Age at first mating affects parental effort and fecundity of female Mongolian gerbils

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We examined effects of age at first mating on both parental effort and fecundity of female Mongolian gerbils, *Meriones unguiculatus*. We found that, with increasing age at first mating and resulting age at first parturition, female gerbils: (1) were more likely to retrieve young removed from the nest, (2) spent more time both in contact with and nursing young, and (3) provided an environment in which pups grew more rapidly. Older mothers were also less likely to become pregnant than were younger mothers and, if successful in delivering a second litter, showed longer interlitter intervals and delivered smaller second litters. Between delivery and weaning of first litters, older mothers lost more weight than did younger mothers. We discuss these findings as consistent with the prediction from life-history theory that parental effort should increase with age-related decreases in residual reproductive value. Furthermore, and as predicted by parental investment theory, older mothers delivered reliably more male-biased second litters than did younger mothers. Because of the different sex ratios of litters born to older and younger dams, we anticipate discovery of differences in reproductive and parental behaviours of offspring of dams of varying ages as a result of differences in the intrauterine exposure of their young to testosterone.

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Because fitness costs of raising young vary with parental age, life-history theory predicts greater parental effort by older than by younger parents. Older parents, with relatively little opportunity for future reproduction, should be selected to make greater parental investment (Trivers 1972) in current offspring than young parents that have a lifetime of potential reproduction before them (e.g. Williams 1966; Pianka & Parker 1975; Charlesworth & Leon 1976; Clutton-Brock 1991).

Although there is correlational evidence from field studies consistent with the hypothesis that older parents make greater parental investment than do younger parents (e.g. Clutton-Brock 1984; Pugesek 1984, 1990, 1995; Pugesek & Diem 1990; Voland & Gabler 1994), we could find no experimental studies comparing parental behaviour of old and young animals. We undertook the present laboratory investigation of effects of age at first parturition on parental effort and residual reproductive value to determine both the relative reproductive effort and relative fecundity of female Mongolian gerbils, *Meriones unguiculatus*, mated for the first time when 35–120 days of age.

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We studied only primiparous females because, in many vertebrate species, reproductive behaviour changes with successive parturitions (Galef 1983; Nur 1984). Consequently, disassociating female reproductive experience from female age is necessary if conclusions are to be drawn concerning effects of age per se on parental effort.

The ages that we selected for studying reproduction in Mongolian gerbils spanned the range of ages at which more than 90% of female gerbils that are paired at weaning with unfamiliar, reproductively proven males deliver their first litters in the laboratory (Clark et al. 1986). In the wild, female Mongolian gerbils breed either in the year of their birth (if they are born in spring or early summer) or in the spring of the following year (if they are born late in the breeding season; Leont'ev 1962; Krylova 1978; Orlenev & Pereladov 1981). Consequently, in natural circumstances, ages of females at first reproduction should be bimodally distributed.

