Can captivity lead to inter-species mating in two *Mesocricetus* hamster species?

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**Abstract**

In two closely related species, females generally prefer conspecific males over heterospecific males. We found that estrous (but not diestrous) female Syrian hamsters *Mesocricetus auratus* prefer the odors of conspecific males to odors of Turkish hamsters *Mesocricetus brandti*. However, female Syrian hamsters are not aggressive toward male Turkish hamsters and will readily mate with them. We hypothesize that many generations in captivity led to a reduction in females’ ability to avoid inter-species mating, possibly related to the heightened sexual receptivity observed in *Mesocricetus* hamsters in captivity. To test this hypothesis, we replicated a study carried out with female Turkish hamsters soon after the current laboratory stock of this species was established. In that study, female Turkish hamsters showed lordosis toward male Syrian hamsters in only 20% of interactions and attacked heterospecific males in 80% of the pairings. Using animals descended from that original colony (after many generations in captivity and certain episodes of inbreeding), 100% of female Turkish hamsters mated with heterospecific males and none showed aggression toward heterospecific males. Thus female avoidance of interspecific mating may be affected by captive rearing conditions.

**Introduction**

Captivity associated with inbreeding may negatively affect several aspects of reproduction by females in a variety of species (Ryan, Lacy & Margulis, 2003). However, we are not aware of any studies showing that captivity may reduce the ability of females to prefer males of their own species (conspecific males) over males of a different species (heterospecific males). Failure of females to prefer conspecific males may result in inter-specific mating, to the detriment of the female’s reproductive success, because inter-specific mating normally results in no or sterile offspring. Inter-specific mating between closely related species long kept in captivity may be due to the decrease in aggression and/or the increase in sexual receptivity normally associated with captivity (Künzl et al., 2003). When the level of female receptivity in response to males increases in a captive population, the discrimination against some types of males (e.g. heterospecific males) may be overcome by heightened sexual receptivity.

To analyze the effect that multiple generations in captivity may have on species discrimination, we conducted three experiments using captive and inbred laboratory populations of Syrian hamsters *Mesocricetus auratus* and Turkish hamsters *Mesocricetus brandti*. Laboratory Syrian hamsters were descended from a single pair of siblings that were captured in 1930 (Murphy, 1985; Gattermann et al., 2001) and thus laboratory hamsters are highly inbred (Fritzsche et al., 2006). Such a high level of inbreeding and/or decades of living in captivity affect male reproductive success in hamsters. When females were paired sequentially with both a laboratory male and a wild-type male, wild-type males fathered 87% of the pups. However, lordosis duration was similar in laboratory and wild-type females (Fritzsche et al., 2006). Body mass, body measurements and several organ weights did not differ between wild-derived and laboratory Syrian hamsters maintained under the same conditions (Gattermann et al., 2002).

In our first experiment, we investigated preferences of female Syrian hamsters for odors of conspecific versus heterospecific males. In 1977, receptive female hamsters spent more time in close proximity to a conspecific male than to a heterospecific male when both males were behind barriers (Murphy, 1977). Thus we predicted that sexually receptive females (but not necessarily unreceptive females) would show a preference for odors of conspecific males over those of heterospecific males. In our second experiment, female Syrian hamsters in estrus were paired with a conspecific male and then with a heterospecific male (or vice versa). We predicted that female Syrian hamsters would show lordosis and copulate only with conspecific males, but would not show lordosis in the presence of heterospecific males. We also predicted that females would be aggressive toward heterospecific males but not toward conspecific males. In our third experiment, we used a similar design with female Turkish hamsters. We took advantage of the existence of a previous study conducted in the 1970s with female Turkish hamsters soon after the first laboratory colony of this species was established (Murphy, 1978). That
 colony was founded with 29 animals, and, as with Syrian hamsters, has never been outbred to the best of our knowledge (Lyman & O'Brien, 1977). In Murphy’s study, the subjects were among the first generations of captive-bred individuals. In only 20% of the trials did females show lordosis toward heterospecific (Syrian hamster) males, whereas in 80% of interactions females aggressively attacked heterospecific males. We replicated that study using animals descended from the original colony to test the hypothesis that prolonged captivity leads to heightened sexual receptivity and inter-specific mating.

