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Environmental enrichment improves mating success in fruit flies

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Environmental enrichment, defined as housing conditions that include a combination of complex inanimate and social stimulation, has strong positive effects on brain and behaviour in various species. We extended previous studies to evaluate how enrichment affects mating success. In a series of experiments, we found that male fruit flies, *Drosophila melanogaster*, reared in an enriched environment were twice as successful in acquiring mates as were males from standard rearing conditions. The dominant factor increasing mating success was the larger space available per fly. Flies from enriched and standard environments showed no significant behavioural differences, leading us to suggest that different social environments at high and low per capita spaces are associated, on average, with either subtle behavioural differences or distinct pheromonal profiles to which females are sensitive while choosing mates.

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Environmental enrichment refers to laboratory housing conditions that include a combination of complex inanimate and social stimulation (van Praag et al. 2000). For example, the standard laboratory housing for rats and mice consists of a small barren cage in which an animal is kept in isolation. In contrast, an enriched cage is larger and contains several individuals to allow social interactions. The cage also contains various objects such as toys, which stimulate exploratory behaviour, and a running wheel for exercise. Research on mammals, pioneered by Hebb (1947), has documented that environmental enrichment improves learning and memory, increases brain size, neuron size, dendritic branching and synapses per neuron, and alters the expression of genes linked to neuronal structure, synaptic plasticity and transmission (Rosenzweig 1966; Greenough & Volkmar 1973; Kolb & Whishaw 1998; van Praag et al. 2000; Rampon et al. 2000; Wurbel 2001).

In fruit flies, *Drosophila melanogaster*, environmental enrichment, which includes large cages, items of various colours, odours and shapes, and occasional vibration, is associated with up to a 20% increase in the volume of parts of the mushroom body (Technau 1984; Heisenberg et al. 1995). Adult flies reared in constant light have a significantly larger mushroom body, central complex and optic lobe compared with flies reared in constant darkness (Barth & Heisenberg 1997; Barth et al. 1997b). Flies reared under a normal light:dark cycle have a mating advantage over flies reared in constant darkness when competing for females reared under a normal light:dark cycle. Moreover, the latency to copulation is shorter with male and female pairs reared under similar rather than different light regimes (Hirsch et al. 1995; Barth et al. 1997a). Other studies have also documented effects of experience on fly mating behaviour (reviewed in Spieth & Ringo 1983; Hirsch & Tompkins 1994).

Most research on environmental enrichment has focused on evaluating effects on either the brain or individual behaviour. It is unclear, however, whether conspecifics discriminate between individuals from enriched and standard environments. Such information is important because enrichment may influence behaviours affecting fitness, such as mating success. The large effects of environmental enrichment on the fly brain and the data indicating effects of adult experience on mating success suggest that environmental enrichment may confer a mating advantage. We tested this prediction in a series of experiments with fruit flies. Specifically, we asked (1) whether environmental enrichment increases the mating success of male fruit flies, (2) what behavioural parameters are most affected by enrichment and (3) what enrichment factor is most important for mating success. Our initial protocol for enrichment was modified from the fly studies by Technau (1984) and Heisenberg et al. (1995) and included large cages and coloured pipe cleaners, which provided visual and tactile stimulation.

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Larger space and items that provide extra sensory stimulation are two of the most common enrichment factors used in experiments and animal husbandry (van Praag et al. 2000). After documenting an overall enrichment effect, we conducted further experiments to test which enrichment factor was the most important.

GENERAL METHODS

We used a wild stock of *D. melanogaster* flies initiated from approximately 50 flies collected in Vancouver, British Columbia, Canada, in early summer 2001. The stock was expanded and subsequently kept in four population cages containing several thousand individuals inside an environmental chamber at 25°C, 70% RH on a 14:10 h light:dark cycle with lights on at 1000 hours. Each of the population cages was 20 × 20 × 35 cm and contained numerous pipe cleaners of five colours (white, black, blue, green, red) flush-taped to the walls and attached vertically and haphazardly to two 10 × 10-cm cork sheets at the centre of the floor. Each cage contained two standard 200-ml food bottles, each containing 50 ml of food; the bottles were replaced once a week. One litre of food medium contained 83 ml of molasses, 83 g of cornmeal, 34 g of yeast, 12 g of agar, 10 ml of propionic acid solution, 0.12 g of penicillin, 0.3 g of streptomycin and distilled water. The populations were augmented weekly with flies grown at a low density of approximately 200 larvae per standard food bottle. The low density was regulated by counting and removing eggs.

All flies used for the experiments developed at a low density of approximately 200 larvae per standard food bottle. Before virgin collection, we mixed flies from approximately 24 bottles in a single cage. Then small numbers of flies were anaesthetized with CO_2, sexed and placed in single-sex experimental containers for 4 days, housed in the same environmental chamber as the stock. Fly mortality during these 4 days was negligible (<5%). In experiments 1–6, each bottle or cage had a petri dish 35 mm in diameter containing 6 ml of standard food with live yeast on top. This amount of food is several orders of magnitude larger than the food that 100 flies consume over 4 days. All experiments were conducted in a blind fashion and the data were recorded via a laptop computer programmed in C. That is, the experimenters recorded the flies by colour and/or vial number and did not know what treatments the flies belonged to. All statistical analyses were done on arcsine-transformed proportions of matings and log-transformed latencies; all the averages reported are least squares means. All statistical tests involved either regular or repeated measures analysis of variance (ANOVA).

