

RESEARCH ARTICLE

Differential physiological responses of the German cockroach to social interactions during the ovarian cycle

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SUMMARY

In many animal species, social interactions can influence the morphology, physiology and behavior of individuals, including their rate of development and reproduction. Reproduction in cockroaches is regulated by juvenile hormone III (JH), and social interactions have been shown to accelerate female reproduction in the German cockroach, *Blattella germanica* (L.), by stimulating JH production. However, it is not clear in this or any other insect species whether social facilitation of the reproductive rate occurs throughout the ovarian cycle or only at certain stages. We compared the effects of social interactions during the pre-oviposition period (when JH production is high) and during gestation (when little JH is produced), as well as during the first ovarian cycle (when females are virgin) and the second ovarian cycle (after females had mated). Social interaction with one conspecific female was sufficient to accelerate JH production and oocyte maturation, but this effect was reversed by crowding. Social interactions also accelerated the onset of sexual receptivity in virgin females. However, social interactions failed to shorten gestation, suggesting that social cues stimulate JH production only when the corpora allata (CA) are active and not when CA activity is suppressed by the central nervous system. Females were most responsive to transient social isolation and transient social interactions when 2–3 days old, suggesting that they are particularly sensitive to social interactions when their CA become active. Overall, these results show that all JH-dependent events in the reproductive cycle of *B. germanica* females are under the strong influence of social interactions.

Key words: social interaction, *Blattella germanica*, oocyte maturation, sexual receptivity.

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INTRODUCTION

The onset and pace of reproduction in most animals are dependent upon specific internal and external stimuli. Internal stimuli include the animal's nutritional state, blood composition and osmolarity and whether it has mated. External stimuli include environmental conditions (e.g. temperature, humidity, photoperiod and host plants) and social interactions (Engelmann, 1970; Gilmore and Cook, 1981). In many animal species, social interactions can affect fitness by altering the morphology, physiology and behavior of individuals, thus influencing growth, development or reproduction (Krause and Ruxton, 2002; Wilson, 1971). Behavioral modulation of reproduction is expected in social animals. For example, in some vertebrate social systems only dominant individuals mate (e.g. Hodge et al., 2008) and in some eusocial insects the queen uses pheromones to inhibit reproductive maturation in workers (reviewed in Le Conte and Hefetz, 2008).

Social facilitation or social suppression of reproduction is less common in facultatively gregarious or solitary animals. Nevertheless, here too, some examples of reproductive effects due to the presence of conspecifics have been documented. For example, interaction of adult females with sexually mature males has been shown to accelerate the onset of reproduction in a number of vertebrate species and some insects (reviewed in Rekwot et al., 2001). Social interactions dramatically shift the desert locust from solitary to gregarious phase, and social interactions also influence female reproduction; solitary females are preferentially attracted to

vegetation containing eggs of gregarious females, resulting in phase-dependent gregarization of their progeny (Bashir et al., 2000). Moreover, in some insect species, reproduction can be accelerated in females by the presence of individuals of various developmental stages, both genders, and even different species (e.g. Lees, 1967; Bradley, 1985) (reviewed in Braendle et al., 2006; Allen, 2010).

Previous studies on social facilitation in cockroaches have focused on nymphal development, where social interactions greatly accelerate the pace of development (Roth and Willis, 1960; Wharton et al., 1968; Izutsu et al., 1970; Woodhead and Paulson, 1983; Lihoreau and Rivault, 2008). Little is known, however, about socially facilitated physiological changes in adult cockroaches. This phenomenon has been investigated only in the German cockroach, *Blattella germanica*, and the brownbanded cockroach, *Supella longipalpa*. Whereas females of both species respond to mating stimuli with faster oocyte maturation, only *B. germanica* females, and not *S. longipalpa* females (Chon et al., 1990), respond to social interactions with a shorter preoviposition period and hence faster reproduction (Gadot et al., 1989b; Holbrook et al., 2000).

The German cockroach represents a unique system for studies of insect reproduction. Females exhibit a reproductive pattern that is functionally intermediate between oviparity and ovoviviparity (Roth and Stay, 1962). Females undergo a period of sexual maturation after eclosion, followed by mating. Like oviparous cockroaches, the female ovulates and extrudes an egg case. However,

unlike oviparous species, the egg case is then rotated 90° and retained externally by the female, attached at the vestibulum (an outer chamber that lies above the enlarged seventh abdominal sternite), until the nymphs hatch approximately 21–22 days later at 27°C (Roth and Stay, 1962). This incubation period is functionally similar to gestation in ovoviviparous cockroaches, as the corpora allata (CA) are inhibited in both groups, suppressing juvenile hormone III (JH) production (Tobe and Stay, 1985; Gadot et al., 1989a; Gadot et al., 1989b; Gadot et al., 1991) (for reviews, see Schal et al., 1997; Treiblmayr et al., 2006). As the major gonadotropic hormone, JH is produced and released by the CA in a stage-specific manner and it paces the female's reproductive rate. The JH biosynthetic rate increases after eclosion, stimulating vitellogenesis and its uptake by the oocytes, declines through ovulation and remains low during gestation (Belles et al., 1987; Gadot et al., 1989a). Juvenile hormone also (1) stimulates the production and release of the attractant sex pheromone blattellaquinone (Liang and Schal, 1994; Nojima et al., 2005) and a courtship-inducing contact sex pheromone blend (Eliyahu et al., 2008), (2) stimulates the production of accessory gland proteins that form the egg case (Burns et al., 1991) and (3) controls the expression of sexual receptivity in the female (Schal and Chiang, 1995).

