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Juvenile Hormone Biosynthesis and Oocyte Development in Adult Female *Blattella germanica*: Effects of Grouping and Mating

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The roles of grouping and mating in modulating the activity of the corpora allata (CA) in adult female cockroaches were investigated using the in vitro radiochemical assay of juvenile hormone (JH) biosynthesis. Isolated virgin females have longer, asynchronous cycles of CA activity and oocyte maturation than do isolated females mated on day 8. Three factors were identified as the major contributors to this difference: 1) an experimental artifact of selection for sexually receptive females, 2) a positive effect of grouping on JH synthesis and oocyte maturation, and 3) a positive effect of copulation on oviposition and retention of the ootheca. Mated females constitute a subpopulation of receptive females that differ significantly from other females by having higher rates of JH synthesis prior to mating. The relative importance of such selection is substantial when the rate of mating is low, as in experiments with isolated females that are exposed to males for a short period of time. Long-term exposure of females to males introduces a grouping effect, which obscures any additional effect of mating on CA activity and oocyte development. However, mating influences ootheca formation and its retention. The effect of grouping can be mimicked in isolated females by transection of the nerves connecting the CA–corpora cardiaca complex to the brain, suggesting that in this insect isolation causes brain inhibition of the CA, and grouping provides disinhibitory stimuli that release the CA from brain inhibition.

Key words: brain disinhibition, receptivity, ootheca, cockroach

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INTRODUCTION

Juvenile hormone-III biosynthesis by the CA,* as assayed in vitro by the radiochemical assay, is precisely regulated during the gonotrophic cycle of adult female cockroaches [1,2], including the oviparous cockroach *Blattella germanica* [3–5]. The activity of the CA in the adult female is both dependent on and modulated by integrated internal and external stimuli. These stimuli may include regulatory factors associated with the ovary and ootheca [1], feeding and/or the resulting chemical composition of the internal milieu, mechanical and chemical stimuli associated with mating and insemination, photoperiodic signals, and various social stimuli [2,6]. The degree of dependence of CA activity on any one of these factors varies widely and appears to be species specific even in closely related species with similar patterns of reproductive behavior. For example, in cockroaches, mating is obligatory for activation of the CA in *Diploptera punctata* [7], it has an accelerating effect on CA activity in *Nauphoeta cinerea* [8], it slightly accelerates oocyte maturation in bisexual *Pycnoscelus indicus* [9], and has no effect on oocyte maturation in parthenogenetic *Pycnoscelus surinamensis* mated with *P. indicus* males [9].

The elucidation of the specific effects of these stimuli is complicated by the fact that many experimental designs involve a combination of different stimuli, as was pointed out in the case of social stimuli and feeding [6]. Sexual receptivity of the female in several cockroach species, including *B. germanica*, is thought to be regulated by JH [10,11]. In these species, copulation is possible only by active participation of the receptive female. Therefore, elucidation of the role of copulation in the regulation of CA activity is complicated by the dependence of copulatory readiness on CA activity.

In this paper, we demonstrate the complexity of the mutual relationships among mating, grouping, and sexual receptivity with regard to the regulation of the CA. Important among these is the direct effect of grouping on CA activity and oocyte development, which we show here for the first time in an adult cockroach. Based on nerve transection experiments, we suggest that stimuli associated with grouping are mediated by the brain, acting as disinhibition agents in similar pathways as proposed for other external stimuli (e.g., mating in *D. punctata* [7], feeding in *Periplaneta americana* [12].

MATERIALS AND METHODS

Blattella germanica nymphs were reared at 27°C under a 12L:12D photoperiod with Purina dog food and water provided ad libitum. Newly emerged females (day 0) were isolated daily and maintained under the same environmental conditions either individually or in groups. Mature males were introduced on day 8 to individually reared females and discarded after mating was observed, as were females that failed to mate. Virgin females were kept isolated at all times, except as indicated in specific experiments. For studies of the second ovarian cycle, mated females were isolated at least 2 weeks prior to hatching

*Abbreviations used: CA = corpora allata; CC = corpora candiaca; EC = egg case; JH-III = juvenile hormone-III; NCC = nervi corporis cardiaci.

of the first ootheca, and the progeny were removed within 24 h of hatching. Grouped females were kept without males or were mated at specific ages, as indicated.