EXPERIMENT 1: MATING SUCCESS OF ENRICHED VERSUS STANDARD MALES

Methods

In this experiment, we tested whether rearing in an enriched environment for the first 4 days of adult life would affect male mating success. The experiment had 12 replicates. Each replicate consisted of a set-up on day 1 and mating trials on day 5. In the set-up, we placed 100 virgin males into each of two enriched Plexiglas cages and each of two standard food bottles. We also placed 60 virgin females into each of three enriched Plexiglas cages and each of three standard food bottles. All cages and bottles were kept inside the environmental chamber at 25°C and 70% RH on an LD 14:10 h cycle with lights on at 1000 hours.

The enriched cages consisted of five walls of 5-mm-thick transparent Plexiglas and an opening covered with a sleeve of fine mesh. Each cage had a volume of 1331 cm^3, with inside dimensions of 11 × 11 × 11 cm. Each of the three walls and the top of the cage had five 10-cm-long pieces of pipe cleaner taped flush. The floor was covered with a thin cork sheet with five 7-cm-long pieces of pipe cleaners extending vertically at haphazard locations. The pipe cleaners were of five colours (white, black, blue, green, red) arranged on each wall in random order. The small food dish was placed at the top of a 6-cm-long glass vial at the centre of the cage.

The standard bottles were of semitransparent polypropylene and had a volume of 200 cm^3. They were about 0.5 mm thick and 10 cm high, and had a diameter of 6 cm at the bottom and 4 cm at the top. The bottles were placed upside down with the food dish on a standard cap at the bottle opening. A 15-mm hole cut in the bottom of each bottle and covered with a fine mesh provided additional air circulation.

Overall, the enriched cage was approximately seven times larger than the standard bottle, its transparent walls increased visual stimulation, it had inanimate objects that provided tactile and visual stimulation, the larger overall space allowed uninterrupted flights over longer distances, and the space per fly was approximately seven times larger. That is, the number of flies per container, and hence the amount of food per fly, were identical in the enriched cage and standard bottle, but the space available per fly was greater in the enriched cage than in the standard bottle.

On day 4 of each replicate, we removed the food dishes from all cages and bottles and verified that they were free of eggs (male containers) and larvae (female containers). We then placed fresh dishes with food medium and live yeast in the female cages and bottles. We also placed fresh food dishes with abundant live yeast saturated with either red or blue food colouring into the male cages: one enriched male cage and one standard male bottle received red food colouring, and one enriched male cage and one standard male bottle received blue food colouring. This marking technique does not affect either male behaviour or female choice (Ashburner 1989).

On the morning of day 5, we conducted the mating trials. There were four types of trials (two male–colour combinations, two female treatments) conducted in random order. Each trial involved chilling flies on ice, then placing one enriched male and one standard male of distinct food colouring and either an enriched or standard female inside a transparent vial 9.5 cm long and 2.5 cm in diameter. The order of male placement

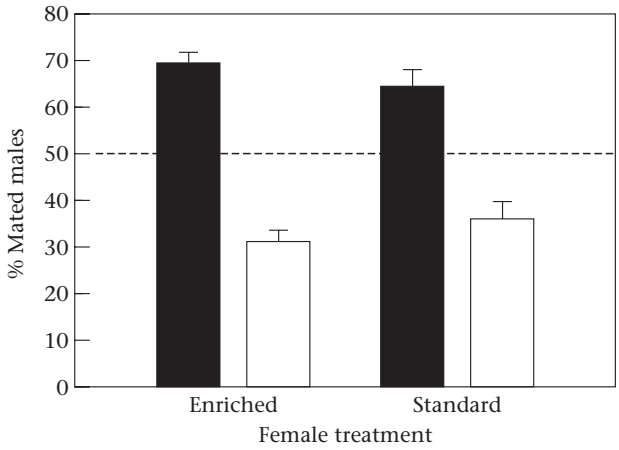

Figure 1. Mean±SE percentages of enriched males (■) and standard males (□) that mated with enriched and standard females in experiment 1 (N=1039 trials with a single female and one male of each treatment per trial).

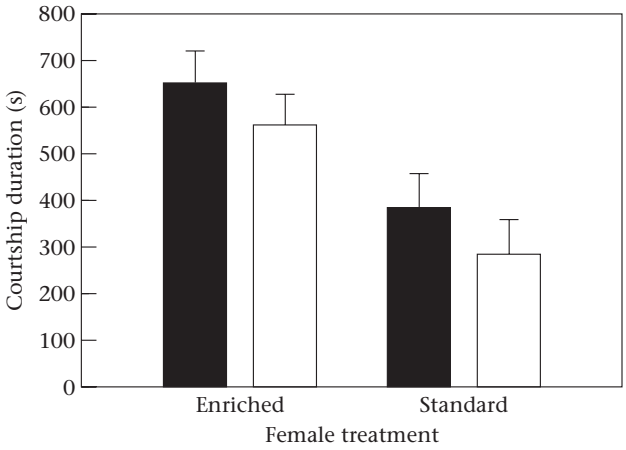

Figure 2. The total time that enriched males (■) and standard males (□) courted enriched and standard females in experiment 2 (N=160 trials involving one male and one female per vial).

alternated between trials, with the enriched males placed first in half the trials and the standard males placed first in the other half; the female was always placed last. Immediately after placing flies into 50 vials, we started scanning the vials for matings. Once mating occurred, we inspected the colour of the mated male under a dissecting microscope and recorded the data on a computer. The computer recorded the identity of the mated male and the latency to mating, defined as the time elapsed from trial commencement to recording.

