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Food Intake in *Blattella germanica* (L.) Nymphs Affects Hydrocarbon Synthesis and Its Allocation in Adults Between Epicuticle and Reproduction

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The causal relationship between food intake and hydrocarbon synthesis was examined in vivo and in vitro. Fed *Blattella germanica* (L.) nymphs synthesized hydrocarbons in a stage-specific manner, with high rates occurring in the first 6 days of a 13-day last stadium, in relation to feeding. A similar pattern was exhibited in vitro by sternites and tergites from fed nymphs. In contrast, starved nymphs synthesized hydrocarbons at normal rates for the first 2 days, but then synthesis declined and ceased by day 6. Their abdominal sternites and tergites displayed a similar biosynthetic pattern in vitro, showing that starved tissues lost the capacity to synthesize hydrocarbons, even when provided appropriate nutrients. Synthesis resumed within 2 days of being fed on day 6, reaching a maximum rate 6 days later. Some hydrocarbon appeared on the nymphal cuticle, but almost 4-fold more hydrocarbon was internal in hemolymph lipophorin, fat body, and the developing imaginal cuticle. Because most hydrocarbon synthesized in nymphs provisions the adult, and synthesis is related to food intake, we examined trade-offs in allocations in food-limited insects. Nymphs provided with insufficient quantities of food allocated normal amounts of hydrocarbons to the nymphal epicuticle, but molted into smaller adults with significantly less internal hydrocarbons. These cockroaches directed nearly normal amounts of hydrocarbons to their epicuticle, oocytes, and oothecae, at the cost of internal hydrocarbon reserves for repair and subsequent gonotrophic cycles. Hydrocarbons, thus, appear to serve an important cross-stadial resource and the object of competition among several nymphal and adult tissues. Arch. Insect Biochem. Physiol. 41:214–224, 1999. **© 1999 Wiley-Liss, Inc.**

Key words: hydrocarbons; feeding; starvation; synthesis; parental investment; German cockroach; *Blattella germanica*

Abbreviations used: GLC = gas liquid chromatography; HC = hydrocarbon

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INTRODUCTION

Cuticular lipids, especially hydrocarbons (HC), form the main waterproofing barrier of insects, including the German cockroach, *Blattella germanica* (L.) (for reviews see Hadley, 1981, 1994; Nelson, 1978; Nelson and Blomquist, 1995). Insects unable, for whatever reason, to interpose this waterproofing layer of wax between themselves and unsaturated air soon succumb to desiccation (Ebeling, 1976; Wigglesworth, 1946, 1958). The rigid, confining nature of the arthropod exoskeleton requires periodic molts if growth is to occur, which exposes the growing immature stages to successive episodes of water stress. Teneral insects are extremely susceptible to desiccation and have behavioral adaptations (concealment, choice of a moist microhabitat during and following eclosion) to minimize the risk of dehydration until the cuticle is tanned. The molting insect emerges from its shed exoskeleton with an adequate waterproofing layer already in place, as one would expect given the lethal consequences of dehydration (Guo and Blomquist, 1991; Young and Schal, 1997; Young et al., 1999).

A prevalent theme in animals with discontinous development, including insects, is that some specialized molecules are synthesized in one life stage and mobilized for tissue development in another life stage. Because insects appear to lack a mechanism for reclaiming HC from the shed cuticle (Guo and Blomquist, 1991; Dwyer et al., 1986), periodic molts require synthesis of lipids for the new cuticle. In a number of holometabolous and hemimetabolous insects, it has been shown that after each ecdysis the amount of internal HC declines sharply, suggesting that the internal HC serve as a reservoir for the cuticle of the next developmental stage. The rate of HC synthesis increases dramatically after each molt, during the feeding stages, and falls to low levels during the wandering stages (Dwyer et al., 1986; Cripps et al., 1988; Guo and Blomquist, 1991; Young and Schal, 1997). Similarly, adult females synthesize HC in a stage-specific pattern that is related to food intake, and they transfer HC to the next generation, the oocyte, via the reproductive cycle (Schal et al., 1994, 1998).

The German cockroach is an opportunistic, long-lived, insect that is often resource-limited nutritionally as a result of unpredictable food availability. Kunkel (1966) showed that withholding food from nymphs, followed by synchronous re-feeding, was an effective way to synchronize the development of variously aged individuals in a laboratory colony. As little as 12-h access to food after a period of cuticle tanning was sufficient for nymphs to successfully complete the next molt cycle. Later work determined that adequately nourished immatures would commit to the next molt relatively early in the stadium (approximately 50% developmental time) and would successfully molt even when subsequently deprived of food (Kunkel, 1975). These experiments illustrated the importance of the internal accumulation of all of the nutritional resources required to complete the next molt.

An important question in physiological ecology is how a given resource (here, hydrocarbons) is allocated between somatic maintenance (here, epicuticular HC) that ensures survival of the individual, and reproductive effort (here, internal HC bound to the imaginal cuticle and ovaries). Our objectives were to relate patterns of HC synthesis to food intake, quantify the amounts of HC transferred to the nymphal epicuticle and those retained internally for adult development, and to assess the allocation of HC in malnourished adult females among the epicuticle and ovaries.