In *B. germanica*, oocyte development is cyclic and interrupted by a protracted gestation. Its panoistic paired ovaries each comprise approximately 20 ovarioles, and only a single basal oocyte in each ovariole (i.e. the next to be oviposited) matures during the preoviposition period (Roth and Stay, 1962). At oviposition, the penultimate oocyte becomes the new basal oocyte, but its growth is suppressed during most of the 21-day gestation period. This suppression is due to central nervous system (CNS) inhibition of CA activity, originating from mechanosensory signals associated with the presence of the externally incubated egg case in the genital vestibulum (Roth and Stay, 1962; Liang and Schal, 1994; Schal and Chiang, 1995). Thus, during gestation, JH production remains low, but at the end of gestation, before egg hatch, JH titers slightly increase and the basal oocytes grow slightly as well (Gadot et al., 1989a; Gadot et al., 1989b). The average length of the basal oocytes is a reliable measure of the reproductive stage because all basal oocytes mature synchronously and their length correlates well with both the activity of the CA (as measured by their JH biosynthesis *in vitro*) and with JH titer in the hemolymph (as measured by GC-MS) (Sevala et al., 1999; Treiblmayr et al., 2006).

In the present study, we sought to determine whether social interactions could hasten the reproductive rate throughout the ovarian cycle or only during certain limited stages of the cycle. We hypothesized that the ovarian cycle of *B. germanica* would be affected most by social interactions when the CA are active and produce JH and not when the CA are practically inactive during gestation. First, we showed that both JH production and basal oocyte growth were density-dependent; social interactions increased both, but crowding at high female density reversed these effects. We also found that social interactions stimulated oocyte maturation in both virgin and mated females. In order to determine if only JH-dependent events are influenced by social interactions we investigated the effects of social interactions at different periods of the ovarian cycle.

MATERIALS AND METHODS

Insects

A laboratory colony of insecticide-susceptible German cockroaches (American Cyanamid strain) was maintained at 27±1°C, ambient relative humidity (40–70%), a photoperiod of 12 h:12 h L:D, and

provided with shelter, water and food pellets (LabDiet 5001 Rodent Diet, PMI Nutrition International, Brentwood, MO, USA). This large, developmentally synchronous colony permitted the use of many newly eclosed females from a single cohort in most experiments. Newly eclosed (day 0) adult females of similar size and degree of sclerotization with intact wings were selected for each experiment. Experiments were conducted under the same conditions as described above: controlled temperature and photoperiod, with water and food available *ad libitum*.

Oocyte dissection and measurements

Females were ice-anesthetized and dissected under cockroach saline (Kurtti and Brooks, 1976), and the ovaries were removed. Each ovary contains approximately 20 ovarioles, and only a single basal oocyte in each ovariole takes up yolk proteins. Because all 40 basal oocytes in the two ovaries grow synchronously, 10 randomly selected basal (vitellogenic) oocytes were measured with an ocular micrometer in the eyepiece of a dissecting microscope. Measured oocyte lengths were averaged for each female but, because all basal oocytes of *B. germanica* mature synchronously, there was little variation in oocyte length within each female.

Effects of female density on oocyte maturation and JH biosynthesis

The effect of female density on oocyte development was investigated by placing 1, 2, 8 or 20 newly eclosed females in a plastic Petri dish (90 mm diameter, 15 mm high; Fisher Scientific, Pittsburgh, PA, USA) with food and water for 6 days. Females were carbon dioxide-anesthetized on day 6, their CA were incubated *in vitro* to measure JH biosynthetic rates, and their basal oocytes were measured. We set up 40 females per treatment (40 dishes of one female each, 20 dishes of two females, five dishes of eight females and two dishes of 20 females), but some replicates were lost because of the difficulty of dissecting and incubating the CA. Only females from which we could measure both parameters were used, resulting in 26–33 females per treatment.

JH biosynthesis was measured *in vitro* using a radiochemical assay (Pratt and Tobe, 1974; Gadot et al., 1989b; Holbrook et al., 2000). Briefly, the CA–corpora cardiaca complexes were dissected and incubated in modified methionine-free TC-199 medium (Specialty Media, Lavalette, NJ, USA), supplemented with 100 µmol l⁻¹ L-[³H-methyl]-methionine (NEN, Wilmington, DE, USA), 5 mmol l⁻¹ CaCl₂ and 20 mg ml⁻¹ Ficoll type 400. Incubation and extraction conditions followed previous methods (Holbrook et al., 2000).

Time course of oocyte maturation in isolated and paired females in the first and second ovarian cycles

Newly eclosed (day 0) females were either socially isolated or paired in plastic Petri dishes and provisioned with food and water. Cohorts of virgin isolated and paired females were dissected daily for 6 days and their oocytes were measured. Sample size was 10 females per treatment on each day.

B. germanica females may obtain and store enough sperm when first mated to last their entire reproductive life of several months (Cochran, 1979). Thus, the first ovarian cycle is unlike subsequent ovarian cycles as females mature sexually, become sexually receptive, and mate during the first preoviposition period (Schal et al., 1997). Because the first ovarian cycle requires social interaction with males, we hypothesized that the female's endocrine system would be affected by social interactions during the first, but not subsequent, ovarian cycle. For the second ovarian cycle, we reared

newly eclosed females in large groups in plastic cages (185×130 mm, 105 mm high; Althor Products, Windsor Locks, CT, USA) with egg carton shelters and allowed them to mate on day 6. Only mated females with a fully rotated egg case were selected on day 9 and monitored in a separate plastic cage during gestation. Just before the end of the gestation period (day 28), we removed the egg case, thus synchronizing the start of the second ovarian cycle among all females. Females were housed in social isolation or in pairs during the second ovarian cycle. Oocyte length was measured daily in cohorts of females 1–4 days after the removal of the egg case (day 29–32).

