Age at puberty in male African striped mice: the impact of food, population density and the presence of the father

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Summary

1. The time at which animals enter puberty and become sexually mature is a significant life-history trait, influencing lifetime reproductive success. Great variation exists both between and within species.
2. The proximate mechanisms regulating the time at which a male enters puberty are not well-understood. Environmental cues are predicted to provide the relevant information on resource availability and opportunities for reproduction. When these are good the onset of puberty begins whereas at other times investment in survival becomes more important.
3. Male African striped mice (Rhabdomys pumilio) demonstrate large variation in the age at which they enter puberty, with grassland populations starting at 4 weeks old and semi-desert populations at over 10 weeks old.
4. We predicted that differences in the availability of food, social organization and population density could explain these differences.
5. Using data on 170 individual males from 4 years of field studies in a semi-desert population, we found that males became scrotal at a younger age when no breeding male was present in their group and when food was abundant, while population density had no effect.
6. In laboratory experiments we demonstrated that males fed with poor protein food, that regularly encounter larger unfamiliar males (mimicking high population density), and that live in family groups with their father present, become scrotal at a significantly later age, independent of their growth rate.
7. Males housed in family groups have lower testosterone but higher corticosterone levels than singly housed males, indicating they are sexually suppressed. When they become scrotal in their family group, their testes are only half as large as those of their singly housed brothers, and they contained significantly less sperm.
8. We conclude that male striped mice have a flexible response to the onset of puberty, and that the onset of sexual maturity is dependent on several environmental cues. Our results indicate that there is no threshold body mass, which, when reached, would automatically trigger puberty in male striped mice.
9. Male helpers in some species are reproductively suppressed, but ours is the first study that demonstrated the importance of different ecological factors in the timing of puberty in male helpers in a facultative cooperatively breeding species.

Key-words: adolescence, fast-slow continuum, maturation, group living, solitary living, cooperative breeding, communal breeding
The striped mouse (*Rhabdomys pumilio*) occurs in many habitats in southern Africa. In the present study it was studied in the Succulent Karoo semi-desert, where it shows high social flexibility. The mice in the photograph are individually marked with hair dye, permanently marked with ear tags, and carry radio-transmitters.

**Introduction**

The age at which animals reach sexual maturity is critical in determining an individual's lifetime reproductive success (Oli & Dobson 2003). If sexual maturity is reached too soon, resources that are more critical for survival might be wasted because reproduction at the animal's current state is unlikely or too costly (Berner & Blanckenhorn 2007). In contrast, if sexual maturity is reached too late, important opportunities to reproduce might be missed (Berner & Blanckenhorn 2007). The optimal time to reach sexual maturity can differ between species, as demonstrated by the slow–fast continuum differentiating species that mature, reproduce and die fast and species that take longer (Kraus *et al.* 2005; Read & Harvey 1989). Variation also exists within species (Waterman 2002; Yamamoto 1993) including humans (Belsky *et al.* 1991; Ellis & Garber 2000; Gluckman & Hanson 2006). Environmental and social stimuli might be important in determining the age at sexual maturity. However, the proximate mechanisms underlying the trade-off between the age at which to start puberty (also called maturation or adolescence) and the investment of resources into survival are poorly understood (Berner & Blanckenhorn 2007).

One species that shows large variation in the age at which males reach puberty and become sexually mature is the African striped mouse (*Rhabdomys pumilio*; Fig. 1). This species inhabits a variety of habitats from moist grasslands to semi-deserts. In grasslands, males can become scrotal (testes descended within the scrotal sac), indicating that they are sexually mature (Brooks 1982), at an age of only 4–6 weeks and a body mass between 20 and 30 g (Schradin & Pillay 2005b), whereas in the Succulent Karoo semi-desert scrotal males typically weigh more than 40 g and are more than 10 weeks old (Schradin & Pillay 2004, 2005b). Scrotality is necessary for the correct temperature for normal spermatogenesis (Hughes & Acerini 2008) and scrotal males are generally regarded as being in breeding condition and having viable sperm (for muroid rodents see Scheibler *et al.* 2006; Saltzman *et al.* 2006; for the striped mouse see Brooks 1982; Jackson & Bernard 1999; Jackson & Bernhard 2005; Perrin 1980a; Willan & Meester 1989). The age at which striped mouse males become scrotal for the first time is regarded as their age of onset of sexual maturity (Perrin 1980a; Brooks 1982; Willan & Meester 1989), but sexually mature adult striped mouse males are non-scrotal during the non-breeding season, when spermatogenesis is not necessary (Perrin 1980a; Willan & Meester 1989; Jackson & Bernard 1999; Jackson & Bernhard 2005; Schradin & Pillay 2005a). Population differences in the age of becoming scrotal are thought to be due to ecological and social differences between the populations (Schradin 2005).