We attempted to conduct 100 trials per each of the 12 replicates, but failed to reach that number on the first few days. Therefore, we had a total of 1039 trials. The main statistical analysis was done on the frequencies of mating for each male treatment (two types) male colour combination (two) and female treatment (two) for each of the 12 replicates, and the ANOVA model included male treatment, male colour and female treatment as the independent factors.

Results

The enriched males mated twice as much as the standard males ($F_{1,91}$=23.7, P<0.001; Fig. 1). The effects of female, food colouring and the male by female interaction were all nonsignificant. Approximately half the trials (51%) ended in mating and the latency to mating was similar with enriched and standard males ($\bar{X} \pm$ SE=1549 ± 47 s and 1652 ± 67 s, respectively; $F_{1,522}$=1.6, P=0.21).

EXPERIMENT 2: BEHAVIOURAL OBSERVATIONS WITH ONE MALE AND ONE FEMALE PER VIAL

Methods

To understand the differential mating success observed in experiment 1, we quantified differences in courtship behaviour between the enriched and standard males through direct observation. The set-up for the experiment was similar to that of experiment 1, with observations conducted on day 5. The observations were replicated four times a day over 2 days. Each replicate consisted of chilling flies on ice, then setting up 20 vials, each containing one female and one male.

Two randomly selected replicates on each day included enriched females and the other two replicates included standard females. Ten of the vials of each replicate included enriched males, and the other 10 vials included standard males. We randomly placed five vials of each male type in a plastic stand, and each of two observers recorded the behaviour of males (courtship activity and mating latency) in one stand of 10 vials for 45 min or until mating ended (N=160 males, or 40 trials for each of the four male × female treatment combinations). For each male, we calculated the total courtship duration, which included 'following', 'wing vibration' and 'mounting attempts'. If mating occurred, we calculated the duration from mounting until disengagement (Cobb et al. 1985). The main statistical analysis was done on the courtship duration of the males that courted and included the main factors of male treatment (two types), female treatment (two), observer (two), whether the pair mated (yes or no) and replicate (eight).

Results

The average total courtship duration was similar for enriched males and standard males ($F_{1,126}$=1.7, P=0.2), but the enriched females were courted twice as long as the standard females ($F_{1,126}$=14.2, P<0.001; Fig. 2). A separate analysis revealed that among the mated females, enriched females and standard females were courted for similar durations ($\bar{X} \pm$ SE=381 ± 55 s and 320 ± 47 s, respectively; $F_{1,70}$=0.7, P=0.4), but among the unmated females, enriched females were courted more than twice as long as standard females (880 ± 95 s and 342 ± 93 s, respectively; $F_{1,51}$=16.6, P<0.001). The male by female interaction in courtship duration was nonsignificant (P=0.96; Fig. 2).

Neither the male nor the female treatments differed in the latency to court, latency to mate or mating duration (ANOVAs: $F_{1,126}$, NS). Overall, similar proportions of enriched and standard males courted (81% and 85%, respectively) and mated (46% and 49%), and similar proportions of enriched and standard females were courted (90% and 76%) and mated (55% and 40%).

EXPERIMENT 3: BEHAVIOURAL OBSERVATIONS WITH TWO MALES AND ONE FEMALE PER VIAL

Methods

To evaluate further possible behavioural differences between enriched and standard flies, we conducted behavioural observations with two males and one female per vial. This experiment required a different type of marking, because the food colouring is not visible to the naked eye. On each of the two set-up days, we collected 200 virgin males and used a fabric writer to mark the thoraxes of 60 of these males with white dots. We then arranged the males in four groups (N=15 marked males and 35 unmarked males each). Two of these groups were placed in enriched Plexiglas cages (50 per cage) and the other two groups were placed in standard bottles (50 per bottle). We also placed 50 virgin females into each of two enriched cages and each of two standard bottles.

In the morning of day 5, we chilled the males and separated the marked and unmarked males into distinct cages. Each trial involved placing one enriched male and one standard male with a female in a vial. There were four types of trials: (1) enriched marked male and standard blank male with enriched female, (2) enriched marked male and standard blank male with standard female, (3) enriched blank male and standard marked male with enriched female and (4) enriched blank male and standard marked male with standard female. Each of two observers watched one vial at a time for 15 min and recorded all courtship activity by each male. We recorded 'following', 'wing vibration', 'mounting attempts' and 'mating' (Cobb et al. 1985). Overall, we observed 40 vials over 2 days, with 10 vials per each fly combination. Because all four behavioural categories were highly positively correlated, we report the results as total courtship duration. We focus on the vials where both males courted the female, because only these vials allowed us to treat the female as a repeated measure.

Results

There was no significant difference in courtship duration between enriched and standard males in the 25 vials where both males courted the female. The mean ± SE courtship duration was 131 ± 31 s for the enriched males and 152 ± 31 s for the standard males (repeated measures ANOVA: $F_{1,24}$=0.6, NS). Marked males courted significantly longer than did blank males ($F_{1,24}$=7.7, P=0.01). Enriched females were courted for 171 ± 27 s and standard females were courted for 112 ± 36 s, respectively (P=0.2). We observed no direct interactions between the males. Overall, 12 enriched males and three standard males mated, and there was no significant difference in courtship duration between trials ending in mating (N=15) and trials ending with no mating (N=50). Finally, eight enriched and seven standard males did not perform courtship at all.

