

## Age of maturation and behavioral tactics in male yellow ground squirrel *Spermophilus fulvus* during mating season

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**Abstract** Life-history theory predicts that in hibernators age of maturation is related positively to body size and negatively to the duration of active season aboveground. Yellow souslik is a large-sized ground squirrel with long hibernation, which suggests late maturation. We used four-year field observations of marked individuals to determine the age of maturation in males through analysis of age-dependent variation in body size, mass, androgen status, timing of spring emergence, ranging patterns and social behavior during the mating season. Yearling males were smaller, lighter, had lower level of fecal testosterone, emerged later and had smaller home ranges than older males. Social activity and the number of females encountered did not differ between age classes. After the second hibernation none of the studied parameters varied with age. Cluster analysis revealed two behavioral tactics: “active” males (adults only) emerged earlier, ranged more widely, initiated more contacts, encountered more females and were heavier than “passive” males (both yearling and adult). Thus, males of *S. fulvus* reached adult size and matured after two hibernations, which is relatively fast for such a big species with short active period. Indirect evidence for copulations and high variation among yearlings in all parameters suggest that some of them might successfully compete with adults. Active tactic of wandering and searching for females is energetically costly, and probably only adult males in good physical condition can afford it, whereas passive tactic of residing is energy saving and good for adults in poor condition and for yearlings that are continuing to grow [Current Zoology 60 (6): 700–711, 2014].

**Keywords** Age of maturation, Mating system, Reproductive tactics, Ground squirrel, *Spermophilus fulvus*

Age at first reproduction is one of the key parameters in the studies of life-history, evolution of social systems and population dynamics (e.g., Charnov, 1990; Ferriere and Clobert, 1992; Stearns, 1992; Saether et al. 2013). Numerous case studies and theoretical models demonstrated strong relationships between maturation rate and various life-history traits, such as lifespan, mortality, senescence, fecundity (Harvey and Zammuto, 1985; Charnov and Berrigan, 1990; Promislow and Harvey, 1990; Ferriere and Clobert, 1992; Møller, 2006). Time of switching from growth to reproduction, growth rate, and body size at maturity are important characteristics to understand somatic-reproductive trade-offs (Kozłowski, 1992) and “fast-slow continuum” of life-history variation (Read and Harvey, 1989; Promislow and Harvey, 1990).

Also, the timing of first reproduction is a pivotal trait of social and mating systems (Blumstein and Armitage, 1998). In particular, presence or absence of non-reproductive males or females in a social group determines the number and diversity of demographic roles, i.e.,

social complexity (Blumstein and Armitage, 1997; 1998). Age at first reproduction can affect the distribution of reproduction among individuals in a population, i.e., the reproductive skew (Emlen, 1999), while sex differences in the age at first reproduction can influence the operational sex ratio (OSR), and, consequently, mating strategies of males and females (Emlen and Oring, 1977).

Ground-dwelling squirrels are the perfect model group for studying social systems, reproductive strategies and life-history trade-offs. Most of them are diurnal, burrowing, obligate hibernators living in open landscapes and active for a short season aboveground (Michener, 1984; Waterman, 2007; Ahmad, 2008). Thus, they are ideal subjects for the long-term individually-based studies that allow to monitor the development and life histories of individually recognizable animals (Clutton-Brock and Sheldon, 2010). Ground squirrels exhibit a wide diversity of mating systems (Dobson, 1984; Waterman, 2007) varying from obligate monogamy (e.g., in Alpine marmots *Marmota marmota*, Ar-

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nold and Dittami, 1997; Blumstein and Armitage, 1999 Hackländer et al., 2003) through various forms of polygyny as in *M. flaviventris* (Armitage, 1986; Armitage, 1998) or prairie dogs *Cynomys gunnisoni*, *C. ludovicianus*, *C. parvidens* (Foltz and Hoogland 1981; Armitage, 1986; Hoogland, 2007) to promiscuity, as in *Ictidomys tridecemlineatus* (Schwagmeyer and Wootner, 1986). At the same time, they demonstrate high interspecific variation in growth rate and age of the first reproduction (Armitage, 1981; Ahmad, 2008). Variation in mating pattern and in developmental rate appeared to be interrelated; in promiscuous species both males and females start to reproduce as yearlings after their first hibernation (e.g., *I. tridecemlineatus*, McCarley, 1966; *Otospermophilus beecheyi*, Dobson and Davis, 1986; *Urocitellus richardsonii*, Michener and Koepl, 1985), whereas polygynous and monogamous species delay reproduction to 3–4 years old and exhibit sex differences in time of recruitment (e.g., *Urocitellus columbianus*, Neuhaus et al., 2004). In some species, delayed maturation and recruitment were explained by extremely short active season and, thus, young need several years to reach adult status (Armitage, 1981).

In males, besides reproductive maturation *per se*, successful recruitment depends also on their competitive ability, and physiological development (e.g., androgen status) is associated with changes in behavioral patterns (Boonstra et al., 2001; Strauss et al., 2007; Scantlebury et al., 2008). Several individual life-history and behavioral characteristics were found to affect male competitive ability and mating success in ground squirrels. In species that exhibit female defense polygyny (e.g., *C. ludovicianus*, Hoogland, 1995; *U. columbianus*, Raveh et al., 2010), males of larger size had an advantage in access to females and defended them more successfully from competing males. In *I. tridecemlineatus*, which exhibits scramble competition polygyny, males that ranged more widely searching for females had more chances to encounter female in estrous (Schwagmeyer and Wootner, 1986).