In grasslands, striped mice are solitary, population density is low and the breeding season is 6 months long (Perrin 1980a; Willan & Meester 1989). Thus, males born during one season can leave their natal group at 4–6 weeks of age and are able to start independent breeding (Schradin 2005). In contrast, in the Succulent Karoo, population density is high and the breeding season is only 3 months long. Here, striped mice typically form extended family groups with one breeding male, up to four breeding females, and adult philopatric males and females that do not breed in the group (Schradin & Pillay 2004). Groups can contain up to 30 adult individuals of both sexes (Schradin & Pillay 2004). Therefore males might not have the space to disperse in the same breeding season in which they were born due to the high population density. Moreover, in the Succulent Karoo the breeding season is typically only three months long (Schradin & Pillay 2005a), which does not allow much time for independent breeding in the breeding season a male is born. Additionally, young males might be suppressed by the dominant breeding male of the group, which can weigh more than 80 g (Schradin & Pillay 2004, 2005a), as well as by aggressive encounters with neighbouring territorial males and females (Schradin 2004; Schradin & Pillay 2004). Reproductive activity in striped mice is not influenced by the photoperiod (Jackson & Bernard 1999) but rather by food availability. In the Succulent Karoo, mice breed in spring when the photoperiod is relatively short, while grassland mice breed in summer, when the photoperiod is long (Schradin 2005). Thus, the variation in the timing when sexual maturity is reached might be caused by a combination of several factors which we tested using long-term field data and by conducting experiments in captivity. In particular, we tested the influence of the following three factors:

1. **The influence of protein**, which is known to affect growth and sexual maturity in many species (White 1993). Reproduction in female striped mice is restricted to the period of availability of protein-rich food, such as insects in grasslands (Perrin 1980b) and young plant growth in the Succulent Karoo (Schradin & Pillay 2006). The protein content of food might also be an important cue for males regarding whether or not the breeding season continues, because females breed only during periods when protein rich food is abundant (Schradin & Pillay 2006). Thus, we predicted that males with a protein poor diet would become scrotal at a later age than males with a protein rich diet.

2. **The influence of social stress induced by high population density** (Christian 1971; Rödel *et al.* 2004). When population

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**Fig. 1.** The striped mouse (*Rhabdomys pumilio*) occurs in many habitats in southern Africa. In the present study it was studied in the Succulent Karoo semi-desert, where it shows high social flexibility. The mice in the photograph are individually marked with hair dye, permanently marked with ear tags, and carry radio-transmitters.
density is high, small non-scrotal males are more likely to encounter larger aggressive territorial males. This could lead to increased levels of stress hormones which can suppress reproduction (Christian 1971; Wingfield & Sapolsky 2003). Thus, we predicted that males which are stressed by repeated encounters with larger, aggressive males would become scrotal at a later age than unstressed control males.

3. The potential of reproductive suppression within social groups. Philopatric males are known to have much higher corticosterone levels than breeding males (Schradin 2008b; Schradin et al. 2009), which could indicate that they are physiologically castrated (Reyer et al. 1986; Creel 2001; Wingfield & Sapolsky 2003). Thus, we predicted that males housed in families would become scrotal at a later age than singly housed males.

Materials and methods

FIELD STUDY

Field studies were conducted from 2005 to 2008 in Goegap Nature Reserve near Springbok in the Northern Cape Province, South Africa (29°41′56″S, 18°16′00″E). The area is arid, with an average rainfall of 160 mm p.a. The vegetation type is classified as Succulent Karoo (Cowling et al. 1999).