Effects of social isolation and social interactions on sexual receptivity

Blattella germanica females undergo several days of sexual maturation before they become sexually receptive, and the onset of sexual receptivity is regulated by JH (Schal et al., 1997). We hypothesized that in the presence of conspecifics, females would become sexually receptive faster than females raised in social isolation. Newly eclosed females were either isolated or paired with other females in plastic Petri dishes. On day 6, all isolated females were transferred into a cage (300 mm diameter × 90 mm high; Althor Products) with sexually mature males (9–10 days old), and all paired females were likewise transferred into a second cage and provided with males from the same cohort. We removed copulating pairs every 15–30 min for 3 h and placed them in a refrigerator for later dissection. Copulation averages ~90 min in this species, so this design guaranteed that successful copulations would be discovered. We then measured oocyte length in all four treatment groups: (a) isolated mated; (b) isolated unmated; (c) paired mated; and (d) paired unmated. Because few socially isolated females mated on day 6, this assay was repeated on day 7 with another cohort of females and males. Sample size was 36 females per treatment on each day.

Differential responses of females to duration of social interactions

To test whether females are differentially responsive to social interaction during successive stages of their preoviposition period, we housed individual newly eclosed females in isolation, transiently paired some females with another 10–12-day-old female (marked by clipping its wing tips) for only 24 h on either day 0, 2, 4 or 6, and then again socially isolated the females until the beginning of day 7 (to allow day 6 pairing to be completed), when they were dissected and their basal oocytes were measured (see design in Fig. 4A). Other females were treated in the same way, but they were transiently paired with other females for 48 h instead of 24 h on either days 0–1, 2–3 or 4–5; these females were dissected at the end of day 6 (see Fig. 4B).

Opposite experiments were also conducted, with newly eclosed females socially paired, then transiently isolated for 24 h or 48 h, and again paired for the remainder of the experiment (see design Fig. 4A,B). Sample size was 17–21 females per treatment.

Responses to social interactions during gestation

If social interactions during gestation were to accelerate reproduction during the ~21-day gestation period, as they do in the preoviposition period, then gestation and embryogenesis would be disrupted. We therefore hypothesized that the female's reproductive physiology would be unresponsive to social interactions during gestation. Nevertheless, because JH biosynthesis is known to increase slightly before egg hatch, and the basal oocytes grow slightly as well (Gadot et al., 1989b; Schal et al., 1997; Treiblmayr et al., 2006), we

hypothesized that social interactions in the late phase of gestation might influence the second ovarian cycle.

To examine the effects of social isolation and social interactions on the duration of gestation, newly eclosed females were reared in large groups in plastic cages, as before. Males were introduced to 6-day-old females. We monitored for mating every 15 min during a 2 h period and, because copulation in *B. germanica* lasts for approximately 90 min (Schal and Chiang, 1995), we are confident that only mated females were retained for these experiments. To generate a synchronous cohort of gestating females, only females with a fully rotated egg case on day 9 were either socially isolated or housed in pairs during the ensuing gestation period. Egg hatch was monitored daily at the end of gestation. Sample size was 41 isolated and 42 paired females.

To investigate the influence of social isolation or interactions during the first gestation period on the development of second-cycle oocytes, the experimental procedure described above was repeated. The effect of these treatments was assessed by measuring the length of the basal oocytes on day 24 (day 15 of gestation = 6 days before hatch = 71% embryogenesis time), and again on day 29 (day 20 of gestation = 1 day before hatch = 95% embryogenesis time). Sample size was 10–15 females per treatment.

Statistical analyses

Data were analyzed with Student's *t*-test to compare two-sample means and with one-way ANOVA for multiple comparisons using SAS[®] 9.1.3 software (SAS Institute Inc. 2002–2003, Cary, NC, USA). The least significant difference (LSD) test was used for comparisons of means and PROC GLM for residuals from adjusted model as well as to test whether residuals met the assumption of independence and homogeneous variances among the treatments. For unbalanced data, we used least squares means (LSMEANS). The significance level for rejecting the null hypothesis was $\alpha=0.05$. Variation around the mean is represented by the standard error of the mean (s.e.m.).

RESULTS

Effects of female density on oocyte maturation and JH biosynthesis

Socially isolated virgin females matured their oocytes slowly, from ~0.5 mm at eclosion to only 0.90±0.04 mm ($N=26$) by day 6 (Fig. 1). Females housed with a single conspecific female, on the other hand, exhibited significantly faster oocyte maturation (day 6: 1.30±0.03 mm, $N=26$) and this effect was even more pronounced at the higher density of eight females per Petri dish (1.44±0.02 mm, $N=33$). However, crowded females, at 20 females per Petri dish, exhibited significantly stunted oocyte maturation (day 6: 1.14±0.06 mm, $N=29$) (ANOVA, $P<0.001$) (Fig. 1).

The patterns of oocyte maturation matched the activity of the CA in these females, as expected (Fig. 1). Because paired females demonstrated clear social facilitation of reproduction (i.e. faster oocyte development) by day 6 (ANOVA, $P<0.001$), this minimal experimental design (socially isolated vs pair-housed) was used in all subsequent experiments.

