The Journal of Experimental Biology 209, 645-655
Published by The Company of Biologists 2006
doi:10.1242/jeb.02026

Effects of larval nutrition on the endocrinology of mosquito egg development

Aparna Telang[1,*], Yiping Li[2], Fernando G. Noriega[2] and Mark R. Brown[3]

[1]*University of Arizona, Department of Biochemistry and Molecular Biophysics, Tucson, AZ 85721, USA,* [2]*Florida International University, Department of Biological Sciences, Miami, FL 33199, USA and* [3]*University of Georgia, Department of Entomology, Athens, GA 30602, Greece*

*Author for correspondence at present address: University of Georgia, Department of Entomology and Center for Tropical and Emerging Global Diseases, Biological Sciences Building, Athens, GA 30602, USA (e-mail: atelang@bugs.ent.uga.edu)

Accepted 30 November 2005

Summary

Reproduction by female mosquitoes is dependent on energy resources but modulated by hormones. Our study focused on blood-meal-dependent, anautogenous *Aedes aegypti* and autogenous *Ochlerotatus atropalpus* that rely on larval-derived nutrient stores to develop eggs. To determine how larval nutrition affects the endocrinology of egg development in these females, we manipulated the quantity of larval food and measured *in vitro* production of juvenile hormone (JH) by corpora allata (CA) and ecdysteroids by ovaries. Newly emerged *A. aegypti* contain lower larval-derived protein reserves, and their CA produce high amounts of JH, in comparison with similarly staged *Oc. atropalpus*. Ecdysteroid production was initiated in newly emerged *Oc. atropalpus* females, which have higher protein reserves and which develop eggs without a blood meal, which is required by *A. aegypti* females to complete egg development.

Key words: autogeny, anautogeny, juvenile hormone, ecdysteroid, corpora allata, ovary, *Aedes aegypti*, *Ochlerotatus atropalpus*.

Introduction

The search for physiological mechanisms underlying egg development in mosquitoes has focused on blood-feeding species, largely due to their potential as disease vectors. Females of mosquito species that must ingest blood meals for the first and subsequent ovarian cycles are considered anautogenous. Our knowledge of the endocrinology of mosquito oogenesis is based mainly on the anautogenous yellow fever mosquito *Aedes aegypti* (Klowden, 1997; Raikhel et al., 1999). Soon after female *A. aegypti* eclose, the corpora allata (CA) begin secreting juvenile hormone (JH), which induces differentiation (competency) of ovaries and fat body. Once this pre-vitellogenic phase is complete, oogenesis is arrested until a blood meal is obtained. The act of feeding and presence of blood in the midgut initiate an endocrine cascade that ultimately results in egg maturation and oviposition. A steroidogenic gonadotropin released from the brain stimulates *A. aegypti* ovaries to secrete ecdysteroids (Klowden, 1997). Ecdysteroids then act through nuclear hormone receptors to increase expression of the vitellogenin genes in the fat body to support yolk synthesis (Raikhel et al., 2002).

In addition to endocrine regulation, products of blood meal digestion play a role in activating anautogenous oogenesis. The concentration of total free amino acids in the haemolymph of the female house mosquito *Culex pipiens pallens* reaches a maximum level 18 h after blood feeding (Uchida et al., 1990), and infusion of a balanced mixture of amino acids into the haemolymph activates ovarian development in a number of mosquito species (Uchida et al., 2001). In *A. aegypti*, vitellogenic response by the fat body increases substantially in the presence of both amino acids and 20-hydroxyecdysone, and this response is transduced by the target of rapamycin (TOR) pathway (Hansen et al., 2004). In addition to a female's reliance on blood meal nutrients for anautogeny, nutritional support is also present at eclosion (teneral) in the reserves she carries from her larval stage. For *A. aegypti* and several *Anopheles* species, the nutritional environment experienced by a female larva dictates her adult body size and resulting teneral reserves (Briegel, 1990a; Briegel, 1990b). Teneral reserves affect important female reproductive processes, such as utilization of reserves, fecundity, longevity and blood meal consumption and utilization (Briegel, 1990a; Briegel, 1990b; Briegel et al., 2002; Naksathit et al., 1999a; Naksathit et al., 1999b; Takken et al., 1998; Zhou et al., 2004). Thus, both larval-derived teneral reserves and a blood meal in anautogenous females can serve as sources of yolk precursors and as stimuli for hormonal regulation of egg maturation.

Other mosquito species, such as the rock pool mosquito, *Ochlerotatus atropalpus*, are autogenous, as they do not require a blood meal for at least the first ovarian cycle and instead utilize teneral reserves as adults to produce eggs. Autogeny has a strong genetic component, but its expression is highly responsive to certain environmental factors, such as nutrition and density in larval stages and host availability,

mating and sugar-feeding in the adult stage (Corbet, 1964; Corbet, 1967; Eberle and Reisen, 1986; O'Meara, 1979; Russell, 1979a; Russell, 1979b; Su and Mulla, 1997b). When *Oc. atropalpus* females were given high amounts of food as larvae, they emerged with a large body size and teneral reserves and produced a greater number of eggs compared with females fed less food as larvae (Telang and Wells, 2004). Studies of *Aedes albopictus* and *Culex tarsalis*, species with autogenous and anautogenous strains, have found that the key difference between strains is the greater level of teneral metabolic reserves found in autogenous females (Chambers and Klowden, 1994; Su and Mulla, 1997a).

While nutrition fuels oogenesis, hormones play a role in regulating this process. How the nutritional condition of a female mosquito influences hormones involved in oogenesis has not been adequately studied. The nutritional condition of anautogenous females is determined by larval-derived reserves, a sugar meal and a blood meal, whereas larval-derived teneral reserves primarily determine that of autogenous *Oc. atropalpus*. To understand how stage-specific nutrition affects endocrinology of oogenesis in autogenous and anautogenous mosquitoes, we manipulated larval nutrition of *Oc. atropalpus* and *A. aegypti* and measured their teneral nutrient reserves and production of JH and ecdysteroids for the first few days of adult emergence. During this time, sugar ingestion also affects female reproduction (Foster, 1995) and so its influence on hormonal activity was also examined. Past studies have used ablation and implantation methods to determine the contribution of endocrine tissues (Fuchs et al., 1980; Lea, 1963; Lea, 1964; Lea, 1970) and ecdysteroids (Kelly and Fuchs, 1980; Masler et al., 1980) to autogenous egg maturation. However, our study is the first to measure CA biosynthesis of JH and ovarian ecdysteroidogenesis during autogenous egg development in response to larval and adult nutrition. The first few days of emergence are of interest given that oocytes are arrested in anautogenous females but complete development in autogenous females over this time period. Our study shows that differences in nutritional and hormonal profiles exist between anautogenous and autogenous females during this critical time period of emergence.

Materials and methods

Animals

A laboratory colony of *Ochlerotatus atropalpus* (Coquillett) (Bass Rock strain) was maintained in an insectary at 27±1°C, 65% relative humidity with a daily photoperiod of 16 h:8 h light:dark, using established procedures (Telang and Wells, 2004). Females of our colony strain are 100% autogenous for their first ovarian cycle, as measured by primary follicles advancing beyond Christopher's stage II, similar to Clement's stage 2b without the need for a blood meal (Christophers, 1911; Clements, 1992). Routine colony maintenance of *Aedes aegypti* (L.) (Rockefeller strain) was previously described (Zhou et al., 2004). Adult females feed *ad libitum* on 3% sucrose for 4–5 days prior to a blood meal. Females are fed using an artificial blood feeder in which porcine blood, supplemented with 100 mmol l^{-1} ATP per 1.0 ml blood for phagostimulation, is added to a Parafilm^{TM}-lined glass vessel. Feeding stations are maintained at 37°C with the aid of a circulating water bath.

