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Cuticular hydrocarbons as maternal provisions in embryos and nymphs of the cockroach *Blattella germanica*

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SUMMARY

Cuticular hydrocarbons of arthropods serve multiple functions, including as barriers to water loss and as pheromones and pheromone precursors. In the oviparous German cockroach, *Blattella germanica*, long-chain hydrocarbons are produced by oenocytes within the abdominal integument and are transported by a blood lipoprotein, lipophorin, both to the cuticular surface and into vitellogenic oocytes. Using radiotracer approaches, we tracked the location and metabolic fate of ^{14}C- and ^{3}H-labeled hydrocarbons through vitellogenic females and their embryos and nymphs. A considerable amount (~50%) of radiolabeled maternal hydrocarbons was transferred to oocytes and persisted through a 20-day embryogenesis and the first two nymphal stadia. The maternal hydrocarbons were not degraded or lost during this protracted period, except for significant losses of cuticular hydrocarbons starting with the first-to-second instar molt. Thus, although embryos and nymphs can produce their own hydrocarbons, maternal hydrocarbons provide a significant fraction of the cuticular and hemolymph hydrocarbons of both stages. These results show, for the first time in any insect, that a mother provides a significant complement of her offspring's cuticular hydrocarbons. Further research will be needed to determine whether provisioning hydrocarbons to eggs is a general strategy among insects and other arthropods or if this strategy is limited to taxa where eggs and early instars are susceptible to desiccation.

Key words: cuticular hydrocarbons, maternal investment, waterproofing, communication, cockroach, *Blattella germanica*.

INTRODUCTION

Maternal investment in offspring ranges from minimal provisions of yolk before ovulation to substantial expenditures in resources and energy after ovulation, resulting in considerable overlap in time with the offspring generation and a consequent cost to the female's future reproduction. Investment in most oviparous animals is restricted to a vitellogenic period during which all maternally derived yolk protein precursors are sequestered in the maturing oocytes (Speake and Thompson, 1999). Material provisions of oviparous females in eggs are nevertheless extensive and costly because all provisions are gathered, processed and sequestered within a short pre-ovulatory period. The greatest investment is represented by proteins – mainly vitellogenin and other glycolipoproteins – that are metabolized during early embryogenesis to release products that are used to biosynthesize embryonic and neonate tissues. In marine invertebrates, a group in which egg-size evolution has been extensively researched, protein content of the egg increases in direct proportion to egg size (Jaeckle, 1995), highlighting the pivotal role of proteins in embryogenesis.

Nevertheless, large amounts of maternal lipids are also sequestered in oocytes (Kunkel and Nordin, 1985; Speake and Thompson, 1999; Ziegler and van Antwerpen, 2006), and lipids too, like proteins, are metabolized during embryogenesis to serve as components of membranes (i.e. phospholipids) and as metabolic fuel sources (i.e. most neutral lipids, such as di- and triacylglycerols). There are few quantitative studies on the provisioning and metabolic fate of apolar lipids in eggs, and this is especially surprising in insects, where all life stages require extensive deposition of long-chain hydrocarbons on the cuticle to prevent desiccation. Indeed, a recent minireview of lipid uptake by insect oocytes (Ziegler and van Antwerpen, 2006) makes no mention of hydrocarbon uptake. In some marine invertebrates, lipid biomass (mainly wax esters) is higher than expected in large eggs, and the adaptive value of increased lipids is thought to involve greater buoyancy and higher energy content (Emlet, 2001; Emlet and Hoegh-Guldberg, 1997). But the embryonic strategies involved in processing maternal lipids are poorly understood. In this report, we investigate the patterns of maternal provisioning of long-chain hydrocarbons in oocytes and the metabolic fate of the maternal hydrocarbons in embryos and nymphs of a terrestrial insect. We show that, unlike other maternal nutriments, hydrocarbons are not metabolized as an energy source, but rather are conserved for waterproofing the cuticle across several larval molts.