EXPERIMENT 4: BEHAVIOURAL OBSERVATIONS WITH TWO FEMALES AND ONE MALE PER VIAL

Methods

To evaluate possible differences between enriched and standard females, we conducted behavioural observations with two females and one male per vial. On each of the two set-up days, we collected 200 virgin females and used a fabric writer to mark the thoraxes of 60 of these females with white dots. We then arranged the females in four groups (N=15 marked females and 35 unmarked females per group). Two of these groups were placed into enriched Plexiglas cages (50 per cage) and the other two groups were placed into standard bottles (50 per bottle). We also placed 50 virgin males into each of two enriched cages and each of two standard bottles.

In the morning of day 5, we chilled the females and separated the marked and unmarked females into distinct cages. Each trial involved placing one enriched female and one standard female with a male in a vial. There were four types of trials: (1) enriched marked female and standard blank female with enriched male, (2) enriched marked female and standard blank female with standard male, (3) enriched blank female and standard marked female with enriched male and (4) enriched blank female and standard marked female with standard male. Each of two observers watched one vial at a time for 15 min and recorded all courtship activity towards each female. Overall, we observed 40 vials over 2 days, with 10 vials per each fly combination. Because all male and female behavioural categories were highly positively correlated with total courtship duration, we report only total courtship duration. Furthermore, we focus on the vials where both females were courted by the male, because only these vials allowed us to treat the male as a repeated measure.

Results

In the 23 vials in which the male courted both females, the enriched and standard females were courted for similar durations ($\bar{X}$ ± SE=110 ± 26 s and 143 ± 23 s, respectively; repeated measures ANOVA: $F_{1,22}$>1.4, NS). Overall, 12 enriched females and 12 standard females mated, and there was no significant difference in courtship duration between trials ending in mating (N=24) and trials ending with no mating (N=55). Finally, 12 enriched and 13 standard females did not receive courtship at all.

EXPERIMENT 5: WHAT CONSTITUTES AN ENRICHED ENVIRONMENT? DECORATION VERSUS CAGE VOLUME

Methods

The enriched treatment in experiments 1–4 involved both decoration and a larger cage than the standard fly bottle. In this experiment, we tested which of these two factors affected male mating success. We thus compared the mating success of enriched males to that of males grown in three types of containers: standard bottles, decorated bottles and undecorated cages. The standard bottles were as in the previous experiments, the decorated bottles were standard bottles decorated with five coloured pipe cleaners and the undecorated cages were 11 × 11 × 11-cm Plexiglas cages with no decoration and with the food dish placed on the bare floor.

The experiment was replicated four times. Each replicate consisted of a set-up on day 1 and 120 mating trials on day 5. The general methods were similar to the ones in experiment 1. Briefly, in the set-up, we placed 80 virgin males into each of three enriched Plexiglas cages, one standard bottle, one enriched bottle and one undecorated cage. We also placed 80 virgin females into each of three enriched Plexiglas cages and each of three standard food bottles. All cages and bottles were kept inside an environmental chamber as described above. On day 4, we replaced the food dishes and provided the enriched males with one food colouring and the other three male treatments with the other colouring. The colour combinations were changed randomly and counterbalanced between replicates. The main statistical analysis was done on the arcsine-transformed proportions of mating for each male pairing (three types) and female treatment (two) for each replicate (four). A preliminary analysis revealed no effect of type of food colouring.

Results

The enriched males mated twice as much as did both the standard-bottle males and the decorated-bottle males, but did not mate more than the undecorated-cage males ($F_{2,18}$=9.6, P<0.001 for the difference between the three trial combinations; Fig. 3). Separate ANOVAs for each of the three trial combinations revealed a strong mating advantage of enriched males over the standard-bottle males and decorated-bottle males (P<0.001) but not undecorated-cage males (N=160 for each trial combination). The effects of female, and male by female interaction, were nonsignificant. Overall, 74% of the trials ended in mating, and the latency to mating was similar between the four male treatments.

EXPERIMENT 6: WHAT CONSTITUTES AN ENRICHED ENVIRONMENT? CONTAINER MATERIAL AND SPACE PER FLY

Methods

In addition to the volume differences between the cages and bottles, they also differed in material (Plexiglas versus polyethylene). Furthermore, the flies could have been affected either by the overall volume of the container or by the space available per fly. To test for the effects of material and space, we compared the mating success of enriched males to that of males grown in three types of containers: standard bottles, low-number bottles and small Plexiglas cages. The standard bottles were as in the previous experiments and contained 50 flies each (4 cm^3 per fly). The low-number bottles were standard bottles containing eight flies each (25 cm^3 per fly). Finally, the small Plexiglas cages were 6 × 6 × 6-cm Plexiglas cages with no decoration and with the food dishes placed on the cage floor. Each small Plexiglas cage contained 50 flies (4.3 cm^3 per fly). The enriched males were as in the previous experiments, with 50 males placed per cage, creating individual space of 26.6 cm^3 per fly.

The experiment was repeated four times. Each repetition consisted of a set-up on day 1 and 150 mating trials on day 5. The general methods were similar to the ones in experiment 1. Briefly, in the set-up, we placed 50 virgin males into each of six enriched Plexiglas cages, two standard bottles, and two small Plexiglas cages. We also placed eight virgin males into each of 12 standard bottles. Finally, we placed 75 virgin females into each of four standard food bottles. This experiment involved only standard females because we found no significant effects of female origin (enriched versus standard) on male mating success in all the preceding experiments.