Manipulation of nutrition for larval Oc. atropalpus and A. aegypti

Larval nutrition was manipulated in the same manner for both species using rearing procedures previously described (Telang and Wells, 2004). Specifically, 24-h-old larvae of both species were placed in plastic trays (27×16×6.5 cm) containing 1.0 litre of tapwater at a low larval density of 50 per pan. For experiments, larvae were fed only 10% bovine liver powder according to a schedule outlined in Table 1. The feeding schedule for *A. aegypti* and *Oc. atropalpus* was different to accommodate their different larval development periods, but the species received the same total number of feedings and amount of food for each treatment over their respective larval stage. Earlier examination of larvae of both species reared under the 'low food quantity' treatment showed that this amount of food was regularly depleted but still supported growth and development to pupation whereas the 'high food quantity' treatment provided nourishment in excess of that required for maximal growth. The majority of females began to pupate 1–2 days following the last day of feeding by larvae, and only females eclosing from these pupae were examined. For both species, the low food quantity treatment always produced some larvae that underwent an extended developmental period, and these were not included in our measurements. This experimental design uses a low larval density to remove any effects of crowding while only varying food quantity and availability.

Quantification of nutrient reserves

Newly emerged females (0–6 h old) reared as larvae on the two diet regimens were immediately frozen for analysis of metabolic reserves. Whole-body homogenates of 8–10 newly emerged females were made to extract glycogen, storage lipids and proteins using a procedure first described by Van Handel (1965) and modified for *Aedes aegypti* (Zhou et al., 2004),

Table 1. *Schedule for feeding Oc. atropalpus and A. aegypti larvae*

Days after hatch		Amount of food given per diet treatment (mg)	
Oc. atropalpus	*A. aegypti*	High	Low
1	1	100	100
3	3	100	
6	5	100	
10	6	100	100
		Total: 400	200

Larval food consisted of 10% bovine liver powder suspension.

except that we omitted sections of the protocol leading to the isolation of a sugar fraction. Fractions of glycogen, lipid and protein were frozen until they could be quantified using colormetric-based assays. The amount of storage lipid, triacylglycerol, was determined by a modified vanillin reagent assay (Van Handel, 1985b). Total amount of glycogen was determined using a modified anthrone-based assay (Van Handel, 1985a). Protein was quantified using the BCA protein assay reagent kit (Pierce, Rockford, IL, USA). Complete details of our assay procedures were previously described (Telang and Wells, 2004). All nutrients are reported on a microgram per mg dry mass basis. Three replicates of each experiment were conducted for *Oc. atropalpus* (N=12) and two replicates of each experiment were conducted for *A. aegypti* (N=8).

Body size and fecundity

Another group of females from each replicate larval diet regimen was used to measure body size and fecundity. Egg production in relation to larval food amount was quantified for individual sugar-fed, mated *Oc. atropalpus* females at 72 h post-emergence. Ovaries were dissected, and the number of matured primary follicles was counted using a dissecting microscope (N=36). Egg production in response to larval food amount was quantified for individual *A. aegypti* females 72 h after blood feeding (N=28). In both species, the number of mature primary follicles was used because we were interested in a measure of potential fecundity. In preliminary experiments with both species, we determined that there was no significant difference between the number of mature follicles dissected and the number of eggs oviposited (data not shown). The same females from which we obtained fecundity data were also used to measure body size. Wing length was used to assess body size (Nasci, 1990; O'Meara and Krasnick, 1970) and was measured from the point of attachment to the wing tip, not including fringe, under a dissecting microscope using an ocular micrometer.

In vitro radiochemical assay for CA activity

For both species, females emerging from larvae given high or low amounts of food were maintained on either water or 3% sucrose, in association with males. Corpora allata (CA) complexes consisting of CA + corpora cardiaca (CC) + aorta + brain + head capsule were isolated from five females of each larval and adult diet treatment at specific times after emergence (0–6, 12, 24, 36 and 48 h). For female *Oc. atropalpus*, this time course covers an ovarian cycle that ends at 72 h post eclosion with mature eggs ready to be oviposited. For female *A. aegypti*, this time course reflects her previtellogenic phase, during which her primary follicles develop to a point of arrested growth awaiting a blood meal. Full details of assay methods and reagents were previously described (Li et al., 2003) and will only be summarized here; the same assay conditions were applied to both *Oc. atropalpus* and *A. aegypti* experiments. Briefly, CA complexes from individual females are transferred and pre-incubated in tissue culture medium without methionine, so that intraglandular methionine is consumed prior to assay. Complexes are then transferred and incubated for 4 h in fresh medium containing ^{3}H-labelled methionine. Under assay conditions, the incorporation of ^{3}H-labelled methionine into JH III was linear for at least 6 h in both *Oc. atropalpus* and *A. aegypti* (data not shown). After extraction and separation by thin-layer chromatography, the JH III band is removed, placed into scintillation cocktail and assayed for ^{3}H. The quantity of JH produced is calculated from the specific activity of the ^{3}H-labelled methionine in the medium and averaged for one hour. One replicate of this experiment was conducted.

Pre-vitellogenic follicle development in A. aegypti

Early follicular development in response to larval (high *vs* low food amounts) and adult nourishment (water *vs* sugar) was measured in *A. aegypti* 72 h post-emergence. Primary follicles were staged and their lengths were measured under a dissecting microscope using an ocular micrometer (N=60 for each larval and adult diet combination).

Ovarian ecdysteroid production in vitro, haemolymph ecdysteroid titer and the ecdysteroid radioimmunoassay

Oc. atropalpus females subjected to both nutritional regimens as larvae were given access to males and given water or 3% sucrose prior to the dissection of ovaries at different times after eclosion (0–6, 12, 24, 36 and 48 h). For the *in vitro* bioassay, four ovary pairs from identically staged and treated females were dissected in saline solution (128 mmol l^{-1} NaCl, 4.7 mmol l^{-1} KCl and 1.9 mmol l^{-1} $CaCl_2$) (Riehle and Brown, 1999) and then transferred to and incubated in 60 μl of buffered medium (139 mmol l^{-1} NaCl, 4.05 mmol l^{-1} KCl, 1.85 mmol l^{-1} $CaCl_2$, 12.5 mmol l^{-1} Hepes, 2.5 mmol l^{-1} trehalose, 0.3 mmol l^{-1} $MgCl_2$ and 0.9 mmol l^{-1} $NaHCO_3$; pH 6.5, adjusted with NaOH) (Riehle and Brown, 1999) in a polypropylene tube lid for 6 h at 27°C. After incubation, 50 μl of medium was collected and analyzed for ecdysteroid content using a radioimmunoassay (RIA) with an ecdysteroid antiserum at a 1:45 000 final dilution (Sieglaff et al., 2005). Haemolymph was collected from the same set of females prior to ovary removal for the *in vitro* bioassay. To collect haemolymph, the last two abdominal segments of four females were excised while abdomens were immersed in 75 μl of saline solution on ice, and then gentle pressure was applied to each female to facilitate haemolymph diffusion into the solution. After 5 min incubation, 50 μl of the saline solution was removed and stored at –80°C for the ecdysteroid RIA.

For each experiment, triplicates of four ovary pairs and four body haemolymph collections were analyzed for all time points and for each nutritional regimen in the same RIA. Each experiment was replicated with females from three different cohorts. Values for each tissue sample types are reported as 'ecdysteroid pg', because the secreted ecdysteroid species are unknown. Values reported are means of triplicate treatments from three experiments (N=9 per treatment).

Data analyses

Individual dry mass and percent dry mass protein, lipid and glycogen (calculated as μg nutrient per mg insect mass) were analyzed using analysis of variance (ANOVA), with larval diet, species and an interaction term included in our statistical model. Both wing length and egg production were analyzed using ANOVA, with larval diet as the explanatory variable. Follicle length was analyzed using two-way ANOVA and we incorporated larval diet, adult diet and an interaction term in our model. The biosynthesis of JH was analyzed within each species using two-way ANOVA, with the inclusion of diet treatment, time post-eclosion and an interaction term in our statistical model. Ecdysteroid production by ovaries and ecdysteroid levels in haemolymph were analyzed using two-way ANOVA with the inclusion of diet treatment, time post-eclosion and an interaction term in our statistical model. When necessary, differences between means were further analyzed using linear contrasts of treatment effects, and only tests found to be non-significant, based on $P>0.01$, are reported in this paper. All data were statistically analyzed using JMP IN (version 4.0.3, SAS Institute Inc.). Adjusted mean values (± standard errors of mean) were obtained from statistical models and used in all graphical illustrations.