We observed mice from 28 different focal groups, with 9–20 different focal groups per year. From each group, at least one individual was fitted with a radio-collar (Holohil, Canada) to establish the sleeping sites of the groups (Schradin & Pillay 2005b). Trapping was done around nesting sites at least twice a month as described elsewhere (Schradin & Pillay 2004). Each individual was trapped approximately 12 times a month at different nests. Trapped mice were weighed and sexed, males were recorded as scrotal (testes descended into the scrotal sacs; Brooks 1982) or not (testes inside the body), and all individuals were permanently marked with ear tags (National Band and Tag Co., USA) and hair dye (Rapido, Pinetown, South Africa) for individual recognition during field observations. We have no indication that males retracted their testes during handling in our study, that is, males that were regarded as scrotal when taken out of the trap, then weighed and marked, were still scrotal at the end of the procedure. We observed all nests at the field site and determined the group identity of each mouse (Schradin & Pillay 2004), and whether groups had a breeding male or not. Only males that weighed less than 25 g (approximately 4–5 weeks old) when first trapped and for which we knew the natal group were included in the study.

Population density was estimated from the numbers of individuals trapped at the field site divided by the size of the field site. Population density varied dramatically over the years of study due to differences in food abundance, drought and predation. Population density at the start of the breeding season (September) varied from 6·5 to 19·0 mice/ha (average: 14·4 ± 12·9 mice/ha). We observed at least nine groups every year (between 9 and 20). Due to changes in population density, the study area varied from 2·3 to 9·5 ha (average: 6·5 ± 3·0 ha). The sex ratio was determined by dividing the number of scrotal males by the number of sexually mature females (females with a perforated vagina or females that had already bred) trapped at the field site each month. Date of birth was estimated from the start of the breeding season (September) varied from 6·5 to 19·0 mice/ha (average: 14·4 ± 12·9 mice/ha). We observed at least nine groups per year. From each group, at least one individual was trapped and weighed, then marked, were still scrotal at the end of the trapping season.

To obtain information about food availability, we non-destructively sampled eight plots each of 2 × 2 m within the home ranges of eight different groups on the 15th of each month using standard protocols (Braun-Blanquet Method; Werger 1974). All plant species present in each plot, their abundance and their vegetative status (i.e. green, flowering or death) were recorded as well as number of seedlings per plot. Thus, we obtained a measure of abundance of protein rich seedlings and of the number of food plants for each month. For analysis, we used the mean values of the eight plots.

Statistics

Data are presented as mean ± SD. A general linear mixed model (LMM) was fitted to normal data using REML (SAS, proc MIXED). Error degrees of freedom (df) were calculated using the Satterthwaite method. Effects were tested using type III (simultaneous) modelling, that is, in multiple effects models parameters for each independent variable were corrected for all other fixed effects in the model. For 170 males, we could determine at which age (in weeks) they became scrotal. To avoid pseudoreplication, we took mean values for males from the same litter or those that were raised together at the same time (males from litters of two or more cooperatively breeding females), as these males experienced the identical social and biotic environment. This resulted in a sample size of 104 litters. We fitted a LMM with the age at which males became scrotal as the response variable. To reach normal distribution of the dependant variable (tested as Shapiro–Wilks and Kolomogorov–Smirnov tests), age had to be transformed by –1/age. As covariates, we entered population density, sex ratio, abundance of seedlings and food. As a fixed categorical effect we included the social situation under which the male was housed before becoming scrotal (1 = with breeding male present in the group or 2 = with no breeding male present in the group). We entered group ID as a random factor as for some groups several litters were included, and we tested whether significant effects were the same over different groups. None of the preliminarily calculated interactions were significant and were thus excluded from the analysis and are not presented in the results section.

CAPTIVE STUDIES UNDER NATURAL WEATHER CONDITIONS

These studies were conducted at our research station in the Goegap Nature Reserve (29°37′S; 17°59′E), South Africa, which is situated in the natural habitat of the study species. The study took place from 2004 to 2007. The colony was started in 2001 with founder pairs trapped at the field site. Test subjects were housed on the veranda of the research station and were therefore exposed to the normal light/dark cycle and temperatures. Test subjects were protected from wind, rain and direct sunshine by shade cloth.

