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A co-housing strategy to improve fecundity of mice in timed matings

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Abstract

Timed matings of mice are often carried out to obtain offspring of a precise age when required for a study. Timed matings involve housing male and female mice together for a limited time period, typically overnight. A limitation of this practice is that many mouse pairs fail to mate during the brief co-housing period. The authors co-housed each breeding pair in the same cage but separated by a transparent partition for 3 d before carrying out timed matings. This co-housing strategy resulted in increased copulation during the timed mating period and also significantly increased the average number of pups produced per breeding pair. The authors suggest that co-housing likely permits male urine-borne pheromones to induce female estrus and also enables the expression of male and female mating behaviors.

It is common practice to breed laboratory mice in timed matings when offspring of precisely known age are required for experiments. There are several applications of this technique: (i) the identification of the day of embryonic alteration or lethality in genetically engineered mice¹; (ii) the collection of the thymus from embryos on embryonic day (E) 14–16 for fetal thymic organ culture to study T-cell development²; and (iii) the production of precisely age-matched mice to be compared in experimental groups or controls. Most timed mating experiments involve housing one male with several females for one night only and, the next day, isolating any females that bear a vaginal plug, a product of male secretions that is used as a visual marker of copulation. The day after a vaginal plug is observed is considered E1. The efficiency of impregnation in timed matings often can be limited, meaning that many pairings may be needed to yield sufficient offspring for a study.

Male urine contains pheromones that advance estrous and synchronize the follicular phase in previously anestrus adult group-housed female mice (a phenomenon called the Whitten effect³), by inducing an increase in gonadotropin-releasing hormone (GnRH), the release of leutinizing hormone (LH) and ovulation^{4–7}. Accompanying these processes is a parallel increase in female expression of sexual receptivity behaviors, including lordosis⁸, upon exposure to males. Additionally, female mouse urine possesses pheromone activity, induces LH surges that promote testosterone secretion in male mice^{9,10} and causes sexually experienced male mice to emit ultrasonic vocalizations that facilitate mating behavior¹¹. Some of these observations have been made in experiments that involved placing male and

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female mice on opposite sides of a transparent cage partition⁹. We therefore hypothesized that housing male and female mice in the same cage but separated by a transparent cage partition for some time before carrying out timed matings might increase their receptivity to mating. We carried out experiments to determine the extent to which prior partitioned co-housing of male and female mice affects mating and pup production in timed matings.

METHODS

Mice

We purchased C57BL/6J mice from The Jackson Laboratory (Bar Harbor, ME). All females (8–9 weeks old) were virgin when they arrived at our facility. Some males (8–9 weeks old) were virgin whereas others (26–35 weeks old) were retired male breeders. All work involving animals was done in accordance with the standards in the *Guide for the Care and Use of Laboratory Animals*¹² and was approved by the IACUC of Mayo Clinic. The animal care and use program and facilities at Mayo Clinic meet all federal regulations and guidelines and are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Housing

Mice were housed in plastic shoebox cages (189 mm × 297 mm × 128 mm with 451 cm² floor space; Allentown, Inc., Allentown, NJ) with Shepherd's Cob Plus bedding (Shepherd Specialty Paper, Cleveland, OH) and were maintained on a 12-h:12-h light:dark cycle in a temperature-controlled (20–26 °C) and humidity-controlled (30–70%) facility. Cages were changed weekly. We provided the mice with *ad libitum* access to breeder diet (Picolab 5058, Purina Mills, LLC, St. Louis, MO) and municipal water. Mice were housed in a specific pathogen-free facility, and infectious agent monitoring was done by Charles River Research Animal Diagnostic Services (Wilmington, MA) on samples from sentinel mice. Over the course of these experiments, the sentinels from the rooms involved tested negative for all monitored infectious agents.

Cage partitions

Cage partitions were constructed of 6-mm-thick transparent poly(methyl methacrylate) (PMMA; plexiglass acrylic) by Mayo Clinic's Biomechanical Shop (Fig. 1a). The material was laser-cut and joined during assembly using a standard solvent-based acrylic adhesive agent (Weld-On 3, Weld-on Adhesives, Inc., Compton, CA). We did not include perforations in the partitions because holes can allow some tactile social contact between mice (such as nose-to-nose touching) across the partition¹³, and the extent to which this might affect the expression of preferences for novel social partners is unclear¹⁴. The partitions can withstand multiple washes in a standard automated cage washer with a maximum temperature of 180 °C.