In hibernating ground squirrels, both males and females emerge asynchronously, and males usually start to emerge first, while females emerge later (Michener, 1984). Females in most species come to estrous immediately after emergence and stay fertile for a very short period (only for a few hours) (Lacey et al., 1997; Hoogland, 1998a; Michener, 1998). Thus, OSR varies strongly during the mating season, and males face different levels of male-male competition depending on the date of emergence. In the beginning of the mating

season, OSR is strongly male-biased; therefore, early emerging males face stronger competition and, in addition, suffer from bad weather conditions. On the other hand, males emerging early benefit from a prolonged mating season and lower risk of missed opportunity to encounter female in estrous. Indirectly, time of emergence may indicate maturation and individual competitive ability in ground squirrels. In species, where males or females start to reproduce after their first hibernation, the date of emergence does not vary with age within sexes (Michener, 1983b). In contrast, in species which delay breeding until two or more years of age, non-breeding cohorts emerge significantly later than the same-sex breeders (*U. columbianus*, Michener, 1984). Therefore, population partitioning by time of spring emergence can be good evidence for the presence of non-reproductive cohorts.

The yellow ground squirrel (or yellow souslik, *Spermophilus fulvus*, Licht.) is a diurnal obligate hibernator. The body mass of *S. fulvus* (up to 2,000 g for males before hibernation; Vasilieva et al., 2009) is one of the largest among ground squirrels (excluding marmots); while the active season is one of the shortest (about 3 months in adults and 2–2.5 in juveniles), which suggests slow growth and late maturation. In species with longer active period (*U. richardsonii*, *U. parryii*, *I. tridecemlineatus*), males mature after their first hibernation (Michener, 1984; Holekamp, 1984; Boonstra and MacColl, 2000). In medium-sized ground squirrel species that exhibit similar to *S. fulvus* short active period (*U. beldingii* and *U. columbianus*), males reach adult size and mature only after 2–3 hibernations (Michener, 1984; Murie and Harris, 1984). However, previous studies showed that young *S. fulvus* grow fast, females start to reproduce as yearlings and demonstrate similar reproductive patterns to adults (Popov et al., 2006; Vasilieva et al., 2009). Much less is known about maturation and recruitment of males. Some data suggest that they may or may not reproduce as yearlings. Shubin and Kydyrbayev (1973) found bimodal distribution of testicular sizes in one of the populations and speculated that testes of smaller sizes belonged to yearling males. In the other population, the distribution was unimodal, suggesting that at least some yearlings reproduced. However, they were unable to estimate the age of autopsied individuals. Accurate aging is one of the advantages of the long-term individually-based field studies of recognizable individuals, which is essential for estimating life-history parameters including age of maturation and recruitment (Clutton-Brock and Sheldon, 2010).

In this study, we used long-term field observations of individually recognizable individuals with known age and life-history characteristics to determine age of physical, physiological and behavioral maturation in male yellow sousliks through analysis of age-dependent variation in body mass, body size, androgen status, emergence pattern, ranging, social behavior and behavioral tactics during the mating period.

## 1 Materials and Methods

The study was performed in spring (from mid-March to mid-April) 2004–2007 and in May–June 2005–2007 in a natural colony of yellow ground squirrel *Spermophilus fulvus orlovii* (Ogn. 1937) in Saratovskaya oblast', Russia, in the vicinity of village Dyakovka (50°43'88"N, 46°46'04"E). In yellow souslik, like in the most ground squirrels, males emerge from hibernation first, and females start to emerge 7–10 days later. Spring sessions started at the beginning of the male emergence from hibernation, and thus we could estimate the date of emergence for each male. We considered the day of emergence of the first female in the colony the starting point of the mating season. We controlled the core area (cca 40 ha) of the entire colony (cca 60 ha) partially isolated from the other colonies by the village, the Yerulan river and the Dyakovskii Forest, which is the southern-most forest in European Russia. The habitat within the study site is a patch of hemipsammophilic steppe under moderate grazing pressure from cattle with sparse bushes and few trees. We started permanent individual marking in the colony in 2001 (Shilova et al., 2006; Vasilieva and Tchabovsky, 2009), therefore we knew the exact age of nearly all the males.

### 1.1 Study species

The yellow (or long-teeth) souslik, *Spermophilus fulvus*, occupies desert and semi-desert habitats in Central Asia, Iran and China. In Russia, it inhabits sandy dry steppes on the left bank of the mid and lower Volga River (Shilova et al., 2014). Yellow sousliks typically live at low population density (approximately 3–10 ind./ha) in patchily distributed dispersed colonies on sandy soils, and are considered solitary animals (Bokstein et al., 1989). However, within colonies, philopatric females form spatial kin-clusters and matriline; therefore, *S. fulvus* can be assigned to the second grade according to the gradation of sociality of ground squirrels by G. Michener's (1983a).