MATERIALS AND METHODS

Insects

Cockroaches used in these experiments were from an American Cyanamid insecticide-susceptible strain raised in glass jars at 27°C and ambient relative humidity on a 12:12 light:dark cycle. Purina Rat Chow no. 5012 (Purina Mills, St. Louis, MO) and water were provided ad libitum. All nymphs were taken from the colony within 2 h of ecdysis into the last stadium, in mid-photophase, before they had eaten their exuviae. Pairs of female nymphs were placed in clean 100 × 15 mm plastic Petri dishes with water and chow at 27°C and 50% relative humidity. Chow portions larger than what would normally be consumed in the last stadium contained no detectable HC.

Chemicals

Sodium [1-^{14}C]propionate, 1.9 GBq/mmol (51 mCi/mmol), was obtained from NEN Research

Products (DuPont Co., Boston, MA) or American Radiolabeled Chemicals Inc. (St. Louis, MO). All other chemicals were from Sigma (St. Louis, MO), Bio-Rad (Richmond, CA), or Fisher Scientific (Pittsburgh, PA).

Hydrocarbon Quantification

All insects were killed by freezing at –20°C overnight. External lipids were extracted from the cuticle by immersing a pair of thawed insects in 2 ml of *n*-hexane containing 15 μg *n*-hexacosane as internal standard, mixing gently for 5 min, decanting the solvent into a clean vial, repeating the wash, then rinsing the vial and insects with 1 ml hexane. The HC fraction was eluted from a silica gel Pasteur pipette column (Biosil A, Bio-Rad) with 7 ml hexane.

Internal lipids, including those in the developing imaginal cuticle, were extracted by a modification of the procedure of Bligh and Dyer (1959). The pairs of insects previously extracted for external lipids were homogenized 30 s (Polytron, Brinkmann Instruments, Westbury, NY) in chloroform:methanol:water (2:2:1.8 ml) containing 30 μg *n*-hexacosane as internal standard and the resulting homogenate vortexed and centrifuged at 1,500*g* for 5 min. The chloroform layer was transferred to a clean vial and the vortex-centrifuge extraction of the methanol:water phase was repeated twice more with the addition of 2 ml chloroform each time. The lipid extract was then taken up in hexane and fractionated as above. Both external and internal HC fractions were injected into a splitless injector leading to a 25 m × 0.32 mm × 1 μm Hewlett-Packard (Avondale, PA) HP-1 capillary column. A Hewlett-Packard 5890II gas-liquid-chromatograph (GLC) was interfaced with a 3365II Chemstation. The extraction procedures have been validated and the chromatographic conditions have been previously described (Young and Schal, 1997).

Hydrocarbon Synthesis In Vivo

Methylmalonyl-CoA, derived from propionate, acts as a methyl-branch donor in the synthesis of methyl-branched HC in *B. germanica* (Chase et al., 1990). After being anesthetized by chilling on an ice bath 10–20 min, insects were injected with 4,033 Bq sodium [1-^{14}C]propionate in 1 μl *B. germanica* iso-osmotic saline solution (Kurtti and Brooks, 1976) as described in Young and Schal (1997) and Young et al. (1999). Insects were freeze-killed 8 h later, external and internal HC were extracted as above, and the HC fractions dissolved in Scintillene cocktail (Fisher Scientific, Pittsburgh, PA) and analyzed on a Beckman LS-5801 (Fullerton, CA) liquid scintillation spectrometer.

Hydrocarbon Synthesis In Vitro

Abdominal sternites and tergites were removed with forceps, cleaned of adhering fat body, and rinsed 3× with saline. The pronotum was likewise removed from the thorax. Sclerites were incubated in 0.5 ml *B. germanica* saline solution (BG-SSA, pH adjusted to 7.2, osmotic pressure 410 mOsm) (Kurtti and Brooks, 1976) at 27°C with 4,657 Bq sodium [1-^{14}C]propionate. An orbital waving shaker (The Waver, VWR) was used to keep the sclerites oxygenated (Katase and Chino, 1982). Previous results showed linear ^{14}C incorporation into HC for at least 6 h (Young et al., 1999). After 3 h, 1 ml of methanol was added and the sample was vortexed and stored at –20°C. Thawed samples and medium were extracted with 3 ml of hexane with a sonicator probe (Micro Ultrasonic Cell Disrupter, Kontes, Vineland, NJ), centrifuged at 2,000*g* for 10 min, and the HC in a 2-ml aliquot of the hexane were analyzed as above.

Determination of Food Intake

Insects used in the starvation-refeeding experiments were paired in Petri dishes as above with the following changes: Insects were provided with water but no food for the first 6 days post-ecdysis and weighed daily. On the 6th day the cockroaches were offered ground and sieved (Tyler mesh no. 25) rat chow in a plastic tissue-culture cell (330 μl volume). The chow and plastic cup were preconditioned in the rearing incubator in a Petri dish containing a water tube but no insects.

Limited Food Provision for Last-Instar Females

Females were weighed immediately after ecdysis into the last stadium, fed 3–4 days, weighed again, and then returned to a Petri dish with water but no food. All nymphs were paired through adult eclosion. The age at eclosion, adult body mass, and the ingestion or removal of exu-

viae were all recorded. A second set of females was similarly taken through the last stadium, mated on days 5–6 post-eclosion, and their oothecae were removed within 12 h of oviposition (day 9 post-eclosion), weighed, and extracted for external and internal HC as above, as were the adult females in both sets of experiments.