Timed matings

We set up 24 timed matings with prior partitioned co-housing of female mice ($n = 24$) and male mice with no prior breeding experience ($n = 8$) or male mice with prior breeding experience ($n = 16$). We also set up 26 timed matings with no prior co-housing between

female mice ($n = 26$) and male mice with no prior breeding experience ($n = 10$) or male mice with prior breeding experience ($n = 16$).

To set up the breeding cages for the timed matings with prior partitioned co-housing, we placed a clean partition in each clean cage with fresh bedding. Food was placed on the floor of the cage, and water bottles were placed in the hoppers on both sides of the partition (Fig. 1b). We placed one male mouse and one female mouse on either side of the partition and housed them together for 72 h, beginning between 4:00 pm and 6:00 pm. After the 72-h period of partitioned co-housing, we removed the partition and one of the water bottles, and we moved all food from the cage floor to the hopper. We then housed each breeding pair together overnight to allow mating to occur. We set up the timed matings with no prior co-housing at the same time that the partitions were removed from the cages of the breeding pairs that had prior partitioned co-housing so that all matings occurred on the same night. The following morning, we removed the males from all of the breeding cages and checked the females for vaginal plugs. Then we returned the females to their breeding cage and to general housing. Between E21 and E28, we checked the cages for litters and counted any pups.

Statistical analysis

We used Prism (GraphPad) software to calculate arithmetic means and standard errors (s.e.) and to carry out one-tailed χ^2 tests and one-tailed Student's *t*-tests. We used StatMate (GraphPad) software to carry out post hoc Power analysis ($P = 0.05$, one-tailed).

RESULTS

Presence of vaginal plugs

A greater number of females had vaginal plugs after timed matings with prior partitioned co-housing (13 of 24, 54%) than after timed matings with no prior co-housing (8 of 26, 31%; one-tailed χ^2 test $P = 0.047$; Fig. 2a). We observed the same trend among breeding pairs that included a virgin male (4 of 8 with prior partitioned co-housing (50%) compared with 3 of 10 with no prior co-housing (30%); Fig. 2b) and among breeding pairs that included a retired breeder male (9 of 16 with prior partitioned co-housing (56%) compared with 5 of 16 with no prior co-housing (31%); Fig. 2c), but the differences were not statistically significant in these subsets (one-tailed χ^2 test $P > 0.10$ and $P > 0.10$, respectively).

Litter production

The percentage of females with vaginal plugs that produced litters was not statistically different after timed matings with prior partitioned co-housing (8 of 13, 61%) and after timed matings with no prior co-housing (4 of 8, 50%; one-tailed χ^2 test $P > 0.10$; Fig. 3a). (No females that lacked vaginal plugs bore litters in these experiments.) For breeding pairs that included a virgin male (Fig. 3b) and those that included a retired breeder male (Fig. 3c), there were also no significant differences in the percentages of females with vaginal plugs that produced litters after timed matings with prior partitioned co-housing and after timed matings with no prior co-housing (3 of 4 with prior partitioned co-housing (75%) compared with 1 of 3 with no prior co-housing (33%) for pairs that included virgin males; 5 of 9 with

prior partitioned co-housing (56%) compared with 3 of 5 with no prior co-housing (60%) for pairs that included retired breeder males; one-tailed χ^2 test $P > 0.10$ for each comparison).

Number of pups

The average number of pups produced per breeding pair was significantly greater after timed matings with prior partitioned co-housing (1.5 ± 0.51 ; 36 pups produced from 24 matings) than after timed matings with no prior co-housing (0.31 ± 0.17 ; 8 pups produced from 26 matings; one-tailed Student's t -test $P = 0.013$; Fig. 4a). Post hoc Power analysis showed that the pooled data had 75% power to detect this difference in means ($\alpha = 0.05$, one-tailed). Among breeding pairs that included virgin males (Fig. 4b), the average number of pups produced per breeding pair was significantly greater after timed matings with prior partitioned co-housing (2.25 ± 1.16 ; 18 pups produced from 8 matings) than after timed matings with no prior co-housing (0.2 ± 0.2 ; 2 pups produced from 10 matings; one-tailed Student's t -test $P = 0.035$). Among breeding pairs that included retired breeder males (Fig. 4c), there was also a greater average number of pups produced per breeding pair after timed matings with prior partitioned co-housing (1.13 ± 0.50 ; 18 pups produced from 16 matings) than after timed matings with no prior co-housing (0.38 ± 0.26 ; 6 pups produced from 16 matings), but this difference was not significant (one-tailed Student's t -test $P = 0.096$).