Long-chain cuticular hydrocarbons waterproof insect cuticle and serve as pheromones or as pheromone precursors (Gibbs, 1998; Howard and Blomquist, 2005; Nelson and Blomquist, 1995; Schal et al., 1998; Schal et al., 2003). In the oviparous German cockroach, *Blattella germanica* L., hydrocarbons are biosynthesized only by specialized cells (oenocytes) of the abdominal integument and they accumulate in the hemolymph and the cuticular surface of all life stages (Fan et al., 2003; Gu et al., 1995). In the adult female cockroach, surprisingly large amounts of hydrocarbons are found within the ovaries, and because the ovaries do not biosynthesize hydrocarbons, all ovarian hydrocarbons are delivered to the maturing oocytes by lipophorin, a high-density hemolymph

lipoprotein (Fan et al., 2002; Gu et al., 1995; Schal et al., 1994; Schal et al., 1998). The adaptive value of hydrocarbons on the external surface of the eggs is apparent: the specific hydrocarbon blend on the eggs has a melting temperature that is 15.4°C and 21.5°C higher than the melting temperature of female's own cuticular hydrocarbons and lipophorin-bound hydrocarbons, respectively (Young et al., 2000). However, the hydrocarbons within the oviposited eggs are similar to those of the mother. Although the location, metabolic fate and adaptive value of maternally derived hydrocarbons within the developing embryos are unknown, it is likely that, as hypothesized for wax esters in marine invertebrates, hydrocarbons may serve physical functions (i.e. waterproofing) or as nutrients and energy reserves. We now provide the first empirical support for the proposal that maternal hydrocarbons are conserved during embryogenesis, they coat the embryonic and nymphal cuticles and are slowly lost, mainly during successive molts, as nymphs ingest more food and are able to produce their own hydrocarbons.

MATERIALS AND METHODS

Chemicals

Sodium [1-^{14}C]propionate (2.035 GBq mmol l^{-1}; New England Nuclear Research Products, Boston, MA, USA) was used as a methyl branch donor to track methyl-branched hydrocarbons. [11,12-^{3}H]3,11-Dimethylnonacosane (2.22 TBq mmol l^{-1}) was obtained by reductive tritiation (PerkinElmer Life and Analytical Sciences, Boston, MA, USA) of 3,11-dimethylnonacos-11-ene (Biofine International, Vancouver, Canada). L-15B medium (Specialty Media, Lavallette, NJ, USA) and all the remaining chemicals were purchased from Sigma (St Louis, MO, USA). Gas chromatography (GC)-grade organic solvents were obtained from Fisher Scientific (Pittsburgh, PA, USA).

Insect colony

The German cockroach, *Blattella germanica* L., colony was maintained at 27±0.5°C, ~50% relative humidity and 12 h:12 h light:dark photoperiod and was provided with a continuous supply of rat chow (Purina Mills, St Louis, MO, USA) and water. Newly emerged adult females were separated from the colony on the day of adult eclosion (day 0), and maintained in separate plastic cages. Females were always maintained in groups of 10–50 individuals because solitary females are reproductively repressed (Gadot et al., 1989; Holbrook et al., 2000). Females were mated on day 6, they oviposited on day 9, and embryos hatched from egg cases when females were 29 days old, or 20 days after oviposition under these conditions. During the 20-day embryogenesis, the proximal part of the egg case is held in the female's genital vestibulum and is provisioned with water (Mullins et al., 2002).

Ovarian, embryonic and cuticular hydrocarbons

Surface lipids were extracted from egg cases as described previously (Young and Schal, 1997). Each egg case was immersed in 2 ml *n*-hexane containing 15 μg *n*-hexacosane as an internal standard, agitated gently for 5 min, and the solvent was decanted into a clean vial. This procedure was repeated and the egg case was subjected to a final rinse with 1 ml hexane. The three hexane extracts were combined and the hydrocarbons were purified and quantified by GC.

Internal lipids were extracted by a modified Bligh and Dyer (Bligh and Dyer, 1959) procedure (Gu et al., 1995). For samples to be analyzed by GC, 15 μg *n*-hexacosane was added as an internal standard. Lipids were extracted from various tissues by homogenization in water for 30 s (Kontes micro ultrasonic cell disruptor, Vineland, NJ, USA) in a glass vial, and the homogenate was extracted with hexane/methanol/water (2:1:1). Samples were vortexed vigorously and centrifuged at 2000 ***g*** for 10 min. An aliquot of the hexane phase was loaded on a ~500 mg silica gel (100–200 mesh, type 60A; Fisher, Fairlawn, NJ, USA) mini-column in a Pasteur pipette, and hydrocarbons were eluted with 6 ml hexane. The solvent was reduced with a gentle stream of N_2, and hydrocarbons were quantified by GC or by liquid scintillation spectrometry (LSS; LS5801; Beckman, Fullerton, PA, USA).

For GC analysis, the hexane was reduced to 1–2 μl with N_2 and analyzed on a HP5890II GC (Agilent, Palo Alto, CA, USA) equipped with a flame-ionization detector and interfaced with a HP ChemStation (Rev. A.09.03). Splitless injection was made into a 30 m×0.32 mm×1 μm HP-5 capillary column operated at 100°C for 2 min, increased at 20°C min^{-1} to 150°C, then at 5°C min^{-1} to 310°C and held at this temperature for 5 min. The injector and detector were held at 300°C and 310°C, respectively.