### 1.2 Trapping

Yellow ground squirrels are very shy and cautious towards humans making them difficult to capture. We

trapped squirrels with non-commercial self-made snare loops of 1 mm cable or wire cage traps (50 × 50 × 50 cm) without bait. The cage traps and loops were equipped with signal flag and placed in the entrance of the burrow. As the animal moved through the loop or into the trap, the flag became dislodged and fell signaling about the capture. The snare and cage traps were set for a specific individual once seen aboveground after emergence from hibernation. The set traps were constantly observed, and when captured, the squirrels were immediately removed from the traps, sexed, weighed (to 1 g) and individually marked with microchips (Bayer AG, Leverkusen, Germany) and a urzol black D dye (P-Phenylenediamine) (Rhodia, Paris, France) unique mark on the fur for visual identification. We also measured the zygomatic arc breadth (the maximum breadth of the head; to 0.1 cm) with sliding calipers as an estimator of structural size or "body size" (Karels et al., 2004; Skibieli et al., 2009).

A female yellow souslik comes to estrous soon after emergence from hibernation and remains fertile only for several hours. To minimize the interference in the mating process, we processed trapped animals as fast as possible, and the handling time never exceeded 10–15 min.

### 1.3 Observations

We observed the entire colony area daily using 12–30 × binoculars from several elevated (5–15 m in height) hidden observational posts situated in sparse trees and on the fire-observation tower. The grass cover was short enough to allow the reliable recognition of marked individuals. We subdivided the study area into five non-overlapped sections and observed the movements and social interactions of animals daily from 9:00 to 16:00 with the help of trained assistants. We used two procedures to record the movements and contacts of males. First, we observed focal animals for 30–60 min recording their behaviors, relocations and contacts (157 hours of observation in 2005–2007). Second, we applied a scanning procedure recording locations and social contacts of all active individuals present within the colony during the scanning period. We conducted two 30-minute scans a day during the maximal activity of the squirrels. For each active squirrel we recorded the location of the first sighting during the scan. The locations were recorded using land-marks and white flags, and then were mapped by GPS. In addition, we used all occasional sightings of squirrels besides those that were recorded during the scans or focal observations. We also recorded all visits of males to female burrows when a female was

inside. As a result, we produced daily the summarized checklists of all the individuals emerged and present within the colony, as well as the lists of their social contacts, locations and visits to female burrows.

#### 1.4 Hormone assays

*Sample collecting.* We used non-invasive procedure of fecal steroid analysis. Males ( $n = 53$ ) were sampled for feces during the first 7 days after spring emergence from hibernation within 9:00–13:00 hrs, as the squirrels defecated at capture. Collected fresh feces were dried at 30°C to constant mass (for 24 h) and stored at 18°C (Washburn and Millspaugh, 2002; Terio et al., 2002; Pettitt et al., 2007). Storage treatment (drying versus freezing feces) was shown not to affect hormone metabolite concentrations in other ground squirrel species (Pettitt et al., 2007).

*Processing of the fecal samples.* Testosterone metabolites were extracted from feces by a standard method (Jewgenow et al., 2006). Hormone metabolites from dried, powdered, and mixed fecal samples (0.1 g) were extracted for 30 min by shaking with 0.9 ml of 90% methanol and 0.1 g of  $Al_2O_3$ . After centrifugation (10 min at 1,300  $\times$  g) 0.2 ml of the supernatant was transferred into a new clear tube and diluted with 0.2 ml of distilled water.

*Enzyme Immunoassays.* Methanol-extracted samples were analyzed with enzyme immunoassay (EIA) systems. EIA was conducted using flatbed spectrophotometer Multiscan EX (ThermoElectron Corporation), measuring absorbance at 450 nm/620 nm and comparing with the standard mean. The detection of fecal testosterone metabolites concentrations were conducted using commercial kits for testosterone (“Immunotech”, Moscow, Russia). Cross-reactivity values of testosterone antibody with major interfering steroids were low or minimal: 9% for 5-dihydrotestosterone, 1% for 11-hydroxytestosterone, 1% for 5-androsten-3, 17-diol, and <0.01% for all other steroids tested, indicating high antibody specificity. Samples were assayed in duplicate and concentrations are expressed as nanograms/gram of fecal dry matter. The minimum level of detection was 0.058 ng/ml. Intra-assay coefficient of variation was  $3.7 \pm 0.7\%$  (mean  $\pm$  SE,  $n = 44$ ). Interassay coefficient of variation was 13.0% ( $n = 2$ ).

*EIA validation.* Previous studies on fecal steroid analysis have validated radioimmunoassays and EIA for glucocorticoids in many rodents (Harper and Austad, 2000; Kuznetsov et al., 2004; Good et al., 2003; Touma et al., 2004; Mateo and Cavigelli, 2005; Soto-Gamboa et al., 2009) and EIA for female gonadal hormones in