All cages and bottles were kept inside an environmental chamber as described above. On day 4, we replaced the food dishes and provided half the enriched males and half the males of each of the other three treatments with one food colouring. The other half of each of the four male treatments received the other colouring. On the morning of day 5, we conducted the mating trials in two replicates. Each replicate consisted of 25 trials for each of the three mating types. The mating types included one enriched male and one male from either the (1) standard

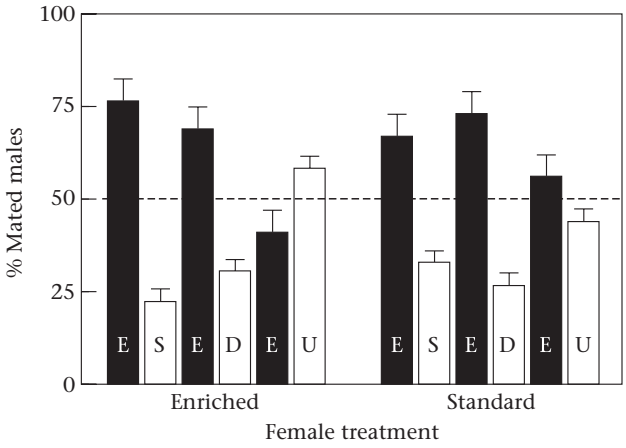

Figure 3. Mean±SE percentages of enriched and other males that mated with enriched and standard females in experiment 5 (N=480 trials). Each pair of black and white bars depicts one type of mate choice trial involving a single female, one enriched male (E, ■) and one male (□) of the standard bottle (S), decorated bottle (D) or undecorated cage (U) treatment.

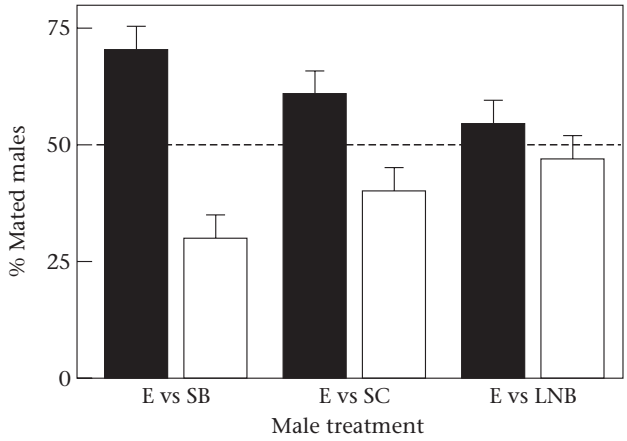

Figure 4. Mean±SE percentages of males of different treatments that mated with females in experiment 6 (N=600 trials). Each pair of black and white bars depicts one type of mate choice trial involving a single female, one enriched male (■) and one male (□) from a standard bottle (SB), small cage (SC) or low-number bottle (LNB).

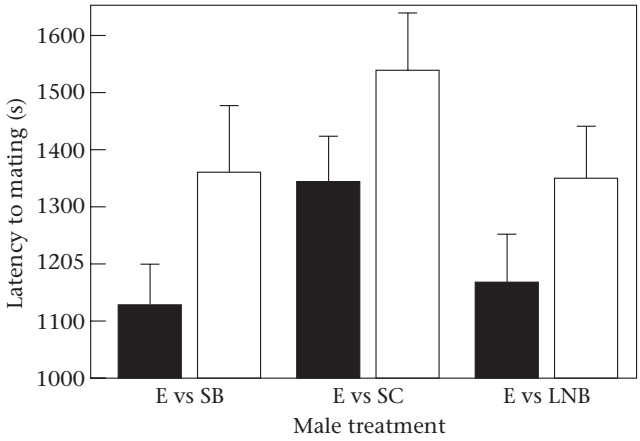

Figure 5. Mean±SE latency to mating in trials that ended in matings in experiment 6 (N=458 trials) involving a single female, one enriched male (■) and one male (□) from a standard bottle (SB), small cage (SC) or low-number bottle (LNB).

bottle, (2) low-number bottle, or (3) small Plexiglas cage treatments.

The main statistical analysis was done on the arcsine-transformed proportions of mating for each male pairing (three types) for each replicate (eight). A preliminary analysis revealed no effect of either food colouring or replicate.

Results

The enriched males mated twice as much as the standard-bottle males, 50% more often than the males from the small Plexiglas cages and at a similar frequency to that of the males from the low-number bottles ($F_{2,42}$=9.6, P<0.01 for the difference between the three trial combinations; Fig. 4). Separate ANOVA analyses for each of the three types of male pairing revealed a strong mating advantage of enriched males over the standard-bottle males (P<0.001) and small-cage males (P<0.02) but not males from the low-number bottles (N=200 for each trial combination). The latency to mating was 15% shorter for the enriched than all other male treatments ($F_{1,452}$=7.3, P<0.01; Fig. 5), with no difference in the magnitude of the enriched-male advantage between the three mating types. Overall, 76% of the trials ended in mating.

EXPERIMENT 7: WHAT CONSTITUTES AN ENRICHED ENVIRONMENT? FURTHER TESTS ON SPACE PER FLY

Methods

In this experiment, we wished to evaluate further whether the space available per fly was the major enrichment factor influencing mating success. We conducted two types of mating trials involving males reared at a similar per capita space in either Plexiglas cages or bottles, and males reared at low per capita space in bottles. The Plexiglas cages were the same 11 × 11 × 11-cm cages that housed the enriched males in all the previous experiments. In this experiment, however, the cages had no decoration and the food dishes were placed on the cage floor. Each cage contained 50 males, creating per capita space of 26.6 cm^3. The low-number bottles were standard bottles containing eight flies each (25 cm^3 per fly). Finally, the high-number bottles were standard bottles containing 50 flies each (4 cm^3 per fly). Each cage and high-number bottle contained a petri dish 35 mm in diameter containing 6 ml of standard food with live yeast on top, and each low-number bottle contained a dish 15 mm in diameter containing approximately 1 ml of standard food and a small amount of live yeast. That is, the per capita food was identical in all three treatments.