METHODS

Subjects

Fifty-five nulliparous female Mongolian gerbils taken from 38 litters born in the vivarium of the McMaster University Department of Psychology (Hamilton,
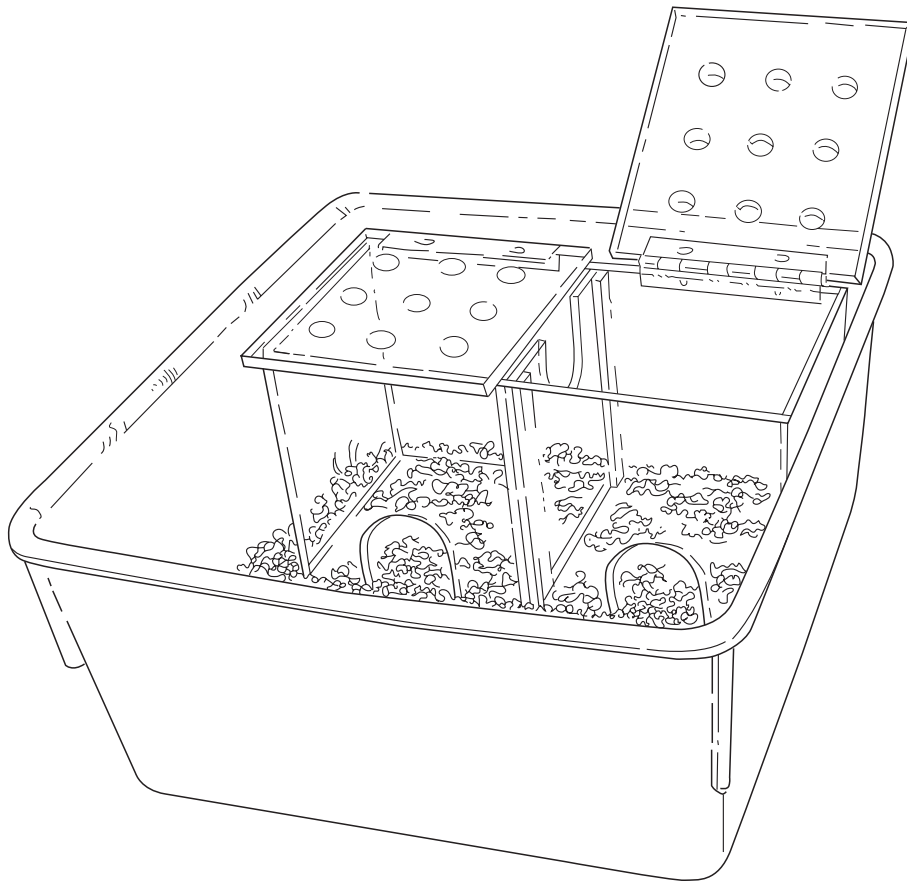
Figure 1. Illustration of apparatus.

Ontario, Canada) and their first two litters served as subjects. The females were third-generation descendants of breeding stock acquired from Charles River Breeding Farms (Wilmington, Massachusetts, U.S.A.). All subjects resided in a single temperature- and humidity-controlled colony room illuminated on a 12:12 h light:dark cycle, and all had ad libitum access to water and Purina Rodent Laboratory Chow 5001.

We maintained females in groups of three or four, in polypropylene shoe-box cages, measuring $35 \times 50 \times 15$ cm, from weaning (when 32 days of age) to breeding. During this period subjects were undisturbed, except for biweekly cage cleaning.

Apparatus

During the experiment, each adult female subject resided in a shoe-box cage identical to those in which subjects had been housed previously. At the appropriate time (see Procedure), we placed in each cage a nestbox (Fig. 1) constructed of clear Plexiglas. The nestbox, described in detail in Clark & Galef (1999, 2000), consisted of two identical compartments in which a female could build a nest. The two compartments were joined by an opening that permitted adult gerbils to move freely between them.

Procedure

Forming breeding pairs

We examined parental behaviour of 48 nulliparous females randomly assigned to four groups of 12 that differed only in the age (35, 70, 90, or 120 days) at which we placed them individually in the cages of reproductively proven adult males from our colony. We assigned only one female from any litter to each of the four groups.

To eliminate aggression between adult males and females meeting for the first time, for the first 24 h that members of a pair shared a cage, we separated them with a screen partition.

If a female failed to become visibly pregnant within 35 days of pairing with a first male, we placed her with a second, and if necessary, with a third potential mate. To observe maternal behaviour in four groups, each consisting of 12 females, we had to try to mate 55 females. Five females in the oldest group failed to become pregnant after pairing with three successive males (and three other females in the oldest group had to be paired twice before they became pregnant), and two females in the youngest group lost their litters shortly after parturition. Although replacement of subjects that fail to reproduce is not ideal, it would not have been reasonable to compare maternal behaviour of females that were unable to reproduce with

maternal behaviour of females that had reproduced, and attribute any differences to the age of females.

Inspection of cages

We examined cages of mated pairs weekly, and if a female became visibly pregnant (in the second or third week of gestation), we removed her mate from her cage. At the same time, we placed in her cage a nestbox (Fig. 1) and 5 g of paper towel to use as nesting material.