In vitro hydrocarbon synthesis by embryos and first-instar nymphs

Methylmalonyl-CoA, derived from propionate, can serve as a methyl-branch donor in the synthesis of methyl-branched hydrocarbons in *B. germanica* (Chase et al., 1990). Since over 80% of *Blattella*'s hydrocarbons are methyl-branched (Jurenka et al., 1989) this allows us to track overall *de novo* hydrocarbon biosynthesis with [1-^{14}C]propionate. An egg case or individual first-instar nymphs were bisected with a sharp razor and incubated in 500 μl L-15B medium [adjusted to 410 mOsm by the addition of 55 mmol l^{-1} NaCl and 40 mmol l^{-1} Hepes, pH 7.4 and sterilized by filtration through a 0.22 μm low protein binding filter (Millipore, Bedford, MA) just prior to use] and 37 kBq [1-^{14}C]propionate. All incubations were at 27°C with constant shaking on an orbital waving shaker (VWR, Atlanta, GA, USA) to oxygenate the tissues. After 3 h, the tissues were removed and analyzed for labeled hydrocarbons. Hydrocarbons were purified and radioactivity in the hydrocarbon fraction analyzed by LSS.

Tracking radiolabeled hydrocarbons

Three-day-old females were injected with 111 kBq sodium [1-^{14}C]propionate in 1 μl *Blattella* saline (Kurtti and Brooks, 1976) and mated on day 6 with 15-day-old males. Another group of 3-day-old females were injected with 3.36 kBq [^{3}H]3,11-dimethylnonacosane in 1 μl *Blattella* saline containing 0.02% Triton X-100 and also mated on day 6. External lipids of embryos and nymphs at different developmental stages were extracted followed by extraction of internal lipids in chloroform/methanol/water (2:1:0.9, v/v). The chloroform phase was dried under nitrogen, hydrocarbons purified and subjected to LSS.

Thin-layer chromatography (TLC) of lipids

Aliquots of 0.336 kBq of extracted lipids were analyzed by TLC (Silica gel 60–F_{254}; Merck, Damstadt, Germany) using hexane/ethyl ether/acetic acid (80:20:2, v/v). Lipid classes were identified by comparing their mobilities with those of authentic standards. Radioactive lipids were scanned with a BioScan system 200 image scanner (Washington, DC, USA).

Statistical analyses

Statistical analyses were performed using SAS statistical analysis software (version 9.1, SAS Institute, Cary, NC, USA).