All data are presented as means ± SEM.

RESULTS

Food Intake and Hydrocarbon Synthesis

Hydrocarbon synthesis in *B. germanica* is related to stage-specific food intake in nymphs and adults (Schal et al., 1994; Young and Schal, 1997). The role of food in HC synthesis was probed by starving newly-molted last instars for 6 days. Figure 1 shows the patterns of food intake and body mass, determined by weighing insects and chow daily. Starved insects maintained their body mass, presumably by remaining inactive and drinking water, throughout the fasting period. They consumed a large ration of food upon being offered chow on day 6, followed by a return to the normal feeding pattern. The nymphs ceased feeding after 6 days, as do insects fed ad libitum after ecdysis (Young and Schal, 1997). Nymphs attained a body mass 2.26-fold their teneral mass (from 26.9 ± 0.6 to 60.9 ± 1.1 mg) at the time food intake ceased in most insects, and by the imaginal molt the nymphs weighed 55.0 ± 1.2 mg. Refed nymphs consumed 19.4 ± 0.6 mg rat chow to attain their eclosion mass.

In normal nymphs, HC were synthesized at increasing rates during the first two-thirds of the stadium, then synthesis ceased 2–4 days prior to the imaginal molt, as determined by incorporation of ^{14}C propionate (Fig. 2A; Young and Schal, 1997). This pattern is related to stage-specific food intake with the majority of the newly synthesized HC being retained internally at all ages. However, a greater proportion of newly-synthesized HC was delivered to the nymphal epicuticle in younger nymphs than it

Fig. 1. Diet consumption and live body mass of *B. germanica* last instar females, starved for 6 days post-ecdysis, then fed ad libitum. Data are means ± SEM of 17 pairs of females; the dashed vertical line indicates the imaginal molt, and a.0 is newly eclosed adult on day 0.

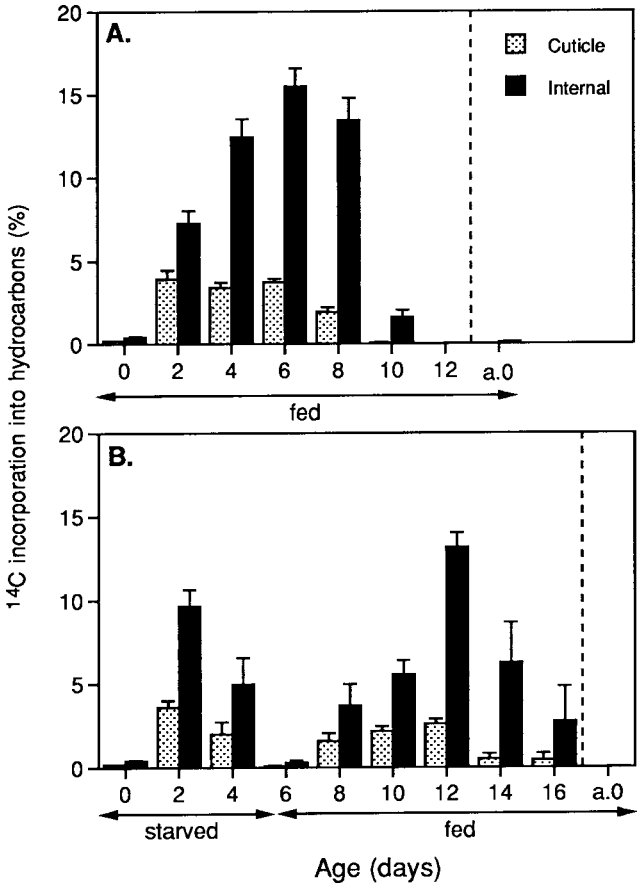

Fig. 2. Incorporation by *B. germanica* females of sodium [1-^{14}C]propionate (4,033 Bq injected into the abdominal hemocoel) into cuticular and internal methyl-branched hydrocarbons. **A:** Insects fed ad libitum (after Young and Schal, 1997; N = 6 females). **B:** Nymphs starved for 6 days, then fed ad libitum through the imaginal molt (N = 5 to 11 females). Bars represent means + SEM, the dashed vertical lines indicate the imaginal molt, and a.0 is newly eclosed adult on day 0.

was in older nymphs. During the first 4 days of starvation, nymphs were able to incorporate labeled propionate into HC (Fig. 2B), presumably by using nutritional reserves accumulated in the previous nymphal stadium. However, HC synthesis declined steadily in starved nymphs until it was nearly undetectable on day 6. Incorporation of label into epicuticular HC declined at a slower rate than did incorporation into internal HC, suggesting that starved nymphs defended their cuticle at the cost of accumulating internal HC reserves. Following feeding on day 6, a normal cycle of HC synthesis ensued, with a peak 6 days later, as in normal nymphs. Much like normal nymphs, in which HC synthesis ceased altogether 2–4 days prior to the imaginal molt, HC synthesis ceased in starved-re-fed nymphs until ecdysis. This relationship was somewhat obscured, however, by our staging procedure, which only allows us to state the time elapsed since feeding resumed, and not necessarily the time remaining until an individual nymph molted (Fig. 2). This apparently gradual tailing-off of feeding is attributable to some individuals who were still synthesizing HC very late in the stadium, presumably as a result of being more retarded than the other insects sampled.

