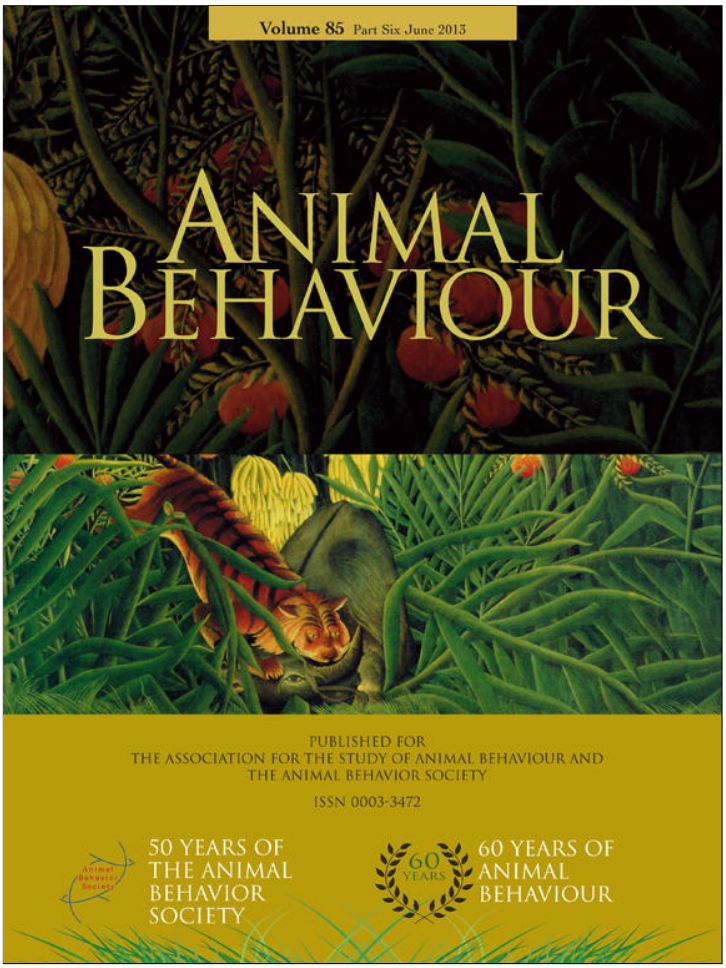

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Social interaction facilitates reproduction in male German cockroaches, *Blattella germanica*

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Sociality can be beneficial for individuals because it may increase access to resources, provide greater protection from predators and create opportunities for mating. In some species, social conditions and experience also influence the physiology and behavioural responses of individuals. Although social facilitation of sexual maturation is poorly understood in insects, the German cockroach has served as an important model system for this phenomenon as group-housed females mature faster than solitary females. However, social modulation of male reproduction has not been investigated in this or any other nonpolyphenic insect species. We investigated the relative effects of social interactions and isolation on endocrine, reproductive and behavioural events during sexual maturation of *B. germanica* males. Results show that social interactions significantly facilitate juvenile hormone biosynthesis as well as protein production by the accessory reproductive glands, whereas isolation suppresses these processes. Other processes, such as accumulation of cuticular hydrocarbons, maturation of the male's courtship readiness and his readiness to copulate, appear to be relatively independent of social experience. However, both courtship and copulation latencies declined with age more in socially paired males than in isolated males. Our study is the first to reveal that social interactions have a profound effect on certain aspects of male reproductive physiology and behaviour in an insect species that does not exhibit phase or developmental polyphenism.

The social environment can exert a powerful influence on an individual's development, growth, metabolism and reproduction (Pulliam & Caraco 1984; Krause & Ruxton 2002). Many examples have emerged of social facilitation of growth and development rates in various insect species (Chauvin 1946; Long 1953; Wharton et al. 1968; Izutsu et al. 1970; Woodhead & Paulson 1983; McFarlane et al. 1984; Weaver & McFarlane 1990; Holbrook & Schal 1998; Lihoreau & Rivault 2008; Ronnas et al. 2010) and on polyphenic development (reviewed in Simpson et al. 2011), where the environment, including the presence of conspecifics, can result in different phenotypes.

There are prominent examples of social facilitation of reproduction, with most describing the effects of male facilitation of female sexual maturation; studies on the social modulation of male reproduction have been limited mainly to vertebrates (reviewed in Rekwot et al. 2001). Surprisingly, however, apart from eusocial species (reviewed in Le Conte & Hefetz 2008), examples of social facilitation of reproduction are considerably scarcer for insects, and most involve behavioural rather than physiological changes in females. Socially facilitated oviposition is most common, as for example in *Aedes aegypti* mosquitoes, where oviposition decisions are governed in part by the presence and density of conspecific eggs and larvae (e.g. Reiter 2007). In *Rhagoletis pomonella* flies, females oviposit more in groups than singly (Prokopy & Reynolds 1998), and egg production and oviposition in blowflies exhibit positive density dependence (Davies 1998). Although group living has been shown to facilitate growth rates in most cockroach species examined to date (Roth & Willis 1960; Woodhead & Paulson 1983; Holbrook & Schal 2004; Lihoreau & Rivault 2008), faster reproduction due to social influence has been documented in females of only one species, the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) (Gadot et al. 1989a). Females of the German cockroach reproduce faster in groups, whereas isolated females show slower oocyte maturation and delayed onset of sexual receptivity (Gadot et al. 1989a; Holbrook et al. 2000; Lihoreau & Rivault 2008; Uzsák & Schal 2012; Uzsák & Schal 2013). Perhaps this is not surprising since German cockroaches live in aggregations (Rust et al. 1995), but females of another commensal cockroach, *Supella longipalpa* (Blattellidae), which also live in aggregations, do not show social facilitation of reproduction (Chon et al. 1990).