Corpora allata–corpora cardiaca complexes were dissected from CO_2-anesthetized females in modified methionine-free TC-199 medium (GIBCO, Grand Island, NY; special formulation after Kikukawa et al. [13]) containing Hank's salts, L-glutamine, 25 mM Hepes buffer, 2% Ficoll, and 5 mM $CaCl_2$. The radiochemical assay was adapted from Pratt and Tobe [14] with modifications after Feyereisen and Tobe [15] and Gadot and Applebaum [16]. In the standard assay for JH-III biosynthetic rate, the CA–CC complex was transferred to 100 μl medium containing 100 μM L-[methyl-^{3}H]methionine (specific activity, 200 mCi/mmol; New England Nuclear, Wilmington, DE) in tissue culture-treated disposable Cell Wells multidishes (Corning, NY). The glands were incubated with gentle shaking in the dark, at 28°C for 2 h. After the removal of the glands, the medium was collected from each well into Eppendorf tubes and extracted with 200 μl isooctane. A 50 μl aliquot of the isooctane phase was assayed directly for radioactivity by liquid scintillation spectrometry (with Scintilene, Fisher), and the values obtained were corrected by a blank incubation for each assay. The validity of this procedure for *B. germanica* was shown elsewhere [5].

Basal oocyte length was measured with an ocular micrometer. The nervi corporis cardiaci (NCC1 and NCC2) were severed as described by Pipa [12].

RESULTS

JH-III Synthesis and Oocyte Maturation in Individually Reared Mated Versus Virgin Females

Females isolated within 24 h of adult emergence (day 0), and kept individually thereafter, were either mated on day 8 or kept as virgins as described in Materials and Methods. Both groups exhibit changes in JH-III synthesis rates that coincide with the gonotrophic cycle (Fig. 1). The magnitude of activity peaks of the CA are not significantly different in virgin and mated females, but their timing is different: Peak activity occurs, on the average, on day 9 in mated females (1 day after mating) and on day 10 in virgin females (Fig. 1). In mated females the rate of JH-III synthesis declines to undetectable levels on day 12, by which time most females ovulate, and complete inhibition of synthesis persists during the next 20 days of "pregnancy" while the egg case is carried externally by the female. In virgin females, the average rate of JH-III synthesis is always above zero during the equivalent period (Fig. 1) because of delayed asynchronous ovulation and a shorter pregnancy in a high percentage of these females (see below).

The differences between the cycles of JH-III synthesis of the two groups are reflected also in the different rates of oocyte maturation. Mated females have basal oocytes that are significantly longer on day 9 (1 day after mating) than the oocytes of 9-day-old virgins (Fig. 2; t test, $P < 0.0001$), and the difference increases in the subsequent days, indicating a faster rate of oocyte maturation in the mated females.

Oocyte length is an accurate predictor of JH-III synthesis rate in vitro, and the relationship between these parameters is the same in mated and virgin

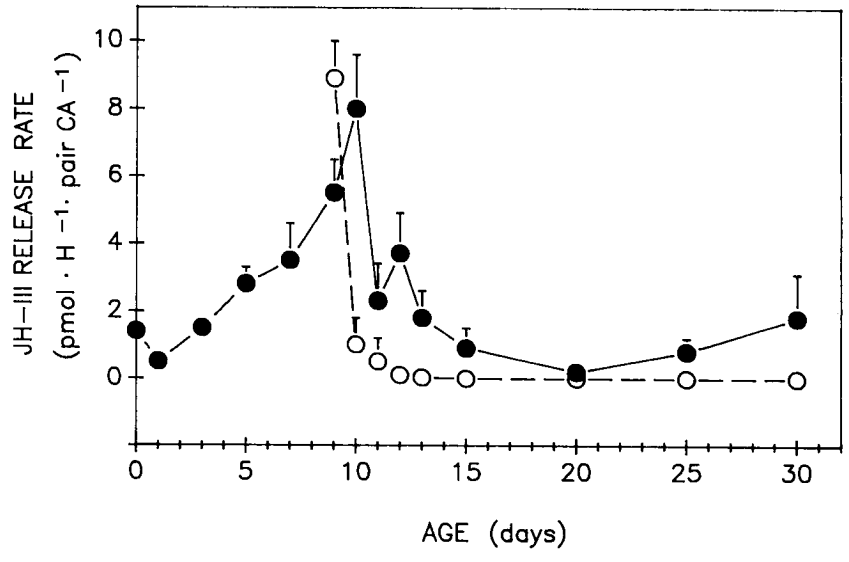

Fig. 1. In vitro rates of JH-III release during the first 30 days of adult virgin (●) and mated (○) isolated females. Females were mated on day 8 and kept isolated thereafter. Each point represents the mean ± S.E. of 4–16 assays.