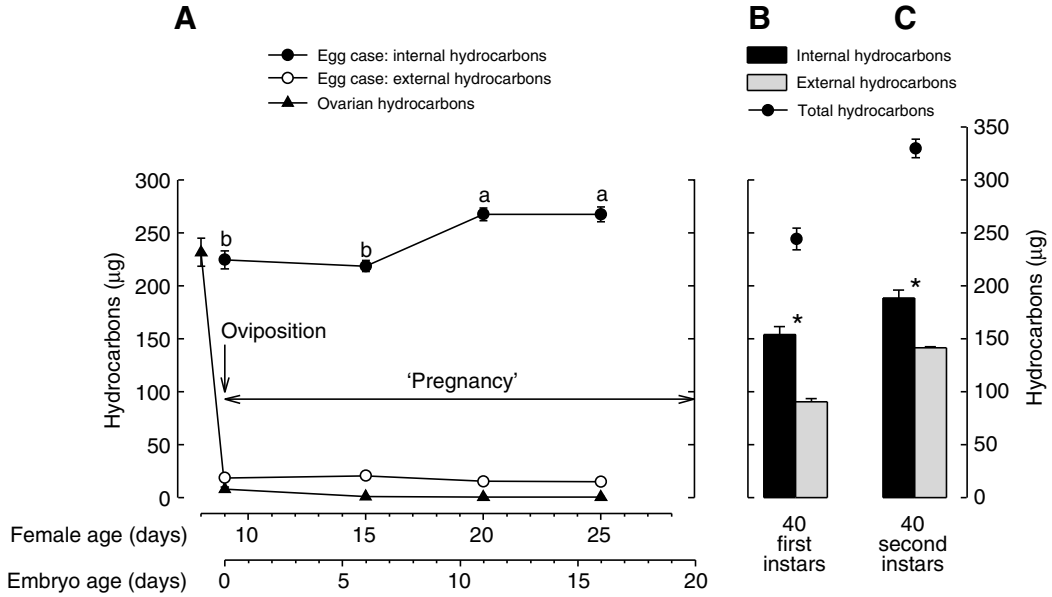

Fig. 1. Changes in hydrocarbons of embryos and nymphs of *B. germanica*. Hydrocarbons of the ovaries and exterior and interior of the egg case (A), and exterior and interior of 40 first-instar nymphs (B) (the equivalent of one egg case) and 40 second-instar nymphs (C) were extracted and quantified by gas chromatography. Data are means ± s.e.m. (N=7). Some s.e.m. are obscured by the symbols. Significant differences among means in A are represented by different letters (ANOVA, LSD, P<0.05) and in B and C by asterisks (paired t-test, P<0.05).

RESULTS

Hydrocarbons during embryonic and nymphal development

In the German cockroach, ~40 basal oocytes synchronously mature between days 0 and 9 of the first ovarian cycle (reviewed in Schal et al., 1997). Females oviposit on day 9, and although *B. germanica* is considered oviparous, females exhibit functional ovoviviparity: fertilized eggs are oviposited into an egg case that remains attached to the genital vestibulum of the female between days 9 and 29 (see Fig. 1A for events of the reproductive cycle). During this 'pregnancy', embryogenesis proceeds and the new basal oocytes in the ovaries are prevented from growing. After hatching, the female resumes a new vitellogenic cycle (not shown).

During the preoviposition period, the female amasses about 450 µg of hydrocarbons internally, about half of which accumulate in the ovaries (Fan et al., 2002). The ovarian hydrocarbons sharply decline at oviposition on day 9 and, subsequently, the ovaries do not accumulate hydrocarbons during 'pregnancy' (days 9–29) (Fig. 1A). While the exterior of the newly formed egg case contained only 18.7±1.20 µg (s.e.m., N=7) hydrocarbons, its interior contained 224.6±8.52 µg (N=7), which approximately represented the difference between ovarian hydrocarbons before and after oviposition (231.6 and 8.2 µg, respectively). Thus, copious amounts of hydrocarbons accumulate in the ovaries and are then transferred into the egg case with the oviposited eggs. Because the ovaries do not synthesize hydrocarbons (Gu et al., 1995), hydrocarbons must be shuttled to the ovaries through the hemolymph.

The external hydrocarbons of the egg case remained relatively constant but with a slight decline towards the end of embryogenesis (Fig. 1A). By contrast, 225 µg of embryonic hydrocarbons were recorded on the first day, they remained unchanged for 6 days (to mother's age 15 days), then significantly increased by ~19% to 268±6.01 µg (N=7) through embryo age 11 days (mother's age=20 days) and remained constant until hatching (one-factor ANOVA, F=15.43, d.f.=3,23, P<0.001).

The elevation in hydrocarbon titer was coincident with the onset of *de novo* hydrocarbon biosynthesis (Fig. 2A) around dorsal closure (day 7–8) (Tanaka, 1976), when embryonic oenocytes become differentiated and competent to produce their own lipids. Hydrocarbon production in embryos increased significantly on day 7 (mother's age 16 days) to a broad peak lasting about 7 days and then gradually declined to non-detectable levels shortly before hatching on day 20. A similar cycle was evident in first-instar nymphs, where hydrocarbon production was low early in the stadium, peaked on days 2–4 and declined to extremely low levels of hydrocarbon synthesis by the final day before the molt (Fig. 2B,C).

Fate of maternally provisioned hydrocarbons

Unlike most maternal chemical provisions in embryos, maternally derived hydrocarbons were not metabolized by either embryos or nymphs. The fate of maternal hydrocarbons was evaluated in two independent experiments. First, sodium [1-^{14}C]propionate was injected into vitellogenic females on day 3. Newly biosynthesized methyl-branched hydrocarbons are thus rapidly radiolabeled (Young and Schal, 1997), transported by lipophorin to the ovaries and then incorporated in embryos and first- and second-instar nymphs. About 7.05% of the injected propionate was used for hydrocarbon production. Some radiolabeled hydrocarbons (58.8%) were retained in the female's interior and transported to the female's cuticular surface, and 41.2% was provisioned into the eggs. Interestingly, maternal (i.e. radiolabeled) hydrocarbons in the embryo remained relatively unchanged throughout embryogenesis (Fig. 3A), indicating that (1) no new maternal hydrocarbons were provisioned to embryos after oviposition, as expected in oviparous reproduction, and (2) little, if any, metabolism of maternal hydrocarbons occurred during embryogenesis.

