Social facilitation of insect reproduction with motor-driven tactile stimuli

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Tactile stimuli provide animals with important information about the environment, including physical features such as obstacles, and biologically relevant cues related to food, mates, hosts and predators. The antennae, the principal sensory organs of insects, house an array of sensory receptors for olfaction, gustation, audition, nociception, balance, stability, graviception, static electric fields, and thermo-, hygro- and mechanoreception. The antennae, being the anteriormost sensory appendages, play a prominent role in social interactions with conspecifics that involve primarily chemosensory and tactile stimuli. In the German cockroach (Blattella germanica) antennal contact during social interactions modulates brain-regulated juvenile hormone production, ultimately accelerating the reproductive rate in females. The primary sensory modality mediating this social facilitation of reproduction is antennal mechanoreception. We investigated the key elements, or stimulus features, of antennal contact that socially facilitate reproduction in B. germanica females. Using motor-driven antenna mimics, we assessed the physiological responses of females to artificial tactile stimulation. Our results indicate that tactile stimulation with artificial materials, some deviating significantly from the native antennal morphology, can facilitate female reproduction. However, none of the artificial stimuli matched the effects of social interactions with a conspecific female.

1. Introduction

Insects exploit multiple sensory modalities, including auditory, olfactory, gustatory and tactile sensing, in a wide range of behavioural contexts such as locating resources, avoiding predators, mate finding and mate choice [1,2]. Antennae are important multi-sensory organs in insects, largely because they are located at the anteriormost position of the insect, they often extend up to several body lengths and sweep and sample nearly 360° of space around the insect, and they can receive information about smell, taste, sound, humidity, temperature and various mechanosensory cues [3]. The antennae are also pivotal sensory organs in social interactions, especially in nocturnal insects.

Mechanoreceptive sensilla are broadly distributed throughout the body surface of insects, with particularly high density on sensory appendages, including the antennae [3,4]. Antennal mechanosensors have been studied most extensively in the contexts of obtaining position information and localization and feature discrimination of obstacles [5]. However, antennal mechanoreception is also important in courtship behaviour, where positional information is integrated with chemosensory signalling between the sexes, as shown for example in cockroaches, crickets and moths [6–10]. In the behavioural phase change of the Australian plague locust, Chortoicetes terminifera, tactile stimulation of the antennae appears to be the sole mechanism that evokes a shift from solitarious to the gregarious state and ultimately swarming [11]. The antennae also appear to be the main organ involved in adult phase change in the desert locust, Schistocerca gregaria [12]. In addition, maternal determination of progeny characteristics has been observed in S. gregaria, where tactile stimulation perceived by their antennae causes females to produce gregarious offspring [12].
In all experiments, newly emerged females from the same cohort as the experimental females were either socially isolated (negative control) or paired for the entire experiment (positive control).

(b) Ovary dissection and oocyte measurements

Test females were ice-anaesthetized, and their ovaries were removed under cockroach saline [25]. In B. germanica, only a single basal (vitellogenic) oocyte matures in each ovariole and all basal oocytes mature synchronously in each of the approximately 40 ovarioles in the paired ovaries. A random sample of 10 basal oocytes of each female was selected, and oocyte lengths were averaged for each female. Measurements were done with an ocular micrometer in the eyepiece of a dissecting microscope.

(c) The effects of tactile cues on the reproductive response to social interactions

The antennae are essential in receiving sensory input, and the tactile stimuli that facilitate oocyte maturation in B. germanica are not species-specific [24]. A Periplaneta americana female was placed into a 15 ml plastic tube, with only the antennae protruding through a small hole in the tube. We covered the head of the cockroach with paraffin, so that only the antennae extended through the hole into a Petri dish (60 mm diameter, 15 mm high, Fisher Scientific, Pittsburgh, PA), where a newly emerged B. germanica female was placed with food and water ad libitum. In an additional treatment—using the same bioassay design—we bilaterally ablated the P. americana antennae to eliminate tactile stimuli by the antennae. P. americana females were briefly anaesthetized with CO2, placed on ice and the flagellum of each antenna was cut with fine scissors just distal to the pedicel. Because P. americana females were necessarily starved and could not drink under these conditions, each female was used for only 2 days and replaced with a fresh female, so three females were used for each dish during the 6 day long experiment. On day 6, the oocyte lengths of 20–23 B. germanica females per treatment were measured.