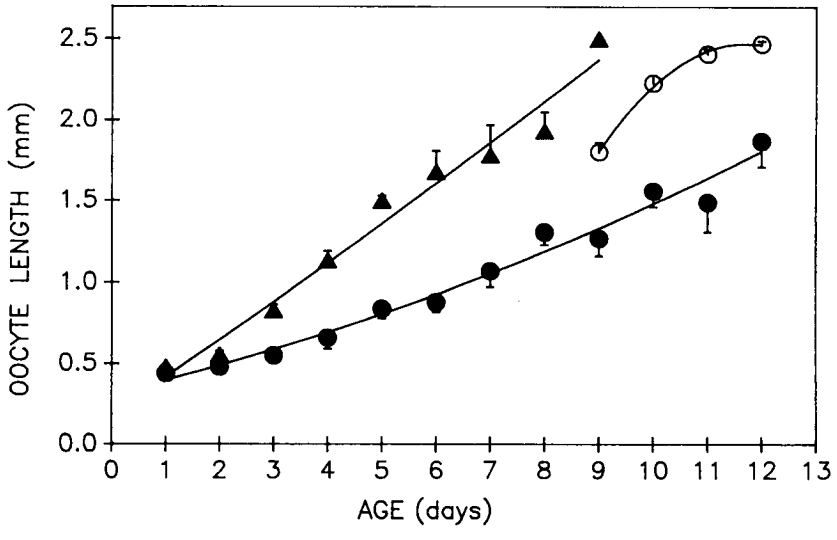

Fig. 2. Oocyte length of isolated virgin females (●), isolated females mated on day 8 (○), and grouped virgin females (▲) in the first reproductive cycle. Each point represents the mean ± S.E. of 9–28 females. Oocytes ovulated were considered to be of maximal length (2.5 mm).

females except for oocytes in the range of 1.7–2.2 mm (Fig. 3). In this range, virgin females have significantly lower rates of JH-III synthesis than do mated females with the same oocyte length (t test, $P < 0.05$). Both mated and virgin females show complete inhibition of JH-III synthesis during ovulation and egg case retention (Fig. 3).

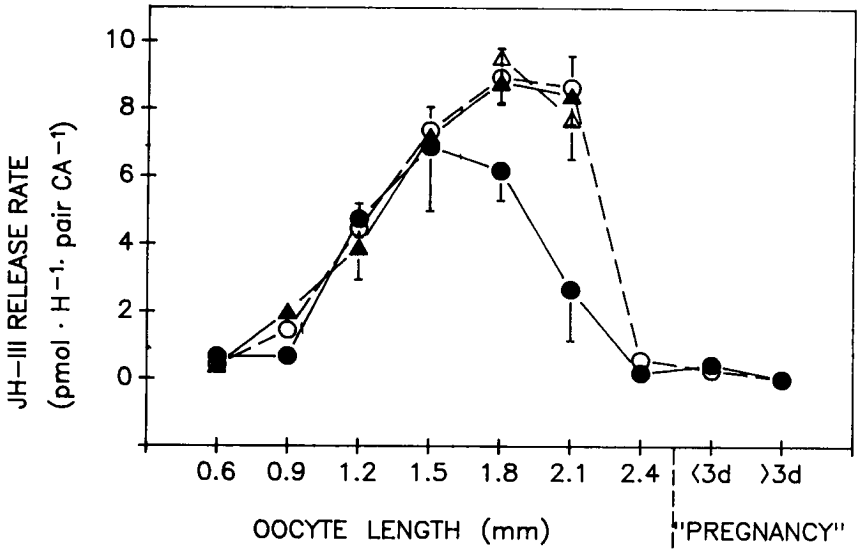

Fig. 3. Rate of juvenile hormone release as a function of oocyte length (± 0.1 mm) in isolated virgin females (●), grouped virgin females (▲), isolated females mated on day 8 (○), and grouped females mated on day 6 (△). Each point represents the mean ± S.E. of 4–20 assays. Data for isolated mated females were combined from the first two ovarian cycles, since no significant difference in the rate of JH-III synthesis of females with similar oocyte length was evident between the two cycles [5]. All other data represent the first cycle of each group. After ovulation (dashed line), glands from females carrying oothecae for <3 days and >3 days were assayed.