The latter suggestion was confirmed by TLC of total lipid extracts at various ages. Hydrocarbons were relatively resilient to degradative processes. Between 4 and 31 days after [1-

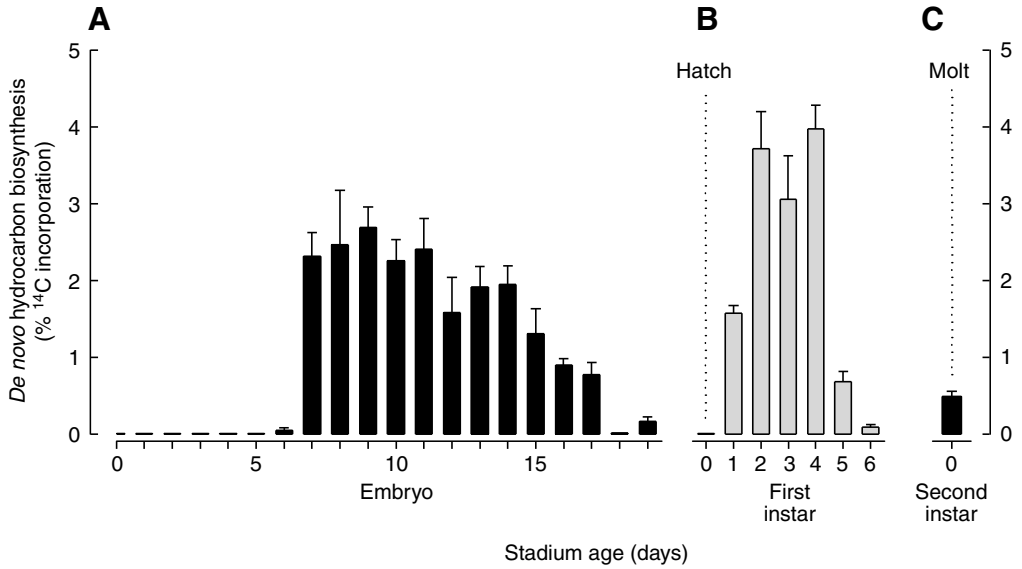

Fig. 2. *In vitro* hydrocarbon biosynthesis by embryos (A), first-instar nymphs (B) and newly molted second-instar nymphs (C) of *B. germanica*. Forty embryos in a single egg case or 40 individual nymphs were bisected with a sharp razor and incubated for 3 h in a medium fortified with sodium [1-^{14}C]propionate. The tissues and medium were extracted in chloroform/methanol/water and the hydrocarbons were then purified on silica gel and quantified by LSS. Data are means ± s.e.m. (*N*=8).

^{14}C]propionate injection into 5-day-old females, >95% of the radioactivity in lipids of embryos and nymphs was associated with hydrocarbons (Fig. 4). Because the propionate pool is rapidly fixed into macromolecules within 4 h of injection (Young and Schal, 1997), transfer of radiolabeled propionate to the oocytes is extremely unlikely. Thus, it appeared that maternal hydrocarbons were not degraded at any embryonic or post-embryonic stage.

Surprisingly little loss of maternally provisioned hydrocarbons was evident during the first instar (Fig. 3A), despite an expected loss at hatching, in association with the molted embryonic cuticle. This is because immediately after hatching the neonates ingest the embryonic cuticle (C.S., personal observation), resulting in a highly efficient recycling of both maternal and embryonic cuticular hydrocarbons (Fig. 1B, Fig. 3A). The first substantial loss of maternally provisioned hydrocarbons occurs during the first-to-second instar molt. About 153.8 μg hydrocarbons was found in internal tissues of newly hatched first instars, while 90.3 μg hydrocarbons (~37% of total) was associated with their cuticular surface (Fig. 1B). By contrast, ~16.0% of the total radiolabeled (maternal) hydrocarbons, representing 38.1±1.5% of first-instar nymph's labeled hydrocarbons, were provisioned to the external cuticular surface, showing that most of the maternal hydrocarbons are retained internally within the first-instar nymph, while only a fraction is externalized. The cuticular hydrocarbons were then shed during the molt to the second instar. The newly molted second-instar nymph, in turn, ingested less of the shed cuticle, resulting in a substantial loss of maternal hydrocarbons. Of the remaining maternal hydrocarbons (34.3% of the total ^{14}C-hydrocarbons), 9.9% was then found on the cuticular surface of the second-instar nymph (equivalent to only 28.9±0.9% of radiolabeled hydrocarbons of the nymph) whereas 24.4% of the total maternal hydrocarbons (71.1±0.9% of radiolabeled nymphal hydrocarbons) were allocated to the internal hydrocarbon pool (Fig. 3A). Because internal – including maternal – hydrocarbons continue to be shunted to the cuticular surface throughout each intermolt period (Young et al., 1999), it is not surprising that ~16.6% of maternal hydrocarbons are lost after the second molt, and this loss is expected to continue in successive molts of well-fed insects. Nevertheless, the steady-state hydrocarbon composition of early nymphs suggests a 'last in/first out' hypothesis, whereby maternal hydrocarbons (first in) tend to be more retained internally than are newly biosynthesized nymphal hydrocarbons, which are more rapidly externalized. Consequently, maternal hydrocarbons appear to be used over many stadia as an internal reserve.