Social isolation might be stressful for nocturnal cockroaches in the absence of shelter. Because the long-term presence of shelters contributes to faster nymphal development, greater adult body mass and greater female fertility (Gemeno et al., 2011), we examined whether shelter ameliorated the effects of social isolation. There was no significant difference between socially isolated females with or without shelters (0.95±0.05 mm vs 0.88±0.06 mm, respectively) (Student's *t*=2.0262, $P=0.3575$); similarly, oocyte maturation in pair-

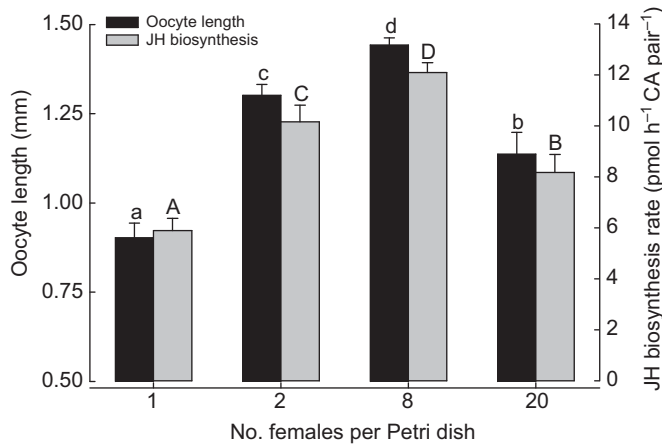


Fig. 1. The effect of female density on reproductive rate in *B. germanica*. Females were housed in Petri dishes for 6 days at densities of 1, 2, 8 or 20 females per dish. Basal oocyte length was measured and juvenile hormone III (JH) biosynthetic rates of the CA were quantified *in vitro*. Sample sizes were 26, 26, 33 and 29, respectively, for 1, 2, 8 and 20 females per dish. Variation around the mean is represented by the standard error of the mean (s.e.m.). ANOVA for oocyte length: $F_{3,110}=32.829$, $P<0.001$. ANOVA for JH synthesis: $F_{3,110}=22.384$, $P<0.001$. Means were compared by LSD, and means not sharing a letter are significantly different ($P<0.05$).

housed females that were provided with either one or two shelters was not significantly different from that of paired females housed without shelter (1.50 ± 0.07 mm, 1.58 ± 0.04 mm, 1.55 ± 0.06 mm, respectively) (ANOVA, $F_{2,54}=0.290$, $P=0.7495$). These results indicate that the differential oocyte growth observed in socially isolated and paired *B. germanica* females is independent of any stress associated with lack of shelters in the remainder of our experiments.

Time-course of oocyte maturation in socially isolated and paired females in the first and second ovarian cycles

For the first 2 days after adult eclosion, oocyte maturation was similar in solitary *B. germanica* females that remained socially isolated and in females paired with a conspecific female (Fig. 2). Subsequently, however, paired females diverged significantly from isolated females, and by day 6 their oocytes were 1.76-times longer than the oocytes of isolated females (1.63 ± 0.05 mm vs 0.92 ± 0.07 mm) (Student's t -test: $t=2.1009$, $P<0.0001$, $N=10$ each) (Fig. 2).

In the second ovarian cycle, mated females proceed directly into vitellogenesis and rapid oocyte maturation, bypassing the processes of sexual maturation and mating that they experience in the first ovarian cycle. Therefore, their basal oocytes grow faster during the second ovarian cycle. Still, a significant difference in oocyte length of socially isolated females and paired females could be seen as early as day 2 in the second ovarian cycle, and these differences increased during the subsequent 2–3 days (Fig. 2).

Effects of social isolation and social interactions on sexual receptivity

Social isolation significantly delayed not only oocyte maturation but sexual receptivity as well. The basal oocyte length of 1.25 mm is the approximate stage for the onset of sexual receptivity (Schal and Chiang, 1995). Only 8.1% of the socially isolated females mated on day 6, whereas 66.6% of the paired females mated on day 6. All the socially isolated females had oocytes <1.25 mm, whereas 58.3%

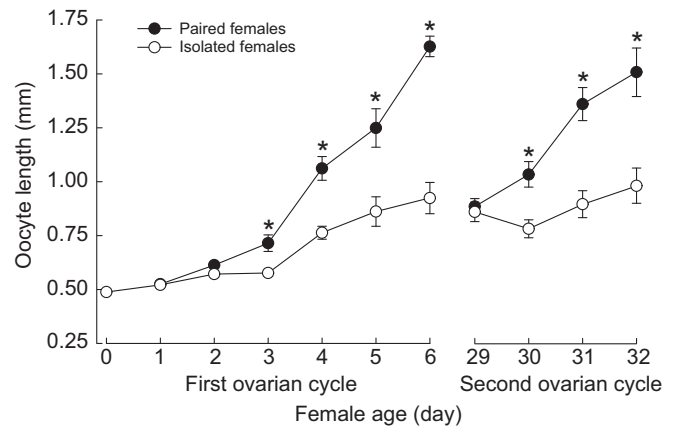


Fig. 2. Time-course of oocyte maturation in *B. germanica* females during the preoviposition period of the first and second ovarian cycles. For the first ovarian cycle, newly eclosed (day 0) females were socially isolated or housed in pairs in Petri dishes. For the second ovarian cycle, females were reared in groups after adult emergence, mated on day 6 and reared in groups during gestation. The egg case was removed on day 28, 2 days before the end of gestation, thus synchronizing their reproductive cycle. Females were then socially isolated or housed in pairs. Basal oocyte length was measured on each day indicated. Each point shows the mean \pm s.e.m. of 10 females in the first ovarian cycle and 10–15 females in the second cycle. Significant differences between treatments are shown with an asterisk (Student's t -test: $t=2.1009$, $P<0.05$).

of the socially paired females developed their oocytes to >1.25 mm (Fig. 3A). Similar results were obtained with a separate cohort of females on day 7, but with 58.3% of the socially isolated females mating (85.8% with oocytes >1.25 mm) compared with 91.7% of paired females (100% with oocytes >1.25 mm) (Fig. 3B). These results show that all JH-regulated events – both physiological and behavioral – appear to be under the strong influence of social interactions.