Density dependence of male reproductive behaviour appears to be common and is most often expressed as a lower sexual threshold in isolated adult males, as shown for example in *Drosophila paulistorum*

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courtship (Kim & Ehrman 1998). Social facilitation of male reproductive physiology, on the other hand, is a rare and relatively uninvestigated phenomenon, limited to cases of phase and developmental polyphenism (e.g. aphids, crickets, locusts). The best studied case involves locusts, in which sexual maturation of males can be socially facilitated or retarded, as part of a general density-dependent phase polyphenism that affects a wide range of phase characteristics including anatomy, morphology, coloration, behaviour, ecology, development and reproduction (reviewed in Pener & Simpson 2009). The colour of adult *Schistocerca gregaria* changes with crowding, and both males and females sexually mature faster in the presence of sexually mature older males (Norris 1954). On the other hand, *Locusta migratoria migratorioides* males reared in isolation mature faster than those reared under crowded conditions (Norris 1964).

Socially facilitated reproduction may be mediated through direct contact between conspecifics, or via visual, olfactory, gustatory or auditory cues associated with group members (e.g. Uzsák & Schal 2013). However, the mechanisms that underlie social facilitation of insect sexual maturation are poorly understood. The juvenile hormones (JHs) are pivotal gonadotropic hormones in insect reproduction (Raikhel et al. 2005), and in German cockroach females JH-III is produced and released by the corpora allata (CA) in a stage-specific manner: JH biosynthesis increases and stimulates oocyte maturation, declines through ovulation and remains low during gestation (Bellés et al. 1987; Gadot et al. 1989b; Sevala et al. 1999; Treiblmayr et al. 2006). JH controls and paces many processes associated with reproduction in *B. germanica* females, including the synthesis of yolk and oothecal proteins, sexual receptivity, production of sex pheromones, mating and the time course of oviposition (reviews: Schal et al. 1997; Treiblmayr et al. 2006). Sensory cues associated with social interactions in females significantly affect CA activity, demonstrating that social facilitation of reproduction is coupled to the endocrine system (Uzsák & Schal 2012). It is not known, however, whether *B. germanica* males have a similar response to social interactions as do females.

Although JH release rates are considerably lower in males than in females in *B. germanica*, JH production in males increases steadily after adult emergence, as in females (Piulachs et al. 1992). This pattern suggests that JH might be important in male reproduction as well. Indeed, JH has been found to regulate male accessory reproductive gland (ARG) development during sexual maturation in *B. germanica*: CA removal resulted in lower total protein content of the ARG, and administration of JH restored normal ARG development (Piulachs et al. 1992). Furthermore, as the spermatophore forms during copulation, conglobate gland (a part of the ARG) proteins are depleted and JH production by the CA declines; but within several hours after copulation JH synthesis rates are elevated and proteins accumulate in the conglobate gland (Vilaplana et al. 1996b). The amount of a specific ARG protein, *Blattella germanica* allergen 4 (Bla g 4), increases gradually after adult emergence and reaches a plateau by day 14 (Fan et al. 2005). This age-modulated production of Bla g 4 is regulated by JH, and topical administration of JH results in elevated Bla g 4 levels (Fan et al. 2005). Thus, ARG size and protein content appear to be reliable measures of reproductive maturation in males, just as oocyte size and yolk content are in females, to investigate effects of social facilitation on reproduction.

Other *B. germanica* male physiological and behavioural events that might be socially facilitated during reproductive maturation include the accumulation of cuticular hydrocarbons (CHCs), courtship and copulation readiness, and the ability to successfully inseminate females. After adult emergence, CHCs accumulate on the cuticular surface and in the haemolymph of *B. germanica* females (Schal et al. 1994), so it is plausible that production of CHCs might follow a similar pattern in males. Moreover, accumulation of CHCs might be affected by social experience, as shown in *Drosophila melanogaster*, where isolation during development leads to greater quantities of CHCs (Kim et al. 2004) and the genotypic composition of adult social groups affects the pattern of male pheromone (CHC) accumulation on the cuticle (Krupp et al. 2008). JH controls the expression of sexual receptivity in the female German cockroach; the CA must be present and active for females to become sexually receptive and for copulation to occur (Schal & Chiang 1995). Males, however, become sexually receptive even if their CA are extirpated (allatectomized), but sexual readiness is delayed; conversely, administration of JH accelerates sexual readiness in males (Schal & Chiang 1995). It is conceivable that the influence of JH on sexual maturation in males might be mediated by social cues, as in females.

Here, we investigated the relative effects of social isolation and social interactions in *B. germanica* males on (1) JH biosynthesis by their CA, (2) protein content of their ARG, (3) the onset age of courtship, (4) copulation readiness (latency to copulate) and (5) sperm maturation, measured indirectly as the frequency of eggcase abortion. We conclude that group living facilitates reproduction in adult males, as it does in adult females, but this social facilitation is of considerably lower magnitude in males than in females.

METHODS

Insects

Adult male *B. germanica* were collected from a laboratory colony of insecticide-susceptible German cockroaches (American Cyanamid strain, also called Orlando strain, obtained in 1989) reared at 27 ± 1 °C and 40–70% ambient relative humidity on a 12:12 h light:dark regime, with unlimited access to water and food pellets (LabDiet 5001 Rodent Diet, PMI Nutrition International, Brentwood, MO, U.S.A.). Newly emerged adult males (day 0) of similar size, degree of sclerotization and with intact wings were selected for all experiments. Individuals were either isolated or randomly assigned to pairs or groups, housed in plastic petri dishes (90 mm diameter, 15 mm high, Fisher Scientific, Pittsburgh, PA, U.S.A.), provisioned with food and water ad libitum, and maintained and observed under the environmental conditions described for the colony above. We use 'paired' to denote pair-housed males (i.e. a *B. germanica* male housed in the same dish with another male).