We examined cages of pregnant females daily for presence of a litter. On the day of birth of a litter, after completing both the unobtrusive measurement of a dam's maternal behaviour and the test of retrieval (described below), we determined the number of pups in her litter and the sex and body weight of each pup.

Observations of undisturbed females and litters

Each day from the day of birth of a litter (day 1), until the litter was 20 days of age, an observer who was unaware of the group assignment of individual dams observed their behaviour. Starting 4 to 6 h after light onset, the observer determined, once every 20 s for 15 min, whether a dam was either huddled over her pups in a nursing posture, or was in physical contact with one or more of her pups, but not huddled over them.

Test of retrieval

On day 1, immediately after the unobtrusive measure of a female's interaction with her litter had been completed, we removed the dam from the apparatus, weighed her, and moved her pups from the nestbox compartment that contained the litter and nest to the other nestbox compartment. We then placed the dam in the open area of her home cage, and determined whether she returned her litter to the original nest site within 30 min.

Equating litter sizes and sex ratios

It has been shown previously that, in Mongolian gerbils, both litter size (Elwood & Broom 1978; Waring & Perper 1980) and the sex of pups in a litter (Clark & Galef 1992) can influence parental behaviour of their dam. Consequently, following testing of females on day 1, we culled litters born to females assigned to the four groups so that, across groups, litter sizes were equated. Litters within each of the four groups ranged in size from two to eight and had a mean (± 1 SE) of 5.1 ± 0.5 pups. We also equated across groups, in so far as possible given the sexes and body weights of pups delivered, the mean sex ratios and body weights of litters on the day of their birth (mean sex ratios of litters in a group: range from 50.1 ± 8.6–52.8 ± 8.9; analysis of variance, ANOVA: $F_{3,44}=0.09$, NS; mean body weights of individual pups in litters in groups: range 3.0 ± 0.1 to 3.1 ± 0.1; ANOVA: $F_{3,44}=0.24$, NS).

By equating litter size, sex ratio of litters and initial body weight of pups dams reared, we hoped to impose equivalent demands on dams in the four groups so that any effects of dams' ages on maternal behaviour and fertility would not be confounded by systematic variation in the litters that they reared.

Reweighing mothers and pups

On days 10 and 30 postpartum, we weighed both mothers and pups.

Remating females

After we weaned pups on day 30 postpartum, we placed each of their dams in the cage of an unfamiliar, reproductively proven, adult male from our colony. Again, to prevent aggression between unfamiliar males and the females that we introduced into their cages, we separated pair members with a screen partition for the first 24 h that they shared a cage.

Pairs remained together undisturbed for 50 days except for: (1) weekly inspection to determine whether females were pregnant, (2) daily inspections of pregnant females to determine the date of birth of any litters, (3) biweekly cage cleaning, and (4) removal of males when a female was visibly pregnant.

If a female was unlikely to mate with the first male with whom we paired her (as indicated by her attacking her partner after the partition separating the pair was removed), we paired her with a second and, if necessary, a third male.

Data Analysis

To detect effects of maternal age on reproductive performance, maternal behaviour and development of pups we used trend analyses. Such analyses distinguish between linear and nonlinear contributions to variance. In a study such as the present one, with four groups, the linear component of the analysis has one degree of freedom, and the nonlinear component two degrees of freedom.

RESULTS

Age of Females at First Parturition

Females first bred at 35, 70, 90 and 120 days of age delivered their first litters when on average (± 1 SE), 69.7 ± 1.5, 106.1 ± 2.1, 135.8 ± 3.2 and 178.9 ± 4.9 days old.

Probability of Females Giving Birth

Age at pairing had an effect on the probability that virgin females would be impregnated by the first male with whom they were paired (chi-square test: $\chi^2_3=16.8$, $P<0.001$). This relationship reflects the fact that all 12 females in the youngest group gave birth following their first pairing, whereas only four of 12 females in the oldest group did so.