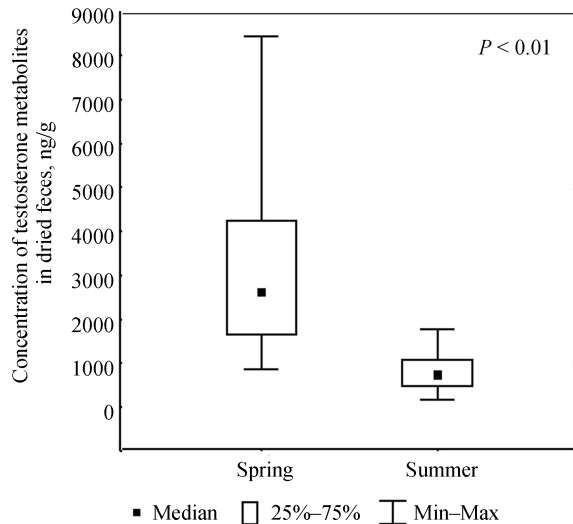
ground squirrel species (Pettitt et al., 2007). Yellow ground squirrel is an obligate hibernator, and we could not find the method to keep them in captivity without altering their natural life cycle and activity, especially, their hibernation and reproduction, which is essential for biochemical or physiological validation (Palme, 2005). We also were not able to collect blood samples in the wild colony during the spring. Quick blood sampling is impossible in yellow souslik because of very high blood coagulability. Therefore, in yellow souslik, blood sampling is a stressful procedure, requiring anesthesia, and could severely disturb the behavior of males and the mating process. Consequently, for correct measurement of the behavioral parameters of males during the mating season we had to exclude this invasive procedure to minimize the interference in animal activity in spring. Instead, we performed correlation test to verify the relationship between serum testosterone level and testosterone fecal metabolite concentration based on samples collected in summer. The correlation was positive and significant ( $r_s = 0.76$ ,  $P = 0.03$ ,  $n = 8$ ).

In addition, the testosterone assay was validated by the parallelism of the serial dilution binding curve of fecal extracts (from neat to 1:128) with the testosterone standard curve (0–10 ng/mL).

To validate biologically the fecal testosterone assay, we collected fecal samples from 10 adult wild males ( $\geq 2$  hibernations) at different stages of the active season: 1) during the mating season (March–April) and 2) before immergence to hibernation (in June). The concentrations of fecal testosterone metabolites were substantially and significantly higher ( $3,295 \pm 2,403$  ng/g) in spring, than in summer ( $839 \pm 524$  ng/g;  $t$ -test for dependent samples,  $t = 3.4$ ,  $df = 9$ ,  $P = 0.008$ , Fig. 1). Increased gonadal hormone level in plasma during the mating season is the trusted indicator of reproductive system activation in a seasonal breeder including squirrels (Licht et al., 1982; Barnes et al., 1988; Place and Kenagy, 2000; Boonstra et al., 2001; 2011). High immunoreactivity of antibody to testosterone in feces during the mating season as compared with the pre-immergence period provides reliable evidence for the validity and physiological relevance of fecal testosterone analysis for yellow ground squirrel males.

#### 1.5 Data analysis

Males with a known year of birth were assigned to one of the two categories: yearlings or adults; the latter were subcategorized as two, three and four or more years old according to the number of hibernations survived.



**Fig. 1** Concentration of testosterone metabolites in dried feces from adult males in spring (mating season) and in summer (before immergence). Repeated measures, Student's *t*-test.

It was impossible to distinguish sexually mature and immature males by external examination. In particular, neither scrotum pigmentation nor testis size was indicative of active reproductive status. To estimate the age of physiological and physical maturation in males, we analyzed age-dependent variation in the level of fecal testosterone metabolites during the mating season and in physical condition (body mass and body size measured within the first 5 days after emergence), respectively.

To determine the age of behavioral maturation and recruitment of males, we could not use the direct measures of mating success because yellow ground squirrels mate underground, and matings are difficult to record. Thus, we used indirect measures of behavioral maturation and recruitment analyzing age-dependent variation among males in ranging patterns, date of emergence, social activity, competitive ability in intrasexual conflicts and the number of potential female mates.

Home range size of males during the mating season was estimated using the minimum convex polygon method (100% MCP). All males recorded during less than 5 days and with fewer than 6 locations were excluded from the analysis. To calculate MCP, we used ArcView 3.3 (© Environmental Systems Research Institute, Inc. 1992–2000), Animal Movement v. 1.2 (© P. N. Hooge and B. Eichenlaub, 1997 Alaska Science Center - Biological Science Office, U.S. Geological Survey, Anchorage, AK, USA).

To measure social activity, we calculated the fre-

quency of all types of social contacts (number of contacts per 100 min) initiated by male aboveground: agonistic (chasing, fights), neutral (sniffing), and sexual (anogenital inspections of females and sexual pursuits). We averaged contact frequencies obtained from each observational protocol for every male over the mating season. All observations of males prior to emergence of the first female were excluded from the analysis. To assess the number of potential female partners, we used the total number of females with whom a male interacted aboveground during the mating season. All male-female contacts occurred near female winter burrows, and almost all of them (482 of 518, 93%) were initiated by males; among those, we observed only 2 fights (0.4%). Only non-agonistic (neutral or sexual) contacts initiated by a male in the first 7 days after female's emergence from hibernation were used to calculate the number of potential female partners for each male.

To assess the competitive ability of males, we calculated the percentage of conflicts (agonistic contacts) won by each male with chasing a competitor considered a victory (Schwagmeyer and Brown, 1983). Only males with at least 5 male-male agonistic contacts recorded were included in the sample.

### 1.6 Statistical analysis

For variables that conformed to a normal distribution (male body mass and body size and the date of male emergence, Shapiro-Wilk's *W* test,  $P > 0.05$ ), we used Student's *t*-test (for two-group comparisons) or one-way ANOVA followed by Tukey post hoc test (for four groups). All samples were homoscedastic (Levene's test,  $P > 0.05$ ). Home range size, social activity, the number of potential female mates and the level of fecal testosterone metabolites did not conform to the assumptions of normality (Shapiro-Wilk's *W* test,  $P < 0.05$ ) and could not be transformed to normal distribution. Therefore, we used the nonparametric Mann-Whitney *U* test and Kruskal-Wallis ANOVA followed by non-parametric Dunn's post hoc test. To classify males according to their behavioral tactics during the mating season, we performed K-means clustering with three variables (the date of spring emergence, home range size and the number of potential female mates) and two clusters. All variables were standardized before clustering.