Results

The effect of larval nourishment on female teneral reserves and fecundity

The effect of larval food quantity on female body mass and teneral reserve levels was examined for both *A. aegypti* and *Oc. atropalpus*. Given our interest in species differences, quantitative levels of metabolic reserves are presented on a per mg dry mass basis. The effect of larval diet on levels of teneral reserves will be reported for each species first. Female *A. aegypti* derived from high-food larvae were significantly heavier and lipid rich compared with females of low-food larvae (Fig. 1A,B). Whether given low or high amounts of food as larvae, female *A. aegypti* emerged with similar glycogen ($P=0.315$, from a linear contrast) and protein ($P=0.281$, from a linear contrast) reserve levels (Fig. 1C,D). For autogenous *Oc. atropalpus*, females of high-food larvae were significantly heavier (Fig. 1A) and richer in lipid and glycogen levels (Fig. 1B,C) than females of low-food larvae. However, *Oc. atropalpus* females of low-food larvae were significantly richer in protein (Fig. 1D).

Body dry masses attained by both autogenous *Oc. atropalpus* and anautogenous *A. aegypti* females strongly reflected the amount of larval food available (two-way ANOVA, $P=0.005$). While *A. aegypti* and *Oc. atropalpus* females of low-food larvae weighed the same at emergence ($P=1.0$, from a linear contrast; Fig. 1A), *Oc. atropalpus* of low-food larvae accumulated significantly less lipid, but significantly more glycogen and protein, than *A. aegypti* females from low-food larvae (Fig. 1B–D; $P=0.001$, from a linear contrast in all cases). Female *Oc. atropalpus* were significantly (37%) heavier relative to *A. aegypti* when both were derived from high-food larvae (Fig. 1A; $P<0.001$, from a linear contrast). On a per mg dry mass basis, *Oc. atropalpus* females of high-food larvae accumulated similar lipid levels ($P=0.70$, from a linear

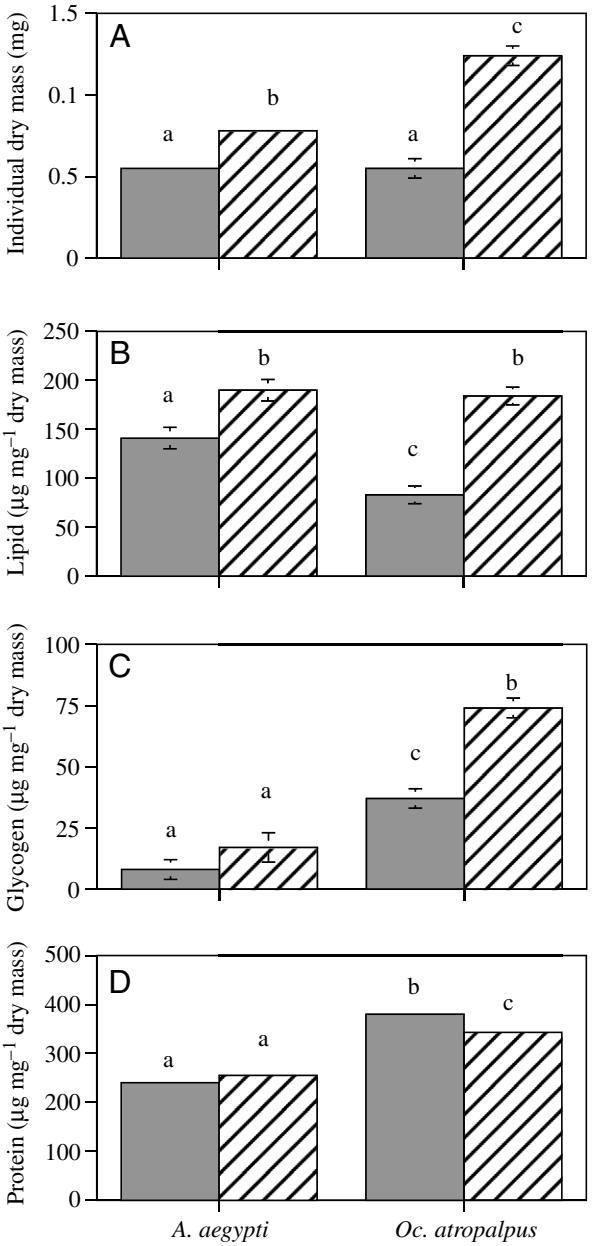

Fig. 1. Effects of larval food quantity on female dry mass and teneral metabolic reserves in both *A. aegypti* and *Oc. atropalpus*. (A) Dry mass (adjusted means ± s.e.m.; $N=30$) of newly emerged adults, (B) lipid amounts in newly emerged adults, (C) glycogen amounts in newly emerged adults, (D) protein amounts in newly emerged adults reared on a high larval food (striped columns) and a low larval food (grey columns) regimen. All nutrient values represent adjusted means ± s.e.m.; $N=12$ for *Oc. atropalpus* and $N=8$ for *A. aegypti*. Within each figure, columns with different letters are significantly different (from a linear contrast $P<0.01$). Error bars represent s.e.m. Lack of bar indicates that the s.e.m. is smaller than column scale.

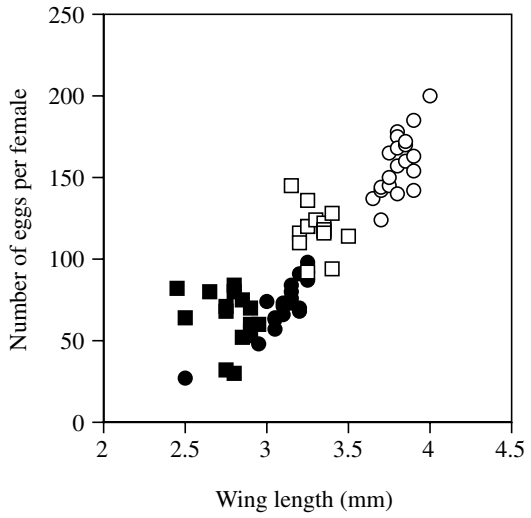

Fig. 2. Egg production as a relation of female wing length in *A. aegypti* given a blood meal (square) and *Oc. atropalpus* given no blood or sugar meal (circle). Females emerged from either a high larval food (open symbols) or a low larval food (filled symbols) regimen. For *A. aegypti*, *N*=28. For *Oc. atropalpus*, *N*=36.

contrast), but significantly more glycogen and protein (P<0.0001, from a linear contrast in both cases), compared with *A. aegypti* of high-food larvae (Fig. 1B–D).

The amount of food given to larvae greatly influenced the relation between body size, as assessed by wing length, and the number of eggs matured by females of both species (Fig. 2). *Oc. atropalpus* females from high-food larvae were significantly larger and more fecund (mean, 162 eggs) than smaller females from low-food larvae (mean, 70 eggs) (r^2=0.94, P<0.001). Anautogenous *A. aegypti* mature eggs only after ingesting a blood meal, but fecundity levels during their first ovarian cycle were greatly affected by larval food quantity (Fig. 2). Female *A. aegypti* emerging from high-food larvae were larger and, after ingesting a blood meal, matured more eggs (mean, 118 eggs) compared with blood-fed females of low-food larvae (mean, 64 eggs) (r^2=0.58, P<0.001).

Overall, both *A. aegypti* and *Oc. atropalpus* females derived from high-food larvae emerged with a larger size and body mass compared with females from low-food larvae. As a result, both *A. aegypti* and *Oc. atropalpus* females from high-food larvae contained greater total amounts (μg per individual) of lipid, glycogen and protein due to their heavier dry mass. For subsequent sections of our report, females from high-food larvae will be referred to as 'high-reserve' and females from low-food larvae as 'low-reserve' for both species.