JH-III Biosynthesis by the CA

To assess the effect of male density on JH biosynthesis, isolated and grouped males (2, 4, 8, 12, 16, 20 males) were housed for 7 days in plastic petri dishes (200 mm diameter, 20 mm high, Fisher Scientific). JH biosynthesis was measured in vitro using a radiochemical assay developed by Pratt & Tobe (1974), with modifications following Holbrook et al. (2000). This assay is based on the stoichiometric incorporation of radiolabelled methyl group from methionine into JH-III under equilibrium conditions. Briefly, the CA-corpora cardiaca complexes were dissected from cold-anaesthetized decapitated males and incubated in 6×50 mm glass culture tubes in 0.1 ml of modified methionine-free TC-199 medium (Specialty Media, Lavalette, NJ, U.S.A.), supplemented with 100 μM of L-[^{3}H-methyl]-methionine (198 mCi/mmol; NEN, Wilmington, DE, U.S.A.), 5 mM of $CaCl_2$, 25 mM HEPES and 20 mg/ml of Ficoll type 400. After a 3 h incubation, the medium was extracted with 0.25 ml of isooctane, an aliquot of which was assayed with a liquid scintillation counter.

Bla g 4 Protein Content of the ARG

Newly emerged adult males (day 0) were socially isolated or housed in pairs in petri dishes. On day 3 or 10, males were cold-

anaesthetized and the ARG complex was carefully removed, homogenized in 0.25 ml of PBS and centrifuged (10 000 revolutions/min at 4 °C for 20 min). The pellet was rehomogenized and centrifuged again in fresh PBS, and the two supernatants were combined and stored at −80 °C. We used an indirect ELISA that we previously developed and optimized to quantify Bla g 4 protein using recombinant Bla g 4 standards (Fan et al. 2005).

Quantification of Cuticular Hydrocarbons

Newly emerged adult males (day 0) were socially isolated or housed in pairs in petri dishes. Males were freeze-killed at various ages and extracted individually for 1 min in 0.5 ml of *n*-hexane containing 5 μg of *n*-hexacosane as an internal standard. The extract was slowly reduced to 0.1 ml under a gentle stream of N_2 and 1 μl was introduced with an Agilent 7683B autoinjector into the split–splitless inlet (operated at 310 °C in splitless mode) of an Agilent 7890A gas chromatograph (GC). CHCs were separated on a DB-5 column (20 m × 0.18 mm × 0.40 μm film thickness), with hydrogen as the carrier gas at a linear velocity of 40 cm/s, and detected with a flame-ionization detector. Total CHCs were calculated by comparing the summed area of all integrated CHC peaks to the integrated area of the internal standard peak.

Courtship Readiness

Newly emerged adult males (day 0) were housed in petri dishes either isolated or in pairs for 2–3 days. To determine whether paired males attain courtship readiness (capability to court females) before males maintained in social isolation, we used a modification of the 'antenna on a stick' assay developed by Roth & Willis (1952), as recently modified by Eliyahu et al. (2009). An antenna of a cold-anaesthetized 6-day-old virgin *B. germanica* adult female was carefully ablated with fine scissors just distal to the scape, and inserted into a small mass of modelling clay at the end of a glass Pasteur pipette. This antenna was used immediately to stimulate the antennae of test males that were individually housed in small plastic cages. The antennae of each male were gently stroked with the test antenna for up to 2 min, and a positive response and its latency were recorded when the male showed the first courtship response. Courtship is an unmistakable behaviour that occurs only in a sexual context and consists of the male rotating his body relative to the stimulus and raising his wings (Roth & Willis 1952). Eliyahu et al. (2009) used each antenna to stimulate several groups of 10 males each without loss of effectiveness. In our assays, one female antenna was used for three successive assays (<6 min total) with one isolated male, one paired male (one male of the pair was discarded before the assay), and a control group-housed male that was 13–14 days old. The order of assays was randomized, and all assays were conducted during the second half of the scotophase, when copulation is most frequent (Liang & Schal 1993), avoiding the last 2 h of the scotophase. Sample size was 41–74 males per treatment.

Copulation and Mating Success

To determine whether paired males attain greater copulation success (mate at a younger age, successfully inseminate and fertilize females) than isolated males, newly emerged adult males (day 0) were housed in petri dishes either isolated or in pairs. All isolated males were transferred from petri dishes into a plastic cage (185 × 130 mm, 105 mm high, Althor Products, Windsor Locks, CT, U.S.A.) where they were exposed to twice as many sexually mature 6-day-old virgin females. Paired males were treated the same way. We then monitored copulations for 2 h and removed copulating pairs every 15 min and placed them in petri dishes. Because copulation lasts ~90 min in this species (Schal & Chiang 1995), this design guaranteed that we captured all successful copulations. The time to copulation (to within 15 min) was recorded. When the copulating pair separated, males were discarded and females were given food and water and monitored daily for oviposition, hatching of nymphs and eggcase abortions. This experiment was conducted several times with 1-, 2-, 3-, 4-, 5- and 6-day-old males with 18–156 males per treatment. Different males were used each day and compared to 13–14-day-old males.

Data Analysis

Data were analysed with SAS® 9.1.3 software (SAS Institute Inc. 2002–2003, Cary, NC, U.S.A.). We used one-way ANOVA to test for the effects of social condition on JH synthesis rates, and two-way ANOVA to test for the effects of social condition and age on JH synthesis, ARG content and CHC amount. PROC GLM was used to obtain residuals from the adjusted model, and the homogeneity of variances was tested using Brown–Forsythe test to check whether residuals would hold the assumption of homogeneous variances within each treatment. Chi-square logistic regression was used to test for the effects of social condition and age on the frequencies of mating and the frequencies of female abortion. All ANOVA tests were followed by Fisher least significant difference (LSD) post hoc tests. For all tests, the level of significance was set to $P < 0.05$. Variation around the mean is represented by the standard error of the mean.

Ethical Note

We used the most humane methods possible in all experiments. We did not observe any adverse effects from isolation and all insects were properly anaesthetized prior to dissections.