Materials and methods

All animals were born and raised in captivity at Cornell University, Ithaca, NY, USA. Hamsters were weaned at 30 days of age and housed individually in solid bottom polycarbonate cages (45 × 24 × 14.5 cm) with sand-chip bedding material and constant access to water and food (ProLab 1000, Agway, Syracuse, NY, USA). Turkish and Syrian hamsters were maintained in separate rooms with independent air intake and exhaust. Turkish hamsters were maintained on a 16L:8D light–dark schedule with lights off between 10:00 and 18:00 h (Eastern Standard Time). Syrian hamsters were maintained on a 14L:10D light–dark schedule with lights off between 10:00 and 18:00 h (Eastern Standard Time). Experiments were run within the first 3 h of the dark phase (i.e. 10:00–13:00 h). We used dim and indirect light to allow videotaping and observations. When a video camera was used, it was located c. 1 m from the cage. Females were not tested with the scent of a male relative or tested for mating with a male relative.

The estrous cycles of all females were determined several days before a trial. To confirm that a female was in estrus on a specific day, a conspecific male hamster was placed inside the female’s home cage. If lordosis occurred, the female was considered in estrus. If no lordosis was observed, the female was retested on the following days until lordosis occurred. Given the 4-day estrous cycle in Syrian and Turkish hamsters (Lisk, 1985), once the day of estrus was determined, the female was randomly facing one side of the arena (and thus we normalized the data so that for each subject the proportions of time investigating the three glass plates is mutually exclusive; however, the total time that a subject spent investigating each one of the three glass plates is equal 1 (Aitchison, 1986). We used Friedman’s rank sum tests to compare investigation time of the three odors when females were in estrus and when they were in diestrus-2.

Experiment 1

Syrian hamster females (n = 19) were tested twice, once when sexually receptive (estrus) and once when non-receptive (diestrus-2) for their preferences for the odors of male Syrian and Turkish hamsters; the two trials were separated by at least 1 week. The order of the trials was counterbalanced across subjects and there were no order effects (P = 0.54). Average age of females was 276.47 ± 8.19 days (range, 211–321 days). Five females had mated once before testing, whereas the rest (14) were nulliparous. Average age of conspecific males was 248.74 ± 8.96 days (range, 114–309 days). Average age of heterospecific males was 230.32 ± 7.92 days (range, 102–313 days). For both the conspecific and the heterospecific males, half of the males had sexual experience before testing, whereas the other half was sexually naïve. Females were tested in a square arena (46 × 46 cm). A glass plate (6.3 × 6.3 cm) was placed in the middle of each of the four walls. One plate was left unscented, the second plate was scented with a saline solution (control odor), the third plate was scented with flank-gland secretions of a male Syrian hamster (conspecific odor) and the fourth plate was scented with flank-gland secretions of a male Turkish hamster (heterospecific odor). For the ‘conspecific odor’ and the ‘heterospecific odor,’ each flank gland of a male donor was rubbed 15 times (i.e. 30 flank-gland marks per donor) in a circular motion against the glass plate. For the ‘control odor,’ a swab was submerged in a saline container, dried until the swab was not saturated and then rubbed 30 times against the glass plate. All the scented glass plates were prepared immediately before a test trial, and the experimenter tried to cover a similar area of the glass plate. After randomly placing the four glass plates, a female was put in the center of the arena using a 600 mL opaque plastic beaker. The beaker covered her for 5 s before the beginning of the trial. This ensured that the female was randomly facing one side of the arena (and whatever glass plate was on that side) at the beginning of the trial. Each trial lasted 10 min. We measured the time that the female spent sniffing or licking the glass plates with the control, conspecific and heterospecific odors. We did not measure investigation of the unscented glass plate because the glass plate with saline was a better and sufficient control. The time that a subject spent investigating each one of the three glass plates is mutually exclusive; however, the total investigation time for these three glass plates differs among subjects, and thus we normalized the data so that for each subject the proportions of time investigating the three glass plates would equal 1 (Aitchison, 1986). We used Friedman’s rank sum tests to compare investigation time of the three odors when females were in estrus and when they were in diestrus-2. If any of those two Friedman’s rank sum tests yielded a statistically significant result, we used a Wilcoxon
test to compare investigation time of the conspecific and heterospecific odors. That is, we did not conduct biologically irrelevant pairwise comparisons involving the saline in order to reduce the probability of Type I errors.