Test subjects originated from breeding pairs which were housed in type III Lab-o-tec® cages (40 × 15 × 25 cm; made of plastic) under conditions as described below. For all experiments, pairs of brothers from the same litter were used (paired data design) as they were matched in body mass (P > 0·3). One brother was always singly
housed, while the other brother was in one of the four different experimental groups described below. Males were separated from the family groups after weaning at day 16 (Brooks, 1982) and housed on their own in Type II Lab-o-tec® cages (26 × 15 × 20 cm). Hay was provided as litter material and cotton wool and tissue paper as nesting material. All tanks were cleaned weekly. Water was provided ad libitum. Except for mice in the ‘protein low diet group’ (see below), each mouse received a 4-g seed mixture (Marltons Pets & Products, USA; Seeds from Agricol, South Africa) in the morning, a piece of fruit or lettuce at midday, and five sunflower seeds in the afternoon. Food was allocated during the day to prevent obesity and to mimic natural foraging behaviour (Schradin 2006). Starting on day 21, mice were weighed and checked weekly (every 7 days) whether they were scrotal until both the control male and the experimental male were scrotal. Mice were not checked daily to avoid stress, which itself could have influenced the age when males became scrotal (see introduction and results). We conducted four different experiments:

1. The influence of protein on age when becoming scrotal. One brother was fed with low protein food, consisting of apples and carrots (apples 0·3 g proteins per 100 g, carrots 1·1 g proteins per 100 g; DAK nutrition table). In contrast, the control males were fed as described above with protein high food sunflower seeds (peeled sunflower seeds 22·5 g proteins per 100 g; DAK nutrition table). Ten sibling pairs were used for this experiment.

2. The influence of encounters with unfamiliar males on age when becoming scrotal. To simulate high population density in the field, encounters with unfamiliar scrotal males (stimulus males) were staged 5 days a week for 5 min per day between 2:00 and 5:00 pm to resemble territorial encounters. A different stimulus male was used each day. Encounters were done in a neutral arena of 100 × 80 × 65 cm which was cleaned after each presentation. If damaging fights occurred within 5 min of the start of encounters, the experiment was immediately stopped; no mouse was injured. Stimulus males were captive males from the colony kept at the research station in type III Lab-o-tec® cages and fed as described before. Additionally, we gave hay with urine and faeces from different strange scrotal males into the cage 5 days a week at midday when males were fed. Twenty-one sibling pairs were used for this experiment. Control males were housed as in the other experiments, but were also placed into the clean and empty arena five times a week for 5 min each. They did not receive extra hay during midday feeding, so that the main disturbance, a hand over the cage putting food in, was the same for all males.

3. In family. Control males were singly housed as described above, while experimental males remained in their natal family with both parents and siblings of their own litter. We did not control the number or sex ratio of siblings remaining in the family. This could be an interesting future study, but our different results for mother- and father-families (see below) indicate that the siblings have no effect. Fourier sibling pairs were used for this experiment.

4. In mother-family. Control males were singly housed as described above, while experimental males remained in their natal family with only the mother and their siblings of their own litter being present, while the father was removed on D16. Seventeen sibling pairs were used for this experiment.

5. In father-family. Control males were singly housed as described above, while experimental males remained in their natal family with only the father and their siblings of their own litter being present, while the mother was removed at D16. Thirty-three sibling pairs were used for this experiment. This large sample size was due to the fact that this was the last experiment and we continued sampling as long as we had pairs available.

Statistics
Due to small sample sizes (N = 10, N = 21, N = 14, N = 17 and N = 33), all tests performed were nonparametric and two-tailed (Siegel & Castellan 1988) using the statistic software Instat. We compared the age (in weeks) at which brothers became scrotal using the Wilcoxon-matched pair rank sign test (T). Sample sizes for test statistics are given as total sample size minus pairs with the same value, as the Wilcoxon tests excludes pairs that are identical (Siegel & Castellan 1988). We also compared the body mass between males, using the data when each male became scrotal. We did so to control for an effect of the experimental treatment on general development. If the experimental treatment directly influenced the age at onset of sexual maturity, we expected experimental males to be heavier when becoming scrotal than control males. Otherwise, a general slowdown in development, for example, due to competition for food in the family group treatments, could have been the cause for later age when becoming scrotal, because onset of puberty depends on body mass in many species (Berner & Blanckenhorn 2007). Data are presented as means ± SD.