RESULTS

Social Facilitation of JH-III Synthesis in Paired B. germanica Males

The CA of pair-housed males produced significantly more JH on day 7 than did the CA of isolated males (one-way ANOVA: $F_{6,232} = 11.79$, $P < 0.0001$; Fig. 1). This effect was even more pronounced at higher male densities up to 20 males per dish. Because the social facilitation of JH production was already apparent in paired males, we compared the time course of social facilitation using only isolated and paired males. JH production increased with age in both isolated and paired males, but within 3 days of adult emergence, CA activity of paired males began to diverge from that of socially isolated males; by day 7, rates of JH synthesis were significantly higher in paired males (2.23 ± 0.10 pmol/h per CA pair, $N = 58$) than in isolated males (1.73 ± 0.08 pmol/h per CA pair, $N = 59$) (two-way ANOVA: social condition effects: $F_{1,343} = 46.83$, $P < 0.0001$; age effects: $F_{6,343} = 20.70$, $P < 0.0001$; Fig. 2). Interestingly, the differences between paired and isolated males persisted through at least day 19. These results indicate that the rates of JH biosynthesis are socially facilitated in adult males, as previously demonstrated for females.

Social Facilitation of ARG Protein

Because JH release rates influence ARG protein production in males (Piulachs et al. 1992; Vilaplana et al. 1996a; Fan et al. 2005), we examined the effect of social conditions on Bla g 4, a protein produced exclusively in the male ARG. Results showed that 3 days of social interaction with a conspecific male were not sufficient to

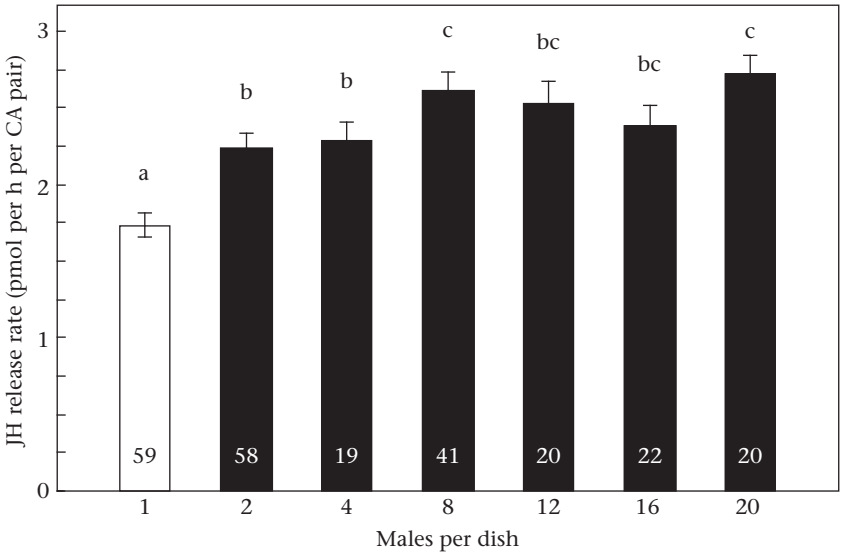

Figure 1. The effect of *B. germanica* adult male density on JH-III biosynthesis on day 7. Bars represent means ± SE. Sample size is indicated within each bar. One-way ANOVA: $F_{6,\ 232} = 11.79$, $P < 0.0001$. Means were compared by LSD, and means not sharing a letter were significantly different ($P < 0.05$).

significantly facilitate Bla g 4 protein production in paired males. However, the Bla g 4 content of the ARG steadily increased, and the ARG of 10-day-old males contained three to four times more Bla g 4 than did the ARG of 3-day-old males (two-way ANOVA: age effects: $F_{1,34} = 47.52$, $P < 0.0001$; Fig. 3); by day 10, paired males produced significantly more Bla g 4 than isolated males (two-way ANOVA: social condition effects: $F_{1,34} = 14.55$, $P = 0.0005$). The pattern of accumulation of Bla g 4 protein by the ARG confirms that its expression is socially facilitated in adult males.

Accumulation of CHCs in Males Is Not Socially Facilitated

The CHCs gradually increased in both isolated and paired males throughout the first 15 days after adult emergence: CHCs increased in isolated males from 77.56 ± 3.74 μg on day 1 to 123.75 ± 5.47 μg on day 15, and in paired males from 79.99 ± 2.42 μg on day 1 to 123.51 ± 5.98 μg on day 15 (two-way ANOVA: age effects: $F_{7,144} = 31.28$, $P < 0.0001$; Fig. 4). However, there were no significant differences in the quantities of CHCs between isolated and paired males on any of these days ($F_{1,144} = 0.96$, $P = 0.3295$). Thus, social interactions with conspecific males do not appear to influence the rate of hydrocarbon accumulation on the cuticular surface.

Effects of Social Conditions on Male Courtship Behaviour

The 'antenna-on-a-stick' elicited courtship behaviour in 100% of 13–14-day-old males within 12.7 ± 1.26 s ($N = 115$; Fig. 5a, b). We used 2- and 3-day-old males to assess the maturation of their courtship response. A surprisingly large percentage of 2-day-old males courted the female antenna, but responses were independent of the males' social conditions. Even more 3-day-old males showed courtship behaviour (chi-square test: age effects: $\chi^2_1 = 9.06$,