In *B. germanica*, HC are synthesized by the integument that underlies the tergites and sternites (Gu et al., 1995; Young et al., 1999). Radiotracer studies showed that abdominal sclerites removed from fed insects had a pattern of HC synthesis in vitro that was remarkably similar to the in vivo pattern shown in Figure 2A (Fig. 3A). The integument underlying the sclerites of continuously starved insects, likewise, exhibited a pattern similar to the in vivo pattern seen in whole nymphs during starvation (Fig. 2B). The rate of in vitro HC synthesis increased for the first 2 days of starvation, but then declined as it did in whole insects (Fig. 2B) to undetectable levels (Fig. 3B). This suggests that when food is unavailable, the biosynthetic tissue suspends HC synthesis, and the presence of nutrients alone in the culture medium is insufficient to restore HC biosynthesis. The tissue might require longer exposure to nutrients and/or specific cues that were absent from the in vitro system.

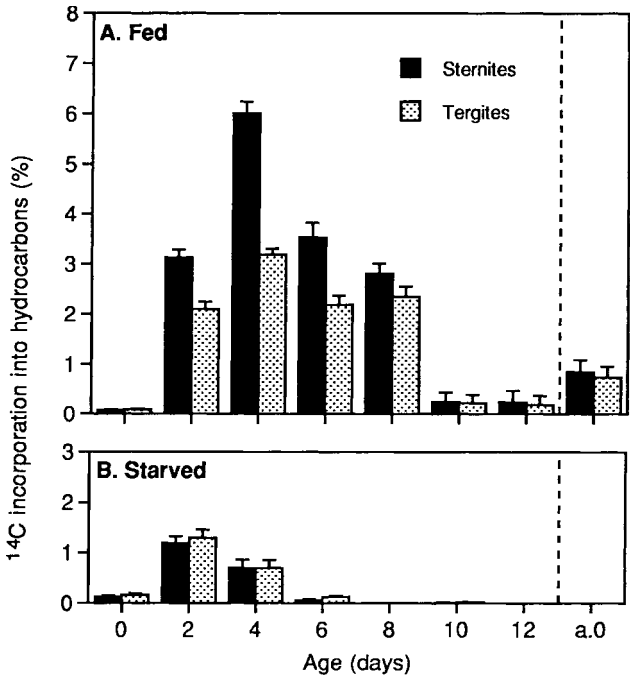

Fig. 3. Incorporation of sodium [1-^{14}C]propionate (4,657 Bq injected into the abdominal hemocoel) into methyl-branched HC by abdominal integument incubated 3 h in vitro. **A:** Control insects, fed ad libitum. **B:** Starved insects. Data are means + SEM, N = 12 females per mean, the dashed vertical lines indicate the imaginal molt, and a.0 is newly eclosed adult on day 0.

Epicuticular and Internal Hydrocarbon Titers

The accumulation of HC, as determined by GLC, is shown in Figure 4. The total amount of internal HC declined during the 6-day starvation from 50.4 ± 2.0 μg on day 0 to 28.5 ± 2.5 μg on day 6 ($P < 0.001$, $t = 5.892$, df = 13) (Fig. 4A) despite the synthesis of HC on days 2–4 (Fig. 2B). In contrast, epicuticular HC increased from 38.5 ± 2.3 μg on day 0 to 64.6 ± 3.6 μg on day 6 ($P < 0.001$, $t = -5.947$, df = 13). During the feeding phase after day 6, epicuticular HC increased only marginally. However, the appearance of HC internally corresponded well with HC synthesis, as in insects allowed to feed ad libitum throughout the last stadium (Fig. 4B). Despite the 6-day starvation, the re-fed insects attained pre-eclosion levels of HC similar to those in control insects both internally (221.6 ± 14.6 and 240.9 ± 12.4 μg, respectively; $P = 0.39$, $t = 0.885$, df = 12) and on the nymph epicuticle (85.1 ± 5.6 and 83.2 ± 5.3 μg, respectively; $P = 0.82$, $t = 0.237$, df = 12). Thus, it appears that while starvation delays the imagi-

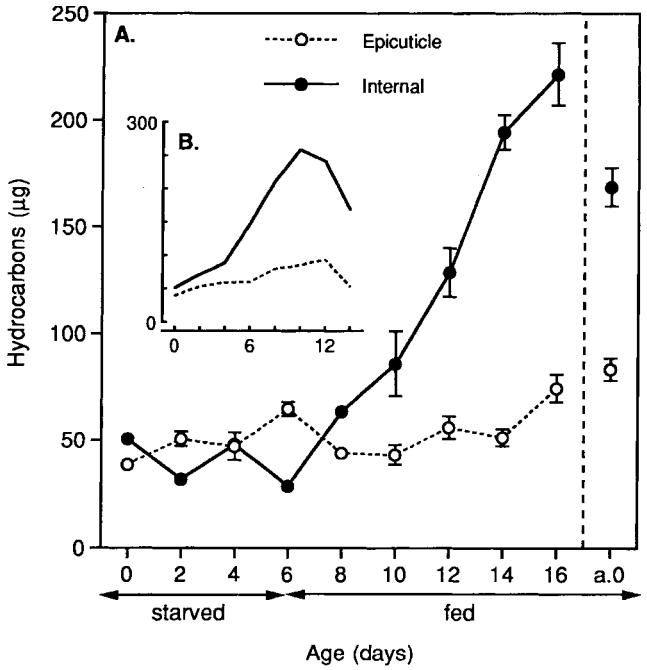

Fig. 4. Hydrocarbon accumulation, determined by GLC, in *B. germanica* females (**A**) starved for 6 days and then fed through adult eclosion, and (**B, inset**) fed ad libitum through adult eclosion (after Young and Schal, 1997). Data are means ± SEM, N = 4 pairs of females, and dashed vertical line indicates the imaginal molt.

nal molt and clearly has significant maladaptive consequences, the nymph is able to readily restore its provision of HC for the adult stage.