JH-III Synthesis and Oocyte Length in Relation to Sexual Receptivity of the Female

Mating may enhance and synchronize oocyte development by elevating rates of JH-III synthesis, or, alternatively, females that mate on day 8 may comprise a subpopulation with higher rates of JH-III synthesis and oocyte maturation. The latter hypothesis assumes that receptive 8-day isolated females have higher levels of CA activity and longer oocytes than nonreceptive 8-day isolated females. Comparison of the basal oocyte lengths of 8-day isolated females that were exposed to males for 4 h and dissected immediately afterwards showed that receptive females (42% of all females; those observed copulating) had significantly longer oocytes than nonreceptive females, which refused copulation (1.54 ± 0.08 mm [these and succeeding values are mean ± S.E.], n = 5; and 0.89 ± 0.13 mm, n = 7, respectively; t test, $P < 0.002$). The average oocyte length of both groups combined was not significantly different from the average oocyte length of 8-day virgin females monitored on other occasions (1.16 ± 0.13 mm, n = 12; and 1.31 ± 0.08 mm, n = 22, respectively; t test, $P > 0.05$). Thus mating screens for receptive females in an advanced stage of oocyte maturation and with relatively high rates of JH-III synthesis, which comprise a more homogeneous subpopulation of all the 8-day-old isolated females.

Effect of Grouping on Oocyte Development and CA Activity

Mating involves social and physical interactions with other insects, to which isolated virgin females are not exposed. Therefore, grouping stimuli may also

contribute to the differences observed between mated and virgin females. Figure 2 shows that virgin females, reared in groups, mature their oocytes faster than either isolated virgin females or isolated females mated on day 8. The effect of grouping on the relationship between JH-III synthesis rates and oocyte length is equivalent to that of mating: Grouped females, either virgin or mated (on day 6) exhibit similar rates of JH-III synthesis to isolated mated females (on day 8) with the same oocyte length; their rates are significantly higher than those of isolated virgin females with oocytes in the range of 1.7 to 2.2 mm (Fig. 3).

However, in these experiments, mating was allowed only at specific ages. It is possible that earlier mating may exert an influence on oocyte development in addition to grouping. Moreover, the complex behavior associated with courtship may also facilitate oocyte maturation. We therefore compared oocyte development in isolated virgin females and females that were reared from adult emergence with 1) two other females (grouped virgins), 2) two normal males (grouped mated), and 3) two phallomerectomized male in which the hook sclerite of the left phallomere was removed (such males are incapable of copulating, although they court normally and induce females to feed on their tergal secretion). Basal oocyte length was determined on days 6–8. On all three days, isolated females had significantly smaller oocytes than grouped females, but no significant differences in oocyte maturation occurred during this period among females housed with other females or with normal or phallomerectomized males (Fig. 4, Duncan's Multiple Range Test). Grouping with either females or males appears to be as effective as mating in regard to JH-III synthesis rates and oocyte maturation.