A second, independent confirmation of this pattern was obtained by injecting [^{3}H]3,11-dimethylnonacosane, a native *B. germanica* hydrocarbon, into vitellogenic females and tracking its metabolic fate in embryos and nymphs. As before, TLC indicated that little hydrocarbon was metabolized (data not shown). The pattern of utilization of maternal ^{3}H-hydrocarbon by embryos and nymphs was nearly identical to that of ^{14}C-hydrocarbon derived from propionate (Fig. 3A,B). Both patterns clearly show that a large fraction of maternal hydrocarbons is provisioned to eggs and subsequently to the interior of nymphs, to serve mainly for coating the external cuticular surface of nymphs at each molt and during the intermolt period.

DISCUSSION

Development is profoundly influenced by maternal factors, most notably maternal resources that are provisioned for embryonic development. In oviparous animals, egg nutrients are the only source of metabolic fuel for the developing embryos. Therefore, the imported maternal lipids and proteins in eggs need to be properly stored and made available when they are needed during embryogenesis and immediately after hatching. Most of the yolk precursors of insects are produced in the fat body and transported to the developing oocytes through the hemolymph (Telfer et al., 1991). In addition to proteins, large stores of lipids are found in mature insect eggs (Chino et al., 1977). Studies with *Manduca sexta*, *Locusta migratoria*, *Rhodnius prolixus* and *B. germanica* have described the lipid transport processes (Arrese et al., 2001; Ryan et al., 1986; Schal et al., 2003; Schal et al., 1998). In *M. sexta*,

1% of the lipid in the oocyte is biosynthesized by the egg itself, while 5% of the lipid is delivered by vitellogenin. Lipophorin – both low- and high-density – is the major vehicle for lipid delivery during vitellogenesis (Kawooya and Law, 1988; Kawooya et al., 1988; Liu and Ryan, 1991; Ziegler and van Antwerpen, 2006).

Maternally provisioned proteins and lipids are metabolized during embryogenesis to release nutrients and a metabolic fuel supply. Along with other lipids, a large amount of hydrocarbons, constituting about 4.85% of egg lipids, is found in newly oviposited eggs of the German cockroach (Fan et al., 2002). The fate and function of maternal hydrocarbons within the embryo have not been investigated in any insect. Our radiotracer results showed clearly the intriguing fact that hydrocarbons were not metabolized during embryogenesis. Rather, maternal hydrocarbons remained intact to aid in waterproofing the cuticles of the first, second and even later instar offspring. Quantification of embryonic hydrocarbons and *in vitro* studies of hydrocarbon production showed that embryos began to make hydrocarbons approximately 30% into embryonic development (Fig. 1). This is also the stage in *B. germanica* when the oenocytes, the only cell type that biosynthesizes hydrocarbons (Fan et al., 2003), become differentiated and when deposition of the first embryonic cuticle is initiated (Rinterknecht, 1985). It is possible that maternal hydrocarbons might be used to coat the embryonic cuticle before the embryo is capable of making its own hydrocarbons. Towards the end of embryogenesis, a second embryonic cuticle – that of the pharate first instar – is deposited. After formation of the pharate first-instar cuticle, the oenocytes show signs of regression (Rinterknecht, 1985), coinciding with a sharp decline in production of hydrocarbons (Fig. 2A) and molting hormone.