Juvenile hormone biosynthesis in relation to female teneral reserves

In vitro biosynthesis of JH by CA isolated from high- and low-reserve females of both species was measured at different times after emergence. From 12 to 48 h post-eclosion (PE), JH biosynthesis by CA from low-reserve female *A. aegypti* was significantly lower in comparison with CA from high-reserve females (two-way ANOVA, P<0.0001), and their CA-JH biosynthesis did not increase when given 3% sucrose (two-way ANOVA, P=0.236; Fig. 3). Levels of teneral reserves did not affect CA activity in *Oc. atropalpus*. No significant changes occurred in CA-JH biosynthesis when either low- or high-reserves females were given a

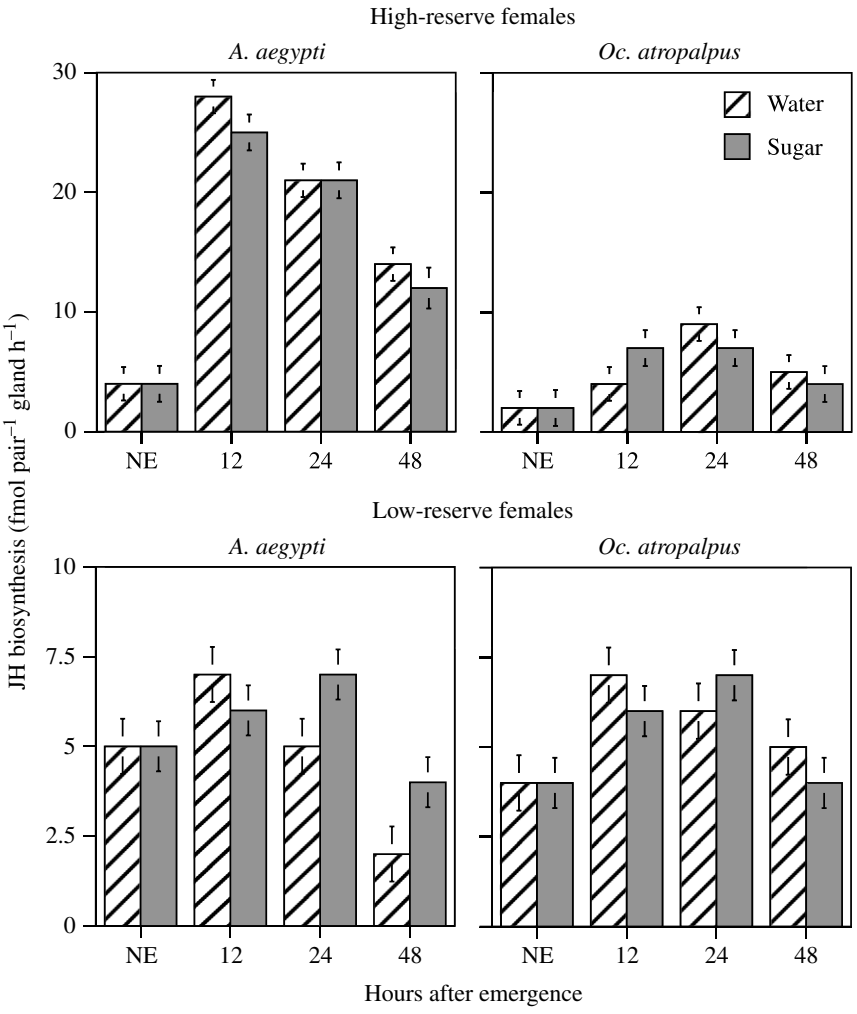

Fig. 3. *In vitro* synthesis of juvenile hormone by female corpora allata (CA) complexes as measured by incorporation of [^{3}H]methionine at different times post-emergence (NE, newly emerged). CA-JH amounts (adjusted means ± s.e.m.; *N*=200) are presented for both *A. aegypti* and *Oc. atropalpus* as a function of high (top graphs) or low (bottom graphs) metabolic reserves and whether adults were given water (striped columns) or 3% sucrose (grey columns) upon eclosion.

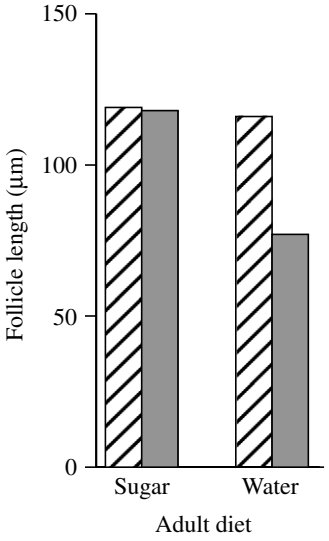

Fig. 4. For female *A. aegypti*, length of primary follicles (adjusted means ± s.e.m.; *N*=240) was measured 72 h post-emergence in response to high (striped columns) or low (grey columns) larval food amount and whether females were given water or 3% sucrose over this same time period. Lack of standard error bar indicates that the s.e.m. is smaller than column scale.

sugar meal (two-way ANOVA, P=0.336). Overall, JH biosynthesis by CA from these autogenous females was significantly lower compared with that observed from anautogenous *A. aegypti* (two-way ANOVA, P<0.0001; Fig. 3).

Pre-vitellogenic follicle development in relation to female teneral reserves in A. aegypti

Follicles in *A. aegypti* high-reserve females reached resting stage independent of adult diet. However, follicles in *A. aegypti* low-reserve females developed to resting stage only if these females were given 3% sucrose (Fig. 4). Follicle development data for *Oc. atropalpus* are not presented, because pre-vitellogenic follicular arrest does not occur. Egg development is continuous in this autogenous species (Masler et al., 1980; A.T., personal observations).

Ovarian ecdysteroid production in relation to teneral reserves in female Oc. atropalpus

An *in vitro* bioassay was used to determine ecdysteroid production by ovaries dissected from female *Oc. atropalpus* with high and low reserves at different times during their first ovarian cycle. In addition, haemolymph was collected from the same set of females before dissection of ovaries used in the *in vitro* bioassay. Levels of ecdysteroid production differed over the first 48 h of post-emergent ovarian development (two-way ANOVA, P<0.0001) and were significantly influenced by levels of teneral reserves in females (two-way ANOVA, P<0.0001) (Fig. 5A). Ovaries of high-reserve females produce a detectable level of ecdysteroids at emergence (Fig. 5A). This capacity increased at 12 h PE, peaked at 62–65 pg at 24 and 36 h PE and fell to a basal level at 48 h PE (Tukey–Kramer HSD, $P\leqslant0.05$). After 12 h PE, yolk uptake is evident in developing oocytes of high-reserve females. As observed with ovarian ecdysteroidogenesis, haemolymph ecdysteroid titre was also strongly influenced by the level of teneral reserves (two-way ANOVA, P=0.008) and showed a similar rise and fall pattern in high-reserve females, with the highest level at 36 h PE (Tukey–Kramer HSD, $P\leqslant0.05$) (Fig. 5B).

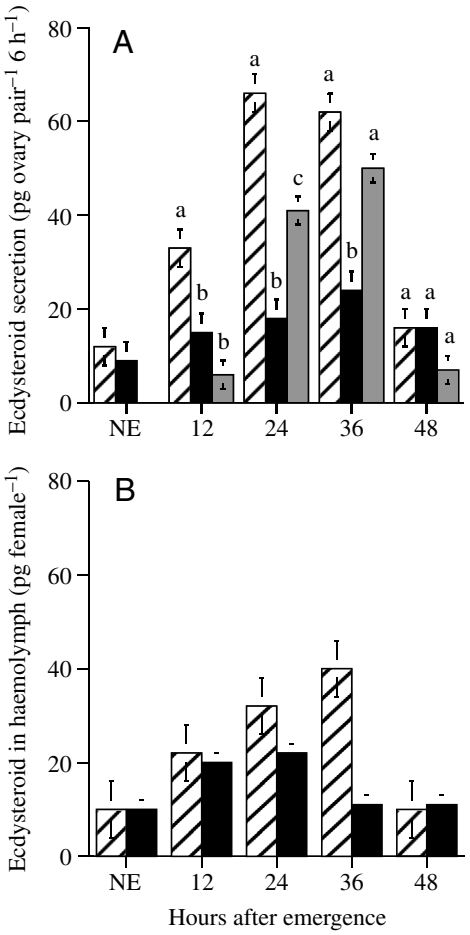

Fig. 5. (A) *In vitro* ecdysteroid secretion by ovary pairs dissected from *Oc. atropalpus* females at different times during autogenous oogenesis and with large teneral reserves fed water as adults (striped columns), low teneral reserves fed water as adults (black columns) or low teneral reserves fed 3% sucrose as adults (grey columns). Values represent adjusted means ± s.e.m.; *N*=126. Within each time point, columns with different letters are significantly different (Tukey–Kramer HSD, $P\leqslant0.05$). (B) Ecdysteroid titre measured in haemolymph collected from *Oc. atropalpus* females at different times during autogenous oogenesis with large teneral reserves fed water as adults (striped columns) or low teneral reserves fed water as adults (black columns). Values represent adjusted means ± s.e.m.; *N*=90. NE, newly emerged.