Blood samples
Blood samples for hormone measurements were taken from males in the 17 sibling pairs that differed in the age when they became scrotal. Blood was taken twice from each male, when the first male became scrotal, and when the second male became scrotal. In all cases both males were sampled, the order of the sampling being random. The male was taken out of its cage, anaesthetized with methoxyflurane and a blood sample of about 300 μL was taken from the sublingual vein (Heimann 2006). Samples were taken within less than 3 min, a time period short enough to avoid a stress response in striped mice (Schradin, 2008b). Samples were allowed to clot for 1 hour before being centrifuged for 10 min. The resulting serum was frozen in aliquotes at −20 °C.

Measurement of corticosterone and testosterone
All samples were analyzed in the EIA laboratory of the Zoological Institute, University of Zurich. Commercial kits from IBL Hamburg were used for corticosterone and testosterone. We followed the procedures listed in the manuals of the kits, but due to very high
corticosterone levels, samples were diluted 1:50 or 1:100. Validation of assays and inter- and intra-assay variability are reported elsewhere (Schradin 2008b; Schradin et al. 2009).

**Testes development**

All males were euthanized after experiments. From the last 14 brother pairs, testes were dissected directly after euthanasia and combined testes mass (without cauda epididymis) was measured. To ascertain sperm presence/absence and the number of sperm, both cauda epididymides were placed into a test tube with 500 μL of distilled water. The epididymides were homogenized and vortexed for 30 s. A measure of 60 μL of the solution was mixed with 60 μL of 5% gluteraldehyde. Forty micro litres of the solution were placed on a slide and analysed under a microscope at a magnification of 10× for sperm. Sperm were then counted at three random places under a magnification of 40×.

**Statistics**

Due to small sample sizes (N = 17), all tests performed were nonparametric and two-tailed (Siegel & Castellan 1988) using the statistic software Instat. We compared the age (in weeks) and body mass (in g) when brothers became scrotal using the Wilcoxon-matched pair rank sign test (T). Hormone data were compared using a Friedman ANOVA for paired comparisons with Dunn’s post hoc test for selected comparisons. Data are presented as mean ± SD.

**Results**

**FIELD STUDY**

Philopatric males became scrotal at an age of 9·6 ± 7·4 weeks (range: 3·0–43·0, N = 170 individuals) and weight of 31·4 ± 11·5 g (range: 13·0–65·0 g). Philopatric males became scrotal at a significantly later age when they lived together with a breeding male (F_{1,97} = 4·34, P = 0·04; Fig. 2), when food availability was low (F_{1,92} = 15·54, P = 0·0002; Fig. 3a), and when protein rich seedlings were abundant (F_{1,87} = 11·30, P = 0·001; Fig. 3b). This last result was unexpected and it was probably due to some males only becoming scrotal at the beginning of the following breeding season when seedlings are most abundant, and, hence, when these males were already many months old (see outliers in Fig. 3b, right top; with all data N = 105, r = 0·42, P < 0·0001; exclusion of three outliers: N = 102; r = 0·12, P = 0·24). There was a non-significant tendency for an influence of sex ratio on the age at which males became scrotal (F_{1,95·6} = 3·14, P = 0·08), while the effect of population density was not significant (F_{1,89} = 0·28, P = 0·60).

Co-linearity between seedlings and food (r = 0·03, P = 0·76) was very low. In contrast, population density and food availability correlated significantly (r = −0·56, P < 0·0001), and this correlation was negative as population density is highest in the non-breeding season (Schradin & Pillay 2005a) when food availability is lowest (Schradin & Pillay 2006). However, if food was removed from the analysis, population density still did not reach significance (F_{1,90·5} = 3·16, P = 0·08).

