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Proliferating bacterial symbionts on house fly eggs affect oviposition behaviour of adult flies

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Animals commonly leave scent messages by depositing pheromones, faeces, or urine. The intensity of a chemical message may fade over time, but the 'intention' remains the same. We argue that house flies, *Musca domestica* (Diptera: Muscidae), require a message with evolving (sensu changing over time) information content. Gravid females reportedly deploy a pheromone that induces concerted oviposition so that many even-aged larvae ameliorate the resource, such as animal manure. However, continued oviposition by late-arriving females may result in age disparity and cannibalism of larval offspring. Thus, we predicted that house flies have a type of cue that evolves from oviposition induction to inhibition some time after eggs are deposited on a resource. Here we show (1) the existence of such evolving ovipositional cues, (2) the adverse fitness consequences that accrue from ignoring the inhibitory cues and (3) the mechanism by which these cues evolve. The evolving cues depend upon a key bacterial strain, *Klebsiella oxytoca*, which originates with female *M. domestica* and which proliferates over time on the surface of deposited eggs. At a threshold density of this strain, further oviposition is inhibited. By deploying such evolving cues, female *M. domestica* can visit an oviposition site just once and deposit cues that will mediate immediate oviposition induction followed by delayed inhibition, thereby ensuring conditions conducive for offspring development.

Keywords: bacterial symbiont; communication ecology; house fly; microhabitat management; *Musca domestica*; reproductive strategy; signalling

Animals commonly leave scent messages by depositing pheromones, faeces, or urine (Brown & Macdonald 1985; Halpin 1986). Scents can convey diverse information, including sexual receptivity (Ferkin & Li 2005; Nojima et al. 2005), occupancy of a territory (Hurst et al. 1998), and cues to food and shelter (Taulman 1990; Reinhard et al. 2002). While the intensity of chemical messages may fade over time, the 'intention' generally remains fixed. For example, dominant adult male house mice, *Mus domesticus*, mark their territories with scents that deter other males (Hurst et al. 1998). Evaporation (Hurst et al. 1998) or degradation (Höller et al. 1991) of these scents may indicate the time elapsed since placement (Anderson 2002), and may thus influence whether recipients respond to the message. However, while old scents may no longer deter other mice, the intended message will probably not change from deterrence to attraction.

Parent insects that use fleeting and resource-limited habitats for offspring development often deploy scent messages that help prevent intraspecific competition. For example, ovipositing cherry fruit flies, *Rhagoletis cerasi* (Diptera: Tephritidae), and apple maggot flies, *Rhagoletis pomonella*, deposit pheromones that are immediately effective in discouraging further oviposition (Averill & Prokopy 1987; Städler et al. 1994).

Female house flies, *Musca domestica* (Diptera: Muscidae), also lay their eggs on fleeting organic resources (Keiding 1974). Larvae hatch within 24 h and develop through three instars in 5–7 days. After a 5-day pupal period, adult males and females eclose. Mated females lay eggs in batches of about 120, with as many as six batches per lifetime (Hewitt 1914).

Unlike tephritid fruit flies, female house flies preferentially oviposit near freshly deposited conspecific eggs. The many even-aged larvae warm and moisten the organic

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material (Bryant 1970; Barnard & Geden 1993), and curtail growth of competitive fungi (Zvereva 1986). Female house flies, however, should avoid oviposition near old eggs or hatching larvae because offspring may then be cannibalized by older offspring from prior oviposition events, as observed among age-disparate larvae of house flies (K. Lam, unpublished data), Neotropical mosquitoes, *Tichoprosopon digitatum* (Diptera: Culicidae), and alfalfa blotch leaf miners, *Agromyza fronella* (Diptera: Agromyzidae) (Quiring & McNeil 1984a, b; Sherratt & Church 1994; Anderson 2002).

Oviposition near fresh eggs is stimulated by pheromonal and visual cues from ovipositing adults (Collins & Bell 1996; Jiang et al. 2002). However, there is no information on whether, when, or how a shift occurs from oviposition induction to inhibition. A pheromonal message elicits a fixed behavioural response, and therefore is unlikely to mediate a spontaneous shift in oviposition behaviour. In contrast, microorganisms, if consistently associated with house fly eggs, could proliferate over time and thus convey a message with evolving (sensu changing over time) information content.