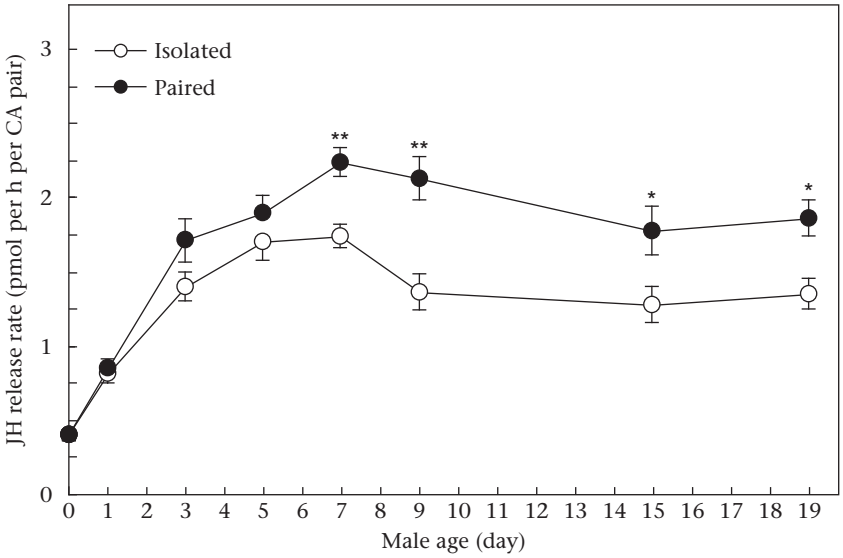

Figure 2. In vitro JH-III release rates during the first 19 days for adult *B. germanica* males. Values represent means ± SE of 20–59 males. Asterisks represent significant differences between the two treatments on the same day (Student's unpaired *t* test: $^*P < 0.01$; $^{**}P < 0.001$).

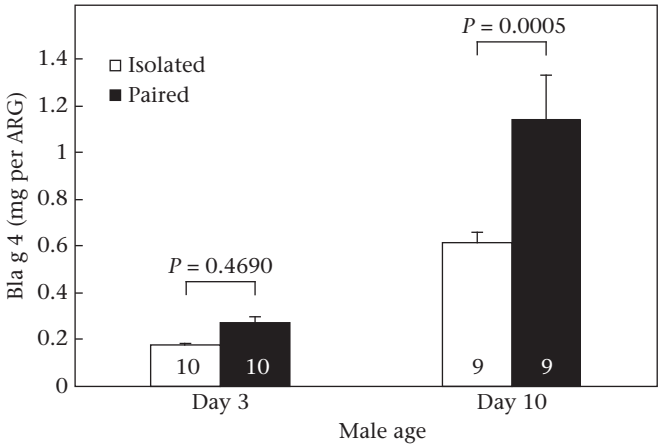

Figure 3. Social facilitation of protein accumulation in the adult male accessory reproductive glands of *B. germanica*. Bars represent means ± SE, sample size is indicated within each bar, and *P* values represent differences between treatments within age group (two-way ANOVA for social condition effects).

$P = 0.0026$), but also independently of their social conditions (Fig. 5a). Notably, however, while paired 3-day-old males responded to female antennae significantly faster than similarly maintained 2-day-old males (53.3% faster; two-way ANOVA: age effects: $F_{1,159} = 13.92$, $P = 0.0003$), the courtship response of 3-day-old isolated males was only 8.7% faster than that of 2-day-old males ($F_{1,159} = 0.23$, $P = 0.6323$; Fig. 5b). These results indicate that the courtship response in males matures much earlier than we suspected, and the only significant social facilitation of this behavioural response was the decline in its latency between days 2 and 3.

Social Facilitation of Copulation Success in Males

The percentage of males that copulated increased with age, with no apparent differences between isolated and paired males. None of 91 tested males copulated on day 1, only 18.6% of 2-day-old males copulated ($N = 311$), 89.5% of the males mated on day 6 ($N = 38$), and 100% of 13–14-day-old males mated ($N = 47$; Fig. 6a). We found significant differences between 2-day-old males and all older males (chi-square test: age effects: $\chi^2_4 = 93.087$, $P < 0.0001$), but not between isolated and paired males at any age ($\chi^2_1 = 1.33$, $P = 0.2500$). We also examined the latency of copulation over the 2 h of observation, and likewise found no significant differences

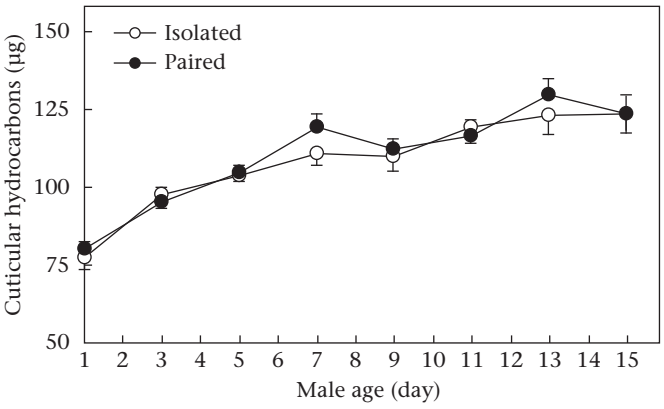

Figure 4. Effects of social isolation and social interactions on the amount of hydrocarbons on the cuticular surface of *B. germanica* adult males. Values are means ± SE of 10 males.