But why does the mother provide later instars with hydrocarbon provisions when they are fully able to biosynthesize hydrocarbon? The answer may lie in the allometric scaling relationship between the cuticle and the fat body, as well as in insects' cyclic loss of competency to make hydrocarbons. It is possible that small nymphs, with a high surface area to volume ratio, are limited in their capacity to make sufficient hydrocarbons to thoroughly coat their cuticle and would experience high evaporative water loss. Maternal hydrocarbons would thus offset such deficiencies and serve to protect young nymphs from excessive water loss. Moreover, only a fraction of each intermolt period is dedicated to hydrocarbon synthesis (Fig. 2B) (Cripps et al., 1988; Young and Schal, 1997) and this process is significantly constrained by both availability of food (Young et al., 1999) and cyclic cellular competency of the oenocytes to produce hydrocarbons (Schal et al., 2003). Interestingly, the first instar of the German cockroach is the only mobile stage that can proceed to the next molt largely independent of food intake (Kopanic et al., 2001). It is quite likely that this lack of dependence upon food intake by the first instar is conferred by the mother's nutrient and hydrocarbon investment in the eggs.

Some marine invertebrates that provision their oocytes with large amounts of nutrients might represent a remarkably similar scenario. While elevated egg proteins free echinoderm larvae from the need to feed, extra provisions of lipid appear not to be utilized during embryonic and larval development. Rather, maternal lipids

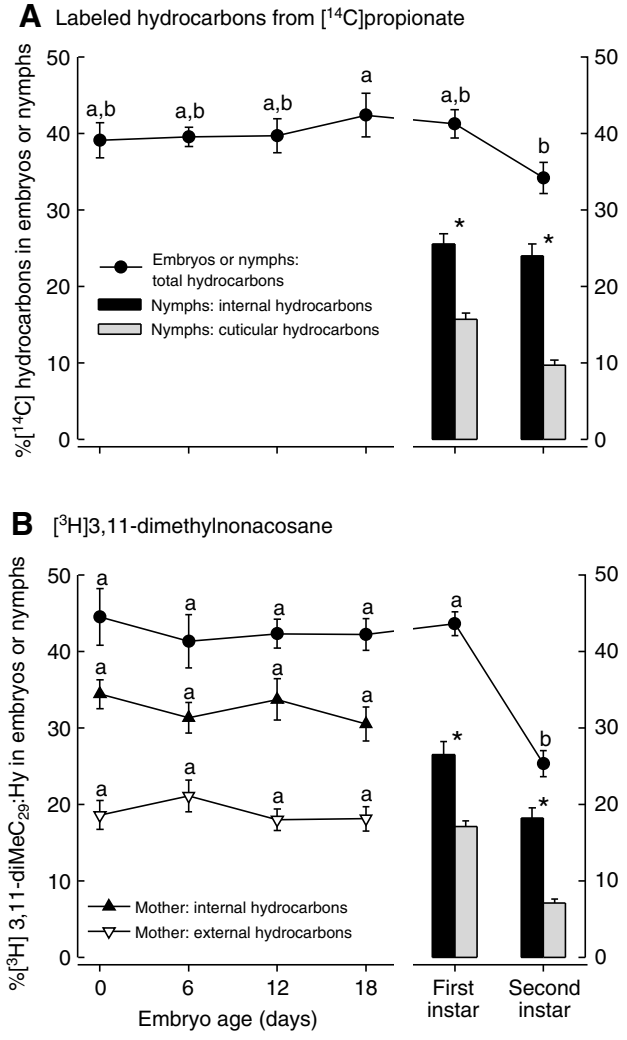

Fig. 3. Maternal transfer of hydrocarbons to oocytes and the fate of radiolabeled hydrocarbons in embryos and first- and second-instar nymphs. Vitellogenic day-3 females were injected with [1-^{14}C]propionate (A) or [^{3}H]3,11-dimethylnonacosane (B), and radiolabeled hydrocarbons were then extracted, purified and quantified on the external surface and internal tissues of the mothers, egg cases (and embryos) and nymphs. Data are normalized to show percentages of the total radiolabeled hydrocarbons in the mother and her offspring. Data are means ± s.e.m. (N=5–8). Significant differences among means within a series are represented by different letters (ANOVA, LSD, P<0.05) and between internal and external hydrocarbons of nymphs by asterisks (paired t-test, P<0.05).

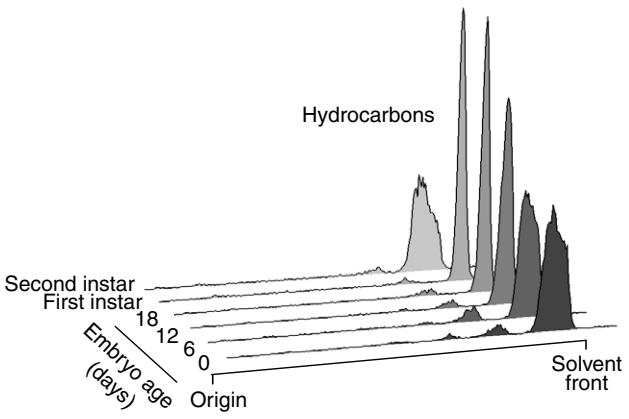

Fig. 4. TLC profiles of lipid extracts showing that >95% of the radioactivity is associated with hydrocarbons. Aliquots of extracts in Fig. 3A were combined for each age group.

