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BRIEF COMMUNICATION

Protein Synthesis Inhibition Alters *Drosophila* Mating Behavior[1]

ANITA PRUZAN

Monell Chemical Senses Center, University of Pennsylvania, Philadelphia, PA 19104

PHILIP B. APPLEWHITE

Biology Department, Yale University, New Haven, CN 06520

AND

MICHAEL J. BUCCI

Division of Natural Science, S.U.N.Y. at Purchase, Purchase, NY 10577

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PRUZAN, A., P. B. APPLEWHITE AND M. J. BUCCI. *Protein synthesis inhibition alters* Drosophila *mating behavior.* PHARMAC. BIOCHEM. BEHAV. 6(3) 355–357, 1977. – Fruit fly *Drosophila pseudoobscura* virgin AR females mate preferentially with AR males when given a choice between AR and *or* males. However, AR females which mated with *or* males when young show a significant change in sexual selection in favor of *or* over AR males in subsequent simultaneous choice tests. Ingestion of food moistened with 4 ml cycloheximide (400 μg/ml) produces 75% protein synthesis inhibition in the female flies. Females exposed to cycloheximide (CXM) immediately after their initial copulation with *or* males resemble virgin flies in their choice of mates, and mate preferentially with AR males. Females exposed to CXM before their initial copulatory experience with *or* males resemble *or*-experienced but untreated (no CXM) flies in their choice of mates, and mate preferentially with *or* males. The change in sexual preference shown by females with prior copulatory experience resembles learning in that it is subject to disruption by CXM in ways analogous to those reported in the literature.

Cycloheximide Memory Fruit fly *Drosophila* Double Mating Sexual selection

RECENT reports have demonstrated that the fruit fly *Drosophila* has the ability to learn [7, 11, 13]. The potential importance of these findings is that the extensive knowledge of the genetics of *Drosophila* can be applied to study the biological bases of learning.

Pruzan [9] and Pruzan and Ehrman [10] showed that 11-day-old virgin *Drosophila pseudoobscura* Arrowhead (AR) females mate preferentially with AR males when given a choice between AR and *or* (Standard with orange eye autosomal recessive marker) males. However, 11-day-old Arrowhead females which mated with *or* males when 3-day-old virgins conferred a mating advantage to *or* males when given a choice between AR and *or* males at age 11 days. This change in mate selection represents modification of behavior as a result of experience, and may represent learning [8]. Administration of cycloheximide, an inhibitor of protein synthesis, leads to a loss of memory of learned tasks in several groups of organisms [1, 6, 12]. If the change in mating preference shown by *Drosophila* is learning, it should be subject to the same type of disruption by cycloheximide as has been found in other organisms.

METHOD

Two naturally occurring karyotypes of *Drosophila pseudoobscura*, Arrowhead (AR) and Standard, homozygous for an autosomal recessive marker (*or*) were used in these experiments (full description of the species in [4]). The flies were maintained in the laboratory in halfpint bottles containing food (Formula 4–24, Carolina Biological Supply Co.) and fresh yeast. New cultures were initiated by serial transfer. Virgin flies were obtained by clearing stock

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bottles of all adults and separating by sex the newly emerged adults within 12 hr.

When the flies were 24 hr old they were subjected to one of the following treatments:
(1) AR females were placed in halfpint bottles containing 2.5 g *Drosophila* medium moistened with 4 ml cycloheximide solution (400 μg/ml CXM, treated medium) for 12–15 hr. The females were then placed together with *or* males and allowed to mate in bottles containing untreated medium for 28 hr.
(2) AR females and *or* males were placed and allowed to mate in halfpint bottles containing untreated medium for 28 hr. Females only were then placed in halfpint bottles containing treated medium (see above) for 12–15 hr.

After treatment, the females were placed in individual vials containing untreated medium for seven days to deposit eggs. *Drosophila* females engage in subsequent matings only after the supply of sperm from the previous mating is depleted. Presence of larvae in the vials indicated that mating with *or* males had taken place, and only these females were used in further tests.