(d) Motorized tactile stimulation system

To investigate features of tactile stimuli that ultimately accelerate oocyte maturation, we designed a motorized system that could stimulate isolated females with substrates that mimicked cockroach antennae. The system was comprised of a controller and 20 parallel stepper motors. The controller consisted of a microcontroller (PIC18F4520, Microchip Technology, Chandler, AZ), speed and direction controls, an LCD display showing the motor angular velocity and 10 microstepping bipolar stepper motor drivers (A4988, Pololu, Las Vegas, NV; figure 1). Each motor driver controls two stepper motors (ROB-09238, SparkFun Electronics, Boulder, CO). The output of the microcontroller could generate angular velocities of 0.1–30 revolutions per minute (r.p.m.), and each motor was set to rotate in a clockwise direction. Each motor was securely attached to the underside of a 6-mm thick acrylic sheet that held 10 motors. A Petri dish (90 mm diameter, 15 mm high) was placed on the acrylic sheet with a hole at its centre aligned with the hole in the acrylic sheet. The motor shaft thus penetrated into the Petri dish, and materials simulating insect antennae were attached onto the shaft. The acrylic sheet effectively thermally insulated the Petri dishes from the motors, and a room fan was used to ensure rapid dissipation of heat from the electronics.

(e) Validation and use of the motorized system

First, we determined whether the rotating motors influenced oocyte growth by producing heat, vibration or other confounding stimuli. Newly emerged females were either socially isolated or
paired in Petri dishes and placed either on the rotating motors or beside them with food and water ad libitum. Because social interactions stimulate oocyte growth only during the scotophase, when females are active, and not during the photophase when females aggregate in close contact with each other [24], the motors were turned on during the entire scotophase (12 h) at a maximum speed of 30 r.p.m. and with a bare motor shaft. The basal oocytes of 10 *B. germanica* females per treatment were measured on day 6. The temperature within similarly deployed Petri dishes was measured at 1 min intervals for 6 days with HOBO UX100-003 data loggers (Onset Computer Corp., Bourne, MA) positioned above motors running only during the 12 h scotophase at 0, 1 and 30 r.p.m. and in a Petri dish adjacent to the motors.

Movement of the antennae is necessary to glean tactile information from the surroundings [26]. The cockroach antennae are flexible and they regularly sweep through space, vibrate, palpate rhythmically and get deflected when the insect comes in contact with conspecifics, suggesting that movement is an important feature of the tactile cue. To determine the most effective motor speed and the optimal duration of stimulation, newly eclosed females were socially isolated in Petri dishes and placed on the motors with food and water ad libitum. Artificial tactile stimulation was provided by a commercially sanitized natural white Cul De Canard feather from the back of a duck (CDC201, Wapsi Fly Inc., Mountain Home, AR) attached to each motor shaft. Each set of females received stimulation for different durations (3, 4.5, 6, 7.5, 12 h (scotophase only) and 24 h) and at different motor speeds (0, 1, 5, 10 and 30 r.p.m.). Sample size was 16–20 females per treatment.

In addition to movement, some physical properties of the antenna such as the structural complexity may influence the reproductive rate of *B. germanica* females. Complexity of the antennal morphology was tested by manipulating duck feathers: either an intact duck feather, feather with its barbs cut to 1 mm, or bare feather with its barbs completely removed. Sample size was 10–18 females per treatment.

(f) Statistical analyses
Data were analysed with one-way ANOVA for multiple comparisons using SAS 9.1.3 software (SAS Institute Inc. 2002–2003, Cary, NC). We used PROC GLM to test for the effects of social conditions on oocyte length as dependent variable. PROC GLM was also used to obtain the residuals from adjusted model and test whether residuals held the assumption of homogeneous variances within each treatment. Because data were unbalanced, LSMEANS and least significant difference test was used to compare means at 0.05 significance level. Variation around the mean is represented by the standard error of the mean (s.e.m.).