– mainly wax esters – appear to be reserved for the postmetamorphic juveniles (Emlet and Hoegh-Guldberg, 1997; Rinterknecht, 1985; Villinski et al., 2002), and it is thought that provision of maternal lipids to postlarval stages is an adaptive response to a particularly vulnerable juvenile stage in a complex biphasic life cycle (Byrne and Cerra, 2000). Unfortunately, however, because the studies on marine invertebrates utilized mass measurements, microscopy and GC, they did not examine *de novo* lipid production nor clearly differentiated between maternal and newly biosynthesized embryonic and larval lipids. Nevertheless, the convergence of maternal lipid provisioning strategies of marine invertebrates and terrestrial insects suggests that maternal waterproofing of neonates has played a central role in the evolution of oviparity. If proven correct, it would appear that wax esters in marine invertebrates, in addition to serving in buoyancy, as previously suggested, might also be used for waterproofing.

The evolution of the amniotic/cleidoic egg of reptiles and birds also represents a case where the transition of embryogenesis to a terrestrial environment required adaptive changes, including prevention of desiccation and greater maternal yolk provisions (Speake and Thompson, 1999). As in marine invertebrates, greater yolk provisions allow embryos of vertebrates, for example precocial birds, to develop to a relatively advanced, active state, independently of the parents. Bird vitellogenin is limited in its ability to deliver lipids to oocytes, and, as in the cockroach, the plasma lipoprotein system is recruited to accomplish this task. But in addition to providing energy, some maternal provisions – for example the 18-carbon polyunsaturated fatty acid α-linolenic acid and docosahexenoic acid, a 22-carbon fatty acid derived from it – are preferentially transferred from yolk to the embryo in birds, or across the placental membrane in mammals (Speake and Thompson, 1999); they are required for neuronal and retinal development and cannot be synthesized by the embryo. Both fatty acids, like hydrocarbon provisions in the cockroach, resist metabolic degradation in the embryonic tissues; the fatty acids are instead targeted for delivery to the developing embryonic brain.

A potential implication of our findings is that, as in the cockroach, some social insects might endow their embryos with cuticular hydrocarbons as trophic provisions. In many social insects, hydrocarbons play important roles in species and nestmate recognition (Howard, 1993), and nestmates exchange and share cuticular hydrocarbons through mutual licking and passive contact (Soroker et al., 1994). These exchanges create a colony-specific odor, the so-called gestalt chemical signature [ants (Crozier and Dix, 1979); bees (Breed and Julian, 1992)]. Thus, studies of hydrocarbon transfer among social insects have concentrated on the homogenization of colony odors (i.e. hydrocarbons) through reciprocal exchanges among sterile workers. It is possible that the demands of egg production by queens also require that hydrocarbons biosynthesized by workers might also be vectored to the queen, to serve as maternal trophic provisions in eggs. Consequently, transfer of hydrocarbons throughout the colony might serve to both homogenize nestmate recognition cues and deliver hydrocarbons to the queens to provision to eggs. Therefore, it is important for researchers on cuticular hydrocarbons in arthropods, especially in social insects, to consider the multiplicity of hydrocarbon functions. For hydrocarbons to serve solely as recognition cues, only nanogram amounts would be needed per individual. Yet, small insects weighing just several mg may carry on their cuticular surface and internally 2–5 orders of magnitude more hydrocarbons than are needed as recognition signals. Clearly, waterproofing and trophic contributions to progeny have played important roles in the evolution of cuticular hydrocarbon profiles.

A number of intriguing issues remains to be addressed. First, how are maternal hydrocarbons provisioned into oocytes? Second, how are hydrocarbon reserves stored and partitioned in the embryo without interfering with growth and cellular differentiation and without undergoing chemical cleavage, like other maternal provisions? Third, what is the metabolic fate and function of maternally provisioned hydrocarbons? Fourth, what happens to hydrocarbon-deficient embryos? And finally, are there other maternal provisions that withstand embryonic catabolism? To our knowledge, such provisions have not been described in any animal or plant, but these questions are readily tractable with radio- and mass-labeling approaches. Also, it will be of interest to determine whether interference with maternal provisions might constitute a new target for environmentally responsible management of cockroach pest populations, which are now known to be a major source of allergens that cause childhood asthma (Rosenstreich et al., 1997).

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