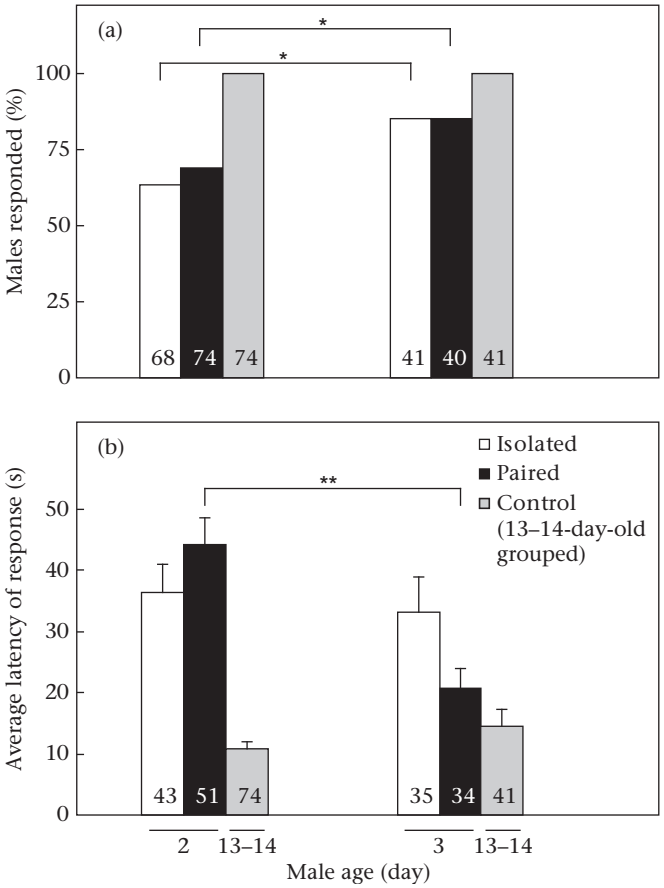

Figure 5. Effects of isolation and social interactions on courtship behaviour in *B. germanica* males. (a) Percentage of males that showed a courtship response (logistic regression and chi-square test). (b) Average ± SE latency of the courtship response (two-way ANOVA). Separate controls of 13–14-day-old males were compared to 2- and 3-day-old males. Sample sizes are shown within each bar. Asterisks represent significant differences between age groups within treatments: $^*P < 0.01$; $^{**}P < 0.001$.

between socially paired and isolated males until day 6; in all groups, most of the males that mated did so within 35 min after sexually mature females were introduced into the arena. Although the average latency to copulation was lower in paired males than in isolated males on day 6 (17.8 ± 1.51 min versus 28.3 ± 3.61 min) this difference was not significant (two-way ANOVA: social condition effects: $F_{1,202} = 2.13$, $P = 0.1460$). Overall, in paired males the average latency to copulation declined 47.3% from 33.8 ± 3.94 min on day 2 to 17.8 ± 1.51 min on day 6 (two-way ANOVA: age effects: $F_{1,202} = 6.39$ $P = 0.0122$), whereas in isolated males the latency varied little, from 34.2 ± 3.41 min on day 2 to 28.3 ± 3.61 min on day 6 (two-way ANOVA: age effects: $F_{1,202} = 0.82$, $P = 0.3667$; Fig. 6b). Thus, the ability of males to copulate appeared to vary with male social conditions. It is possible, however, that female discrimination of males also contributed to differential latency to copulation.

Unmated *B. germanica* females, as well as females that fail to receive sperm during copulation, oviposit unfertilized eggs into an eggcase and subsequently abort the eggcase (Roth & Stay 1962). Therefore, abortions provide an indirect but reliable measure of male competence to transfer sperm to the female and produce viable progeny. Only 2 of 47 (4.3%) females that mated with 13–14-day-old males aborted their eggcase, whereas more than 60% of females that mated with 2-day-old males aborted their eggcases (Fig. 6c). Significantly more females aborted the eggcase when