Ovaries of low-reserve females, given only water upon eclosion, exhibited basal levels of ecdysteroid production at eclosion (Fig. 5A). This capacity incrementally increased at 12 h and 24 h PE to peak at 36 h PE before returning to a basal level at 48 h PE, though differences between time points were

not significant (Tukey–Kramer HSD, $P \leqslant 0.05$). By contrast, when low-reserve females were given 3% sucrose throughout their first ovarian cycle, ecdysteroid production by ovaries was significantly greater at 24 and 36 h PE compared with low-reserve females given only water ($P<0.0001$, from a linear contrast; Fig. 5A). By 36 h PE, ovaries of sugar-fed, low-reserve females produced ecdysteroids at levels comparable to ovaries of high-reserve females (Tukey–Kramer HSD, $P \leqslant 0.05$; Fig. 5A).

Discussion

The nutritional environment experienced by larvae strongly influences female fitness-related traits such as body size, teneral metabolic reserves and fecundity for both anautogenous and autogenous mosquitoes (Briegel, 1990a; Briegel, 1990b; Telang and Wells, 2004; present study). Although *A. aegypti* females require a blood meal to provision and mature eggs, fecundity for the first ovarian cycle is determined by reserves derived from larval nourishment. High-reserve female *A. aegypti* produced significantly more eggs, after a blood meal, than blood-fed low-reserve females. Autogenous *Oc. atropalpus* do not need blood and, instead, utilize larval-derived nutrient reserves to mature the first egg batch. *Oc. atropalpus* females of high-food larvae attained a larger body size and greater levels of metabolic reserves that allowed for much higher fecundity compared with blood-fed *A. aegypti* females. Although similar in dry mass, low-reserve *A. aegypti* females required a blood meal to produce a similar number of eggs to that produced by low-reserve *Oc. atropalpus* females without a blood meal (Fig. 2). Overall, *Oc. atropalpus* females accumulated significantly more metabolic reserves compared with *A. aegypti* females. Therefore, one crucial element that may favour anautogeny in *A. aegypti* is low larval-derived reserves, so females must rely on a blood meal to complete oogenesis. In *Oc. atropalpus* females, reserves are sufficient at adult emergence to enable oogenesis within 12 h thereafter.

Because egg development is dependent on a female's reserve of nutrients, it is important that she does not proceed with oogenesis until sufficient nutrients are available. Presumably, the endocrine and nervous systems monitor these reserves, and accordingly regulate physiological, developmental and behavioural processes that rely on these reserves. Our study focused on the effect of larval nutrition on hormonally regulated oocyte maturation in mosquitoes. To start, we examined the effect of high and low levels of larval-derived metabolic reserves on adult CA biosynthesis of JH in *A. aegypti* and *Oc. atropalpus*. The highest level of JH biosynthesis after eclosion was by CA from high-reserve *A. aegypti* females, and high levels of JH biosynthesis are also reflected in whole-body extracts during pre-vitellogenesis (Shapiro et al., 1986). In addition, we cannot rule out the possibility of JH biosynthesis by ovaries and other tissues in this species (Borovsky et al., 1994). Using the same *in vitro* assay for JH synthesis, but a different larval rearing procedure, Caroci et al. (2004) measured comparably high levels of JH biosynthesis by CA isolated from large *A. aegypti*. In the present study, JH biosynthesis by CA from low-reserve female *A. aegypti* did not increase when given 3% sucrose, but in the Caroci et al. study, it did so in small females given 15% sucrose (Caroci et al., 2004). High JH levels may serve a regulatory role of halting oocyte maturation in anautogenous females and may be a second key element maintaining anautogeny.

In comparison to *A. aegypti*, JH biosynthesis by CA was significantly lower in autogenous *Oc. atropalpus* regardless of her level of larval-derived reserves or adult sugar feeding. Results for *Oc. atropalpus* indicate that low levels of JH biosynthesis by CA after eclosion do not inhibit the activation of oogenesis, but we cannot exclude the possibility of higher JH biosynthesis by CA during the pharate adult stage.

In contrast to CA activity, *in vitro* ecdysteroid production by ovaries is activated in *Oc. atropalpus* at eclosion and rises and falls within 48 h in both high-reserve females and in sugar-fed, low-reserve females. Ecdysteroid titres in haemolymph from high-reserve females showed a similar rise and fall pattern and were present at a physiological range of $2–9\times10^{-8}$ mol l^{-1} [assuming 1 μl total haemolymph volume per female (Shapiro et al., 1986) and calculated from results in Fig. 5B]. Yolk uptake is evident in oocytes of these females after 12 h. By comparison, ovarian ecdysteroid secretion is low but detectable in non-blood-fed *A. aegypti* females derived from well-nourished, standard colony-reared larvae, but for ovaries taken from females at 18 h post-blood meal, *in vitro* ecdysteroid production ranged from 100 to 140 pg per 6 h (Sieglaff et al., 2005).

Ovaries of low-reserve *Oc. atropalpus* females exhibited only basal levels of *in vitro* ecdysteroid production when these females were denied a sugar meal. As observed in these females, yolk uptake in the developing oocytes is delayed by 12–24 h in comparison with high-reserve females, but low-reserve females still mature and deposit viable eggs autogenously (Telang and Wells, 2004). Low levels of *in vitro* ecdysteroid production have been found to occur by non-ovarian tissues in blood-fed *A. aegypti* (Sieglaff et al., 2005); the possibility that low-reserve *Oc. atropalpus* females rely on ecdysteroid production by extra-ovarian tissues to produce viable eggs will be investigated.

Ovaries of sugar-fed, low-reserve *Oc. atropalpus* females showed a similar capacity for *in vitro* ecdysteroid production as that of non-sugar-fed, high-reserve females (Fig. 5A). Activation of ovarian ecdysteroid production in low-reserve, sugar-fed females may be explained by two hypotheses. First, sugar feeding increased energy availability, which supplemented that possibly obtained from low teneral glycogen and lipid reserves. Second, abdominal distention occurring with sucrose ingestion may have accelerated the timing of ovarian secretion of ecdysone similar to that of high-reserve females. Support for the latter hypothesis comes from a study on *A. aegypti* that showed that a blood meal triggers release of a head factor important for activating ovarian

ecdysteroidogenesis, and its release is accelerated by abdominal distention (Klowden, 1987).

In our present report, *in vitro* CA biosynthesis of JH was directly measured in autogenous *Oc. atropalpus* for the first time. JH biosynthesis by CA from female *Oc. atropalpus*, regardless of larval or adult nourishment, was found to be at lower levels throughout her autogenous cycle. On the other hand, ecdysteroid production by ovaries from *Oc. atropalpus* was triggered soon after eclosion in both high-reserve and sugar-fed, low-reserve females. Additional support for an early role of ecdysteroids in autogenous egg production comes from a study of *A. detritus* and *A. caspius* females (Guilvard et al., 1984). These researchers observed a peak of ecdysteroids at 40 h PE, followed by a peak of juvenile hormone 8 h later, in whole-body extracts of both autogenous species. Vitellogenesis was initiated in these species when ecdysteroid levels began to rise and yolk uptake continued during JH increase. Only one other study has quantified ecdysteroid production by ovaries in *Oc. atropalpus* (Birnbaum et al., 1984). Ovarian ecdysteroid production was minimal in females decapitated soon after emergence, but normal levels were attained in decapitated females given a physiologically high dose of JH. Given that the CA is considered to be the primary source of JH in insects (Feyereisen, 1985) and its role in the synthesis of JH in mosquitoes has been well established (Li et al., 2003), it is significant that in our study *in vitro* CA biosynthesis of JH remained at low levels throughout egg production by autogenous *Oc. atropalpus*.