3. Results
(a) Antennal contact mediates the social facilitation of reproduction
To confirm the importance of antennal contact in social facilitation of female reproduction, each isolated *B. germanica* female was allowed to interact only with the antennae of a *P. americana* female whose body was bound in a tube outside the dish; these *B. germanica* females developed their oocytes significantly faster (1.29 ± 0.05 mm, *n* = 23) than females that were fully isolated (0.99 ± 0.07 mm, *n* = 22) or females that interacted with a *P. americana* female whose antennae were ablated (0.87 ± 0.06 mm, *n* = 20; ANOVA, *p* < 0.0001; figure 2). It is difficult to disentangle the relative significance of ‘giving’ social stimuli (touching) versus ‘receiving’ social stimuli (being touched). Nevertheless, it appears that active tactile sensing of social stimuli is crucial for accelerating oocyte maturation, because interaction with an immobile (freshly killed) female fails to stimulate oocyte growth, whereas tactile stimulation of the female with ‘prosthetic’ antenna-like artificial materials in place of the antennal flagellum induces faster oocyte growth [24].

(b) Validation of the motorized tactile stimulation system
We implemented artificial tactile stimulation with stepper motors, which have some intrinsic features that might confound...
Figure 2. Effects of interaction with *P. americana* antennae only on oocyte maturation of *B. germanica* females. Newly emerged adult females were either socially isolated (negative control), or paired for the entire 6 day experiment (positive control; grey bars). An American cockroach female was bound in a plastic tube outside the dish with only its antennae protruding into the dish to interact with the otherwise isolated *B. germanica* female. In an additional treatment, the American cockroach female antennae were carefully ablated, eliminating antennal contact. Mean basal oocyte length ± s.e.m. Different letters above the bars indicate significant differences among treatments (ANOVA, $F_{3,48} = 21.78$, $p < 0.0001$; LSD, $p < 0.05$).

Figure 3. Validation of the motorized tactile stimulation system. Newly emerged adult females were either socially isolated or paired in Petri dishes and placed either on the rotating motors or beside them. The motors were powered during the entire 12 h scotophase at a speed of 30 r.p.m. and with no substrate attached to their shafts. The basal oocytes of *B. germanica* females were measured on day 6 and sample size was 10 females per treatment. Mean basal oocyte length ± s.e.m. Different letters above the bars indicate significant differences among treatments (ANOVA, $F_{3,36} = 4.99$, $p = 0.0054$; LSD, $p < 0.05$).

(c) Stimulus features related to artificial ‘antenna’ movement

We recently found that 2 h of daily social interactions with another female in the middle of the scotophase were sufficient to induce faster oocyte maturation in *B. germanica* [24]. To determine the optimal conditions for artificial tactile stimulation, we fixed a duck feather to each motor and assessed the effects of various motor speeds and durations of daily stimulation on reproductive development in *B. germanica* females. The results again confirmed that movement is an important stimulus feature, because static feathers continuously present in the Petri dishes for 6 days failed to stimulate oocyte growth (figure 4, black bar). With dynamic tactile stimulation, oocyte maturation was inversely correlated with both motor speed and duration of stimulation (figure 4). Rapid tactile stimulation at 30 r.p.m. failed to accelerate oocyte maturation at all the stimulus durations that we tested (average oocyte length across all stimulus durations: $0.80 \pm 0.05$ mm, $n = 59$, similar to $0.95 \pm 0.05$ mm, $n = 49$, in females that were isolated during the entire experiment). Decreasing motor speed to 1–5 r.p.m. or decreasing stimulation duration to 3–6 h resulted in maximal oocyte growth. In our 6 day assays, females responded with the fastest oocyte development (1.28 ± 0.09 mm, $n = 20$) to 6 h stimulation at 1 r.p.m. (figure 4). This combination was used in subsequent experiments.
Numerous animal behaviour studies have used fabricated models to delineate key elements (stimulus characters) of relevant stimuli, dating back to Tinbergen’s [27] elegant and original stimulus-dissection models to delineate key elements (stimulus characters) of relevant stimuli, dating back to Tinbergen’s [27] elegant and original stimulus-dissection models. The challenge is substantially even more imposing when trying to identify the salient tactile features that elicit behaviour, including shape, fine structure, texture, friction, force and various spatiotemporal features of antennal contact. Moreover, tactile cues often involve complex and context-dependent active movements of both the signaller’s and receiver’s head and antennae, and unlike most communication messages that operate through a medium (air, water), the signaller in tactile communication can also be the receiver, as for example in reciprocal antennal fencing where the antenna both touches and is touched and processes both proprioceptive and exteroceptive information [3]. Finally, the insect antenna is endowed with multiple types of mechanosensory structures (e.g. hairs) that are differentially distributed across various antennal segments, and arranged as single sensilla or as specialized arrays of mechanosensory units (e.g. hair plates, chordotonal organs) [4], making it even more difficult to determine where and how socially relevant tactile stimuli are received.

