; mean age in days  $\pm$  SEM), and the number of females reaching this status (Nb O) are given

	% O	Latency	Nb O	<i>n</i>
CB	1.7 $\pm$ 1.3	163 $\pm$ 17	2	15
CNB	0.6 $\pm$ 0.4	112 $\pm$ 45	2	14
TB	3.2 $\pm$ 1.3	133.3 $\pm$ 18.1	6	15
TNB	3.9 $\pm$ 1.6	133.5 $\pm$ 12.7	6	15
Climate	$P = 0.0547$	$P = 0.93$	$\chi^2 = 5.124, P = 0.039$	
Parents	$P = 0.7396$	$P = 1$	$\chi^2 = 0.006, P = 1$	

*n*, No. females in each group; CB, cold conditions and breeder odours; CNB, cold conditions and no breeder odours; TB, temperate conditions and breeder odours; TNB, temperate conditions and no breeder odours.

Climate: *P* values of the comparison between cold groups and temperate groups.

Parents: *P* values of the comparison between TB and TNB groups.



**Fig. 4.** Variation in body mass (mean  $\pm$  SEM) as a function of age in: (a) males and (b) females in the four experimental groups (cold conditions and breeder odours: black circles; cold conditions and no breeder odours: white circles; temperate conditions and breeder odours: black triangles; temperate conditions and no breeder odours: white triangles).

As observed in males, the body mass of females (Fig. 4b) increased with age (ANOVA, age effect:  $F_{16,912} = 25.42, P < 0.001$ ). The major difference in the body mass of females occurred between the two T groups (ANOVA: group  $\times$  age effect:  $F_{1,28} = 6.63, P = 0.016$ ). Females of the TNB group were always heavier than females of the TB group. Variation in body mass differed significantly between C group females and T group females (ANCOVA, group  $\times$  age effect:  $F_{16,912} = 2.32, P < 0.001$ ), but this statistical difference was caused mainly by the differences between the two T groups, with the two C groups being inserted between the two T groups (Fig. 4b).

#### Reproduction after isolation

Of the 39 females placed in pairs to estimate reproductive potential, 24 females had already reproduced or were pregnant after 1 month of pairing. The proportion of reproducing females was higher in the T groups (17/20) than in the C groups (7/19) ( $\chi^2 = 9.55, \text{d.f.} = 1, P = 0.003$ ). No effect of the presence of reproductive adult odours in the T groups was detected (TB: 8/10; TNB: 9/10;  $\chi^2 = 0.39, \text{d.f.} = 1, P = 1$ ). In 79% of the pairs, gestation began during the first week of cohabitation.

#### Discussion

According to the strong link between seasonality and reproduction observed in mound-building mice in the field (Naumov 1940; Pisareva 1948; Murariu 1981; Orsini *et al.* 1983; Duryadi 1993), one may have expected environmental factors and, particularly, daylength to be a major cue for timing reproduction. Data from the present study showed that replicating winter conditions (i.e. fluctuating short daylength and cold ambient temperature) did not significantly affect the reproduction of breeding pairs of mound-building mice given food and water *ad libitum*. As observed in house mice (Bronson 1979; Pelikán 1981; Barnett and Dickson 1989),

mound-building mice appear to be able to reproduce under winter conditions, at least when food intake is sufficient. Nevertheless, there is no field data reporting reproduction of mound-building mice during mild winters. Adults seem to disappear before the beginning of winter and only autumnal individuals living inside mounds appear to survive under winter conditions (Duryadi 1993; Garza *et al.* 1997; Milishnikov *et al.* 1998). The sister species, *Mus macedonicus* (Bonhomme *et al.* 1983; Boursot *et al.* 1993), is found in Eastern Europe and in the Middle-East in a Mediterranean climate and it does not build mounds during the winter (Orsini *et al.* 1983; Guénet and Bonhomme 2003). Therefore, the building of mounds appears to be a behavioral adaptation to the more continental climate encountered by *Mus spicilegus*.

As expected, autumnal individuals placed in replicated winter conditions did not initiate reproduction, and the increased light period in Period 3 did not promote reproduction either. Moreover, autumnal individuals placed in constant long photoperiod and temperate conditions also did not reproduce. Thus, we concluded that neither energy expenditure caused by cold ambient temperature nor an inhibition based on decreased light periods were sufficient to explain the delay in reproduction in autumnal animals placed in trios. Reproduction occurred only after dislocation of trios, 1 month of isolation and pairing with an unfamiliar mate. According to testis position, most males were sexually mature at 2 months of age. Most males (55/60) had reached, at least once, scrotal plus position of the testes, and 56% of males had reached scrotal plus position before they were 2 months old. The capacity of males to reproduce, however, was never directly tested. Yet, vaginal aperture was seldom observed in females placed in trios. During the fourth month of the experimental period, a vaginal aperture was never observed in more than 80% of females (50/60). Thus, we conclude that the lack of reproduction is mainly caused by the females.