Experiment 2

Estrous female Syrian hamsters \((n = 10)\) were tested twice, once with a conspecific male and once with a heterospecific male (Turkish hamster). All females were tested while in estrus in two consecutive estrous cycles (i.e. the two tests were separated by 4 days). We counterbalanced the order of the two tests. At the beginning of a test, a conspecific or a heterospecific male was placed into the female’s cage. For 5 min we video-recorded and later measured (1) the latency to lordosis; (2) the total duration of lordosis; (3) the number and duration of female attacks. Lordosis is a stereotypical position in Mesocricetus spp. in which the female arches down her back, spreads her legs, raises her tail and remains immobile from seconds to minutes. We considered that an attack occurred if we observed any attempted biting or tumbling fight. To avoid female pregnancy affecting the second test, the experimenter prevented the occurrence of ejaculation (in both the first and second tests) by pulling the loose skin on the male’s rear. This method did not stop lordosis and did not seem to disturb the male’s behavior, but it interfered with the normal pattern of male copulatory behavior. Thus we did not analyze male copulatory variables (other than occurrence or not of mating). No pregnancy occurred as a result of the pairings in this experiment. Average body mass and age of females were 132.4 ± 5.08 g (range, 108–157 g) and 94.8 ± 3.74 days (range, 81–114 days), respectively. All females were nulliparous before testing. Average body mass and age of conspecific males were 134.1 ± 4.08 g (range, 110–151 g) and 96.2 ± 4.28 days (range, 72–121 days), respectively. None of the conspecific males had sexual experience before testing. Average body mass and age of heterospecific males were 128.3 ± 2.77 g (range, 110–137 g) and 180.4 ± 16.34 days (103–227 days), respectively. Three heterospecific males had mated once before testing, whereas the other seven males were sexually naïve. We used paired \(t\)-tests to compare latency to lordosis, total duration of lordosis and number and duration of female attacks between the conspecific and heterospecific conditions.

Experiment 3

Estrous female Turkish hamsters \((n = 10)\) were paired with a conspecific male and then with a heterospecific male (Syrian hamster; \(n = 5\)), or with a heterospecific male and then with a conspecific male \((n = 5)\). The two tests occurred during the same day. Females were paired with the first male for 5 min in the female’s home cage. The male was then removed and after 10 min the second male was placed in the female’s cage. There was no interference with male copulatory behavior. For each trial we recorded whether the female showed lordosis and/or behaved aggressively toward the male (Murphy, 1978).

Experiment 3 was designed and run after completion of Experiment 2. We did not use the same methodological design in Experiment 3 as in Experiment 2 because we were more interested in comparing results of Experiment 3 to the study by Murphy (1978) than in comparing results of Experiments 2 and 3. Average age of females was 143.3 ± 30.54 days (range, 83–311 days). All females except two were nulliparous before testing. Age of all conspecific males was 307 days. Four conspecific males had mated once before testing, whereas the other six conspecific males were sexually naïve. Average age of heterospecific males was 318 ± 1.07 days (range, 312–320 days). All heterospecific males were sexually naïve.

Results

We found that estrous (but not diestrous) female Syrian hamsters investigated the odors of conspecific males longer than the odors of males of a closely related species (Experiment 1). However, estrous female Syrian hamsters were not aggressive toward heterospecific males and were as likely to mate with conspecific males as with heterospecific males (Experiment 2). Estrous female Turkish hamsters were also not aggressive toward heterospecific males and mated as frequently with conspecific males as with heterospecific males (Experiment 3) in contrast to a similar previous study (Murphy, 1978).

Experiment 1

When Syrian hamster females were not sexually receptive, they investigated odors of conspecific and heterospecific males for similar durations \((W = 193, P = 0.73; \text{Fig. 1})\). Receptive females, however, spent more time investigating odors of conspecific males over those of heterospecific males \((W = 257, P = 0.027; \text{Fig. 1})\).

Figure 1 Female Syrian hamsters Mesocricetus auratus investigated odors of conspecific and heterospecific males (Turkish hamster Mesocricetus brandti) for similar durations when females were not in estrus (non-receptive females). Sexually receptive females, however, investigated the male conspecific odor significantly more than the male heterospecific odor. \(* P = 0.027\). Values are shown as mean ± SEM.
Experiment 2
All female Syrian hamsters showed lordosis and copulated with both conspecific and heterospecific males. The latency to lordosis did not differ depending on the type of male (conspecific male, 22.82 ± 3.4 s; heterospecific male, 32.41 ± 7.13 s; \( t_9 = -1.28, P = 0.23 \)). Lordosis was maintained longer when females were paired with conspecific males (231.53 ± 7.09 s) than with heterospecific males (177.25 ± 21.73 s; \( t_9 = 2.38, P = 0.04 \)). No female attacks occurred in any trial, that is, females showed no aggression or any signs of avoidance toward heterospecific males.