Females which showed evidence of mating with *or* males were subsequently given a choice between AR and *or* males in simultaneous sexual selection tests. Twenty females were placed without etherization with ten *or* and ten AR males in an Elens-Wattiaux observation chamber (for full description of the apparatus and its use see [5]) for 2 hr. The numbers and type of males mating were recorded. The males used here were all four days old and at the peak of their sexual activity. Four replicates of each treatment group were observed. The results were compared with the data obtained by Pruzan [9] for eleven-day-old virgin females raised on untreated medium.

Determination of Protein Synthesis Inhibition

Twelve flies were placed in each of two halfpint bottles in the above dry medium to which was added either 4 ml of water or 4 ml of the above CXM solution for a period of 12 hr. Then, 100 μl of H^3-leucine (specific activity 65 Ci/mM) was added to both sets of flies in new media for an incubation period of another 12 hr. At this point, the flies were removed from the bottles, washed with distilled water to remove surface radioactivity and each set of flies homogenized in 0.8 ml of distilled water in a Potter-Elvehjem grinder. This was followed by centrifugation for 5 min at 1300 xg to remove the largest particles. A 100 μl aliquot of the supernatant homogenate from each set was placed on a filter paper disc, air-dried for 5 min, and then subjected to trichloracetic acid extraction to measure protein synthesis [3]. Discs were run in triplicate and counted for 4 min each. The experiments described above were performed three times.

RESULTS

A. Biochemical

The results of the three protein synthesis inhibition experiments demonstrate that the sets of flies receiving no CXM had a mean incorporation rate of 9,462 counts per minute (cpm), but with CXM the mean rate dropped to 2,328 cpm. This is a 75% inhibition of protein synthesis.

Since the measured amount of H^3-leucine taken up by *Drosophila* in all the experiments was essentially the same (within 15%), it was not necessary to correct the TCA precipitable amounts of radioactivity for changes in the free radioactive leucine pool. Protein synthesis inhibition is based upon whole flies, so no statement can be made about inhibition specific just to the nervous system.

B. Behavioral

As shown in Table 1, 11-day-old *Drosophila pseudoobscura* Arrowhead (AR) females mate preferentially with AR males when the females tested were virgins, and when the females tested were fed CXM immediately after their initial copulatory experience with *or* males (Treatments 1 and 4). Both untreated females (no CXM) which were allowed to mate with *or* males and females fed CXM before their initial mating with *or* males mated preferentially with *or* males.

We have previously described a method called log odds for evaluating the relative mating success (RMS) between the two types of males in a simultaneous choice test [9]. Log odds is the natural logarithm of the ratio of the number of each of the two types of males observed mating (X and Y). It is normally distributed under the null hypothesis with 0 mean and SD $=\sqrt{\frac{1}{x}+\frac{1}{y}}$ and provides a direct measure of differences in the relative mating success of the males in the treatment groups.

The RMS values are presented graphically in Fig. 1. Results of Z tests reject the null hypothesis of equal mating success for AR and *or* males, (Z = 4.15, 3.50, 3.31 and 3.71 for conditions 1–4 from top to bottom; $p<0.01$) for all conditions. When lines, each representing RMS and the standard error for each treatment are clearly separated, the differences between isolation indices are significant. Lines which wholly overlap indicate no significant differences, but lines which overlap only partially need to be evaluated further statistically:

$$\text{Difference RMS} = \Delta = \frac{\text{RMS condition}_1 - \text{RMS condition}_2}{\text{S. D. of difference} = \sqrt{\text{variance}_1 + \text{variance}_2}}$$

It is evident from Fig. 1 that both AR virgins and females ingesting CXM after their initial mating with *or* males subsequently confer a mating advantage to AR males ($\Delta>0.05$). In contrast, females which mated with *or* males (no CXM) and females which ingested CXM before their initial mating with *or* males subsequently conferred a mating advantage to *or* males ($\Delta>0.05$).

DISCUSSION

In the fruit fly *Drosophila pseudoobscura*, it is the female which either rejects or accepts a male in mating. It is possible, therefore, to observe the effects of different experimental treatments to the female on sexual selection. We have previously shown [9,10] that 11-day-old virgin Arrowhead females confer a mating advantage to Arrowhead males in simultaneous choice experiments between AR and *or* males. However, when 3-day-old females were given a no choice mating experience with *or* males and then retested for mate selection at 11 days of age they showed a statistically significant preference for *or* males. If this change in behavior as a result of experience represents learning, then inhibition of protein synthesis should produce amnesia.