Lack of onset of reproduction in females was not directly linked with replicated winter conditions. Autumnal females did not initiate reproduction even when they were placed under long light period and temperate conditions. Nevertheless, seasonality may be caused by other factors (Bronson and Heideman 1994). An internal clock has been suggested in some rodent species (e.g. Ambid and Berges 1986; Landau and Holmes 1988). This explanation is rather improbable as adult pairs placed in both conditions (i.e. cold or temperate) continued to reproduce; only one female in a group of three individuals initiated reproduction at the end of the grouping period, at 6 months of age. Moreover, reproduction in our breeding stock under laboratory conditions was constant. Nevertheless, we must test this hypothesis by renewing the experiment during the natural breeding period from April to August.

Seasonality of reproduction might be caused by the presence or absence of a specific nutrient (e.g. Nelson and Shiber

1990; Meek *et al.* 1995). However, as the diet remained constant throughout the experimental period, including the period when reproduction occurred, we may reject this explanation. Thus, we concluded that the observed delay in reproduction appeared to have been caused by grouping itself.

Familiarity and kinship were inseparable in our experimental design, but this is the usual situation inside the mounds (Garza *et al.* 1997). In the field, reproduction begins when autumnal animals leave the mounds and disperse in the spring (Naumov 1940; Pisareva 1948; Murariu 1981; Orsini *et al.* 1983; Duryadi 1993; Milishnikov *et al.* 1998; Gouat *et al.* 2003). In our experiment, the onset of reproduction in autumnal individuals appeared to be a consequence of the isolation period following the over-wintering in trios and because of pairing with an unfamiliar mate. Isolation of females is known to induce oestrus in house mice (Vandenbergh 1994) and promotes vaginal opening in mound-building adult female mice (Féron and Gheusi 2003). In house mice, the estrous cycle is stimulated by adult male cues (Vandenbergh 1994) and the presence of an adult male is required by adult female mound-building mice to enter into oestrus (Féron and Gheusi 2003). In our experiment, autumnal females were in direct contact with one or two males who appeared sexually mature according to the scrotal plus position of their testes. Nevertheless, only one successful mating occurred. During the period of cohabitation in trio, male cues appear to have been ineffective in stimulating female reproduction. Familiarity and kinship may have either inhibited the stimulating role of male cues or prevented the production of such cues in males. In groups in which breeder odours were introduced twice a week, autumnal females did not display more vaginal aperture. These results suggest that these females are unable to react to male cues.

Familiarity is known to play a major role in the social structure of the mound-building mouse (Patris and Baudoin 1998, 2000). Familiar animals are very tolerant and display cohesive behaviour, although mound-building mice are very aggressive towards unfamiliar conspecifics even during female–female encounters (Suchomelová *et al.* 1998, Patris *et al.* 2002). In our experiment, no animals were wounded, and no agonistic behaviour was observed during sporadic observations held during the dark photoperiod. On the contrary, animals displayed cohesive behaviour and used a common nest. Familiarity appeared to be a key factor. In addition to the present experiment, a preliminary study was done on two groups of nine autumnal individuals (five males and four females) from three unrelated litters. They were placed in C group conditions inside a plastic arena of 1 m<sup>2</sup> with sawdust and cotton wool, and given food and water *ad libitum*. They built a common nest and no reproduction occurred although the animals were from unrelated litters. Nevertheless, we clearly need new experiments to better understand the respective roles of familiarity and kinship on the delay of reproduction in autumnal individuals.

In our experiment, cold conditions did not clearly affect the increase of body mass in autumnal individuals. If males from T groups tended to be heavier than males of C groups, no such difference was observed between the two groups of females. There were also differences between the two T groups in both males and females, but in opposite directions according to BNB odour treatment. Thus, it is difficult to attribute differences between groups solely according to differences in ambient temperature. It is rather probable that mound-building mice are able to compensate for the increase in energy demand caused by low temperature by increasing their food intake, as has been shown in the house mouse (Manning and Bronson 1990). The climatic conditions of the C groups had a more definite effect on sexual state. Males of T groups displayed more testes in the scrotal plus position than C-group males. Among C-group males, the position of the testes seemed to be linked with ambient temperature; abdominal position being observed more often when the temperature was low (Periods 2 and 3). In females, the number of individuals showing vaginal aperture was higher in the T group than in the C group. Surprisingly, the differences between the two groups of females were still distinguishable after 1 month spent under similar temperate conditions. The proportion of females initiating reproduction in the T groups was twice that of the C groups. As the experiment had to be stopped after 1 month of life in pairs, we do not know whether or not the difference between the two groups would have remained and if the C-group females would have begun to reproduce after a longer period.

Our results revealed that neither climatic conditions nor olfactory cues from reproducing adults were the proximal factors that delayed reproduction in autumnal individuals. The delay in reproduction in autumnal individuals was a consequence of the social effects of living in groups, in the same cage in our experiment and inside the mounds in natural conditions. We must now understand why and how mound-building mice alternate their social organization from dispersed reproductive units in spring and summer to common nesting of juvenile animals inside mounds in autumn and winter.

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