**STUDIES UNDER NATURAL WEATHER CONDITIONS**

1. Males fed protein poor food became scrotal at a significantly later age than males fed protein rich food (T = 0, N = 9, P = 0·004; Fig. 4a) and at a significantly lower body mass (T = 4, P = 0·03, Fig. 4b).

2. Interactions between young males and stimulus males were amicable (grooming) in 26·5 ± 25·9% of the cases, aggressive without contact (avoiding, chasing) in 51·1 ± 25·8% of the cases and aggressive with contact (fighting or biting) in 22·4 ± 10·3% of the cases. Young males were never injured. Males that were exposed to unfamiliar large males became scrotal at a significantly later age (T = 5·5, N = 13, P = 0·002, Fig. 4a) and at a significantly higher...
body mass ($T = 25, N = 16, P = 0.03$; Fig. 4b) than singly housed males.

3. Family housed males became scrotal at a significantly later age ($T = 0, N = 11, P = 0.001$, Fig. 4a) and at a significantly higher body mass ($N = 13, P = 0.01$; Fig. 4b) than singly housed males.

4. Males that lived in mother-families without the father became scrotal at a similar age ($T = 7, N = 6, P = 0.56$, Fig. 4a) and at a significantly lower body mass than singly housed males ($T = 35, N = 17, P = 0.05$; Fig. 4b).

5. Males that lived in father-families without the mother became scrotal at a later age ($T = 0, N = 18, P < 0.0001$, Fig. 4a) and at a significantly higher body mass than singly housed males ($T = 142, N = 32, P = 0.02$; Fig. 4b).

**CAPTIVE STUDIES UNDER CONTROLLED LABORATORY CONDITIONS**

As in the study conducted in South Africa, males in Zurich that were family housed became scrotal at a significantly later age ($T = 0, N = 17, P < 0.0001$, Fig. 4a) and at a significantly higher body mass ($T = 23, N = 24, P < 0.0001$; Fig. 4b) than singly housed males.

**Hormone measurements**

Males differed significantly in their corticosterone levels ($Fr = 12.459, N = 17, P = 0.006$). Non-scrotal family housed males did not differ in corticosterone levels from their singly housed brothers when the latter became scrotal ($P < 0.05$; Fig. 5a). However, the week they were found to be scrotal, family housed males had significantly higher corticosterone levels than their scrotal singly housed brothers ($P < 0.05$; Fig. 5a). Singly housed males decreased their corticosterone levels over time ($P < 0.01$; Fig. 5a), in contrast to family housed males ($P > 0.05$).

Males differed significantly in their testosterone levels ($Fr = 29.000, N = 16, P < 0.0001$). Non-scrotal family housed males had significantly lower testosterone levels compared to their singly housed brothers when the latter became scrotal ($P < 0.05$; Fig. 5b). The week they were found to be scrotal, family housed males still had significantly lower testosterone levels compared to their singly housed scrotal brothers ($P < 0.001$; Fig. 5b). Testosterone levels did not change significantly over time for neither family nor singly housed males ($P > 0.05$).

**Testes development**

The testes of scrotal family housed males were $0.66 \pm 0.3\%$ of their body mass while the testes of their singly housed brothers were $1.89 \pm 0.6\%$ of their body mass ($T = 0, N = 14, P = 0.001$; Fig. 6). Of the 14 family housed males, 11 had no sperm present in the smears (79%), while 6 of their singly housed brothers had no sperm (43%). Family housed males had $14 \pm 38$ sperm and singly housed males $153 \pm 295$ sperm...
Using long-term data we have shown that free-living male striped mice in the Succulent Karoo semi-desert vary in the age when they reach puberty and become scrotal from 3 to 43 weeks, and a body mass from 13 to 65 g. Our study highlights the importance of long-term studies over a variety of ecological conditions, since in a previous one year study on the same population males were reported to become scrotal only when reaching a body mass of more than 40 g (more than 10 weeks old; Schradin & Pillay 2005b).