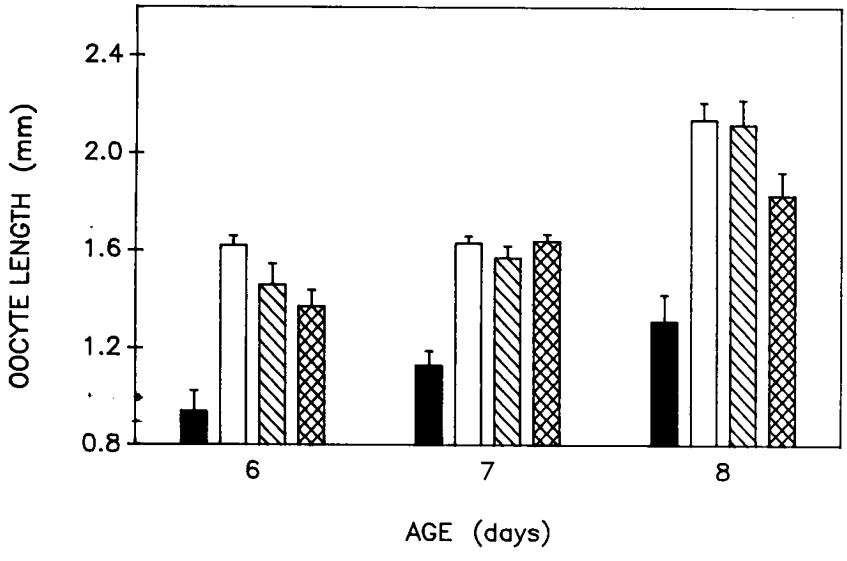

Fig. 4. Oocyte length of 6-, 7- and 8-day-old females reared in isolation (closed bars), with two other females (open bars), with two males (striped bars), and with two phallomerectomized males (hatched bars). The bars above the columns represent the S.E. of the mean of 10–20 females.

Effects of Grouping and Mating on Oviposition and Retention of the Ootheca

Grouping also exerted a dramatic effect on the timing of oviposition. None of the isolated virgin females (n = 59) formed an ootheca before day 10, and more than 50% of the females did not oviposit before day 12 (Fig. 5). Five females that did not oviposit by day 30, when the experiment was terminated, had average basal oocytes of 0.67 ± 0.07 mm, and resorption bodies were evident. In contrast, all grouped females oviposited by day 11. The timing of oviposition was not different between females housed with other females (n = 76) or with phallomerectomized males (n = 65, Fig. 5, χ^2, $P > 0.05$), and all grouped virgin females were combined for further analysis. A significant effect of mating on the timing of oviposition was found when virgin and mated females reared in groups were compared (Fig. 5, χ^2, $P < 0.001$); more than 60% of the mated females (n = 64) oviposited by day 8, and 100% did so by day 9, while approximately 20% of the virgin females (total n = 141) oviposited by day 8 and more than 20% oviposited on day 10.

Absence of fertilization affects the duration of "pregnancy" in the first cycle of virgin females. Infertile oothecae were aborted prematurely: 41% were dropped within 24 h of extrusion, and only 37% were retained for more than 3 days (n = 27). This was also shown in females that were allowed to copulate for only 10 s, an insufficient period for transfer of the spermatophore. Premature abortion of oothecae contributes to the asynchrony in the subsequent ovulation cycle of virgin females, because the second cycle of oocyte maturation begins at different ages in different individuals.

Virgin females in the second ovulation cycle retain their oothecae for 23.7 ± 0.9 days (n = 9), a period similar to the first and second "pregnancies" in mated females (22.1 ± 0.1 days, n = 65, and 23.1 ± 0.5 days, n = 11, respec-

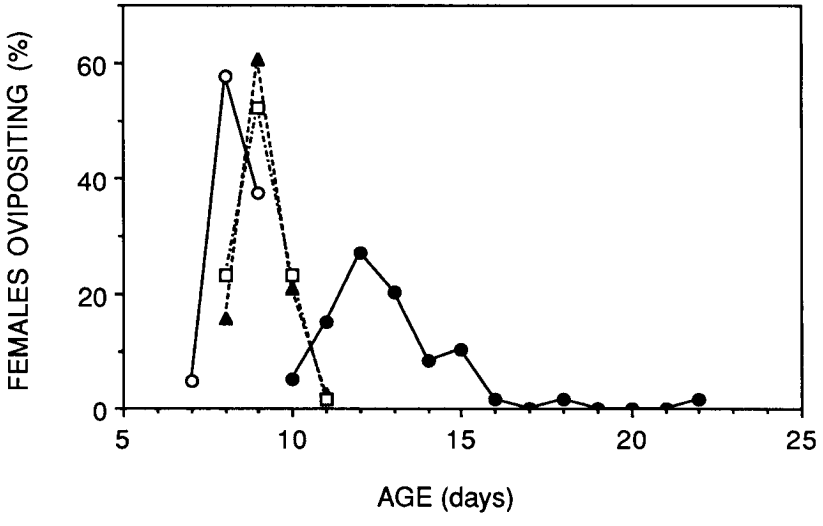

Fig. 5. Oviposition pattern in isolated females (●) and grouped females housed with other females (▲), with males (○), or with phallomerectomized males (□). Each point represents the percentage of females that ovulated on each day.

tively). The difference in patterns of ootheca retention in virgin females in the first and second cycles is unexplained.