Compensation for Sub-Optimal Food

Because starvation elicited significant delays in development with concomitant suppression of HC synthesis, we hypothesized that limiting food would partly suppress HC synthesis but allow adult development. The adults would then either compensate for what was not allocated by the last instars (i.e., increase reproductive effort), or provision less HC to the cuticle and oocytes. The developmental effect of withholding food for progressively longer periods in the last stadium is shown in Figure 5. Most nymphs allowed to feed ad libitum through the imaginal molt or for only 5 or 6 days molted in the usual time, their last larval stadium lasting 12 or 13 days. Nymphs starved after 4 days of feeding exhibited significant developmental delays, and only 85% molted. Slightly more than one-third (35%) of insects fed only 3 days were able to molt, but only after a delay of several days. Nymphs fed only 1 or 2 days eventually died without molting.

Nymphs that were fed only 4, 5, or 6 days were used to generate a population of variably provisioned adult females. Females were weighed at eclosion and their eclosion body mass, as a function of last stadium duration, is shown in Figure 6. Cockroaches that molted later accumulated less body mass, with a 3.7 mg weight deficit associated with every day molting was delayed. The lightest insects tended to be those that had fed only 4 days (not shown). These newly eclosed adults were extracted for cuticular and internal HC. Cuticular HC varied little with weight and were within a relatively narrow range of 53–91 µg per insect (Fig. 7). The mean, 78.7 ± 3.0 µg, was not significantly different from that of insects fed ad libitum ($P = 0.43$, $t = 2.578$, df = 46). Internal HC, as well, varied with body mass, at an average rate of 3.4 µg HC per mg of live body mass. The range was greater than that of cuticular HC, from 121 to 308 µg. Those fed only 4 days as nymphs had significantly lower mean quantities of internal HC at eclosion than did nymphs fed ad libitum (166.6 ± 29.7 and 207.4 ± 36.0 µg, respectively; $P = 0.001$, $t = 4.229$, df = 46). It, thus, appears that nymphs allocate HC to the developing imaginal cuticle at the cost of provisioning HC internally, presumably for oocyte maturation.

The effects of nymphal malnutrition upon adult reproduction were examined by feeding

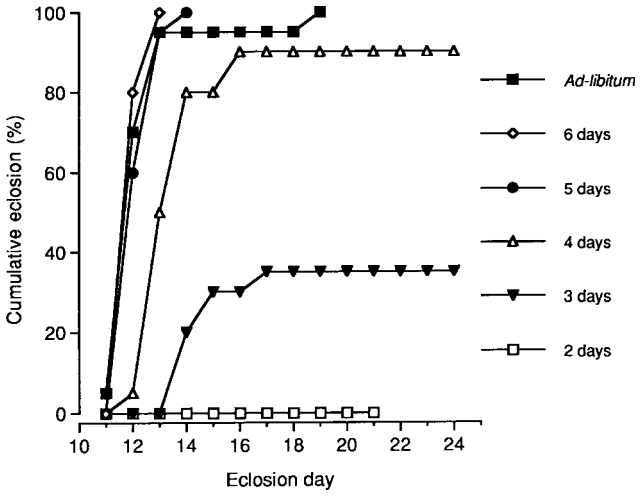

Fig. 5. Cumulative adult *B. germanica* eclosion as a function of length of the last larval stadium. Insects were allowed to feed for varying periods, as indicated by the number of days next to each curve, following the last larval ecdysis. N = 20 females per treatment for ad-lib, 6, 5, 4, and 3 days of feeding; N = 10 females for 2 and 1 days of feeding.

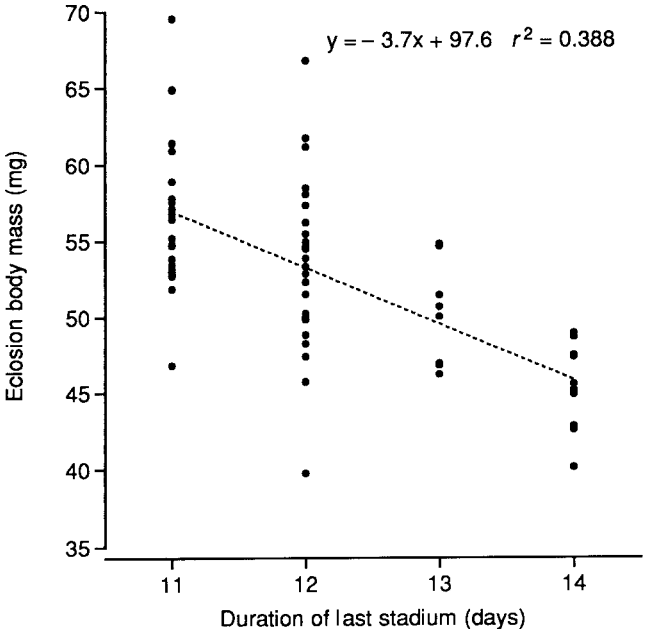

Fig. 6. Body weights of adult *B. germanica* at eclosion as a function of the duration of the last instar. Female nymphs were allowed to feed either 4, 5, or 6 days. N = 70 females.