In many insects, the CA synthesizes and secretes JH to regulate egg production in response to nutrition levels (Wheeler, 1996). In these insects, JH activates and regulates oocyte maturation and vitellogenesis, whereas ecdysteroids secreted by ovaries regulate final stages of egg maturation such as chorionation and oviposition (Davey, 1997; Schal et al., 1997; Strambi et al., 1997). In dipterans studied to date, JH may prime fat body machinery for vitellogenin synthesis, but ovarian ecdysteroids are the principal regulator that increases the rate of vitellogenin synthesis and release into the haemolymph. In dipteran females that take a protein meal, such as a blood meal for anautogenous mosquitoes and stable flies, release of ecdysteroids from ovaries and a corresponding rise in haemolymph ecdysteroid levels is triggered in response to a protein meal (Adams et al., 1985; Adams et al., 1988; Chen and Kelly, 1993; Kelly and Chen, 1997; Kozlova and Thummel, 2000; Schwartz et al., 1989; Yin et al., 1990). These studies have shown both ovarian and haemolymph ecdysteroid levels to correlate with vitellogenesis. Likewise, our data suggest that the greater protein reserves present at eclosion in autogenous *Oc. atropalpus* trigger early ecdysteroid production. This early ecdysteroid stimulation seems to be sufficient to spur continued oocyte development in *Oc. atropalpus* and other autogenous species in which ecdysteroid levels have been examined (Guilvard et al., 1984).

Based on the results reported in this paper, the following model is offered to explain how a female's nutritional condition and hormones interact to regulate oocyte development in autogenous and anautogenous mosquitoes (Fig. 6). The nervous and endocrine systems, ovaries and fat body are known to play key roles in mosquito egg development, and these tissues likely use diverse molecules to monitor and manipulate nutrient reserves. In this model, high levels of glycogen and protein (presumably stored or processed in the fat body) surpass a threshold set in the nervous system that activates ovarian ecdysteroid production and inhibits JH biosynthesis by the CA, which together allow vitellogenesis and egg maturation. When glycogen and protein levels are below this threshold, the CA secretes high JH levels, ovarian ecdysteroid production is low, and egg maturation is delayed or arrested.

Two results from our research support the concept of a nutrient-based threshold for these processes. First, female *Oc. atropalpus* from high-food larvae eclosed with greater levels of glycogen and protein and a larger body size than similarly treated *A. aegypti* females. *Oc. atropalpus* females exhibited low CA biosynthesis of JH, high ovarian production of ecdysteroids, and completed egg development soon after eclosion, but ovaries in *A. aegypti* females with reversed endocrine processes entered pre-vitellogenic arrest (Fig. 6). These respective trends are also evident in low-reserve *Oc. atropalpus* and *A. aegypti* females. The second and most important result is that these two groups of females had a similar body size (Fig. 1A), but the comparatively greater levels of glycogen and protein in *Oc. atropalpus* (Fig. 1C,D) apparently exceeded the threshold to inhibit CA biosynthesis of JH and activate a lower ovarian ecdysteroid production that delayed but did not arrest egg maturation (Fig. 6). Providing low-reserve *Oc. atropalpus* with a low percent sugar meal boosted ovarian ecdysteroid production, maintained the inhibition of JH biosynthesis of the CA, and restored the period of egg maturation to that of high-reserve females. Low-reserve *A. aegypti* females given water or a low percent sugar meal showed low JH biosynthesis and the expected follicular arrest. Notably, the CA from low-reserve *A. aegypti* females given a concentrated sugar meal exhibited the same level of JH secretion as the CA from high-reserve females (Caroci et al., 2004).

This threshold is particularly evident in anautogenous females – only a blood meal initiates the low rate of JH biosynthesis and high ovarian ecdysteroid production required to complete oogenesis in *A. aegypti*. In high-reserve *A. aegypti*, teneral glycogen and protein reserves are below the threshold, and other studies, including a recent one (Sieglaff et al., 2005), report that ovarian ecdysteroid production is generally low in non-blood-fed females. As found in this study, JH synthesis by CA is high, and oocyte development is arrested in high-reserve females. After females in this state ingest a blood meal, its protein presumably exceeds the threshold and, as reported in a related study, both JH biosynthesis and haemolymph titres drop to undetectable levels (Li et al., 2003; Shapiro et al., 1986), while ovaries are stimulated to produce high levels of ecdysteroids (Sieglaff et al., 2005), all of which lead to the activation of vitellogenesis in the fat body and the completion of egg maturation.