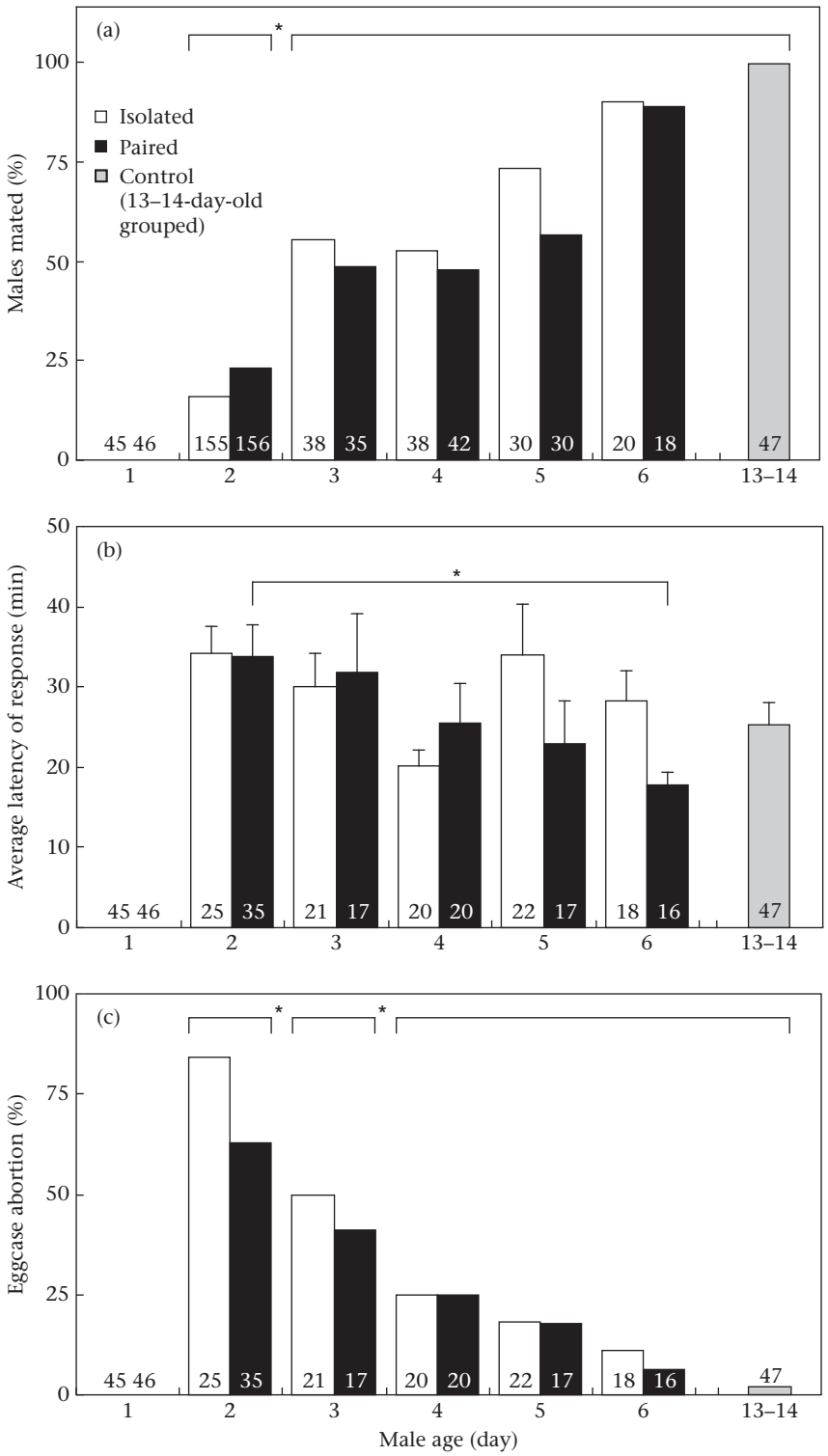

Figure 6. Effects of social isolation and social interactions in *B. germanica* adult males on the frequency and latency of copulation, and the frequency of eggcase abortion. (a) Percentage of males that copulated. (b) Latency to copulation (two-way ANOVA). (c) Percentage of mated females that aborted their eggcases in each male treatment. Sample sizes are shown within each bar. Data in (a) and (c) were analysed by logistic regression and chi-square test for model fit. An asterisk represents a significant difference between age groups ($P < 0.05$).

mated with 2-day-old males (chi-square test: age effects: $\chi^2_4 = 38.51$, $P < 0.0001$) and 3-day-old males ($\chi^2_4 = 5.00$, $P = 0.0300$) than when mated with older males. The frequency of abortions was 33.6% higher in females that mated with 2-day-old isolated males than in females that mated with pair-housed males, but there were no significant differences between these two treatments for males of any age ($\chi^2_1 = 2.15$, $P = 0.1400$). Therefore, male competence to successfully fertilize eggs might be socially facilitated, but these effects are slight and obscured by age-related sexual maturation.

DISCUSSION

Our study revealed that several aspects of male reproductive physiology and behaviour are profoundly socially facilitated in the German cockroach, while others are not. Although social facilitation of male sexual maturation has been demonstrated in phase-polyphenic species (e.g. locusts; reviewed in Pener & Simpson 2009), to our knowledge, this is the first demonstration of social facilitation of reproductive processes, specifically endocrine function, in a nonpolyphenic insect species. Like German cockroach females, males undergo an endocrine-regulated sexual maturation after adult emergence, and social interactions facilitate this process by stimulating higher JH production at a younger age, which in turn stimulates faster production of accessory reproductive secretions and more rapid maturation of sexual receptivity and sexual readiness. Nevertheless, some associated events, such as accumulation of hydrocarbons on the cuticular surface, appear not to respond to social conditions and thus may be relatively independent of JH.

JH regulates the rate of female reproduction in the German cockroach (Bellés et al. 1987; Gadot et al. 1989b; Burns et al. 1991; Schal & Chiang 1995; Schal et al. 1997), and we recently showed that social interactions in *B. germanica* females can modulate the rate of JH production (Uzsák & Schal 2012). Our current results with adult males are consistent with previous reports that JH release rates in males are considerably lower than in females (Piulachs et al. 1992), but the patterns of CA activity in males suggest a role for JH in male sexual maturation, as in females. Also similar to females, we showed that JH synthesis reached higher rates in males that were allowed social interaction with other males, and this response was maximal at a density of 8–20 individuals per cage. With both sexes, however, social facilitation of JH production was evident at densities as low as two paired individuals. Still, two major differences are apparent between the responses of males and females to interactions with conspecific individuals. First, JH production was suppressed in females under crowded conditions (20 females per dish) (Uzsák & Schal 2012), whereas male CA appeared less responsive to crowding under the same conditions. Second, although CA activity was lower in isolated females than in paired females, ultimately females in both treatments oviposited infertile eggs and their CA reached similar activity levels (Gadot et al. 1989b). In males, on the other hand, the differences in JH production persist for at least 19 days, well beyond the period of sexual maturation. It is important to note that all our study subjects were group-reared throughout their 35-day nymphal development, and either isolated or paired only in the adult stage. It is possible that isolation during nymphal development might further magnify the effects of isolation in the adult stage, and the results suggest that differences between grouped and isolated males may persist and affect physiological and behavioural events beyond the period of sexual maturation.

ARG proteins are involved in spermatophore formation, protection of sperm, facilitating sperm transfer to the female, sperm storage within the spermatheca, sperm competition and in modulating female physiology and behaviour (reviewed in: Happ 1984; Gillott 2003; Avila et al. 2011). JH regulates ARG development during male sexual maturation in many insect species, but in some, ARG development appears to be independent of JH (reviewed in: Happ 1984; Gillott 2003). In male *B. germanica,* ARG protein content increases gradually following adult emergence, and this age-related production is modulated by JH (Piulachs et al. 1992; Vilaplana et al. 1996a, b; Fan et al. 2005). We used Bla g 4, an adult male ARG-specific protein that is incorporated into the spermatophore and transferred to the female during copulation (Fan et al. 2005), as a measure of the influence of social conditions on the male's reproductive physiology. The results demonstrated a clear social facilitation of ARG maturation: the Bla g 4 content of pair-housed males was significantly higher than that of socially isolated males on day 10, reflecting the difference in JH production. ARG proteins are depleted during mating, and elevated JH synthesis rates after mating induce the accumulation of new proteins (Vilaplana et al. 1996a). Therefore, it would be interesting to know whether isolated males, because they produce less JH, are also less capable of replenishing their ARG secretions than are socially grouped males, regardless of their age.