Effect of NCC Transection on Oocyte Development in Grouped and Isolated Virgin Females

Transection of the NCC1 and NCC2 in newly emerged females caused a significant acceleration of oocyte development in isolated virgin females, but not in grouped virgin females, as compared with sham-operated and intact females (Table 1, Duncan's Multiple Range Test). The acceleration of oocyte maturation caused by denervation of the CC–CA complex is similar to that caused by grouping, as demonstrated by comparing NCC-transected isolated females with intact grouped females (Table 1; t test, $P > 0.05$).

DISCUSSION

The effect of mating on oocyte maturation and CA activity has been the subject of many investigations [2,6]. Although mating is not obligatory for oocyte maturation and oviposition in *B. germanica,* Roth and Stay [10] implied that it significantly accelerates the rate of oocyte maturation. However, the combined effects of grouping and of selection for sexually receptive females, which occur in mated females but not in isolated virgin females, may, as we show in this study, account for this presumed mating effect.

Sexual Receptivity

Isolated virgin females show significant delays in both peak activity of the CA and oocyte development as compared with mated females (Figs. 1, 2). However, since sexual receptivity increases as oocyte length increases in *B. germanica* (as in several other cockroaches [11]), receptive females comprise a selected subpopulation that differs significantly from both unreceptive females and unselected isolated virgin females. A relatively high percentage of the isolated females were not receptive to males on day 8 so that selection of mating (and thus for sexually receptive females) resulted in substantial, unintended selection for females with longer oocytes. Roth and Stay [10] compared *B. germanica* virgin females with females mated on day 9 and showed a significant acceleration of oocyte development after mating. Lack of data on the frequency of mating at this age and whether the females were housed in groups or in isola-

TABLE 1. Effect of NCC1 and NCC2 Transection on Oocyte Development in Grouped and Isolated Virgin Females*

	Oocyte length (mm)[a]	
	Grouped females	Isolated females
NCC transected	1.41 ± 0.17 (8)	1.57 ± 0.10 (9)
Sham operated	1.70 ± 0.04 (12)	1.07 ± 0.07 (13)
Intact	1.65 ± 0.06 (22)	0.88 ± 0.06 (24)

*Newly emerged females were either NCC1 and NCC2 transected or sham operated and reared in groups or in isolation thereafter. Females were dissected on day 6 for determination of oocyte length.

[a]Mean ± S.E., numbers of insects in parentheses.

tion prevent any comparison with our data, but their experimental design suggests that in their results the effect of selection for sexually receptive females may have been substantial.

Thus far we have been unable to establish any unobtrusive behavioral or morphological correlates, other than mating, that will predict sexual receptivity of females. Pipa [12] considered vigorously courted *P. americana* females to be receptive, but this appears to be more closely related to pheromone release than to receptivity. Mounting the male and feeding from its tergal secretion was used in other cockroach species to indicate sexual receptivity [11, 17], but, based on numerous observations in *B. germanica*, we found that mounting does not necessarily lead to mating, and it often occurs in nymphs, males, and starved females, which are known to be unreceptive.

Grouping

Oocyte maturation is faster in grouped virgin females than in isolated females (Fig. 2), but mating results in no greater acceleration in the rates of CA activation and oocyte maturation than grouping alone (Figs. 3, 4). When their oocytes are 1.7–2.2 mm long, isolated virgin females have significantly lower rates of JH-III synthesis than grouped females or isolated mated females (Fig. 3). This may explain the delayed and asynchronous oviposition in isolated virgin females (Fig. 5), which may suffer from insufficient levels of JH-III, resulting in resorption of partially matured oocytes in some individuals. Thus, grouping and mating directly or indirectly maintain high levels of JH-III synthesis until late stages of oocyte maturation. The similar effects of mating and grouping may be unrelated, or they may be a direct consequence of the brief social interaction not experienced by isolated virgin females. Until the exact nature of the grouping effect is elucidated, it is not possible to reject either of these possibilities. Moreover, the inherent difficulty of determining the state of sexual receptivity of isolated females that are only briefly exposed to males (as discussed above), excludes the possibility of testing the effect of mating in the absence of the grouping effect. These considerations question the validity of any comparison between isolated mated females and isolated virgin females.