Differential responses to duration of social interactions during the first preoviposition period

When females were socially isolated since adult emergence, but then transiently paired with a conspecific female for only 24 h on day 0 or on days 2, 4 or 6 (see design in Fig. 4A), their basal oocytes were not significantly different from the negative controls (socially isolated females during the entire experiment), indicating a lack of response to the transient social interaction (Fig. 5A). Likewise, when females were paired with conspecific females since adult emergence, but then transiently isolated for 24 h on day 0 or on days 2, 4 or 6 (see design Fig. 4A), the length of their basal oocytes was approximately the same as in females that were socially paired during the entire experiment (positive controls) (Fig. 5A). Thus, either social isolation or social interactions for only 24 h during a 6-day oocyte maturation period appears to be insufficient to change the course of the ovarian cycle.

However, when socially isolated females were paired transiently for 48 h (see design Fig. 4B), the basal oocytes of only those females that were paired on days 2–3 were significantly larger than the oocytes of females that were isolated during the entire experiment (Fig. 5B). When pairs of females were split and isolated transiently for 48 h (see design Fig. 4B), there was no significant difference between the oocyte length of females isolated on any two days during the 6-day-long experiment and the positive control (females paired during the entire experiment). Notably, however, oocyte maturation

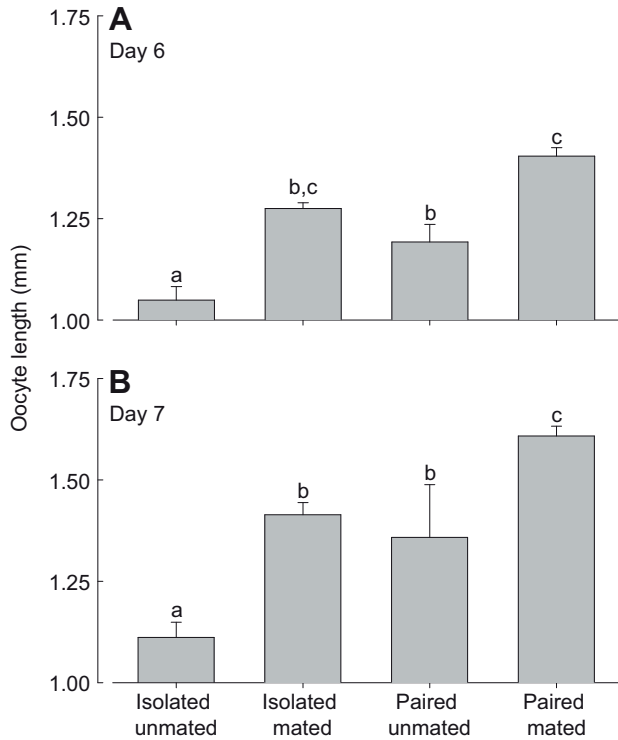


Fig. 3. Effects of social isolation and group-housing on the onset of sexual receptivity in *B. germanica* females. Newly enclosed females were either socially isolated or paired with another female in a Petri dish and allowed to mate on day 6 (A) or day 7 (B). Basal oocyte length was measured in all females ($N=36$ per treatment). Variation around the mean is represented by the standard error of the mean (s.e.m.). ANOVA for day 6: $F_{3,69}=23.670$, $P<0.001$. ANOVA for day 7: $F_{3,68}=43.164$, $P<0.001$. Means were compared by LSD, and means not sharing a letter are significantly different ($P<0.05$).

in paired females that were isolated only on days 2–3 was slightly delayed compared with the positive control (Fig. 5B) and, by comparing them with the standard curve of oocyte growth (Fig. 2), we could estimate that these females experienced a 1-day delay in their ovarian cycle. Overall, the most significant influence on oocyte maturation was transient social interaction or transient social isolation on days 2–3, indicating that females at this age are particularly responsive to social cues (Fig. 5A,B).

Effects of social isolation and social interactions during the gestation period

While social isolation significantly delayed oocyte development and sexual maturation in German cockroach females in both the first and second preoviposition periods, it did not affect the duration of gestation. The mean duration of gestation was the same in socially isolated females (21.49 ± 0.31 days) and females paired with another conspecific female (21.78 ± 0.79 days) (Student's $t=1.7198$, $P=0.0901$). This suggests that when the CA are inactive, social interactions fail to stimulate oocyte maturation.

In 24-day-old females (day 15 of gestation), the length of the basal oocytes was similar whether females were socially isolated (0.50 ± 0.02 mm) or paired with other females (0.52 ± 0.01 mm) ($t=1.7291$, $P=0.2004$) during the gestation period. However, by day 29, one day before normal egg hatch, the oocytes of socially paired females grew significantly larger (0.66 ± 0.02 mm) than in socially

