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Cuticular hydrocarbon synthesis and its maternal provisioning to embryos in the viviparous cockroach *Diploptera punctata*

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Abstract

Embryos of the viviparous cockroach *Diploptera punctata* accumulate large amounts of hydrocarbon (HC) of either maternal or embryonic origin. HC synthesis and its accumulation in maternal and embryonic tissues were measured over the course of gestation. Female abdominal integument was the only tissue that synthesized appreciable amounts of HC in vitro, and did so at an increasing rate from the time of mating to mid-pregnancy, when rates of synthesis declined. The embryos synthesized HC at rates $<1\%$ those of the female, showing that the majority of HC detected in and on embryos was of maternal origin. The brood sac that houses the developing embryos did not synthesize HC in vitro, indicating that HC must be transported from the female abdominal integument to the embryos. The mass of female epicuticular HC was constant at $\sim$183 μg, while her internal HC increased fourfold from mating to mid-pregnancy, then declined until parturition. The decline in internal HC reflected both declining HC synthesis in the female and greater export to the embryos, as embryonic internal HC increased 250-fold prior to parturition. An external HC coating over the oothecal covering and chorion of the embryos increased to mid-pregnancy, then declined. Unlike oviparous cockroaches, *D. punctata* females fed throughout the reproductive cycle, reflecting the nutritional demands of continuously provisioning the developing embryos.

Keywords: *Diploptera punctata*; Hydrocarbons; Viviparity; Maternal investment; Feeding

1. Introduction

Insects display a range of maternal contributions to offspring development. Most are oviparous, investing resources in eggs only prior to ovulation. Some are ovoviviparous, retaining eggs internally and providing water and protection, but not nutrition, until embryogenesis is complete. Viviparity, though uncommon, is widespread among insects and has evolved at least once, sometimes repeatedly, in each of 11 orders (Hagan, 1951). Viviparous females both retain and nourish developing embryos until they reach an advanced state of development. Regardless of reproductive strategy, all females must meet certain requirements to produce viable offspring: embryos must receive sufficient nutrients to support development, and must be protected against desiccation and pathogens. It is of interest to relate the timing, magnitude, and mechanisms of these essential investments to the reproductive mode (i.e., oviparity, ovoviviparity, viviparity) of the female.

The cockroaches (Dictyoptera, Blattaria) offer an excellent opportunity to investigate the evolution of maternal provisioning strategies. Among over 4000 described species of cockroaches, there are many oviparous and ovoviviparous representatives. True viviparity, however, has been found in only one cockroach species, the Pacific beetle cockroach *Diploptera punctata*. In this species, females retain developing embryos in a glandular brood sac during a 2-month gestation. When embryos complete dorsal closure and

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can drink, the brood sac begins to secrete protein-rich milk that supports embryonic growth, such that first instar nymphs at birth have 50 times the dry weight of eggs at oviposition (Stay and Coop, 1973). In 50-day old females (~65% through embryogenesis), milk collected from the brood sac consists of about 46% protein, 5% amino acid, 25% carbohydrate, and 20% lipids—of which half are nutritive substances, and the other half wax or hydrocarbons (Ingram et al., 1977). This milk is undoubtedly responsible for embryo dry weight gain during gestation, and the glycoprotein fraction is secreted by the brood sac itself (Stay and Coop, 1974; Ingram et al., 1977; Williford et al., 2004). The sources of the hydrocarbon components are unclear.

D. punctata embryos also bear a conspicuous waxy coating from the day they are oviposited into the brood sac, but the coating is most abundant during the middle 50% of gestation. This coating consists of 89% hydrocarbons, 6% long chain alcohols, 4% wax esters, and 1% aldehydes, and likely represents another aspect of maternal investment in *D. punctata* (Nelson et al., 2004). All insects, at all developmental stages, bear cuticular lipids that are crucial to water balance and pathogen exclusion (Nelson and Blomquist, 1995). Hydrocarbons (HC) are an important maternal investment in the oviparous cockroach *Blattella germanica*, in which HC provisioned to developing oocytes are not metabolized by embryos, but are retained in the hemocoel and cuticle of the first two nymphal instars (Fan et al., 2002, unpublished data).