The experiment was repeated four times. Each repetition consisted of a set-up on day 1 and 160 mating trials on day 5. The general methods were similar to the ones in experiment 1. Briefly, in the set-up, we placed 50 virgin males into each of three Plexiglas cages and three standard bottles. We also placed eight virgin males into each of 30 standard bottles. Finally, we placed 50 virgin females into each of six standard food bottles. All cages and bottles were kept inside an environmental chamber as described above. On day 4, we replaced the food dishes in the male containers with fresh food dishes containing either red or blue food colouring. On the morning of day 5, we conducted the mating trials in two replicates. Each replicate consisted of 40 trials for each of the two mating types. The mating types included one low-number male and one male from either the Plexiglas cage or high-number bottle.

The main statistical analysis was done on the arcsine-transformed proportions of mating for each male pairing (two types) for each replicate (eight). A preliminary analysis revealed no effect of either food colouring or replicate.

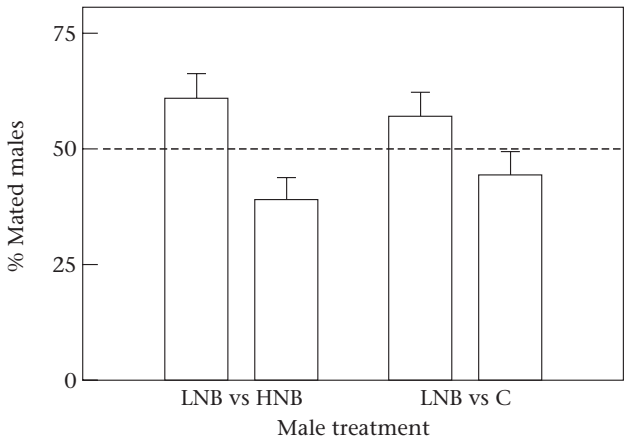

Figure 6. Mean±SE percentages of males from each treatment that mated with females in experiment 7 (N=640 trials). Each pair of bars depicts one type of mate choice trial involving a single female, one male from a low-number bottle (LNB) and one male from either a high-number bottle (HNB) or a cage (C).

Results

The males from the low-number bottles mated 50% more often than the males from the high-number bottles ($F_{1,14}$=15.7, P<0.001) and at a similar frequency to that of males from the Plexiglas cages ($F_{1,14}$=2.5, NS; Fig. 6). The latency to mating was similar between males ($\bar{X} \pm$ SE=1140 ± 100 s, 1191 ± 59 s and 1291 ± 103 s for males from cages and low- and high-number bottles, respectively, NS). Overall, 58% of the trials ended in mating.

DISCUSSION

The Effect of Environmental Enrichment on Mating Success

Male flies from the enriched environment were twice as likely to mate when competing with males from the standard environment. This result was replicated in four distinct experiments (experiments 1, 3, 5, 6; Figs 1, 3, 4) involving more than 2000 mating trials and spanning over 6 months. The enriched males showed a similar advantage over standard males when mated with either enriched or standard females (Figs 1, 3). That is, there was no assortative mating based on experience. The enriched males also had a shorter latency to mating than standard males, although the difference was statistically significant only in experiment 6 (Fig. 5). It is most likely that the difference in latency to initiate mating reflected faster acceptance of the enriched than standard males by the females. This conclusion agrees with our observations that the large difference in mating success between the males was not accompanied by noticeable behavioural differences.

Enrichment also affected females by making them more attractive to males. The males from either treatment were twice as persistent in courting enriched than standard females when only one male was placed with one female (Fig. 2). A similar pattern (although not statistically significant) was observed in experiment 3, in which two males, one enriched and the other standard, were placed into vials with either an enriched or a standard female. In experiment 4, however, in which we observed a single male in vials each containing one enriched and one standard female, we found no difference in courtship duration. Our observations indicated that, when a male was placed with two females, he was indiscriminate and kept switching between the females during and between courtship bouts. This may reflect an artefact created by confinement inside a small vial.

Behavioural Observations

The behavioural observations revealed no noticeable difference between enriched and standard males, either when a single male was placed with a single female in experiment 2 (Fig. 2), or when one male of each treatment was placed with a female in experiment 3. The males were similar in their latency to initiate courtship, courtship duration, latency to initiate mating and mating duration. The courtship duration was nonsignificantly longer for the enriched than standard males in experiment 2 (Fig. 2) but nonsignificantly shorter in experiment 3. Hence, our failure to detect a difference is probably not because of low statistical power. Similarly, we noticed no behavioural difference between enriched and standard females.

What Was the Most Important Enrichment Factor?