We suspected that other aspects of sexual maturation in males would be socially facilitated as well. Surprisingly, however, we found only scant evidence for the effects of social conditions on mating behaviour because rapid age-related maturation within the first 1–3 days after adult emergence appeared to dominate and possibly obscure the effects of social conditions. In investigating the maturation of the male courtship response, we were also surprised to find that, by 3 days after adult emergence, an exceptionally high proportion of the males ($\sim$85%) were able to respond sexually to a sex-pheromone-bearing detached antenna from a sexually mature virgin female. These results contrast with those reported by Nishida & Fukami (1983), where such high sexual responses did not mature until males were 7–8 days old, but the differences most likely resulted from dissimilar rearing conditions. Overall, it appears that male sexual responses mature unusually fast, faster than other physiological processes such as ARG maturation and the male's ability to successfully inseminate the female and fertilize her oocytes (see below). Isolated and grouped males did not differ in courtship attempts and number of matings (Lihoreau et al. 2009), but the latency of the courtship response suggests some limited social facilitation of courtship. Whereas the courtship latency of isolated males changed little between days 2 and 3, in paired males the latency declined by 53.3% in just 1 day, and pair-housed 3-day-old males responded nearly as fast as sexually mature 13–14-day-old males. Further analyses of these fine-scale changes in response to social interactions will require greater sample sizes and possibly slowing the rate of sexual maturation by maintaining males at cooler temperatures.

Successful courtship in *B. germanica* is followed by copulation that lasts $\sim$90 min, during which the male transfers a spermatophore to the female. Young males were either incapable of mating or were rejected by females, but the males' ability to copulate increased with age to 100% success by 2 weeks. Surprisingly, however, about 50% of 3-day-old males mated, whereas in Lihoreau & Rivault (2010) none of the 1–12-day-old males mated with receptive females. Methodological differences, such as the 2 °C difference, and strain differences may be responsible for these divergent results. The only difference we found between isolated and paired males was in the latency to copulation, not in the percentage of males mating, and even this difference was an age-related change rather than an absolute difference between isolated and paired males: the average latency to copulation declined dramatically in grouped males and much less so in isolated males. Moreover, few females mated with 2-day-old males, and most of these females aborted their eggcase, presumably because sperm transfer was ineffective or sperm were inviable. Although the frequency of abortions was much higher in females that mated with isolated males than in females that mated with grouped males, again, this difference was not statistically significant. To unambiguously determine whether the male's competence to successfully mate is socially facilitated will require a larger sample size than the 311 2-day-old males we used.

Finally, we found no evidence of social facilitation of CHCs. CHCs have been investigated as pheromone precursors and as mediators of kin recognition in the German cockroach (Lihoreau & Rivault 2009; Blomquist et al. 2011). While there is no evidence in males

of JH-modulated production of CHC-derived pheromones, as shown in females (reviewed in Blomquist et al. 2011), we hypothesized that social conditions might affect the production of CHCs because of their role in kin recognition. Moreover, as water-proofing agents, more CHCs might be placed on the epicuticle of isolated males to retard water loss. Although the amount of CHCs gradually increased with age, both isolated and grouped males accumulated similar quantities of CHCs. In females, large amounts of CHCs accumulate in internal stores (mostly haemolymph and fat body), more than on the cuticular surface (Schal et al. 1994), so it might be instructive to consider whether social conditions influence the internal accumulation of CHCs in males. Interestingly, in *D. melanogaster,* CHC sex pheromones vary with the circadian clock and social conditions (grouped in single or mixed genotypes), in support of the idea that social context can shape the relationship between an individual's phenotype and genotype (Krupp et al. 2008).

All in all, our results show that social interactions facilitate some aspects of male reproductive physiology, but not others. Maturation of the male's courtship response appears to be relatively independent of social conditions, although its latency as well as the latency to copulation decreased more over time (male age) in grouped males than in isolated males. Likewise, although our results were not significant, eggcase abortion was more common in females that mated with isolated males than those that mated with grouped males. On the other hand, JH biosynthesis and ARG protein production, two critical components of male sexual readiness, were significantly facilitated by group living and repressed by isolation. Indeed, CA activity in isolated males remained lower than in grouped males even beyond the males' sexual maturation. This observation suggests that isolated males may represent a cryptic 'solitarious phase', distinct from 'gregarious' males. It will be important to determine whether these two phenotypes are readily reversible, as shown in females (Uzsák & Schal 2012) and if they differ in their expression of behavioural syndromes, such as aggression, exploration, foraging, sociability and interactions with potential mates, differences that Lihoreau et al. (2009) documented between group-living and solitary nymphs. Unlike locusts, where male sexual maturation and mating behaviour completely depend on the CA and JH, and social conditions affect CA activity (reviewed in Pener & Simpson 2009), sexual maturation in *B. germanica* males is less dependent on both social conditions and JH.

The German cockroach is an exemplary colonist species and an obligatory human commensal. Because populations of *B. germanica* undergo severe bottlenecks and frequent boom-and-bust cycles, it is perhaps not surprising that males of this long-lived species have evolved a sexual maturation system that is relatively independent of social context, compared to females. This difference between the sexes could be related to several life history characteristics of this species. First, *B. germanica* females obtain and store enough sperm from a single copulation to last their entire reproductive life (Lihoreau & Rivault 2010). This demographic feature severely biases the operational sex ratio and would constitute strong selective pressure on males to mature rapidly and more independently of social conditions, and express high sensitivity to female pheromones and low courtship response thresholds as early as possible (Eliyahu et al. 2009). This was apparent as some young males showed courtship responses and mated before they were capable of transferring viable sperm. Second, unlike many insect species, including some cockroaches, unmated German cockroach females do not resorb their oocytes, and instead produce a large and inviable eggcase that requires a significant nutritional and energetic investment. Thus, a virgin female that finds herself socially isolated from conspecifics might reduce oocyte development until a potential mate arrives to prevent or delay maturing an inviable eggcase. On the other hand, it is not costly for males to mature faster, and their reproductive strategy can thus be more independent of social experience. Socially isolated male colonists can rapidly mature sexually and mate soon after adult emergence, yet if receptive females are not available, males are ready for new receptive females or even for the next colonization event. Lastly, socially facilitated reproduction might have also evolved because social interactions with conspecifics offer other benefits such as greater foraging efficacy, protection from predators, or exchange of symbiotic microbes. Females would benefit more from these effects because they consume much more food than males do (Hamilton & Schal 1988) and transfer endosymbiotic microbes to their offspring.

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