All statistical analyses were performed with STATISTICA, v. 8.0 (StatSoft, Tulsa, OK, USA). All tests were two-tailed with the significance level of 0.05. The values are presented as the means  $\pm$  SD; and for non-normal samples, we also presented the median.

Some males appeared more than once in the initial

dataset in subsequent years. To avoid pseudo-replication, we excluded all the repeated measurements of males randomly from each of the subsets for all the analyses.

## 2 Results

### 2.1 Physical condition and growth

The body mass and body size at emergence varied significantly among age classes (Table 1), and were, on average, much smaller in yearlings than in older males ( $445 \pm 93$  vs  $803 \pm 125$  g;  $t = 11.1$ ,  $df = 58$ ,  $P < 0.0001$  and  $40.9 \pm 1.8$  vs  $46.6 \pm 1.8$  mm;  $t = 9.1$ ,  $df = 44$ ,  $P < 0.0001$ , respectively). Males reached adult physical condition by two years old: neither the body mass nor the body size increased with age after the second hibernation (Tukey post hoc test,  $P > 0.1$ ). However, some yearling males exhibited parameters of physical condition similar to those of adults.

### 2.2 Level of fecal testosterone metabolites

The level of fecal testosterone was significantly dependent upon age: on average, in yearling males, it was roughly half (median = 994 ng/g, mean =  $1,457 \pm 1,922$  ng/g,  $n = 17$ ) the level in adults (median = 2,127 ng/g, mean =  $2,731 \pm 1,995$  ng/g,  $n = 29$ ;  $U = 99$ ,  $Z = 3.4$ ,  $P = 0.0008$ , Fig. 2). Individual variation within the age categories was very high and overlapped variation between categories: fecal testosterone concentrations varied from 160 to 8,512 ng/g in yearling males and from 818 to 8,656 ng/g in adults.

### 2.3 Timing of spring emergence

The date of emergence from hibernation was significantly related to age (Table 1), but did not vary with age after the second hibernation (i.e., among adults of different age classes; Tukey post hoc test,  $P > 0.4$ ). Adults emerged on 24 Mar  $\pm 7.8$  days, and, on average, 10 days earlier than yearlings (3 Apr  $\pm 7.3$  days,  $t = -5.7$ ,  $df = 71$ ,  $P < 0.0001$ ). Some yearlings, however, emerged as early as older males.

### 2.4 Ranging

The variation in home range size was extremely high in yearling (Min = 32 m<sup>2</sup>, Max = 42,390 m<sup>2</sup>) as well as

in older males (Min = 209 m<sup>2</sup>, Max = 152,663 m<sup>2</sup>). On average, home ranges were three to four times smaller in yearling males (median = 3,359 m<sup>2</sup>, mean =  $8,218 \pm 13,490$  m<sup>2</sup>,  $n = 14$ ) than in adults (median = 15,418 m<sup>2</sup>, mean =  $29,476 \pm 31,602$  m<sup>2</sup>,  $n = 52$ ; Mann-Whitney  $U$  test,  $U = 144$ ,  $Z = 3.5$ ,  $P = 0.0006$ ), however, some yearlings ranged as widely as did adults.

### 2.5 Social activity

The frequency of contacts initiated did not differ significantly between yearling (median = 0.06 contacts/100 min of activity, mean =  $0.8 \pm 1.2$  contacts/100 min,  $n = 15$ ) and adult males (median = 0.48 contacts/100 min, mean =  $1.5 \pm 3.1$  contacts/100 min,  $n = 37$ ;  $U = 233$ ,  $Z = 0.9$ ,  $P = 0.4$ ). However, percentage of wins was much higher in adult males (median = 0.88, mean =  $0.69 \pm 0.34$ ,  $n = 14$ ) than in yearlings (median = 0.07, mean =  $0.17 \pm 0.23$ ,  $n = 6$ ;  $U = 8$ ,  $Z = 2.8$ ,  $P = 0.005$ ).

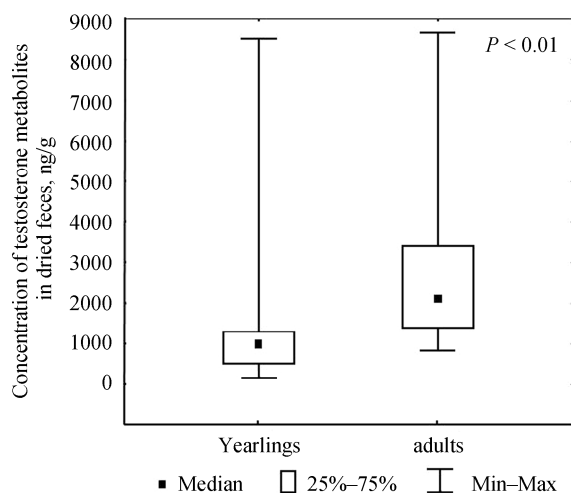
Similarly, we did not find significant differences between yearling and adult males in the number of female interaction partners (median = 1.0 females, mean =  $1.3 \pm 1.1$  females,  $n = 12$  for yearlings; median = 2.0 females, mean =  $2.0 \pm 2.1$  females,  $n = 43$  for adults;  $U = 223$ ,  $Z = 0.7$ ,  $P = 0.5$ ). The number of female partners varied strongly within age categories, from zero to 3 in yearling and to as many as 15 in adult males.