Microorganisms are associated with many insect species. Bark and ambrosia beetles (Coleoptera: Curculionidae) carry symbiotic fungi (Farrell et al. 2001) that help curtail host tree defences (Paine et al. 1997) or serve as a food source (Francke-Grosmann 1939; Klepzig et al. 2001). Microbial symbionts also participate in signalling. For example, attraction of conspecifics to host trees by mountain pine beetles, *Dendroctonus ponderosae* (Colytidae), is inhibited when fungal symbionts oxidize a component of the beetles' aggregation pheromone to produce an antiaggregation pheromone (Ryker & Yandell 1983). Moreover, onion maggot flies, *Delia antiqua* (Meigen), deploy bacteria that produce oviposition stimulants (Hough et al. 1982; Judd & Borden 1992). Conceivably, ovipositing female house flies may deploy microorganisms that multiply and, at some point in time, exceed an abundance threshold above which further oviposition is inhibited.

In this study, we tested the hypotheses that: (1) asynchronous oviposition with ensuing age disparity of larval offspring has adverse fitness consequences for parental females; (2) ovipositing females deploy, together with their eggs, evolving cues that first induce and later discourage oviposition by conspecific flies; (3) egg-associated bacteria are responsible for this shift in oviposition behaviour; and (4) specific bacterial strain(s) increase in abundance over time and thus change oviposition induction to inhibition.

GENERAL METHODS

Experimental Insects

Adult house flies were kept in cages at 50–80% relative humidity (RH), 22–30°C and a 16:8 h light:dark regime, and were provided with water, sugar cubes and skim-milk powder ad libitum. House fly eggs were collected on cotton oviposition sites (OSs; see below) and reared to adult insects on artificial diet prepared from wheat bran (400 g), brewers yeast (15 g), molasses (15 ml) and water (700 ml), with a supplemental protein paste prepared from skim-milk powder and water. The colony had been maintained in the Simon Fraser University (SFU) Insectory for over 30 years and was started with flies captured in British Columbia, Canada.

Design of Oviposition Experiments

In our bioassay procedure for testing the ability of stimuli to induce or inhibit oviposition, each experimental replicate employed a mesh cage (30 × 30 × 45 cm) that contained 50 male and 50 female house flies. Two identical oviposition sites (OSs; see below) were treated with different stimuli and were randomly assigned to opposite corners of the cage. When oviposition had ceased (3–6 h), the eggs on each OS were removed and weighed or counted (~15 000 eggs/g). Eggs were weighed for all experiments except experiments 2–3 and 22–25; for these six experiments, the egg mass stimuli were given time to hatch, at which time the still-intact eggs deposited by bioassay flies were counted.

Oviposition Sites (OSs)

Manure OSs, used in experiments 2–3 and 22–25, consisted of 2.5 g of fresh (<24 h old) chicken manure packed tightly into the bottom half of petri dishes (35 × 10 mm). A 5-mm diameter well was poked in the centre of the manure to serve as a focal point for oviposition. Distilled water (0.2–0.4 ml) was applied to the manure surface to prevent manure desiccation during bioassays. Manure was collected from free-range, organic chickens in Wind's Reach Farm (Langley, British Columbia, Canada) and stirred thoroughly prior to packing.

Cotton OSs were used as a more convenient and consistent alternative to manure OSs in experiments 4–8, and were also used for the collection of egg masses. Cotton OSs consisted of braided cotton rolls (10 × 150 mm) moistened with skim milk and coiled within petri dishes (35 × 10 mm). Agar OSs, used in experiments 9–20, consisted of petri plates (50 × 15 mm) containing sterile agar media consisting of water (15 ml), nutrient broth powder (0.12 g), skim-milk powder (0.24 g) and agar (0.3 g).

Statistical Analyses

For experiment 1, a two-tailed two-sample *t* test was conducted to compare the mean numbers of offspring flies that completed development when rearing medium was inoculated with age-disparate eggs. For experiment 21, a two-tailed matched-pairs *t* test was conducted to determine whether the abundance of *Klebsiella oxytoca* (strain SFU-1) on house fly eggs increased between 0 and 24 h postoviposition.

For experiments 2–20 and 22–25, weights or counts of eggs deposited on treatment and control oviposition sites within each replicate pair were converted to two separate