In most species, growth rate is one major factor determining the onset of puberty (Berner & Blanckenhorn 2007), and differences in growth rate could be the proximate mechanism leading to the observed differences in the age of puberty. However, in testing the influence of two factors we could demonstrate that males not only became scrotal at a later age, but also at a greater body mass. Males stressed by encounters with unrelated males, and males housed with their father in their family, did not reach a threshold body mass of entering puberty at a later age. Instead, they became scrotal at a higher body mass. In contrast to this pattern were males subjected to a poor protein diet; these males grew slower than control males and became scrotal at a later age, but even significantly lower body mass! Similarly, males housed in mother families became scrotal at a lower body mass (but not younger age), possibly due to competition for food within the family. In the field, body mass of males becoming scrotal was highly variable. These results indicate that there is no threshold body mass, which, when reached, automatically triggers puberty in male striped mice.

Typically, organisms must acquire a minimum amount of resources and body mass before they can start puberty (Berner & Blanckenhorn 2007). In our study, some male striped mice entered puberty at an extremely early age of 3 weeks. This indicates that male striped mice can enter puberty shortly after they are weaned (16 days; Brooks 1982). It appears that male striped mice do not have to acquire resources before entering puberty, but rely on environmental information indicating that sufficient resources will be available to go through puberty. Thus, the striped mouse shows extraordinary flexibility in its ontogenetic pathways, enabling it to follow a strategy of rapid maturation and reproduction or a strategy of slow maturation and reproduction.

As predicted, males fed a poor protein diet became scrotal at a later age. One co-factor was that males fed sunflowers had not only access to more protein, but also to more fat, as sunflowers have a much higher fat content than carrots and apples. Thus, it was high quality food that influenced the age when males became scrotal, and several previous studies indicate that it is protein which is important for striped mice. For example, the availability of protein rich food determines the breeding season in striped mice (Perrin 1980b; Schradin & Pillay 2006) and experimental provisioning of food during the non-breeding season (winter) increases testes mass and sperm storage (Jackson & Bernard 2005). In contrast to many other mammals, reproduction and the excretion of hormones related to reproduction are not influenced by photoperiod in striped mice (Jackson & Bernard 1999; Schradin 2008a). Instead, dietary protein might influence endocrine pathways and gonadal function (Schneider 2004; Schoech et al. 2004).
This relationship has been demonstrated in Florida scrub-jays (*Aphelocoma coerulescens*), where artificial provisioning of high protein food leads to higher testosterone and lower corticosterone levels, and earlier onset of breeding (Schoech et al. 2004). Adult male striped mice become non-scrotal during the non-breeding season (Schradin & Pillay 2005a) when they decrease their testosterone levels and increase their corticosterone levels (Schradin 2008b). The same endocrine mechanisms are likely to act in young males.

Males became scrotal at a later age when they experienced social stress by meeting larger unknown males. Five encounters a week was probably much less than what young males would experience under conditions of high population density in the field, when there are more than 150 adult mice per ha (Schradin & Pillay 2005a) and aggressive territorial encounters are frequent (Schradin 2004; Schradin & Pillay 2004). These encounters might lead to increased secretion of glucocorticoids, which could suppress reproduction (Wingfield & Sapolsky 2003).

Family housed males became scrotal much later than singly housed males. In the field, males in groups without a breeding male became scrotal at a younger age than males living with a breeding male. In captivity, when the father was removed but the mother remained, males became scrotal as the same age as their singly housed brothers. In contrast, when the mother was removed but the father remained, males became scrotal later. Thus, results from the field and the laboratory studies indicate reproductive suppression by the father.

From field studies, we know that philopatric males have much higher corticosterone levels than breeding males (Schradin 2008b; Schradin et al. 2009), which could indicate that they are physiologically castrated (Reyer et al. 1986; Creel 2001; Wingfield & Sapolsky 2003). However, non-scrotal males did not have higher serum corticosterone levels than their brothers in the week their brothers became scrotal. Only a few weeks later, when the family housed males also became scrotal, family housed males had nearly twice as high corticosterone levels when compared to their brothers, which had decreased their corticosterone levels in the meantime. Corticosterone levels of family house males were somewhat lower (1200 ng/mL) than those of philopatric free living males (2000 ng/mL; Schradin 2008b), while levels of singly housed males were similar to those of free living roaming and breeding males (Schradin et al. 2009). This indicates that high corticosterone levels alone do not prevent males from becoming scrotal.