### 2.6 Behavioral tactics

One of the two clusters constructed on the basis of similarities in the date of emergence, home range size and the number of female partners (Cluster 1,  $n = 26$ ) included both yearling ( $n = 8$ ) and adult ( $n = 18$ ) males, whereas the second cluster consisted of adult males only ( $n = 8$ , Cluster 2). Clusters differed significantly by all three variables ( $P < 0.02$ ). The members of the second cluster were heavier, emerged earlier, ranged more widely, initiated more contacts, interacted with more females, and won more frequently in conflicts ( $n_1 = 7$ ,  $n_2 = 7$ ,  $U = 8$ ,  $Z = 2.1$ ,  $P = 0.04$ ) than males from the first cluster (Fig. 3). On the whole, males from Cluster 1 won only in 25.5 % of 271 conflicts with other males (yearlings: 28.3 % of 166; adults: 21.0 % of 105; Yates

**Table 1** Body mass, body size and date of emergence from hibernation of males from different age classes

Male's characteristics	Male age, hibernations				Statistics
	1	2	3	$\geq 4$	
Body mass at emergence; g (Min-Max; $n$ )	$445 \pm 93$ (293–602; 20)	$758 \pm 135$ (440–985; 22)	$807 \pm 88$ (697–1,070; 17)	$837 \pm 129$ (646–1,098; 12)	$F_{3,67} = 47.0$ $P < 0.0001$
Zygomatic arch breadth; mm (Min-Max; $n$ )	$40.9 \pm 1.8$ (38–43; 11)	$45.7 \pm 1.8$ (41–48; 16)	$47.0 \pm 1.8$ (44–51; 13)	$47.2 \pm 1.2$ (46–50; 11)	$F_{3,47} = 33.9$ $P < 0.0001$
Date of emergence Min-Max; $n$	3 Apr. $\pm 7.3$ (14 Mar.–15 Apr.; 34)	24 Mar. $\pm 8.8$ (10 Mar.–8 Apr.; 28)	27 Mar. $\pm 6.8$ (17 Mar.–9 Apr.; 14)	18 Mar. $\pm 8.3$ (10 Mar.–31 Mar.; 7)	$F_{3,79} = 13.1$ $P < 0.0001$



**Fig. 2** Concentration of testosterone metabolites in dried feces from yearling and adult males after spring emergence. Mann-Whitney U-test.

corrected  $\chi^2$  test,  $\chi^2 = 1.5$ ,  $P = 0.2$ ). In contrast, members of the Cluster 2 wined in 80.0 % ( $n=284$ ) of male-male agonistic interactions (Yates corrected  $\chi^2$  test,  $\chi^2 = 165$ ,  $P < 0.0001$ ). The concentration of fecal testosterone metabolites did not vary between clusters ( $U = 20$ ,  $Z = 0.3$ ,  $P = 0.8$ ). Yearling and adult members of the first cluster differed significantly in body mass only and were similar in other parameters (Fig. 3).

### 3 Discussion

In male *S. fulvus*, spring body mass, body size and the concentration of fecal testosterone metabolites differed between yearling and older males: yearlings, on average, were much lighter, smaller and had only half the concentration of testosterone metabolites of adults. In other words, yearlings continued growing and did not mature after their first hibernation. At the same time, we did not find any age-dependent variation in physical condition or androgen status in males two years and older. Thus, yellow souslik males reach adult size and mature after two hibernations, which is compatible or even faster as compared to medium- or small-sized ground squirrel species with similarly short active period (Armitage, 1981). The relatively rapid growth and maturation of male *S. fulvus* is an unusual trait for other hibernators in which large body size and short period of activity lead to slow growth, late maturation and recruitment (Armitage, 1986, Turbill et al., 2011), i.e., traits associated with slow paced life history (Promislow and Harvey, 1990).

Measurements of fecal hormone metabolites require physiological, biological and analytical validation. We