Thus, it is not surprising that the relevance of most antennal mechanoreceptive sensilla in social interactions remains largely unexplored, as most investigations have focused on relatively instantaneous non-social behaviours such as steering and course control, obstacle navigation and escape responses [3]. These behaviours depend largely on object localization (i.e. where) and much less so on object discrimination and identification (i.e. what). The effectiveness of tactile stimuli in a social context is profoundly dependent on the receiver being able to identify the source of the stimulus (its shape, texture, biomechanics), and our endeavours to disentangle discrete social tactile stimulus characters are therefore considerably more challenging than in other sensory modalities. The challenge is substantially even more imposing in ‘primer-type’ responses that require multiple days of in situ tactile stimulation whose outcomes are physiological rather than behavioural, as in the slow contact-mediated social facilitation of reproduction in B. germanica. While the behavioural outputs of associative learning experiments can decode the tactile stimulus characters, more sophisticated paradigms are required to unravel the primer-type stimuli that drive slow physiological changes. This is apparent in B. germanica, where 24 h of social interactions on any day during the 6 day pre-oviposition period of otherwise isolated females is insufficient to elicit a ‘grouping effect’, and even 48 h of interactions on days 2–3 minimally facilitate reproduction in females [24].

We established and validated a motorized system that enabled us to investigate the effects of artificial tactile stimuli on reproduction of female cockroaches. B. germanica females recorded and synthesized acoustic stimuli to understand key elements that convey specific information to the receiver, and chemosensory biologists have similarly conducted chemical structure–activity studies with synthetic analogues of odorants and tastants to understand the respective olfactory and gustatory receptors and the behaviours they drive. Near-range sounds and vibrational stimuli, which stimulate mechanoreceptors, have also been investigated, especially in arthropods [3]. Notably, it has been exceptionally challenging to fabricate synthetic tactile stimuli mainly, because the insect antenna is a compound sensory organ in which touch is but one component of a multi-sensory network, unlike the vertebrate whisker or vibrissa which is strictly tactile and inert with receptors located in the follicle at its base [32]. It has also been difficult to isolate and quantify the salient tactile features that elicit behaviour, including shape, fine structure, texture, friction, force and various spatiotemporal features of antennal contact. Moreover, tactile cues often involve complex and context-dependent active movements of both the signaller’s and receiver’s head and antennae, and unlike most communication messages that operate through a medium (air, water), the signaller in tactile communication can also be the receiver, as for example in reciprocal antennal fencing where the antenna both touches and is touched and processes both proprioceptive and exteroceptive information [3]. Finally, the insect antenna is endowed with multiple types of mechanosensory structures (e.g. hairs) that are differentially distributed across various antennal segments, and arranged as single sensilla or as specialized arrays of mechanosensory units (e.g. hair plates, chordotonal organs) [4], making it even more difficult to determine where and how socially relevant tactile stimuli are received.

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![Diagram](image.png)

Figure 5. Evaluation of the significance of the structural complexity of an artificial tactile stimulus on the rate of oocyte development in B. germanica females. Newly emerged adult females were either socially isolated (negative control), or paired for the entire experiment (positive control; grey bars). Other newly emerged females from the same cohort were isolated in Petri dishes and placed on the motors. Each female received artificial tactile stimulation for 6 h at 1 r.p.m. by a duck feather attached to the shaft of the motor. Stimulus complexity was tested with either intact feathers with 18 mm barbs, feathers with short 1 mm barbs or feathers with the barbs completely removed leaving a bare feather rachis. Mean basal oocyte length ± s.e.m. Different letters above the bars indicate significant differences among treatments (ANOVA, $F_{4,85} = 12.21, p < 0.0001$; LSD, $p < 0.05$; figure 5).
respond to social interactions with conspecifics—and even with other insects—by elevating their rate of JH production and accelerating the rate of oocyte maturation, a form of phenotypic plasticity [18,19,21,22]. In a previous study, we established that visual and chemosensory cues play little, if any, role in this social facilitation of reproduction, whereas tactile stimuli alone can elicit faster reproduction [24]. In this work, we demonstrate that ‘antenna’-like structures, driven by stepper motors, can also accelerate the rate of oocyte growth. Notably, however, all of our treatments were significantly inferior to normal social interactions with a conspecific female, indicating that other prominent features of this social facilitation system remain to be delineated. Because limited interaction only with the antennae of either P. americana or B. germanica was not as effective as interaction with a whole conspecific female [24], we infer that non-antennal tactile stimuli are also important facilitators of reproduction.