nymphs for the first 3–4 days of the last stadium, then allowing those that successfully molted to adults to feed ad libitum before being mated on day 6 post-eclosion. Each female and her ootheca was weighed after the ootheca was rotated (day 9); females invested 36 ± 0.04% of their pre-oviposition mass in the ootheca. HC were extracted from the exterior and interior of both mother and egg case. Maternal cuticular HC varied little as a function of eclosion body mass (Fig. 8A), as shown in newly emerged females. However, body mass significantly affected the remaining internal HC at oviposition. The oothecal HC showed remarkably less variation across a 62.5% increase in maternal eclosion mass (Fig. 8B). The internal, embryonic HC varied less than did maternal internal HC, averaging 208.8 ± 5.3 μg per ootheca. These results suggest that adults compensated for malnutrition during the nymphal stage and invested proportionately more HC in the ooth-

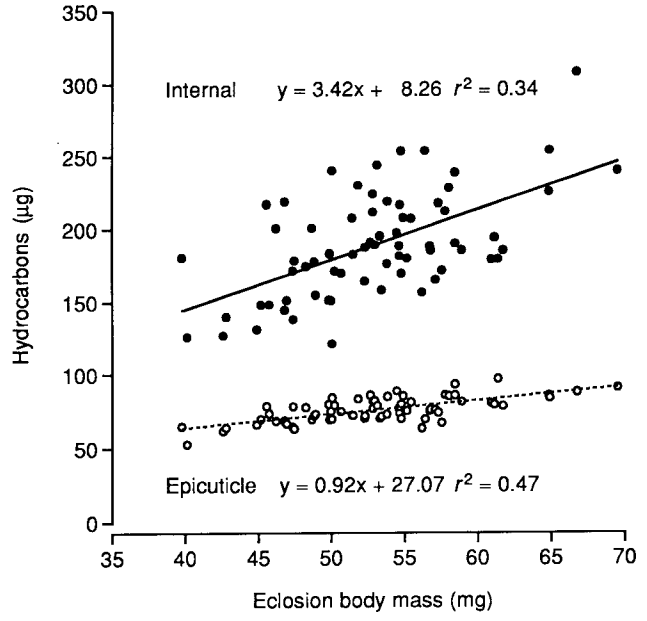

Fig. 7. Hydrocarbon content of the epicuticle and interior of newly eclosed adult *B. germanica,* as determined by GLC. Females shown in Figure 6 (N = 70 females) were used 1 to 2 h after eclosion.

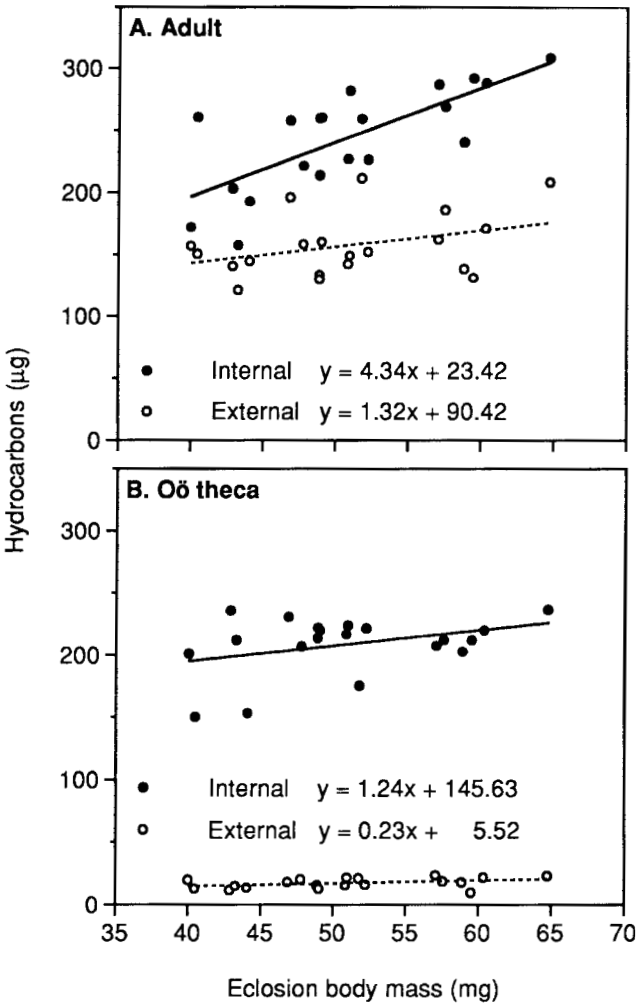

Fig. 8. Hydrocarbon content of the exterior and interior of (**A**) female *B. germanica* immediately post-oviposition, and (**B**) freshly oviposited oothecae. N = 20 insects.

eca than did normal females. As a consequence, such females were left with less internal HC and a second round of compensatory allocation would presumably be required during the second preoviposition period. Consistent with this conclusion is the lack of correlation between post-oviposition maternal HC, which is quite variable (150–300 μg) and total oothecal HC (Fig. 9).