Experiment 3
As with inbred female Syrian hamsters, all female Turkish hamsters allowed heterospecific males to mount and showed no aggression or signs of avoidance toward heterospecific males (Fig. 2). That is, female Turkish hamsters long-kept in captivity behaved similarly toward conspecific and heterospecific males, in contrast to the earlier study by Murphy (1978) using recently captured Turkish hamsters (Fig. 2).

Discussion
Overall, sexually receptive (but not sexually unreceptive) Syrian hamster females preferred odors of conspecific males over those of heterospecific males. This result agrees with a previous study that found that receptive female Syrian hamsters, when presented with a male conspecific and a male Turkish hamster behind barriers, spent 75% of their investigation time with the conspecific male (Murphy, 1977). Similar preferences for conspecific males occur in other species (Doty, 1972; Nevo, Bodmer & Heth, 1976; Pillay, 2000). Thus, in Syrian hamsters many generations in captivity have not resulted in a loss of olfactory preference for conspecific males over males of another species. Female Syrian hamsters also display more sexual advertisement (in the form of vaginal marking) in response to odors of conspecific males than in response to odors of heterospecific males (Johnston & Brenner, 1982). All of the above suggest that females should mate preferentially with conspecific males and thus avoid inter-specific mating. However, in our second experiment, in which female Syrian hamsters were paired with a conspecific male and then with a heterospecific male (or vice versa), all female Syrian hamsters showed lordosis and copulated with both types of males. Females did not avoid or show any aggression toward heterospecific males. These results show that a preference for a given type of male (e.g. conspecific male) does not necessarily mean that females will preferentially or exclusively mate with that type of male. We argue that Syrian and Turkish hamster females mate with heterospecific males due to the heightened sexual receptivity in laboratory hamsters. Females of these species go into lordosis within seconds of being exposed to a male (sometimes odors of a male are enough to elicit lordosis) and can maintain lordosis for minutes in the absence of copulatory behavior.

Unfortunately, we were not able to directly compare the laboratory strain of Syrian hamsters with wild or recently captured Syrian hamsters. Access to wild animals is difficult, and experiments describing mating preferences of Syrian hamster females toward heterospecific males were not conducted near the time of original capture (1930) or the establishment of the existing laboratory stock. However, this was not an issue for Turkish hamsters, because a study was conducted with female Turkish hamsters soon after the establishment of a laboratory colony (Murphy, 1978). Using animals descended from that colony allowed us to make comparisons between animals tested around the time of initial capture (Murphy, 1978) and after many generations (present study). Owing to lack of continuous records, we can only estimate how many generations have elapsed between the two studies. Assuming one generation per year, this estimate is 30 generations. Because the primary difference between Murphy’s 1970s experiment (Murphy, 1978) and our experiment was the number of generations the subjects had been in captivity, we conclude that many generations in captivity may significantly increase female receptivity and decrease female selectivity, in this case leading to inter-specific mating. Inter-specific mating occurred in both nulliparous and uniparous females. We acknowledge the possible existence of methodological differences between the two studies. However, the variables measured (lordosis and aggression) tend to be robust across testing circumstances, indicating that the large differences between both studies are probably not due to methodological differences. One drawback of comparing Murphy’s (1978) study with our results in Experiment 3 is that we cannot tease apart the effects of (a) intentional or unintentional selection for reproductive output, (b) inbreeding and (c) relaxed selection in the captive environment, all of which occur in captive populations.
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(Lacy, 1993; McPhee, 2003; Ryan et al., 2003; Fritzsche et al., 2006). For example, the current colonies of Turkish and Syrian hamsters have undergone severe inbreeding (especially Syrian hamsters), but that does not mean that inbreeding is necessarily a causal factor in the inter-specific mating observed in Experiments 2 and 3. Further controlled experiments would be necessary to tease apart the relative effects of relaxed selection, artificial selection and inbreeding, and ultimately to determine how captivity may lead to indiscriminate mating in Mesocricetus.

Our finding that prolonged captivity may lead to inter-specific mating may be relevant for the reintroduction of captive-bred species, because reintroduction into native habitats may be hampered if receptive females solicit copulations or mate with heterospecific males. One problem with using our results to make this argument is that we paired animals in a confined space, a procedure that forces heterospecific encounters to occur under circumstances that promote mating (i.e. the female is in estrus). We do not know whether encountering heterospecific males in more naturalistic settings might reduce the inter-specific matings observed in this study. Clearly, more research is needed to determine how female discrimination toward heterospecific males may be affected by different rearing contexts.

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References