Present results confirm the findings utilizing mice [6,12] and fish [1]. They found that memory was impaired when CXM was administered 30 min (mice) and one hour (fish)

TABLE 1

NUMBER OF *DROSOPHILA PSEUDOOBSCURA* AR FEMALES MATING. FEMALES WERE 11 DAYS OLD AT TIME OF SIMULTANEOUS CHOICE OBSERVATIONS. THEY VARIED ACCORDING TO PREVIOUS MATING EXPERIENCE AND TREATMENT WITH CYCLOHEXIMIDE (CXM).

FEMALES Treatment	% FEMALES MATING	MATINGS AR Males	or Males	χ^2
Virgins	70	45	11	20.64^{a} *
Mating experience, no CXM ▲	35	11	48	13.64^{b} *
CXM before ▲ mating experience	41	5	28	16.02^{b} *
CXM after ▲ mating experience	40	30	2	28.00^{a} *

* = χ^2 computed against null hypothesis of 1:1 ratio of matings. For 1 df, χ^2 of 6.65 is significant at 0.01 level.
a = significant in favor of AR males
b = significant in favor of or males
▲ = Females were given a "no choice" mating experience with or males when 3 days old.

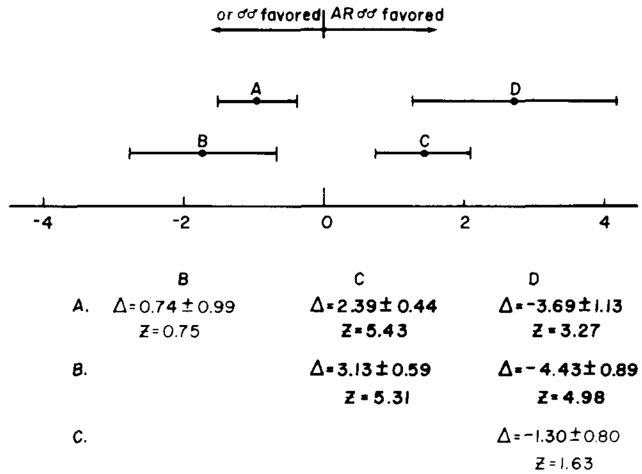

FIG. 1. Relative Mating Success Index and the 95% confidence limit for AR and *or Drosophila pseudoobscura* males observed mating with AR females in simultaneous choice tests. AR females differed according to treatment: A = initial copulatory experience with *or* males (ICE), B = cycloheximide ingested before ICE; C = 11-day-old virgins; D = cycloheximide ingested after ICE. Comparisons between conditions are presented in lower part of the figure. Bold face entries indicate significant differences, $p<0.01$.

after the learning task. The group of AR females which ingested CXM after their initial mating with *or* males showed amnesic effects in subsequent choice experiments. These females conferred a mating advantage to AR males and their choices were statistically indistinguishable from choices made by sexually inexperienced virgin female controls.

Surprisingly, however, AR females which ingested CXM before their initial no choice mating with *or* males failed to show amnesic effects and their choices of mates did not differ from choices made by females which copulated with *or* males but received no CXM (putative learning group). Both groups of females conferred a mating advantage to *or* males. Barondes and Cohen [2] showed normal task retention in mice injected with CXM 5 hr before training. Other studies [1,6] found that CXM administered 30 min before training produced amnesia, in which case training occurred during maximum inhibition of protein synthesis. AR females here ingested CXM before the initial mating. Given the rapid metabolic rate in insects, these females presumably experienced maximal protein synthesis inhibition when isolated from males and protein synthesis may have returned to normal by the time that mating with *or* males took place.

The levels of protein synthesis inhibition obtained by the method of ingestion here (75%) are lower than the inhibition levels in studies utilizing mice (90%), but are consistent with levels of protein inhibition obtained in fish (80%).

The marked amnesic effect could not be attributed to performance decrement, since the per cent of females mating in the second observation was approximately the same for all females with prior copulatory experience. We concluded, therefore, that the change in sexual preference shown by females with prior copulatory experience resembles learning in that it is subject to disruption by CXM in ways analogous to those reported in the literature.

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