Family housed males had much lower serum testosterone levels than singly housed males. Even when they were scrotal, their testosterone levels did not increase, but were three times lower than those of their brothers. Furthermore, scrotal family housed males had much smaller testes than singly housed males, and 79% of scrotal family housed males had no sperm at all. This indicates that family housed males might have been sexually suppressed and physiologically castrated (Reyer et al. 1986; Creel 2001; Wingfield & Sapolsky 2003; Young et al. 2006).

Many studies have demonstrated reproductive suppression in females of cooperatively breeding species (Getz et al. 1983; Abbott 1984; Savage et al. 1988; Moehlman & Hofer 1997; Brant et al. 1998; O’Riain et al. 2000; Clark & Galef, 2001; Clarke, Mithé & Bennett 2001; Solomon et al. 2001; Saltzman et al. 2006), but evidence for reproductive suppression in males is rare. One exception is the Mongolian gerbil (*Meriones unguiculatus*), which lives in family groups with one breeding pair (Agren et al. 1989). Subordinate males typically have small testes and low testosterone levels. Subordinate male gerbils with relatively large testes, complete spermatogenesis and high testosterone concentrations have a high risk of being expelled from the family group (Scheibler et al. 2006). In contrast, subordinate males of the cooperatively breeding highveld mole-rat (*Cryptomys hottentotus*) have relatively smaller testes but not lower testosterone levels than dominant breeders, and most subordinate males have sperm (Rensburg et al. 2003).

In cooperative breeding societies, helpers often have lower androgen levels than breeders (Oliveira et al. 2003, 2008), though not the case in all species. For example male helpers of red-cockaded woodpeckers have high testosterone levels and seem capable of breeding (Khan et al. 2001), as do helpers of azure-winged magpies (*Cyanopica cyanus*, Cruz et al. 2003). Similarly, in the cooperatively breeding cichlid *Neolamprologus pulcher*, a male helper’s reproduction is not suppressed and their androgen levels are similar to those of breeders when kept in captivity (Oliveira et al. 2003). However, for the same species evidence exists from the field that helpers are sexually suppressed, as their testes were much smaller than those of breeders (Fitzpatrick et al. 2006). Male meerkat (*Suricata suricatta*) helpers have high testosterone levels and are capable of breeding, though they do so only with females from neighbouring groups (Young et al. 2005). In cooperatively breeding common marmosets (*Callithrix jacus*), subordinate males do not suffer from suppression of testosterone secretion, even though dominant males typically have the highest testosterone levels (Abbott 1984). Regarding glucocorticoids, the pattern is similarly diverse: in some cooperatively breeding species breeders have high levels, indicating costs of dominance, while in other species helpers have high levels, indicating stress and reproductive suppression (Creel 2001). In sum, variation exists between different cooperatively breeding species in the amount to which male helpers are reproductively suppressed. However, very little is known about the time male helpers reach puberty in cooperative breeding societies, and future studies on other species would be important.

Here we were able to demonstrate different factors that delay the age of becoming scrotal in the striped mouse. However, each experiment taken individually cannot explain the pattern observed in nature, where some males of the Succulent Karoo only became scrotal when their body mass was greater than 40 g (Schradin & Pillay 2005b). The three factors might act together in the field, and males became scrotal at a later age when they lived in family groups with a dominant breeding male, when they experienced numerous aggressive encounters with neighbours, and when protein rich food vanished quickly during the short breeding season. However, when food was plentiful and no dominant breeding male was present, males
became scrotal at a very young age. Thus, the combination of several factors under natural conditions can explain the variability observed in nature.

In conclusion, many factors determine when a male striped mouse becomes scrotal. Striped mice can follow either a mature, reproduce and die fast strategy or a mature, reproduce and die late strategy. It has been demonstrated that the striped mouse shows extraordinary flexibility in its social behaviour, ranging from solitary living to extended family groups with communal breeding and helpers at the nest (Schradin & Pillay 2004, 2005b), enabling it to adapt to different ecological conditions (Schradin 2005). Here we showed that this flexibility is also present in male reproductive maturation. Our study implies that male striped mice are able to use several environmental cues to reach sexual maturity at an optimal age.

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