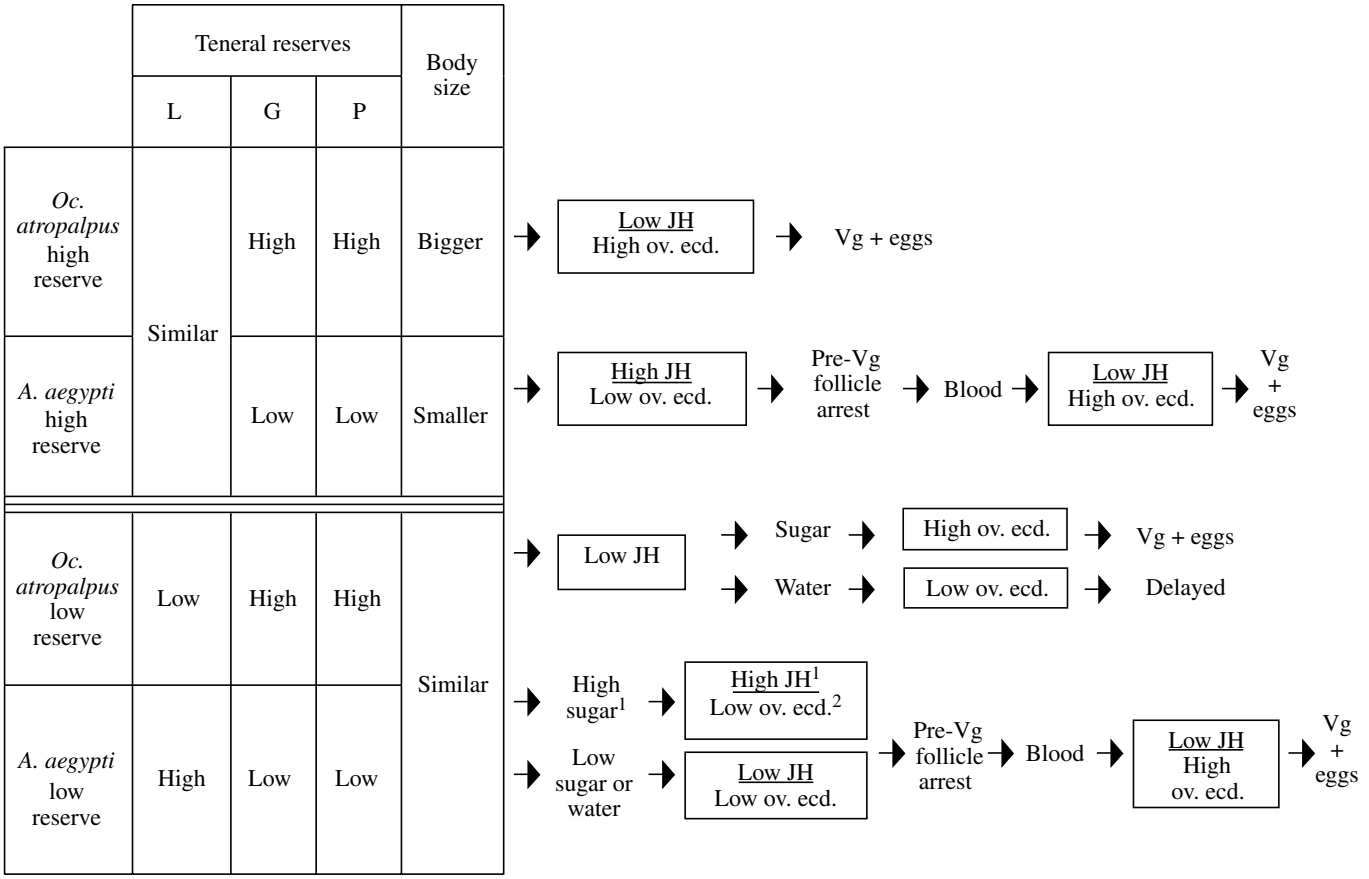

Fig. 6. A model for the interaction among metabolic reserves, hormones and oocyte maturation in autogenous and anautogenous mosquitoes. A summary, from Fig. 1, of teneral reserves of lipid (L), glycogen (G) and protein (P) measured in *Oc. atropalpus* and *A. aegypti* females derived from high-food larvae (termed high reserve) or low-food larvae (termed low reserve) and resulting body size is shown in the table. To the right of the summary table, hormonal responses, presumably as a result of teneral reserves or adult nutrition, are depicted. This model for the first egg development cycle depicts hypotheses generated from the results of experiments presented in this paper, with the assumption that females are mated. In autogenous high-reserve *Oc. atropalpus* females, teneral glycogen and protein levels are sufficiently high to exceed the threshold for stimulation of ovarian ecdysteroid production (high ov. ecd.) and subsequent vitellogenesis (Vg) and egg maturation (eggs). In addition, the biosynthesis of JH by the corpora allata (CA) is low (low JH). In anautogenous high-reserve *A. aegypti*, glycogen and protein levels fall below a threshold needed for ovarian ecdysteroid production and vitellogenesis. Consequently, JH biosynthesis is high (high JH), ovarian ecdysteroid production is low (low ov. ecd.), and oocytes are arrested (pre-VG follicle arrest) until the females take a blood meal (Blood). Hormonal profiles and stage of egg development observed in both autogenous and anautogenous females emerging with low nutrient reserves are depicted similarly. Notes: [1]results from Caroci et al. (2004); [2]results from Sieglaff et al. (2005).

Once sufficient levels of nutrients are available for females to commit to oogenesis, this information must be conveyed from diverse tissues (e.g. fat body or blood-filled midgut) to the nervous system, which then secretes neuropeptides to regulate JH biosynthesis and ovarian ecdysteroid production. In *A. aegypti*, one such neuropeptide, ovary ecdysteroidogenic hormone (OEH), is released from brain neurosecretory cells after a blood meal and directly stimulates ovarian ecdysteroid production (Brown and Cao, 2001; Brown et al., 1998). JH synthesis by the CA is regulated by allatotropins, which enhance synthesis, and allatostatins, which are inhibitory (Noriega, 2004), but evidence for a response to a blood meal is lacking. Presumably, these same neuropeptides with comparable functions are present in *Oc. atropalpus*. Most importantly, studies are needed to discover how the fat body and other tissues convey information about nutrient levels to the nervous system, which then coordinates JH and ecdysteroid production for vitellogenesis and egg maturation in mosquitoes.

This paper benefited from the comments of two anonymous reviewers. This collaborative work was funded by NIH training grant 1 K12 Gm00708 (Center for Insect Science, University of Arizona) to A.T., NIH/NIAID grant AI 45545 to F.G.N. and NIH grant AI33108 to M.R.B.

References

Adams, T. S., Hagedorn, H. H. and Wheelock, G. D. (1985). Haemolymph ecdysteroid in the housefly, *Musca domestica*, during oogenesis and its relationship with vitellogenin levels. *J. Insect Physiol.* **31**, 91-97.

Adams, T. S., Kelly, T. J. and Woods, C. W. (1988). Relationship between the corpus cardiacum allatum complex and ovaries with the haemolymph ecdysteroid profile in the housefly, *Musca domestica*. *J. Insect Physiol.* **34**, 1105-1109.

Birnbaum, M. J., Kelly, T. J., Woods, C. W. and Imberski, R. B. (1984). Hormonal regulation of ovarian ecdysteroid production in the autogenous mosquito, *Aedes atropalpus*. *Gen. Comp. Endocrinol.* **56**, 9-18.

Borovsky, D., Carlson, D. A., Ujvary, I. and Prestwich, G. D. (1994). Biosynthesis of (10*R*)-juvenile hormone III from Farnesoic acid by *Aedes aegypti* ovary. *Arch. Insect Biochem. Physiol.* **27**, 11-25.

Briegel, H. (1990a). Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.* **27**, 839-850.

Briegel, H. (1990b). Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. *J. Insect Physiol.* **36**, 165-172.

Briegel, H., Hefti, M. and DiMarco, E. (2002). Lipid metabolism during sequential gonotrophic cycles in large and small female *Aedes aegypti*. *J. Insect Physiol.* **48**, 547-554.

Brown, M. R. and Cao, C. (2001). Distribution of ovary ecdysteroidogenic hormone I in the nervous system and gut of mosquitoes. *J. Insect Sci.* **1:3**. Available online: insectscience.org/1.3.

Brown, M. R., Graf, R., Swiderek, K. M., Fendley, D., Stracker, T. H., Champagne, D. E. and Lea, A. O. (1998). Identification of a steroidogenic neurohormone in female mosquitoes. *J. Biol. Chem.* **273**, 3967-3971.

Caroci, A. S., Li, Y. and Noriega, F. G. (2004). Reduced juvenile hormone synthesis in mosquitoes with low teneral reserves reduces ovarian previtellogenic development in *Aedes aegypti*. *J. Exp. Biol.* **207**, 2685-2690.

Chambers, G. M. and Klowden, M. J. (1994). Nutritional reserves of autogenous and anautogenous selected strains of *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* **31**, 554-560.

Chen, A. C. and Kelly, T. J. (1993). Correlation of ecdysteroids with ovarian development and yolk protein synthesis in the adult stable fly, *Stomoxys calcitrans*. *Comp. Biochem. Physiol.* **104A**, 485-490.

Christophers, S. R. (1911). The development of the egg follicle in Anophelines. *Paludism* **2**, 73-87.

Clements, A. N. (1992). *The Biology of Mosquitoes*, vol. 1. London: Chapman and Hall.

Corbet, P. S. (1964). Autogeny and oviposition in arctic mosquitoes. *Nature* **203**, 669.

Corbet, P. S. (1967). Facultative autogeny in arctic mosquitoes. *Nature* **215**, 662-663.

Davey, K. G. (1997). Hormonal controls on reproduction in female Heteroptera. *Arch. Insect Biochem. Physiol.* **35**, 443-453.

Eberle, M. W. and Reisen, W. K. (1986). Studies on autogeny in *Culex tarsalis*: 1. Selection and genetic experiments. *J. Am. Mosq. Control Assoc.* **2**, 38-43.

Feyereisen, R. (1985). Regulation of juvenile hormone titer: synthesis. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, vol. 11 (ed. G. A. Kerkut and L. I. Gilbert), pp. 391-429. New York: Pergamon Press.

Foster, W. A. (1995). Mosquito sugar feeding and reproductive energetics. *Annu. Rev. Entomol.* **40**, 443-474.

Fuchs, M. S., Sundland, B. R. and Kang, S. H. (1980). In vivo induction of ovarian development in *Aedes atropalpus* by a head extract from *Aedes aegypti*. *Int. J. Invertebr. Reprod.* **2**, 121-129.

Guilvard, E., De Reggi, M. and Rioux, J. A. (1984). Changes in ecdysteroid and juvenile hormone titers correlated to the initiation of vitellogenesis in two *Aedes* species (Diptera, Culicidae). *Gen. Comp. Endocrinol.* **53**, 218-223.

Hansen, I. A., Attardo, G., Park, J., Peng, Q. and Raikhel, A. S. (2004). Target of rapamysin-mediated amino acid signaling in mosquito anautogeny. *Proc. Natl. Acad. Sci. USA* **101**, 10626-10631.

Kelly, T. J. and Fuchs, M. S. (1980). In vivo induction of ovarian development in decapitated *Aedes atropalpus* by physiological levels of 20-hydroxyecdysone. *J. Exp. Zool.* **213**, 25-32.