An emerging picture from our studies is that antennal movement and morphology are integral components of the tactile stimuli. In support of our in situ studies demonstrating that social facilitation of reproduction is gated with the photocycle and can be achieved with as little as 2 h of contact in the scotophase [24], we now demonstrate that the duration and magnitude of artificial tactile stimulation also impact oocyte growth. The effect of duration of artificial stimulation on oocyte maturation represents an upside-down (concave downward) parabola, with a peak at about 6 h of stimulation in the middle of the scotophase. We suspect that as the quality of the tactile stimulus increases, the minimal required duration of stimulation will diminish. We also demonstrated that the rate of oocyte maturation was inversely related to the frequency of stimulation, represented by motor speed. Thus, oocyte maturation was greatly suppressed by frequent tactile stimulation at 30 r.p.m. but considerably stimulated with slow intermittent contact with a duck feather. Coupled with the observations that reproduction in isolated females is not facilitated by an immobile feather or by the presence of freshly killed (i.e. immobile) females [24], these results indicate that stimuli related to kinematics of the insect antennal motor system and biomechanics and dynamic properties of the antennal flagellum play essential roles in facilitating reproduction.

The morphology of the artificial stimulus also had a bearing on oocyte maturation. Although the bare shaft (rachis) of a denuded feather stimulated oocyte development, greater structural complexity increased its effectiveness. Moreover, some exaggerated stimulus characters appeared to act as super-normal stimuli, as feathers with long barbs were slightly more effective than feathers with shorter barbs, and P. americana antennae were more effective than the native B. germanica antennae [24]. Antennal shape thus appears to be an important feature in tactile social stimulation, in support of our in vivo studies showing that certain insects with non-flagellar antennal morphology were less effective at stimulating oocyte growth in B. germanica [24]. Further support for the importance of antennal morphology, flexibility and texture comes from male courtship behaviour in B. germanica. The female-produced contact sex pheromone readily elicits male courtship when placed on various flagellum-type antennae from a variety of insect species, but not on thick or club-shaped antennae or smooth surfaces such as a nylon line or a human hair [9], suggesting that an important feature of this sexual signal is the integration of chemical and tactile stimuli. The importance of textural cues in determining stimulus identity was also demonstrated in the cockroach P. americana, where cuticular surface properties of a wolf spider trigger escape, whereas touching a conspecific does not, even when both are solvent extracted to eliminate contact chemoreception [33]. The fine shape discrimination capabilities of the cockroach antennae remain to be determined, but it would not be surprising if the diversity and spatial organization of mechanosensory sensilla on the antenna enable feature discrimination comparable with that of rodent whiskers [32].

Our motorized tactile stimulation system represents an essential foundation for further studies of stimulus characters that socially facilitate reproduction in B. germanica and other species. Promising lines of investigation include mounting more realistic engineered jointed ‘antennae’ on robots programmed to execute species-specific and context-dependent behaviours in multiple axes. Decoding the tactile stimulus characters will further allow us to identify neuronal and endocrine circuit elements in the transduction pathway through which mechanoreceptors communicate relevant discrete tactile cues through the antennal nerve to sensory neuropils in the deutocerebrum and to higher brain centres, which ultimately accelerate female reproduction in B. germanica by disinhibiting the activity of the CA. This neuroendocrine pathway has been best described in phase transition in S. gregaria, where tactile stimulation of specific mechanoreceptive sensilla on the hind legs causes an increase in serotonin levels in the metathoracic ganglion which then leads to gregarization [34–36]. It will be fascinating to know whether biogenic amines also respond to social antennal contact resulting in the social facilitation of reproduction in B. germanica females.

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