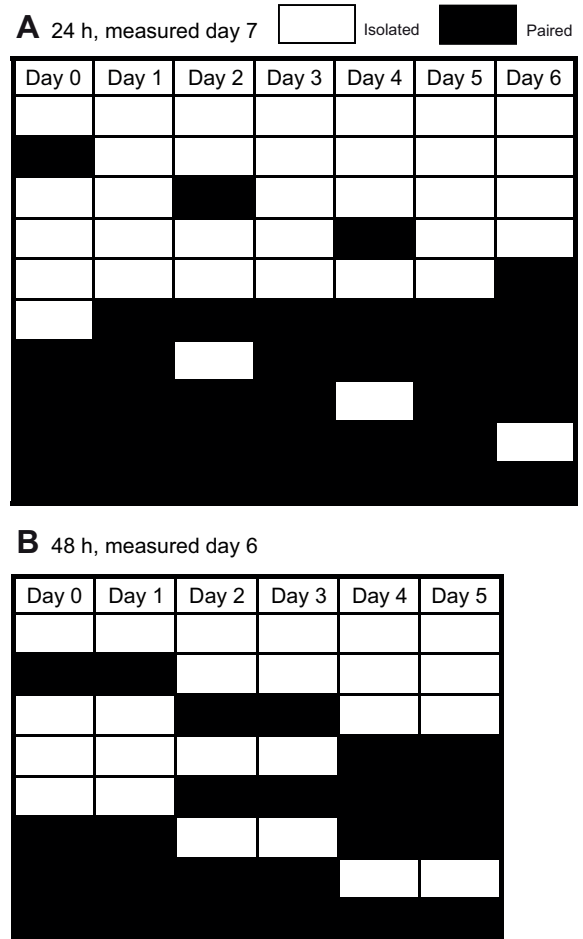


Fig. 4. Experimental design of assays to determine whether certain stages during the preoviposition period are more or less affected by transient social isolation or transient social interactions. (A) Newly enclosed females were either socially isolated and then transiently paired with another female or paired and then transiently socially isolated for 24 h either on day 0, 2, 4 or 6, and their basal oocytes were measured on day 7. (B) Other groups of females were similarly treated but they were transiently paired or transiently socially isolated for 48 h instead of 24 h either on days 0–1, 2–3 or 4–5, and their basal oocytes were measured on day 6. Open rectangles represent social isolation, closed rectangles represent socially paired conditions. Each row corresponds to a treatment (bar) in Fig. 5.

isolated females (0.61 ± 0.02 mm) ($t=1.7081$, $P=0.0314$, $N=12$ and 15, respectively) (Fig. 6). Thus, growth of the basal oocytes is suppressed during most of the gestation period. However, just before the eggs hatch, the basal oocytes begin to grow and their growth rate is affected by social isolation or social interactions.

DISCUSSION

The principal observation emerging from our investigation is that the reproductive rate of adult *B. germanica* females can be modulated by social conditions; social isolation slows both oocyte maturation and the onset of sexual receptivity whereas social interactions, as minimal as between just two females, speed up reproduction. Moreover, our results show that social conditions influence the reproductive rate only when the CA are active or competent to produce JH, the major gonadotropic hormone in cockroaches. Notably, the endocrine-gonadal response to social

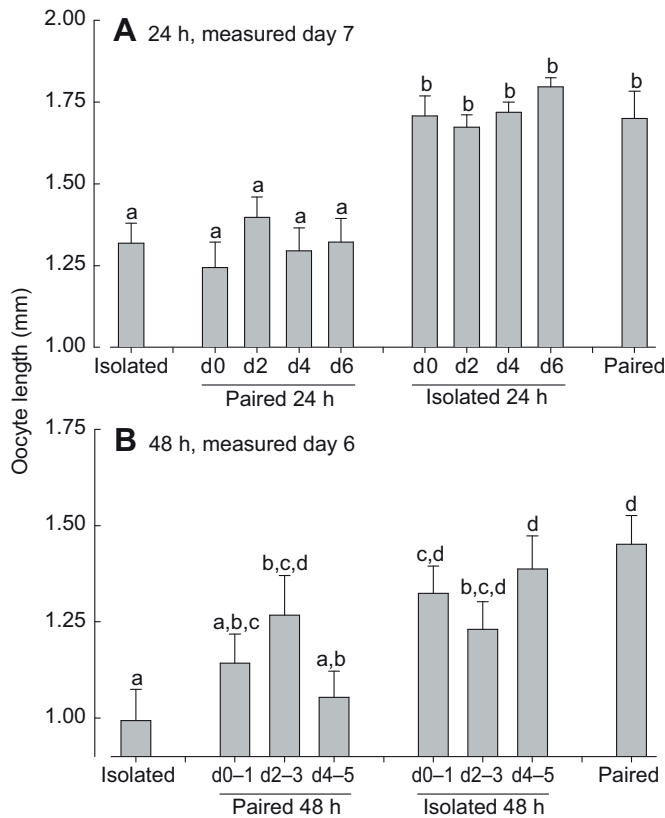


Fig. 5. Differential responsiveness of *B. germanica* females to social interaction and social isolation during the preoviposition period. (A) Newly eclosed females were either socially isolated and then transiently paired with another female or paired and then transiently socially isolated for 24 h either on day 0, 2, 4 or 6, and their basal oocytes were measured on day 7 ($N=19-21$). See design in Fig. 4A. ANOVA: $F_{3,195}=12.27$, $P<0.0001$. (B) Other groups of females were similarly treated, but they were transiently paired or transiently socially isolated for 48 h instead of 24 h either on days 0-1, 2-3 or 4-5, and their basal oocytes were measured on day 6 ($N=17-19$). See design in Fig. 4B. Variation around the mean is represented by the standard error of the mean (s.e.m.). ANOVA: $F_{7,134}=4.02$, $P=0.0005$. Means not sharing a letter are significantly different ($P<0.05$).

interactions was independent of mating status, as both virgin females in the first ovarian cycle and mated females in the second ovarian cycle reproduced faster in response to social cues. Even pregnant females responded to social interactions late in gestation, as their CA became competent to produce and release JH. On the other hand, throughout most of gestation, when CA activity was suppressed by the brain, social interactions and social isolation did not affect the female's reproductive physiology.