Kelly, T. J. and Chen, A. C. (1997). Cycling of ecdysteroid levels in adult female stable flies, *Stomoxys calcitrans* in relation to blood feeding. *J. Insect Physiol.* **43**, 789-794.

Klowden, M. J. (1987). Distention-mediated egg maturation in the mosquito, *Aedes aegypti*. *J. Insect Physiol.* **33**, 83-87.

Klowden, M. J. (1997). Endocrine aspects of mosquito reproduction. *Arch. Insect Biochem. Physiol.* **35**, 491-512.

Kozlova, T. and Thummel, C. S. (2000). Steroid regulation of postembryonic development and reproduction in *Drosophila*. *Trends Endocrinol. Metab.* **11**, 276-280.

Lea, A. O. (1963). Some relationships between environment, corpora allata, and egg maturation in aedine mosquitoes. *J. Insect Physiol.* **9**, 793-809.

Lea, A. O. (1964). Studies on the dietary and endocrine regulation of autogenous reproduction in *Aedes taeniorhynchus* (Wied.). *J. Med. Entomol.* **1**, 40-44.

Lea, A. O. (1970). Endocrinology of egg maturation in autogenous and anautogenous *Aedes taeniorhynchus*. *J. Insect Physiol.* **16**, 1689-1696.

Li, Y., Hernandez-Martinez, S., Unnithan, G. C., Feyereisen, R. and Noriega, F. G. (2003). Activity of the corpora allata of adult female *Aedes aegypti*: effects of mating and feeding. *Insect Biochem. Mol. Biol.* **33**, 1307-1315.

Masler, E. P., Fuchs, M. S., Sage, B. and Oconnor, J. D. (1980). Endocrine regulation of ovarian development in the autogenous mosquito, *Aedes atropalpus*. *Gen. Comp. Endocrinol.* **41**, 250-259.

Naksathit, A. T., Edman, J. D. and Scott, T. W. (1999a). Partitioning of glycogen, lipid, and sugar in ovaries and body remnants of female *Aedes aegypti* (Diptera: Culicidae) fed human blood. *J. Med. Entomol.* **36**, 18-22.

Naksathit, A. T., Edman, J. D. and Scott, T. W. (1999b). Utilization of human blood and sugar as nutrients by female *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* **36**, 13-17.

Nasci, R. S. (1990). Relationship of wing length to adult dry weight in several mosquito species (Diptera: Culicidae). *J. Med. Entomol.* **27**, 716-719.

Noriega, F. G. (2004). Nutritional regulation of JH synthesis: a mechanism to control reproductive maturation in mosquitoes? *Insect Biochem. Mol. Biol.* **34**, 687-693.

O'Meara, G. F. (1979). Variable expressions of autogeny in three mosquito species. *Int. J. Invertebr. Reprod.* **1**, 253-261.

O'Meara, G. F. and Krasnick, G. J. (1970). Dietary and genetic control of expression of autogenous reproduction in *Aedes atropalpus* (Coq.) (Diptera-Culicidae). *J. Med. Entomol.* **7**, 328-334.

Raikhel, A. S., Miura, K. and Segraves, W. A. (1999). Nuclear receptors in mosquito vitellogenesis. *Am. Zool.* **39**, 722-735.

Raikhel, A. S., Kokoza, V. A., Zhu, J. S., Martin, D., Wang, S. F., Li, C., Sun, G. Q., Ahmed, A., Dittmer, N. and Attardo, G. (2002). Molecular biology of mosquito vitellogenesis: from basic studies to genetic engineering of antipathogen immunity. *Insect Biochem. Mol. Biol.* **32**, 1275-1286.

Riehle, M. A. and Brown, M. R. (1999). Insulin stimulates ecdysteroid production through a conserved signaling cascade in the mosquito *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **29**, 855-860.

Russell, R. C. (1979a). A study of the influence of some environmental factors on the development of *Anopheles annulipes* Walker and *Anopheles amictus hilli* Woodhill and Lee (Diptera: Culicidae). Part 1. Influence of salinity, temperature, and larval density on the development of the immature stages. *Gen. Appl. Entomol.* **11**, 31-41.

Russell, R. C. (1979b). A study of the influence of some environmental factors on the development of *Anopheles annulipes* Walker and *Anopheles amictus hilli* Woodhill and Lee (Diptera: Culicidae). Part 2. Influence of salinity, temperature, and larval density during the development of the immature stages on adult fecundity. *Gen. Appl. Entomol.* **11**, 42-45.

Schal, C., Holbrook, G. L., Bachmann, J. A. S. and Sevala, V. L. (1997). Reproductive biology of the German cockroach, *Blattella germanica*: juvenile hormone as a pleiotropic master regulator. *Arch. Insect Biochem. Physiol.* **35**, 405-426.

Schwartz, M. B., Kelly, T. J., Woods, C. W. and Imberski, R. B. (1989). Ecdysteroid fluctuations in adult *Drosophila melanogaster* caused by elimination of pupal reserves and synthesis by early vitellogenic ovarian follicles. *Insect Biochem.* **19**, 243-249.

Shapiro, A. B., Wheelock, G. D., Hagedorn, H. H., Baker, F. C., Tsai, L. W. and Schooley, D. A. (1986). Juvenile hormone and juvenile hormone esterase in adult females of the mosquito *Aedes aegypti*. *J. Insect Physiol.* **32**, 867-877.

Sieglaff, D. H., Duncan, K. A. and Brown, M. R. (2005). Expression of genes encoding proteins involved in ecdysteroidogenesis in the female mosquito, *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **35**, 471-490.

Strambi, A., Strambi, C. and Cayre, M. (1997). Hormonal control of reproduction and reproductive behavior in crickets. *Arch. Insect Biochem. Physiol.* **35**, 393-404.

Su, T. and Mulla, M. S. (1997a). Nutritional reserves, body weight, and starvation tolerance of autogenous and anautogenous strains of *Culex tarsalis* (Diptera: Culicidae). *J. Med. Entomol.* **34**, 68-73.

Su, T. and Mulla, M. S. (1997b). Physiological aspects of autogeny in *Culex tarsalis* (Diptera: Culicidae): influences of sugar-feeding, mating, body weight, and wing length. *J. Vector Ecol.* **22**, 115-121.

Takken, W., Klowden, M. J. and Chambers, G. M. (1998). Effect of body

size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *J. Med. Entomol.* **35**, 639-645.

Telang, A. and Wells, M. A. (2004). The effect of larval and adult nutrition on successful autogenous egg production by a mosquito. *J. Insect Physiol.* **50**, 677-685.

Uchida, K., Ohmori, D., Yamakura, F. and Suzuki, K. (1990). Changes in free amino acid concentration in the hemolymph of the female *Culex pipiens pallens* (Diptera: Culicidae), after a blood meal. *J. Med. Entomol.* **27**, 302-308.

Uchida, K., Oda, T., Matsuoka, H., Moribayashi, A., Ohmori, D., Eshita, Y. and Fukunaga, A. (2001). Induction of oogenesis in mosquitoes (Diptera: Culicidae) by infusion of the hemocoel with amino acids. *J. Med. Entomol.* **38**, 572-575.

Van Handel, E. (1965). Microseparation of glycogen, sugars, and lipids. *Anal. Biochem.* **11**, 266-271.

Van Handel, E. (1985a). Rapid determination of glycogen and sugars in mosquitoes. *J. Am. Mosq. Control Assoc.* **1**, 299-301.

Van Handel, E. (1985b). Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control Assoc.* **1**, 302-304.

Wheeler, D. E. (1996). The role of nourishment in oogenesis. *Annu. Rev. Entomol.* **41**, 407-431.

Yin, C.-M., Zou, B.-X., Yi, S.-X. and Stoffolano, J. G., Jr (1990). Ecdysteroid activity during oogenesis in the black blowfly, *Phormia regina* (Meigen). *J. Insect Physiol.* **36**, 375-382.

Zhou, G., Flowers, M., Friedrich, K., Horton, J., Pennington, J. and Wells, M. A. (2004). Metabolic fate of [^{14}C]-labeled meal protein amino acids in *Aedes aegypti* mosquitoes. *J. Insect Physiol.* **50**, 337-349.