Compared to the standard bottle, the enriched cage provided more visual and tactile stimulation, a larger overall space and a larger per capita space. Per capita food, however, was identical in the enriched cage and standard bottle, indicating that competition for food was not a relevant factor. Experiment 5 suggested that the visual and tactile stimulation provided by the decoration was not important, because enriched males had a mating advantage over males from decorated bottles but no mating advantage over males from undecorated cages (Fig. 3). Experiment 6 suggested that the cage material (transparent Plexiglas versus semitransparent polyethylene) was not important because enriched males had a mating advantage over males from small Plexiglas cages (Fig. 4). In contrast, the enriched males did not have a mating advantage over males reared at low numbers in bottles (Fig. 4). This result suggested that the space available per fly was the most important enrichment factor.

Experiment 7 provided further evidence that the space per fly was the crucial factor influencing mating success. In that experiment, flies reared at similar per capita space (~25 cm^3 per fly) in either large Plexiglas cages (1331 cm^2) or small bottles (200 cm^2) had a similar mating success when competing for a female (Fig. 6). In contrast, flies reared in small bottles at high per capita space (~25 cm^3 per fly) had a mating advantage over flies reared in small bottles at low per capita space (4 cm^3 per fly). Because the flies in each of the three treatments had

similar amounts of food per capita, food-based density effects cannot explain the results. Although the space per fly most strongly influenced fly mating success, we cannot reject the involvement of other factors. Indeed, an enriched environment is usually considered to consist of several factors acting in concert (van Praag et al. 2000), but the large variation associated with fly mating experiments would probably preclude identifying enrichment factors of small effects.

Possible Mechanisms Underlying Enrichment

The inanimate and social environment has strong effects on brain and behaviour in mammals (Kolb & Whishaw 1998; van Praag et al. 2000; Wurbel 2001), insects (Hirsch & Tompkins 1994; Heisenberg et al. 1995; Moore et al. 1995; Barth et al. 1997a; Lomassese et al. 2000; Farris et al. 2001) and other arthropods (Yeh et al. 1996; Carducci & Jakob 2000). There is, however, little information indicating how environmental enrichment affects the way an individual is perceived by conspecifics. Our results suggest that flies were not strongly affected by inanimate parameters. Rather, there seemed to be a difference in the social environment caused by per capita space: males reared at a larger per capita space were more attractive as mates (Figs 1, 3, 4, 5, 6). Moreover, females reared at a larger per capita space were also more attractive as potential mates (Fig. 2), indicating that the per capita space effect is not caused by some unique male–male interaction.

A few studies have documented effects of housing conditions on mating success in *D. melanogaster*. Ellis & Kessler (1975) compared flies reared singly in vials or in groups of 50 in half-pint bottles (230 ml). In one experiment, they found a mating advantage of males housed singly over males housed in groups when paired with either females housed singly or in groups. In another experiment, however, males housed in groups had a mating advantage over males housed singly when competing for females housed in groups. Knoppien (1987) found a nonsignificant mating advantage of single over group-housed flies competing for group-housed females. In another species, *D. pseudoobscura*, Noor (1997) reported that males reared in groups were slower to initiate courtship and courted for shorter durations than males reared singly. Finally, male *D. paulistorum* that were reared in isolation from the first larval instar showed more intense courtship behaviour and higher mating success than did mass-reared males while competing for mass-reared females (Kim & Ehrman 1998). These studies suggested that male habituation in the presence of other males reduces courtship intensity, but this conclusion may not be relevant for our experiments, which neither involved isolated flies nor revealed behavioural differences between males reared in enriched versus standard environments.

Hoffmann & Cacoyianni (1990) reported that *D. melanogaster* males showed territorial behaviour at low numbers of up to 12 flies per cylindrical cage 10 cm in diameter and 4 cm high (314 cm^3). They suggested that a male defends a small food source, which is a likely place to find mates, as long as the fly number is sufficiently low to make territorial defence feasible. It is likely that the conditional territorial behaviour is associated with general differences in neurobiology, physiology and behaviour. For example, territorial males from sparsely populated environments may produce a different composition of pheromones that make them more attractive to females. Social experience has indeed been shown to influence neuronal response to serotonin in crayfish, *Procambarus clarkii* (Yeh et al. 1996) and pheromonal composition in cockroaches, *Nauphoeta cinerea* (Moore et al. 1995). In our mating trials, we never observed aggression between either males or females, perhaps because we used vials with no food. In *D. melanogaster*, aggression associated with access to food has been well described for males (Chen et al. 2002) and exists to a lesser extent in females as well (Ueda & Kdokoro 2002).

To conclude, male fruit flies reared in enriched environment had a strong mating advantage over males from standard environment. The crucial enrichment factor in our studies was per capita space. We suggest that low and high per capita spaces create different social environments associated, on average, with either subtle behavioural differences or distinct pheromonal profiles to which females are sensitive while choosing mates. The strong effect of environmental enrichment on mate choice is highly relevant for research on assortative mating and speciation, in which *Drosophila* has been a prime model system (e.g. Coyne & Orr 1989; Rice & Hostert 1993; Powell 1997; Korol et al. 2000).

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References

Ashburner, M. 1989. *Drosophila: a Laboratory Handbook*. Cold Spring Harbor, Maine: Cold Spring Harbor Laboratory Press.

Barth, M. & Heisenberg, M. 1997. Vision affects mushroom bodies and central complex in *Drosophila melanogaster*. *Learning & Memory*, **4**, 219–229.

Barth, M., Hirsch, H. V. B. & Heisenberg, M. 1997a. Rearing in different light regimes affects courtship behaviour in *Drosophila melanogaster*. *Animal Behaviour*, **53**, 25–38.

Barth, M., Hirsch, H. V. B., Meinertzhagen, I. A. & Heisenberg, M. 1997b. Experience-dependent developmental plasticity in the optic lobe of *Drosophila melanogaster*. *Journal of Neuroscience*, **17**, 1493–1504.