Isolation or grouping effects on CA activity have been shown in several insects, with much ambiguity as to the causes and mechanisms of such effects [6]. Isolation has a clear positive effect on the activity of the CA in *Locusta migratoria* [18], but was implied to restrain CA activity in *P. americana* [19]. However, the latter conclusion is based on comparison between two different sets of conditions in experiments conducted at different times, with conflicting data [19,20]. Weaver [19] concludes that "the reduction in ovarian development which is brought about by total isolation is no greater than that produced by enforced virginity under crowded conditions." In this regard, *P. americana* differs from *B. germanica*, which is much more influenced by isolation than by enforced virginity, as shown here. However, accurate evaluation of the effect of grouping on adults of other cockroaches has not been conducted, and the importance of this factor in modulating CA activity and oocyte development is not known.

It is interesting to note that *B. germanica* females, housed with either phallomerectomized males or with females, developed their oocytes and oviposited infertile oothecae at similar rates (Fig. 5). This indicates that the stimuli result-

ing from grouping are not sex specific and are unrelated to courtship; it remains to be determined whether they are species specific. In a bisexual strain of *Drosophila mercatorum*, such behavioral facilitation of egg production appears to be sex specific and related to presence of males, whereas in a parthenogenetic strain isolated females as well as females housed with either males or females oviposit at similar rates [21].

Mating

Mating exerts a significant but smaller influence on the timing of oviposition than does grouping (Fig. 5). This confirms previous reports [10] but is in apparent contradiction to our results that oocyte development is unaffected by mating. It is possible that mating facilitates ovulation or ootheca formation, and virgin females tend to delay ovulation as compared with mated females. The role of JH-III in this process is unclear, since JH-III levels are low during ovulation (Fig. 3). However, it is clear that mating has differing effects on oocyte maturation and oviposition, and using both parameters interchangeably as indicators for CA activity may be misleading. Oviposition may be regulated by factors other than JH-III, which may be affected by mating.

Mating also affects ootheca formation. The first ootheca of virgin females is imperfect and often aborted prematurely. The second ootheca formed by virgin females is carried for a similar length of time as in mated females. This difference is difficult to explain. A similar pattern was recorded in *Blattella vaga* in the first ovarian cycle, but not in *B. germanica* investigated by Roth and Stay [10]. They reported that virgin females tended to retain the first ootheca for an average period that was similar to that of mated females, and only 17% of virgin females dropped the oothecae earlier than mated females. Environmental or strain differences may account for this discrepancy.

Several other studies have shown specific effects of mating on the rate of oocyte maturation, ovulation, and oviposition behavior in cockroaches [e.g., 22]. Although it is clear that mating influences oocyte maturation in many species, most studies have not controlled for confounding effects of noncopulatory social stimuli. As in *B. germanica*, it is possible that acceleration of the ovarian cycle in mated females is attributable to both social and copulatory stimuli.

Grouping as Disinhibitory Stimuli

Stimuli associated with grouping may be chemical or mechanical. Our data suggest that these stimuli affect the central nervous system (presumably through sensory organs), which converts them into disinhibitory signals relieving the CA from brain inhibition (Table 1). Such a disinhibitory effect was also ascribed to mating in several cockroach species (*D. punctata* [7], *Blaberus* [9], *N. cinerea* [8], and *Leucophaea maderae* [23]), to feeding (*P. americana* [12]), and to long-day conditions in diapausing species (*Leptinotarsa decemlineata* [24] and *L. migratoria* [25]). In all these cases, it is suggested that an inhibitory center in the brain restrains the CA via intact nerves until an appropriate external stimulus is perceived.

The external stimuli that trigger the disinhibition of the CA, may be considered as species-specific "releasing cues," and in the adult female they may be

related to reproductive success. The adaptive values of copulatory and feeding stimuli as "releasing cues" are clear, but it is less obvious how grouping may enhance reproductive success in cockroaches. Since mate-finding in *B. germanica* is dependent on contact sex pheromones [26,27], grouping may enhance the chances of males finding receptive females. Alternatively, grouping itself may have an adaptive value for cockroaches, as is also suggested by its accelerating effect on nymphal development [28,29]. Aggregation in cockroaches is mediated through "aggregation pheromones" originating in the rectal [30] or mandibular [31] regions. It would be interesting to determine whether these factors have any role in the disinhibition of adult CA.

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