Influence of Social Experience on Sexual Readiness of Male House Crickets, *Acheta domestica*

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Summary. Male *Acheta domestica* were deprived of social experience at three different life stages and mated as 2- or 3-day adults. Those deprived from the egg stage were found to copulate more readily than males which had social experience.

Group-rearing has been found to enhance growth and development of crickets[2,3] and development and sexual maturation of locusts[4]. In the present investigation, the effects of group-rearing and of social deprivation were studied with particular reference to copulatory behavior of young male house crickets, *Acheta domestica*.

Materials and methods. The experimental animals were bred from commercially obtained stock and kept in translucent plastic containers at 34 ± 1 °C under a 12 h light/12 h dark lighting regimen, with the dark period beginning at 09.00 h EST. Food and water were continually available. Eggs collected from stock animals were incubated on moist sand singly or in groups of 3. A few days after hatching, nymphs were transferred to 8 × 8 × 13.5 cm high containers. In about the penultimate nymphal instar, they were transferred to 12 × 18 × 22.5 cm high containers. The original group size (1 or 3 animals) was maintained as long as indicated by the experimental design. If a group decreased in size (because of removal of a newly identified female or because of a death), other experimental males of similar experience and size were used to maintain the original size. Growth observations were made daily at the same time of day.

The 60 experimental males were reared as follows: Group I, singly reared from the egg stage; Group II, removed from all male rearing group during last nymphal stadium; Group III, removed from all male rearing group 2–24 h after final molt; Group IV, group-reared throughout life, and kept only with males after the 4th nymphal stadium.

During the cricket's most active period[5], mating behavior of each male was observed at rearing temperature in a lighted area through a mirrored one-way glass for 2 h. The experimental male (2- or 3-day adult) was introduced into a clean, clear plastic mating chamber (floor area = 12 × 13 cm) and left at the observation site 20–30 min before introducing the female (8–15-day adult) directly from her rearing cage.

Results and discussion. A summary of the results is presented in the Table. The data show that young male house crickets deprived of tactual and visual stimulation exhibit signs of sexual readiness more than males with social experience throughout nymphal life (Groups III and IV). The differences are not readily explainable as results of increased aggression, dominance order or sexual 'exhaustion'. Since isolated male crickets are more aggressive than non-isolates[6,7], one might expect singly-reared males to repel femal advances more frequently than males with social experience. The results show that this was not the case. There was little difference between groups in the amount of time the male remained near the female. Furthermore, actual contact by females was apparently not repelled by singly-reared males for they copulated far more frequently (Fisher Exact Probability < 0.01) than males with adult social experience (Groups III and IV).

It is possible that rank in a dominance order affected the sexual responses of males with adult experience. However, this possibility seems remote for the following two reasons. Some males deprived after adulthood was attained had no opportunity to be in an adult dominance order, having been separated before either cage mate became adult. Additionally, all members of this group had been alone before the time of observation for a period equal to or greater than the period of adult social experience. Despite the apparent lack of participation in an adult dominance order, the frequency of copulation of this group (Group III) was much below that of the singlyreared group.

[1] The data were taken from a thesis submitted in partial fulfillment of the requirements for the Ph. D. degree at the City University of New York.

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Effects of deprivation and social experience on male *Acheta domestica*

Group	Total N	Min within 3 cm of female[a]	No. males copulating	Latency to copulation[a]	Latency to spermatophore formation in non-copulating males[a]
I	15	106	12	15.5 (1–102)[b]	30 ($N = 1$)
II	15	102	8	48.5 (3–79)	65 ($N = 5$) (36–89)
III	15	106	4	20.5 (12–38)	69 ($N = 6$) (49–88)
IV	15	101	3	19 (8–72)	87 ($N = 5$) (75–113)

[a] Min median. [b] Numbers in brackets show ranges.

It is also conceivable that the groups with adult social experience were sexually exhausted due to socially induced autosexual or homosexual activities. This possibility cannot be entirely discounted since the latency to spermatophore production in non-copulating group-reared males (Group IV) was significantly higher (Mann-Whitney U $p < 0.04$) than that in the group deprived after adulthood was attained (Group III). However, there was very little difference between the group deprived as nymphs (Group II) and the group deprived in the adult stage (Group III) in this regard. Thus sexual exhaustion does not entirely account for the low performance of all males with social experience as adults.