Reproduction in *B. germanica* females is under the control of JH, which paces the reproductive rate and governs the duration of the preoviposition period, and whose absence enables a protracted gestation (for reviews, see Schal et al., 1997; Treiblmayr et al., 2006). The rate of oocyte maturation in socially isolated, group-housed and over-crowded females reflects the rates of JH production by the respective CA; smaller basal oocytes in isolated and crowded females (20 females per dish) corresponded well with lower rates of JH synthesis, whereas larger oocytes in pair-housed and grouped females (eight per dish) paralleled higher rates of JH synthesis on day 6. These results indicate that social interactions, through yet unknown sensory modalities and CNS integration, strongly influence

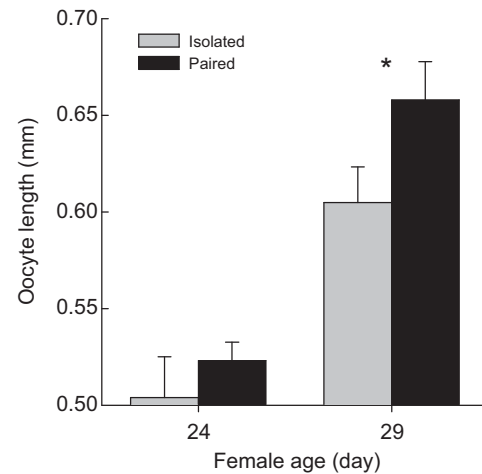


Fig. 6. Effects of social isolation and group-housing on oocyte length at the end of the first gestation period. Females were mated on day 6, and females with a fully rotated egg case on day 9 were either socially isolated or paired with another female during the ensuing gestation period. The effects of these treatments on oocyte length were assessed by measuring the basal oocytes on day 24 (day 15 of gestation), and again on day 29 (day 20 of gestation). $N=10-15$ females. Each bar shows the mean \pm s.e.m. Significant differences between treatments are shown with an asterisk (Student's t -test for day 24, $t=1.7291$, $P=0.2004$; Student's t -test for day 29, $t=1.7081$, $P=0.0314$).

the rates of JH biosynthesis in German cockroach females. They also validate, yet again, that the size of the basal oocytes is an excellent indirect measure of the influence of social conditions on the female's JH biosynthetic rates.

Socially facilitated nymphal development has been documented in all cockroach species that have been rigorously examined (Roth and Willis, 1960; Izutsu et al., 1970; Woodhead and Paulson, 1983; Holbrook and Schal, 2004; Lihoreau and Rivault, 2008). German cockroach nymphs reared in social isolation develop much more slowly than groups of 2-100 individuals; however, there were no significant differences in growth rates among the grouped treatments, presumably because the sizes of the experimental arenas in these tests were adjusted to maintain the same nymphal density (Ishii and Kuwahara, 1967; Izutsu et al., 1970). To test whether social facilitation of reproduction in females is density dependent, we maintained adult females at different densities (1, 2, 8 and 20) in same-size experimental arenas. Oocyte maturation was significantly delayed in socially isolated females compared with pair-housed females, and this effect was even more pronounced with eight females per dish. However, at a density of 20 females per dish, delays in oocyte maturation were observed again. Because crowding induces the production of salivary proteins that also function as repellents for alarm or defensive purposes (Ross and Tignor, 1988), it would be interesting to know whether these proteins are responsible in part for impeding JH production and oocyte maturation.

Faster oocyte maturation in group-housed females may be related to differential food intake by females under different social conditions. However, Holbrook et al. found that although feeding is socially stimulated in adult female *B. germanica* - food consumption was higher in females that were paired with conspecific females since adult eclosion than in females reared in social isolation - oocyte development in these two treatments differed even when all females were provided with the same amount of food

(Holbrook et al., 2000). Therefore, other non-nutritional factors are likely to mediate the social facilitation of reproduction in *B. germanica*.

Physiological responses to stressful conditions are often linked to the circadian clock (for a review, see Boerjan et al., 2010). Therefore, it is possible that stressful conditions, such as absence of a shelter during the photophase of nocturnally active cockroaches, could result in slower oocyte maturation in socially isolated females. This is especially relevant because in large-cage demographic studies of *B. germanica*, the presence of shelters has been shown to contribute to faster nymphal development, greater adult body mass and greater female fertility (Gemeno et al., 2011). However, in our 6-day assays, the presence of shelters did not enhance oocyte maturation in either socially isolated females or in pair-housed females. These results further support the idea that social interactions affect reproduction through specific sensory and neuronal pathways that affect the neuroendocrine system rather than through stress-induced effects.

Four major life history characteristics of *B. germanica* feature in the evolution of social facilitation of reproduction. First, like many pest species, these cockroaches are highly gregarious. Second, unlike some other cockroach species that can reproduce parthenogenetically, German cockroach females must mate in order to successfully reproduce. Third, while females may mate multiple times during their 8–12-month adult lifespan, they obtain and store enough sperm in their first mating to last their entire reproductive life, so multiple matings are not essential (Cochran, 1979). Finally, unlike many insect species, including some cockroaches, *B. germanica* virgin females do not resorb their oocytes, and instead oviposit a large, malformed and inviable egg case that represents a significant nutritional and energetic investment. One hypothesis to account for the evolution of social facilitation of reproduction is that a single colonizer female that finds herself socially isolated from conspecifics ought to slow down her oocyte development until a potential mate arrives, in order to prevent or at least delay ovipositing an inviable egg case. Under these conditions, social interactions would convey to the female the presence of a potential mate, and she should then accelerate her reproductive rate to become sexually receptive faster to maximize her fitness. A corollary of this hypothesis would further predict that females should respond to the inhibitory effects of social isolation only as virgins in the first ovarian cycle, but not in subsequent ovarian cycles after being mated, because mates are no longer essential. This prediction, however, was not borne-out by our results; oocyte maturation in mated females in the second ovarian cycle was also subject to both the inhibitory effects of social isolation and the stimulatory effects of social interactions. Therefore, it is plausible that socially facilitated reproduction evolved because social interactions with conspecifics offer other benefits to the adult female, such as greater foraging efficiency, protection from predators, exchange of symbiotic microbes or even modification of the microclimate.