DISCUSSION

Timed matings of mice can be inherently inefficient, because many mouse pairs fail to mate during the brief mating period. Previous studies have suggested that placing male and female mice on opposite sides of a transparent cage partition likely permits urine-borne pheromones originating from the opposite sex to induce female estrus and to encourage the expression of male and female mating behaviors⁹. We constructed simple, economical, transparent cage partitions and evaluated the effects of their use prior to timed matings on mating and pup production.

Prior partitioned co-housing resulted in increased copulation during the timed mating period. The average number of pups produced per breeding pair was significantly greater after timed matings with prior partitioned co-housing than after timed matings with no prior co-housing. We conclude that co-housing separated by transparent partition can be an effective method to improve the fecundity of mice in timed matings.

The microisolator cages we used are well suited for partitioned co-housing before mating, because passive ventilation may permit some degree of concentration and accumulation of the olfactants that reportedly affect reproductive behavior. The extent to which this method may be compatible with cages mounted in active ventilation systems with greater air turnover has yet to be determined. This question warrants further study.

Also unknown is the extent to which the transparency of the partition might or might not impact the increased copulation and pup production that we observed. Future experiments are needed to empirically determine the effect of the partition's transparency on reproduction by comparing reproductive parameters in timed matings after prior partitioned co-housing with opaque versus transparent partitions.

We found that prior partitioned co-housing can increase copulation and offspring production during timed matings, likely by permitting urine-borne pheromones to induce the expression of male and female mating behaviors. This strategy can be used to improve experimental designs by decreasing the number of breeder mice needed to produce a requisite number of offspring in timed mating experiments.

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FIGURE 1.

Transparent acrylic cage partition. **(a)** Each partition (top) was designed to fit the precise dimensions of the cage (bottom) with ridges to accommodate the cage lid and hopper. **(b)** Once placed inside the cage, with the cage lid in place, the partition allows the separated co-housing of two mice within the breeding cage.

FIGURE 2.

Presence of vaginal plugs after timed matings with prior partitioned co-housing or with no prior co-housing. Shading of bars indicates presence of vaginal plugs. (a) More females had vaginal plugs after timed matings with prior partitioned co-housing than after timed matings with no prior co-housing ($*P = 0.047$, one-tailed χ^2 test). Among breeding pairs that included a virgin male (b) and among breeding pairs that included a retired breeder male (c), the differences in the percentage of timed matings that produced a vaginal plug with prior partitioned co-housing versus with no prior co-housing were not significant ($P > 0.10$ for all comparisons, one-tailed χ^2 test).

FIGURE 3.

Litter production after timed matings with prior partitioned co-housing or with no prior co-housing. Shading of bars indicates production of litters. The percentage of females with vaginal plugs that produced litters was not statistically different after timed matings with prior partitioned co-housing and after timed matings with no prior co-housing among all breeding pairs (a), among breeding pairs that included a virgin male (b) or among breeding pairs that included a retired breeder male (c; $P > 0.10$ for all comparisons, one-tailed χ^2 test).

FIGURE 4.

Number of pups produced per breeding pair after timed matings with prior partitioned co-housing or with no prior co-housing. Among all breeding pairs (**a**) and among breeding pairs that included a virgin male (**b**), the average number of pups produced per breeding pair was significantly greater after timed matings with prior partitioned co-housing than was after timed matings with no prior co-housing ($**P = 0.013$ and $*P = 0.035$, respectively, one-tailed Student's *t*-test). Among breeding pairs that included a retired breeder male (**c**), the average number of pups produced per breeding pair was not significantly different after timed matings with prior partitioned co-housing than after timed matings with no prior co-housing ($P = 0.096$, one-tailed Student's *t*-test).