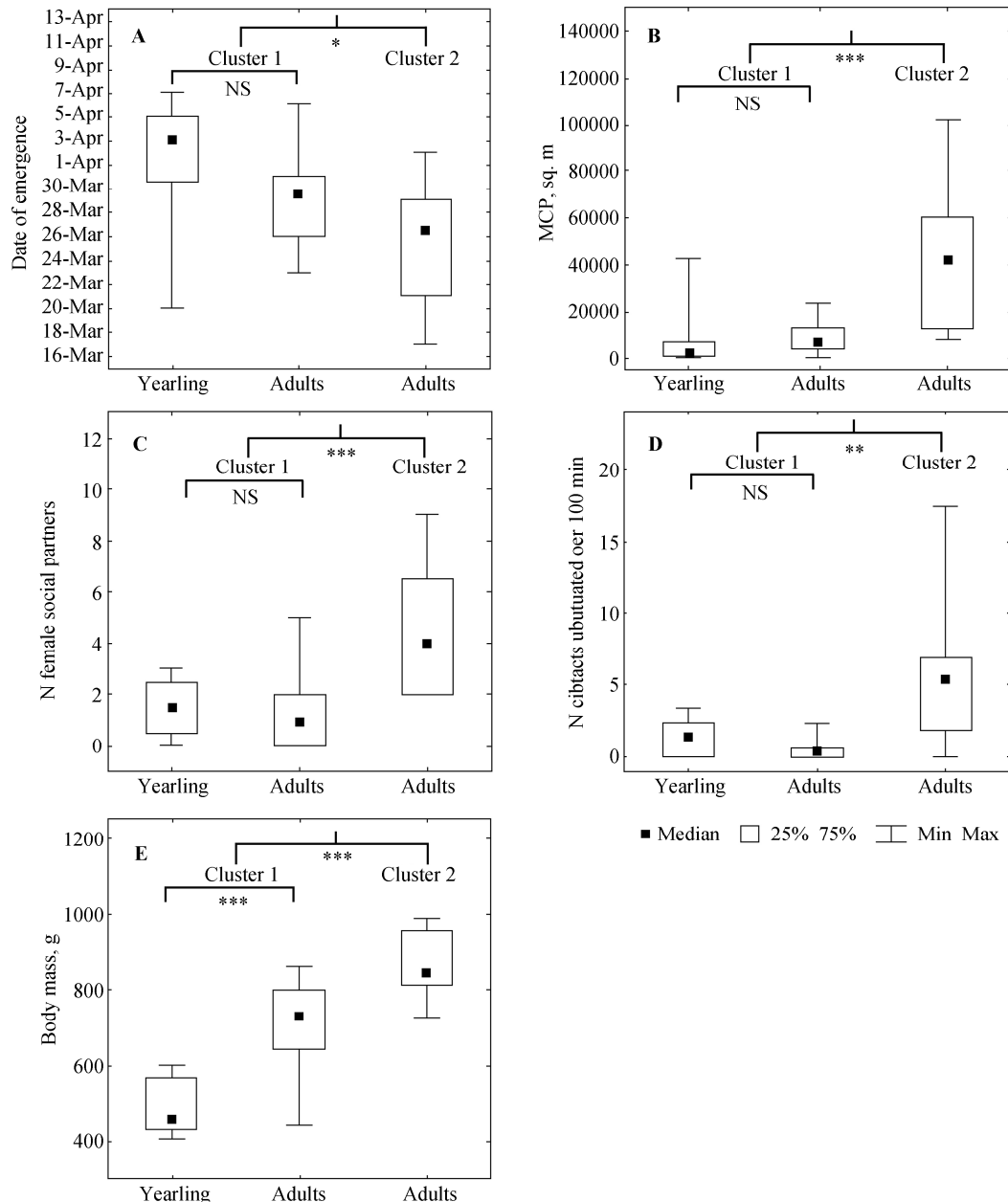
had no possibility to keep animals in captivity, and no yellow sousliks are kept in zoos or breeding centers. Moreover, we had to avoid the invasive procedures in order not to interfere in animal activity. Therefore, like in many other studies of wild animals (Rolland et al., 2005; Wasser, Hunt, 2005; Adachi et al., 2010; Umaphathy et al., 2013), we were forced to limit our efforts to biological validation. In the absence of analytical validation the possibility to measure some other compounds together with testosterone metabolites, as it was shown for primates (Möhle et al., 2002), cannot be absolutely excluded. However, based on the results of biological validation, positive significant correlation between serum testosterone and immunoreactivity in feces, results of ACTH-test (no response seen for testosterone antibodies, Vasilieva, 2011), and parallelism test, we believe that variation in testosterone antibody immunoreactivity in feces corresponded to testosterone levels in souslik blood.

The levels of serum and fecal testosterone are closely associated with the reproductive status of males (Sachser and Prove, 1986; Monfort et al., 1997; Cavigelli and Pereira, 2000; Boonstra et al., 2001; Li et al., 2001). For instance, in European ground squirrels *Spermophilus citellus*, some yearling males breed and demonstrate the peak of testosterone concentration during pre-mating and mating periods in contrast with non-reproductive yearlings with invariably low testosterone concentration. The lower concentrations of fecal testosterone metabolites in non-reproductive males was combined with immaturity of reproductive system, namely, with abdominal testis and unpigmented scrotum, as well as with late emergence and higher growth rate (Strauss et al., 2007). Low fecal testosterone level combined with continuing growth in male yearling yellow ground squirrels suggests that they tradeoff reproduction for growth and do not reproduce as yearlings being recruited only after the second hibernation. This suggestion is supported by the late emergence from hibernation, restricted ranging in the vicinity to the hibernacula burrow and low competitive ability in intrasexual conflicts in yearlings in contrast to adults, who emerged early, moved much greater distances from their burrows searching for potential mates and won in conflicts much more frequently. It also conforms to the data of Shubin and Kydyrbaev (1973) who found high individual variation in testis size in autopsied yellow ground squirrel males and hypothetically attributed it to age differences.