Here we investigate the source of embryonic HC in *D. punctata*. Studies of the cockroaches *Periplaneta americana* and *B. germanica* have implied or demonstrated that HC synthesis is restricted to oenocytes, large cells rich in smooth endoplasmic reticulum and mitochondria, associated with abdominal epidermis in these species (Fan et al., 2003; reviewed in Schal et al., 2003). We can conceive of three plausible sources of the HC layer found on *D. punctata* embryos. First, it may be synthesized either by the glandular cells of the brood sac and provided to the embryos via brood sac milk, or by the subcuticular cells and passed through the brood sac cuticle. In addition to secretory cells and subcuticular cells, the brood sac epithelium, a derivative of abdominal epidermis, contains duct cells. However neither oenocytes nor morphological evidence for the synthesis of HC has been described for the brood sac (Stay and Coop, 1974). Second, HC may be synthesized elsewhere in the female and transported to the brood sac and embryos. Or, third, HC may be synthesized by the embryos themselves. The latter is least likely, as the HC layer is already present on embryos no more than a day old, and HC within these embryos would represent HC provided in yolk. This study distinguishes between these three possible scenarios, and relates patterns of HC synthesis in females and embryos to stages of embryonic development and the temporal pattern of female feeding.

2. Materials and methods

2.1. Insects

Cockroaches were maintained at $27 \pm 0.3\,^{\circ}C$ under a 12:12 h light:dark cycle, and were provided water and Purina rat chow ad libitum. Females mate upon adult ecdysis (day 0), and newly mated females were removed from the colony daily and maintained in separate plastic cages. Under these conditions, oviposition occurs on about day 8 after adult ecdysis, embryonic dorsal closure on day 20, and parturition about days 70–73 (Stay and Coop, 1973; Holbrook et al., 1998).

2.2. In vitro hydrocarbon synthesis

We tested various female tissues (tergites, sternites, thorax, wings, digestive tract, and brood sac) and embryos for in vitro synthesis of HC by incubation with [1-^{14}C]propionate, which serves as a traceable carbon donor in the synthesis of methyl-branched HC, allowing comparison of rates of HC synthesis in various tissues (Chase et al., 1990). This is likely to be a good estimator of total HC synthesis in *D. punctata*, in which both embryonic and maternal external HC consisted of >97% methyl-branched HC (Nelson et al., 2004). Each tissue was incubated in a 4 ml glass vial with 500 µl cockroach saline (Kurtti and Brooks, 1976), adjusted to 360 mOsm L^{-1}, containing 0.0185 MBq (0.5 µCi) [1-^{14}C]propionate (2035 MBq $mmol^{-1}$; New England Nuclear Research Products, Boston, MA) for 3 h at 27 °C with continuous gentle shaking.

To determine which tissue synthesizes HC, three females (days 23 and 30) were dissected and the head, dorsal thorax, ventral thorax, wings, legs, digestive tract, brood sac, and abdominal sternites and tergites were incubated. Sternites were freed of fat body and incubated, with internal surface contacting the medium, in three batches, with sternites 1–2 together, 3–4 together, and 5 to posterior together. Tergites were similarly incubated in three batches, consisting of tergites 1–3, 4–6, and 7 to posterior.

Changes in HC biosynthesis throughout the course of the reproductive cycle were investigated by incubating sternites three and four of the mother, one clutch of 12 embryos (cut into anterior and posterior halves with a sharp razor blade with the internal surfaces contacting the medium) and one brood sac (with hemolymph surface contacting the medium). After 3 h, 0.5 ml methanol was added and the tissue disrupted with a sonicator probe (Kontes, Vineland, NJ) for 30 s. The probe was rinsed into the vial with 1 ml hexane.