Carducci, J. P. & Jakob, E. M. 2000. Rearing environment affects behaviour of jumping spiders. *Animal Behaviour*, **59**, 39–46.

Chen, S., Lee, A. Y., Bowens, N. M., Huber, R. & Kravitz, E. A. 2002. Fighting fruit flies: a model system for the study of

aggression. *Proceedings of the National Academy of Sciences, U.S.A.*, **99,** 5664–5668.

Cobb, M., Connolly, K. & Burnet, B. 1985. Courtship behaviour in the *melanogaster* species sub-group of *Drosophila*. *Behaviour*, **95,** 203–231.

Coyne, J. A. & Orr, H. A. 1989. Patterns of speciation in *Drosophila*. *Evolution*, **43,** 362–381.

Ellis, L. B. & Kessler, S. 1975. Differential posteclosion housing experiences and reproduction in *Drosophila*. *Animal Behaviour*, **23,** 949–952.

Farris, S. M., Robinson, G. E. & Fahrbach, S. E. 2001. Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honey bee. *Journal of Neuroscience*, **21,** 6395–6404.

Greenough, W. T. & Volkmar, F. R. 1973. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Experimental Neorology*, **40,** 491–504.

Hebb, D. O. 1947. The effects of early experience on problem-solving at maturity. *American Psychologist*, **2,** 306–307.

Heisenberg, M., Heusipp, M. & Wanke, C. 1995. Structural plasticity in the *Drosophila* brain. *Journal of Neuroscience*, **15,** 1951–1960.

Hirsch, H. V. B. & Tompkins, L. 1994. The flexible fly: experience dependent development of complex behaviors in *Drosophila melanogaster*. *Journal of Experimental Biology*, **195,** 1–18.

Hirsch, H. V. B., Barth, M., Luo, S., Sambaziotis, H., Huber, M., Possidente, D., Ghiradella, H. & Tompkins, L. 1995. Early visual experience affects mate choice of *Drosophila melanogaster*. *Animal Behaviour*, **50,** 1211–1217.

Hoffmann, A. A. & Cacoyianni, Z. 1990. Territoriality in *Drosophila melanogaster* as a conditional strategy. *Animal Behaviour*, **40,** 526–537.

Kim, Y. K. & Ehrman, L. 1998. Developmental isolation and subsequent adult behavior of *Drosophila paulistorum*. Courtship. *Behavior Genetics*, **28,** 57–65.

Knoppien, P. 1987. Rare-male mating advantage: an artifact caused by differential storage conditions? *Behavior Genetics*, **17,** 409–425.

Kolb, B. & Whishaw, I. Q. 1998. Brain plasticity and behavior. *Annual Review of Psychology*, **49,** 43–64.

Korol, A., Rashkovetsky, E., Iliadi, K., Michalak, P., Ronin, Y. & Nevo, E. 2000. Nonrandom mating in *Drosophila melanogaster* laboratory populations derived from closely adjacent ecologically contrasting slopes at 'Evolution Canyon'. *Proceedings of the National Academy of Sciences, U.S.A.*, **97,** 12637–12642.

Lomassese, S. S., Strambi, C., Strambi, A., Charpin, P., Augier, R., Aouane, A. & Cayre, M. 2000. Influence of environmental stimulation on neurogenesis in the adult insect brain. *Journal of Neurobiology*, **45,** 162–171.

Moore, A. J., Reagan, N. L. & Haynes, K. F. 1995. Conditional signalling strategies: effects of ontogeny, social experience and social status on the pheromonal signal of male cockroaches. *Animal Behaviour*, **50,** 191–202.

Noor, M. A. F. 1997. Environmental effects on male courtship in *Drosophila pseudoobscura*. *Journal of Insect Behavior*, **10,** 305–312.

Powell, J. R. 1997. *Progress and Prospects in Evolutionary Biology: the* Drosophila *Model*. New York: Oxford University Press.

van Praag, H., Kempermann, G. & Gage, F. H. 2000. Neural consequences of environmental enrichment. *Nature Reviews Neuroscience*, **1,** 191–198.

Rampon, C., Jiang, C. H., Dong, H., Tang, Y. P., Lockhart, D. J., Schultz, P. G., Tsien, J. Z. & Hu, Y. H. 2000. Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Sciences, U.S.A.*, **97,** 12880–12884.

Rice, W. R. & Hostert, E. E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution*, **47,** 1637–1653.

Rosenzweig, M. R. 1966. Environmental complexity, cerebral change and behavior. *American Psychologist*, **21,** 321–332.

Spieth, H. T. & Ringo, J. M. 1983. Mating behavior and sexual isolation in *Drosophila*. In: *The Genetics and Biology of* Drosophila (Ed. by M. Ashburner, H. L. Carson & J. N. Thompson), pp. 345–384. London: Academic Press.

Technau, G. M. 1984. Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. *Journal of Neurogenetics*, **1,** 113–126.

Ueda, A. & Kdokoro, Y. 2002. Aggressive behaviours of female *Drosophila melanogaster* are influenced by their social experience and food resources. *Physiological Entomology*, **27,** 21–28.

Wurbel, H. 2001. Ideal homes? Housing effects on rodent brain and behaviour. *Trends in Neurosciences*, **24,** 207–211.

Yeh, S. R., Fricke, R. A. & Edwards, D. H. 1996. The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science*, **271,** 366–369.