DISCUSSION

Relation Between Food Intake and Hydrocarbon Synthesis

HC synthesis and the amount and type of HC on the cuticle may vary with age, with environmental conditions such as temperature, relative humidity and habitat, and with developmental stage (Blomquist et al., 1987a,b; Gibbs and Crowe, 1991; Howard, 1993; Nelson and Blomquist, 1995). In the house cricket, *Acheta domesticus,* the patterns of triacylglycerol, phospholipid, and HC syntheses are similar, with lipogenesis being maximal at mid-stadium and minimal at ecdysis, presumably reflecting the pattern of feeding and water intake (Cripps et al., 1988). In cabbage looper (*Trichoplusia ni*) larvae, the rate of HC synthesis also increases dramatically after each molt, during the feeding stages, and falls to low levels during the wandering stages

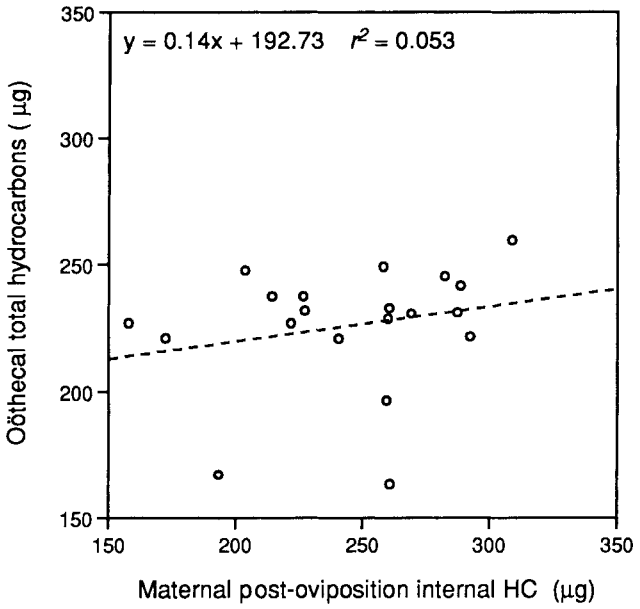

Fig. 9. Total HC invested in oothecae in relation to internal maternal post-oviposition reserves. Females were as in Figure 8. N = 20 female/oothecal pairs.

(Dwyer et al., 1986). Similarly, both nymphs and adults of *B. germanica* exhibit a clear relationship between HC synthesis and food intake (Fig. 2A; Schal et al., 1994; Young and Schal, 1997).

The present study documents a profound link between feeding and HC synthesis. Although last instar females readily withstand a 6-day period of starvation (Fig. 1) and even synthesize HC for the first 2 days of starvation (Fig. 2), it appears that this represents a minimal, indeed barely detectable, addition of HC to the epicuticle or internal tissues (Fig. 4). Presumably, these HC are derived from precursors carried over from the previous stadium, most likely lipids in the fat body, as shown in nutrient deficient and starved cockroaches (Schal et al., 1993). HC synthesis then dramatically declines to undetectable levels. Upon feeding, nymphs resume the normal course of development and attain standard targets of body mass (approximately 61 mg) and internal HC reserves (222 μg), as do normal nymphs, before committing to the molt (Young and Schal, 1997).

The HC synthesizing tissue, the abdominal integument (Young et al., 1999), from starved nymphs failed to synthesize HC in vitro in a nutrient rich medium, whereas the same tissues from fed insects incorporated up to 6% of available radiolabel into HC (Fig. 3). This suggests that HC synthesis was suppressed in starved nymphs not only because nutrients were limiting, but rather in concert with other developmental and metabolic processes associated with the molt cycle. Although HC are critical for survival, they are not a major component of the insect's biomass, there being at most 100 μg on the epicuticle of a nymph weighing 45–60 mg. Thus, lack of nutrients for development to proceed, rather than lack of substrate specifically for HC synthesis may terminate HC synthesis in starved insects. The intrinsic factors that regulate the initiation and termination of HC synthesis are not known.

Differential Allocation of Hydrocarbons Among Target Tissues

Hydrocarbons synthesized in one life stage are, to a large extent, used by the next life stage. Hence, HC synthesized by immatures are used not only on the existing epicuticle, but are internally carried through the molt and appear on the

new epicuticle of the next stadium (Dwyer et al., 1986; Cripps et al., 1988; Guo and Blomquist, 1991; Young and Schal, 1997). Similarly, adult female *B. germanica* accumulate large HC reserves in their hemolymph and fat body, then re-direct HC into the developing oocytes (Gu et al., 1995; Sevala et al., 1999). Consequently, at each stadium and in adults, more HC is transported from the abdominal integument to the interior of the insect than to the epicuticle (Young and Schal, 1997; Schal et al., 1998).

However, a transient increase in the fraction of newly synthesized HC directed to the new epicuticle occurs early in each stadium. Undoubtedly, the newly ecdysed insect is highly susceptible to desiccation, and the immediate benefits of completing the waterproofing layer of the new epicuticle are more important than accumulating internal reserves. Internal HC reserves can be accumulated during the feeding phase of the next stadium.