An alternate explanation to the aforementioned possibilities is that social deprivation increases excitability with reference to both sexual behavior and aggression. Furthermore, such excitability may have a demonstrable physiological basis. Social deprivation has been shown, in aphids, to lead to hyperactivity of the corpora allata[8], which have important physiological functions. The c. allatal hormone stimulates male accessory gland activity and influences behavior in a number of insects[9]. Although these specific mechanisms may not operate in house crickets, their physiology may be modified by social deprivation in a somewhat similar manner. It is proposed that this modification of the physiology involves a lowering of threshold to sexual excitability in singly-reared male house crickets.

In summary, it is concluded that visual and tactual social deprivation from the egg stage enhanced the likelihood of copulation in 2- or 3-day adult male *Acheta domestica* when reared at 34 ± 1 °C.

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Enhanced Inhibition of RNA Synthesis by Amanitins in in vitro Cultured Cells

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Summary. The inhibition of RNA synthesis by α-amanitin on in vitro cultured cells is very slow. The action of various analogues of the toxin was tested and some of them proved more effective. Moreover pretreatment of cell cultures with DEAE-dextran greatly enhanced the effect of β-amanitin.

α-Amanitin is a toxic polypeptide of *Amanita phalloides*. It is a useful tool in biology and biochemistry for its ability to inhibit RNA-polymerase B selectively by binding to the enzyme[2–4].

α-Amanitin brings about a very early inhibition of RNA synthesis in liver and kidney when administered to animals[5,6], but it acts very slowly on in vitro cultured cells[7]. This slow action is probably due to a scarce penetration. This hypothesis receives support from 2 observations. Different types of in vitro cultured cells display different sensitivity to α-amanitin, while RNA synthesis in isolated nuclei is inhibited by equal doses of the drug[8]. Furthermore, the toxicity of amanitin for cultured macrophages increases several times after conjugation of the toxin to albumin which allows amanitin to enter by pinocytosis[9].

A rapid inhibition of the amanitin-sensitive RNA-polymerase B of cultured cells would be required, especially in studies on replication of DNA viruses, in order to try to discriminate between viral and host cell transcription. With the aim at obtaining this effect, we performed two series of experiments on cell cultures. In the first series we examined the effect of a number of naturally occurring and chemically modified amanitins, all known to inhibit RNA-polymerase B in vitro[10–12], in order to compare their transcription-blocking activity with that of α-amanitin. In the second series of experiments we tried to increase amanitin penetration by treating cells with DEAE-dextran, a polycation which is known for its enhancing effect on penetration of several substances into cultured cells. We also tested the effect of the polyenic antifungal antibiotic amphotericin B, which had been reported to facilitate the penetration of α-amanitin[13].

Materials and methods. HEp-2 and BHK cell monolayers were grown for 36 h in 35 mm plastic Petri dishes in Eagle's minimum essential medium (MEM) supplemented with 10% foetal bovine serum. Amanitins were added to the medium at the concentration of 10 or 30 μg/ml, as indicated in the Tables I and II.

To perform pretreatment with DEAE-dextran, monolayers were washed twice with phosphate buffered saline lacking Ca^{++} and Mg^{++} (PBS A), kept in contact with 2 ml/dish of a solution containing 500 μg/ml DEAE-dextran (m.w. 2×10^6, Pharmacia) and 1 mg/ml glucose in PBS A for 15 min at 37 °C, washed 3 times with PBS A and refed with medium. Amphotericin B (Squibb) was dissolved in dimethylsulphoxide (10 mg/ml) and further diluted with the medium.

RNA synthesis was measured by pulse-labelling the monolayers with (^{3}H) uridine (29 Ci/m*M*; the Radiochemical Center, Amersham) at the concentration of 1 μCi/ml of medium in the presence of a 20-fold excess of cold thymidine. After a 30 min pulse, monolayers were

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