Size and Sex Ratio of First Litters

There was a modest tendency in the size of first litters born to females as a function of their ages, with older females giving birth to slightly smaller litters than younger females (linear component: $F_{1,44}=1.94$, NS;

Table 1. Reproductive performance of female Mongolian gerbils of varying ages

|				
Age (days) at first parturition	69.7±1.5	106.1±2.1	135.8±3.2	178.9±4.2
Litter size at birth of first litter	6.3±0.3	6.6±0.5	5.7±0.5	5.7±0.3
Sex ratio at birth of first litter	47.5±7.2	47.6±7.1	51.6±6.8	52.6±6.4
Body weight (g) of dams				
Day 1	66.7±1.3	70.6±1.1	70.0±0.8	73.3±2.3
Day 10	72.9±1.8	74.6±1.4	69.9±0.8	76.6±1.5
Day 30	69.2±1.7	68.9±0.8	68.5±1.1	71.6±1.6
% Change				
Days 1–30	2.9±1.8	−2.2±1.1	−3.3±1.2	−3.1±1.7
Latency (days) of second pairing to second litter	27.3±0.3	28.1±0.3	31.0±1.9	31.9±1.9
Litter size at birth of second litter	7.9±0.4	5.4±0.5	6.4±0.6	5.2±0.6
Sex ratio at birth of second litter	47.1±4.6	42.9±5.4	56.6±7.2	70.5±6.8

Cell entries are means±1 SE.
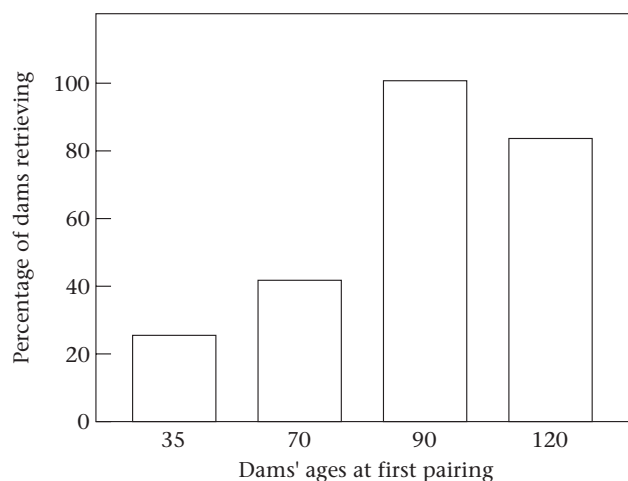
Figure 2. Percentage of females paired for the first time at different ages retrieving pups in 30 min on day 1.

nonlinear component: $F_{2,44}=0.66$, NS; Table 1). There was also no difference in the sex ratios at birth of first litters as a function of mother's age at parturition (linear component: $F_{1,44}=0.39$, NS; nonlinear component: $F_{2,44}=0.02$, NS; Table 1).

Maternal Behaviour

Older females were far more likely to retrieve their pups to the nest than were younger females (Fig. 2; chi-square test: $\chi^2_3=16.30$, $P<0.01$).

Across the 20 days of observation, there was an age-related linear trend in the frequency with which females were observed both in contact with their young (linear component: $F_{1,44}=24.45$, $P<0.001$; nonlinear component: $F_{2,44}=0.50$, NS; Fig. 3a) and huddled over their young in a nursing posture (linear component: $F_{1,44}=20.09$, $P<0.001$; nonlinear component: $F_{2,44}=0.08$, NS; Fig. 3b). On both measures, older females were more maternal than younger females.