At the same time, the level of social activity and the number of female interaction partners did not differ

between yearling and adult males. Male and female yellow ground squirrels copulate underground and do not share burrows during the mating season except for copulation (Vasilieva, Tchabovsky, unpublished data). Thus, a male and a female entering together the same burrow system are likely to copulate. Such behavioral criteria for copulation were used for other ground

squirrels with underground mating (*U. columbianus*, Raveh et al., 2010; *U. parryii*, Lacey et al., 1997). We observed 38 events when a male entered the burrow of a female together with a female in the first days after female emergence from hibernation, i.e., during the likely estrous period, and stayed underground for at least 10 min. Twenty four such visits were performed by 8 adult



**Fig. 3 Individual characteristics of males from Cluster 1 ("passive" males) and Cluster 2 ("active" males)**

A. Date of spring emergence. Cluster 1 ( $n = 26$ ) vs Cluster 2 ( $n = 8$ ):  $U = 54, Z = 2.1, P = 0.03$ ; Yearlings ( $n = 8$ ) vs Adults ( $n = 18$ ) in Cluster 1:  $U = 39, Z = 1.8, P = 0.07$ . B. Home range. Cluster 1 ( $n = 26$ ) vs Cluster 2 ( $n = 8$ ):  $U = 22, Z = -3.4, P = 0.0007$ ; Yearlings ( $n = 8$ ) vs Adults ( $n = 18$ ) in Cluster 1:  $U = 44, Z = -1.5, P = 0.1$ . C. Number of females with whom males interacted. Cluster 1 ( $n = 26$ ) vs Cluster 2 ( $n = 8$ ):  $U = 27, Z = -3.2, P = 0.001$ ; Yearlings ( $n = 8$ ) vs Adults ( $n = 18$ ) in Cluster 1:  $U = 63, Z = 0.5, P = 0.6$ . D. Frequency of social contacts initiated. Cluster 1 ( $n = 23$ ) vs Cluster 2 ( $n = 8$ ):  $U = 24, Z = -2.5, P = 0.01$ ; Yearlings ( $n = 8$ ) vs Adults ( $n = 15$ ) in Cluster 1:  $U = 41, Z = 1.2, P = 0.2$ . E. Spring body mass. Cluster 1 ( $n = 19$ ) vs Cluster 2 ( $n = 6$ ):  $U = 11, Z = -3.0, P = 0.003$ ; Yearlings ( $n = 5$ ) vs Adults ( $n = 14$ ) in Cluster 1:  $U = 6, Z = -2.7, P = 0.007$ . Paired differences are showed by asterisks (NS indicates  $P \geq 0.1$ , \* indicates  $0.05 \leq P < 0.1$ , \*\* indicates  $0.01 \leq P < 0.05$ , \*\*\* indicates  $P < 0.01$ ).



males and 14 by 4 yearling males (some males visited the same female more than once). The samples are not sufficient for any statistical comparisons; however, we can not reject the hypothesis that some yearling males may mate in spite of, on average, great differences in physical condition, hormonal status and date of emergence between age classes. Moreover, some yearlings exhibited androgen level and physical condition, as well as ranging and emergence patterns similar to those of adults. Finally, preliminary unpublished results of microsatellite analysis showed that at least one yearling male shared offspring. Thus, it is not unlikely that at least some yearling males mated with females in the studied population.

If the overt competition took place between males during the mating season it would be difficult for yearlings to compete with adults because of smaller body size, lower body mass and lower fighting ability. However, cluster analysis showed that two behavioral tactics exhibited by males were not strictly related to age: only some of adult males adopted "active" tactic of emerging early and ranging widely, while other adult males as well as all yearling males used "passive" tactic of emerging late and residing near winter burrow. "Active" males were larger in size, heavier, and were more successful in contacting with females and in conflicts with males. "Passive" adult males occupied intermediate position between "active" males and yearlings in terms of date of emergence and body mass, and were similar to yearlings in restricted movements, low social activity, few females encountered and low rank in male-male conflicts. Probably, passive tactic exhibited by some adult males was due to their poorer body condition compared to "active" adults. Active tactic of searching and competing for females in estrous is energetically costly, suggesting that only males in good physical condition can afford it. The passive tactic of residing and waiting for a chance is energy saving and good for adults in poor condition as well as for yearlings that are continuing to grow.

In many mammalian species males are known to adopt various behavioral tactics to achieve mating (Taborsky et al., 2008; Wolff, 2008). In Libyan jirds *Meriones libicus*, older males used active tactic of ranging widely, searching for females and competing for access to them, while younger males resided near particular female waiting for a chance or used sneaking tactic (Chabovsky and Popov, 1989). Among sciurid species, such individual variation in reproductive behavior is known for Cape ground squirrel *Xerus inaurus* (Scan-

tlebury et al., 2008) and gray squirrel (*Sciurus carolinensis*, Koprowski, 1993). In yellow sapsucker, the existence of "passive" adult males in poor body condition might enable some yearlings in a proper physiological condition, to compete with adults despite of smaller body size.

In contrast to promiscuous ground squirrel species, where both sexes start to reproduce as yearlings, a majority of *S. fulvus* males delay maturation in contrast to females, who breed as yearlings at rate similar to older ones (Popov et al., 2006), which skews the overall breeding-season operational sex ratio in favor of females. A female-biased sex ratio among reproductive individuals combined with inequality of males in behavioral tactics suggests a polygynous mating system with weakened male-male competition. Weak direct competition among males is known for some ground squirrel species (*I. tridecemlineatus*, Schwagmeyer and Brown, 1983; *S. citellus*, Millesi et al., 1998) where males use competitive mate searching (Parker, 1978; Alcock, 1980; Schwagmeyer and Woontner, 1986) and allocate time and energy mainly to wandering, but not for female or territory defense. Adoption of such behavioral tactics by ground squirrel males can be favored by extremely short estrus in asynchronously emerging females (Schwagmeyer and Woontner, 1985; Schwagmeyer, 1988).

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