Not surprisingly, based on the modulation of JH production during the ovarian cycle, we found that females were differentially responsive to social cues during the preoviposition period. Transient social interactions, or transient social isolation, for 24 h had no statistically discernible effects on oocyte maturation. However, longer transient social interactions or social isolation for 48 h significantly influenced oocyte maturation, mostly around days 2–3. These observations suggest that females respond maximally to social conditions when their CA become competent to produce JH. Around 2–3 days after eclosion, the CA attain a high competence to produce large amounts of JH, as determined by stimulating the

CA *in vitro* with the JH precursor farnesoic acid (Gadot et al., 1989a). It seems reasonable that females would be most responsive to social/isolation stimuli at times when their CA are most responsive to CNS disinhibition, but we were surprised to find that females with maximally active CA on days 4–6 (Schal et al., 1997) were less responsive to social stimuli than 2–3-day-old females. It is possible that at later stages of oocyte maturation the CA are committed to produce JH at high biosynthetic rates and they are therefore less responsive to CNS directives associated with social stimuli. However, further experiments will be required to confirm our speculations.

Schal and Chiang established that for females to develop and express sexual receptivity the CA must be active and produce JH, and females mate only when the JH titer reaches a certain threshold level (Schal and Chiang, 1995). Although the ovaries are not needed for *B. germanica* CA to become active and for females to mate (Gadot et al., 1991), the inextricable relationship between JH production and the rate of oocyte maturation in normal *B. germanica* females suggests that oocyte size could be used to pinpoint a minimal threshold of JH production below which most females would refuse to mate. Indeed, in ovoviviparous *Leucophaea maderae* females, this threshold was found to be 1.08 ± 0.01 mm (Engelmann, 1960), and most virgin *B. germanica* females accept males when their oocytes are >1.25 mm in length (Schal and Chiang, 1995; Schal et al., 1997). In controlled experiments that examined sexual receptivity and oocyte length in socially isolated females and in females paired with a conspecific female, we found that significantly more paired females mated on either day 6 or day 7 compared with socially isolated females. Moreover, the great majority of paired females that mated on day 6 or day 7 had oocytes >1.25 mm. These results confirm that social interactions not only stimulate oocyte maturation in *B. germanica* females but also hasten the onset of sexual receptivity by at least a day. These results also verify that both physiological and behavioral reproductive events are JH regulated and that these events are strongly influenced by social conditions.

A previous study showed that social interactions accelerate and synchronize the timing of oviposition in virgin *B. germanica* females, whereas social isolation delays oviposition and prompts isolated females to oviposit asynchronously (Gadot et al., 1989b). In addition, Lihoreau and Rivault (Lihoreau and Rivault, 2008) reported that social conditions also influence the number of ovarian cycles (i.e. number of egg cases produced) during the female's lifespan, resulting in significantly fewer cycles per socially isolated virgin female than in socially grouped virgin females. Our results with mated females show that the disparity between socially isolated and socially grouped females is due to different lengths of preoviposition periods and not to differences in their gestation periods; egg hatch occurs approximately 20–21 days after oviposition in both sets of females. This is consistent with the hypothesis that during periods that require the JH titer to remain low for a protracted gestational period, stimulatory social cues, such as those associated with social interactions, are somehow uncoupled from the CNS pathways that activate the CA, rendering females reproductively unresponsive to social cues. Notably, however, slight differences were evident in the maturation of the basal oocytes of socially isolated and socially paired females in the last few days of gestation, corresponding with a slight elevation in the rates of JH production and the JH titer in preparation for the next ovarian cycle (Treiblmayr et al., 2006). This mechanism of integrating social stimuli with endocrine function is adaptively consistent with the strategy of inhibiting vitellogenesis during gestation, because oocyte maturation would result in the abortion of the incubated egg case.

Our study sheds light on the effects of social interactions on JH production, which in turn regulates the rate of female reproduction in the German cockroach. We demonstrated that female density influences oocyte development, indicating that the quantity of social stimulation is an important factor in the social facilitation of female reproduction; the presence of one conspecific female was sufficient to accelerate oocyte maturation, but high female density delayed oocyte maturation through a crowding effect. In aggregate, our results on the stage-specific responses of females to social interactions support the conclusion that social interactions represent a powerful environmental stimulus that modulates the activity of the CA in concert with other extrinsic and internal cues. Therefore, through their effects on the synthesis and release of JH, social interactions indirectly modulate all JH-dependent activities including the synthesis of vitellogenin and other female-specific proteins, attainment of sexual receptivity, production of sexual signals, mating, and the time-course of oviposition.

The sensory modalities that participate in the social facilitation of reproduction and the sensory and CNS pathways that couple social cues and JH production are largely unknown. *B. germanica* is thus a fascinating model system in which to delineate which sensory cues influence JH production and how these cues influence CA activity during vitellogenesis but are somehow uncoupled from influencing the CA during gestation. We are also interested in comparative studies to understand why socially facilitated reproduction has evolved in some cockroach species, such as *B. germanica*, and not in others (e.g. *S. longipalpa*).

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