On the day of birth, body weight of pups did not differ as a function of maternal age (linear component: $F_{1,44}=0.23$, NS; nonlinear component: $F_{2,44}=1.75$, NS; Fig. 4). However, during the first 10 days postpartum, when pups were totally dependent on their mother's milk
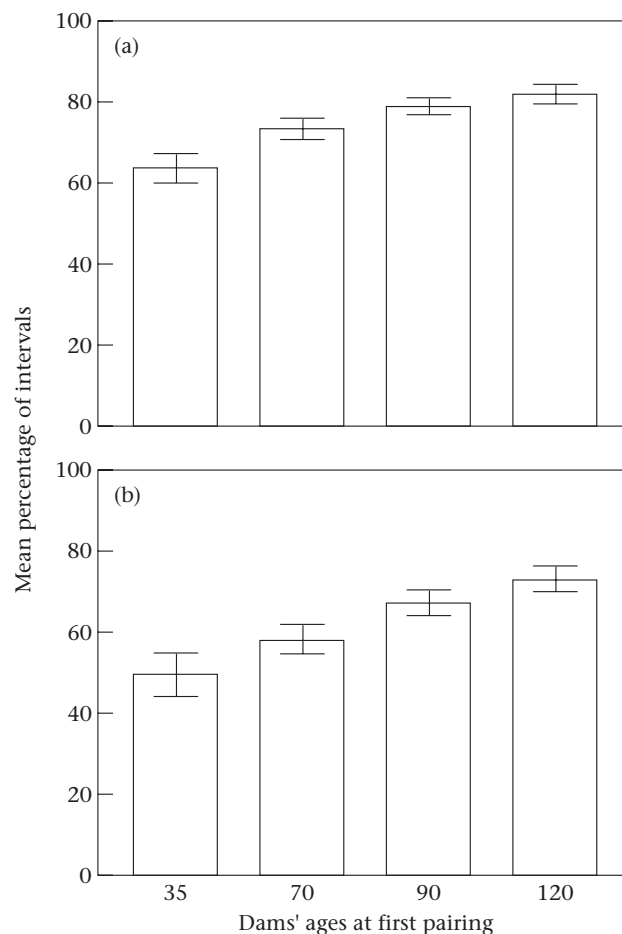

Figure 3. Percentage of observation periods on days 1 to 20 postpartum when females paired at various ages were seen (a) in contact with and (b) huddled over their pups. Error bars indicate standard errors of the mean.

for nutrition, there was a linear trend in the weight gains of pups as a function of their mother's age. Pups reared by older mothers gained more weight than did pups reared by younger mothers (linear component: $F_{1,44}=4.37$, $P<0.04$; nonlinear component: $F_{2,44}=0.73$, NS; Fig. 4). By day 30, approximately 10 days after pups had begun to feed on solid food, there was no longer a relationship between mother's age and change in pup weight (linear
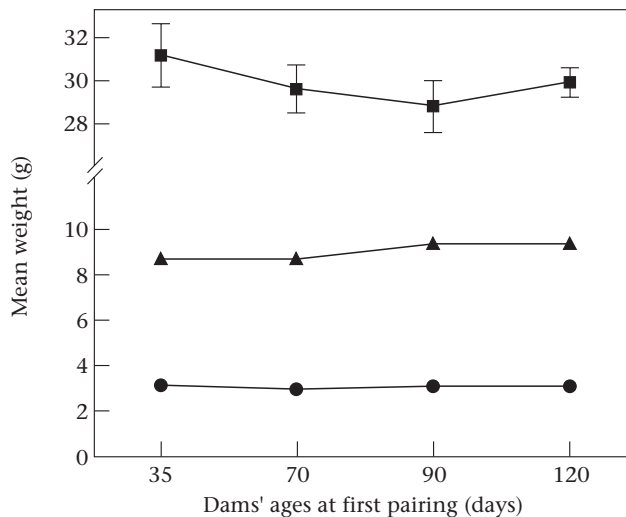
Figure 4. Mean body weights of pups on day 1 (●), day 10 (▲) and day 30 (■) postpartum as a function of dams' ages at first pairing. Error bars indicate standard errors of the mean. When error bars are not visible around a point, they are obscured by the point itself.

component: $F_{1,44}$=3.31, NS; nonlinear component: $F_{2,44}$=0.15; Fig. 4).

During the 30-day period from birth to weaning of the young, throughout which mothers provided their young with milk, there was an age-related linear trend in mothers' body weights, with older mothers showing greater weight loss than younger mothers (linear component: $F_{1,44}$=7.89, P<0.01; nonlinear component $F_{2,44}$=1.62, NS; Table 1).

The effects of age at first pairing on pups' and mothers' body weights could not be attributed to differences in the size of litters resulting from differential mortality of pups. All pup mortality took place in the first few days postpartum and resulted in mean litter sizes for groups ranging from 5.0 to 5.1 pups (linear component: $F_{1,44}$=0.05, NS; nonlinear component: $F_{2,44}$=0.94, NS).