The differential allocation of a finite internal resource among growth, reproduction, and somatic maintenance can result in negative associations among life history traits, as for example egg size and egg number (see Zera and Denno, 1997). When nutrient resources are deficient or limited, resulting in poor HC production, a negative association (i.e., trade-off) might be expected between HC transported to the epicuticle and HC bound for reproduction. We investigated the relative importance of external and internal HC in the reproductive life of female German cockroaches by providing last instars with sub-optimal amounts of food. Withholding food after 3 or 4 days resulted in nymphs that either failed to molt or entered adulthood at a reduced body mass (Fig. 5). Epicuticular HC varied little with body mass, within the usual range of 50–90 μg (mean 78.6 ± 3.0 μg) (Young and Schal, 1997). Internal HC, however, varied much more, and were most notably lower in the lightest insects (< 50 mg) (Fig. 7). Females fed ad-libitum eclose at 50–65 mg (mean 54.2 ± 1.1 mg) and have 180–250 μg of internal HC (mean 207.4 ± 7.1 μg). The smallest females, however, have less than 170 μg of internal HC at eclosion. Although the amount of HC necessary to cover a surface (i.e., epicuticle) should vary less with body mass than the amount needed internally, it appears that transport of HC to the cuticle is favored at the expense of internal HC. This is also evident in starved females: While overall HC production declined in a monotonic fashion, the appearance internally of newly synthesized HC fell off more rapidly than did that of HC directed to the epicuticle (Figs. 2, 4).

In the adult stage, HC can be directed to the epicuticle or oocytes, or retained for use in repair and for later gonotrophic cycles. *Blattella* females must feed during each pre-oviposition period in order to accumulate enough mass for oocyte growth; 90% of a female's internal reserves go into her ootheca (Kunkel, 1966). In order to assess the ability, if any, of adult females to compensate for sub-optimal nymphal nutrition, we allowed them to feed ad libitum post-eclosion, then mate and produce oothecae. After oviposition, 9 days post-eclosion, these stunted females provision their epicuticle, oocytes, and the exterior of the ootheca with comparable amounts of HC as do control females. However, they are left with more heavily depleted internal HC reserves after oviposition. In control females the epicuticular HC comprise 25.6 ± 1.6% of her peak total HC just before oviposition, and 208.8 ± 5.3 μg are provisioned within the ootheca. Undernourished females (less than 50 mg eclosion mass) similarly provision 26.2 ± 1.1% of total HC to the epicuticle. As seen in Figure 9, however, there is no consistent relationship ($r^2 = 0.053$) between oothecal investment of HC, which is usually in the range of 200–250 μg, and internal HC remaining in the mother after oviposition, which had a range almost 3× greater (105–300 μg). This is especially true for the most stunted, smaller females, which appear to make a greater effort to provide the ootheca with a fixed requirement for HC while depleting their own internal HC reserves.

These data suggest, as above, the sequential use of a limited HC resource, which may avoid trade-offs between somatic maintenance and reproduction. The overriding demands of waterproofing the epicuticle make imperative the early allocation of HC to the adult epicuticle and a later maternal allocation of HC to the embryos. Nevertheless, a trade-off (differential allocation of HC) between the first and future reproductive efforts might occur. The female can either internally retain HC for later use, or provision the embryos, leaving the internal HC reserve relatively depleted. She does the latter, presumably because the HC requirement of the competing epicuticle

declines dramatically after the first pre-oviposition period and she will be able to internally retain more HC after each subsequent oviposition.

Still, experimental manipulations indicate that the epicuticle and ovary might indeed compete for HC. When the ovarian sink for HC is removed either through allatectomy, which removes juvenile hormone and ovarian uptake of hemolymph components (including HC), or through ovariectomy, the epicuticle receives more HC and HC-derived sex pheromone (Schal et al., 1994). This suggests that although HC uptake by the ovaries is temporally disassociated from epicuticular uptake in normal adults, the epicuticle can receive greater amounts of HC in the absence of ovarian competition.

In conclusion, HC synthesis in *B. germanica* nymphs is closely coordinated with development. An early urgency to waterproof the epicuticle is followed by accumulation of HC reserves for the next life stage. Starvation suppresses HC synthesis in concert with developmental arrest, but only after the epicuticle has been protected. Upon availability of nutrients, nymphs quickly resume normal development, including the synthesis and accumulation of HC reserves for the next life stage. Adults appear to have evolved a strategy to separate reproduction from an earlier somatic maturation, thus ensuring that internal resources, including HC, can be maximally directed to reproduction. Adult females are flightless, small, opportunistic, and relatively long-lived insects that exploit being relatively independent of seasonal constraints in human-made structures. The female allocates an impressive 90% of her post-eclosion weight gain to the ootheca (Kunkel, 1966), and some somatic demands, such as flight capability, might have been re-directed to reproduction in this species. Although HC do not constitute a major reproductive investment on a mass basis, they are essential for survival in a xeric environment. The female also regulates water balance in the attached embryos by provisioning the outer surface of the ootheca with HC (Fig. 8) and coordinating water flow to the embryos (Roth and Willis, 1955). The stage-specific regulation of HC synthesis in concert with feeding, and the transport and allocation of HC to various sites is of continuing interest to students of insect hydrocarbons.

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