Production of Second Litters

The mean age of a group of mothers at first delivery was correlated with their probability of becoming impregnated a second time (Spearman rank-order correlation r_S=1.00, N=4, P=0.05; Table 1). Furthermore, among those 42 females that did bear a second litter, age of the mother at first pairing was correlated with both latency after pairing to deliver a second litter (linear component: $F_{1,38}$=8.64, P<0.01; nonlinear component: $F_{2,38}$=0.30, NS; Table 1) and the number of pups the female delivered in a second litter; older mothers produced smaller second litters than did younger mothers (linear component: $F_{1,38}$=8.57, P<0.01; nonlinear component: $F_{2,38}$=4.35, P<0.02; Table 1).

Last, we found an effect of mother's age at first pairing on the sex ratio of second litters, with older mothers delivering more male-biased litters than younger mothers (linear component: $F_{1,38}$=9.82, P<0.01; nonlinear component: $F_{2,38}$=1.05, NS; Table 1). Male pups constituted

47.1 ± 4.6 of second litters born to females first paired when 35 days old and 70.5 ± 6.8% of second litters born to females first paired when 120 days of age.

DISCUSSION

Summary

Older gerbil mothers were more likely than were younger gerbil mothers to: (1) retrieve pups on the day of their birth, (2) be in contact with one or more pups, and (3) huddle over pups in a nursing posture, presumably nursing them. Possibly as a consequence of the increased attention provided by older dams to pups, pups born to and reared by older mothers grew more rapidly than did pups with younger mothers and, during lactation, older mothers lost more weight than did younger mothers. Furthermore, in comparison with younger mothers, older mothers had: (1) reduced probability of producing a second litter, (2) greater latency to second reproduction, (3) reduced size of second litters and (4) more male-biased second litters.

Relationship of Findings to Life-History Theory

Both the observations of mothers' interactions with their first litters and our findings regarding mothers' subsequent reproduction are consistent with the hypothesis that older mothers invest more in their offspring and have less residual reproductive value than do younger mothers. However, our findings are open to alternative interpretations. In particular, it is not clear from the present data whether observed age-correlated effects on mothers' production of second litters were a result of reproductive senescence, differential investment in a first litter, or some combination of the two.

Regardless of interpretation of observed correlations between mothers' age at first parturition and parameters of second litter production, the results of the present study offer support for the prediction from life-history theory that females' parental effort should increase with age and consequent declining residual reproductive value. Increased probability of pup retrieval and nursing time and increased rate of pup growth provide evidence of increased maternal effort, possibly mediated by differences in the hormonal profiles of young and old mothers (Matt et al. 1986; Wang & vom Saal 2000). Increased latency to impregnation, decreased probability of impregnation and reduced litter size are all indicative of decreased residual reproductive value.

Sex Ratio of Litters and Parental Investment Theory

The finding that second litters of older mothers were more male biased than were second litters of younger mothers is consistent with Trivers' (1972, 1985) suggestions that selection should favour parents that: (1) invest more in each son than in each daughter, and (2) tend to

raise males when they will invest relatively more and tend to raise females when they will invest relatively less per offspring. On such a theory, and as we found, older parents that make relatively large investments in offspring and have relatively few young per litter should tend to produce a greater proportion of sons than younger parents that invest less in offspring and give birth to more of them.

Predicted Consequences

We have previously described profound effects of the sex of intrauterine neighbours on both testosterone titres and reproductive and parental behaviours of male and female gerbils (for reviews, see Clark & Galef 1995; vom Saal et al. 1999). In the present experiment, mothers' ages predicted the proportion of males in their litters. The proportion of males in a litter affects both a dam's hormonal profile during pregnancy (Clark et al. 1993b) and the probability that any fetus she gestates will occupy an intrauterine position adjacent to a male fetus (Clark et al. 1993a). Consequently, we anticipate discovery of significant differences in hormonal profiles and reproductive and parental behaviours of male and female gerbils delivered by mothers of different ages.

Acknowledgments

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