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The effect of larval and adult nutrition on successful autogenous egg production by a mosquito

Aparna Telang *, Michael A. Wells

Department of Biochemistry and Molecular Biophysics and Center for Insect Science, University of Arizona, BioScience West Building, Room 513, Tucson, AZ 85721, USA

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Abstract

Females of most mosquito species require a blood meal to provision eggs and can be medical problems because of this dependency. Autogenous mosquitoes do not require blood to mature an initial egg batch and, instead, acquire nutrients for egg provisioning as larvae. We studied the importance of larval and adult nourishment for *Ochlerotatus atropalpus* which is obligatory autogenous for its first egg cycle but may ingest blood for subsequent cycles. Larval nourishment strongly influenced autogenous egg production: female larvae that were nutritionally stressed emerged as smaller adults, produced fewer eggs and emerged with less protein, lipid and glycogen stores. Female *Oc. atropalpus* are 100% autogenous, regardless of larval diet quality or whether females are fed sugar or water at emergence. Upon completion of the first egg batch, only females emerging from a poor larval diet ingested blood and produced a second egg batch.

Keywords: Autogeny; Larval nutrition; Hexamerin; Oogenesis

1. Introduction

A thorough examination of how mosquito egg production is regulated must entail how they respond to variable nourishment since female reproductive success depends on adequate nutrient acquisition. Most mosquito species are obligatory blood feeders, termed autogenous, and require ingestion and digestion of blood protein for egg production. Many of these species are medically important because they can feed on humans and therefore have the potential to transmit pathogens (Munstermann, 1995). A smaller percentage of mosquito species are capable of maturing at least their first batch of eggs without a blood meal. Such females are called autogenous, a term defined as "the production of eggs without ingestion of protein by the adult" (Clements, 1992). For autogenous females the role of obtaining nutrients for eggs shifts from the adult to the larva. Among autogenous species there is further variation in the extent of their reliance on larval- and adult-derived nutrients for egg provisioning. Some facultative autogenous species will utilize larval nutrient stores for oogenesis if a blood meal is not available within a particular time period after emergence; however, in such cases, initial egg batch size is considerably smaller compared to that of blood-fed females (O'Meara, 1985). Females of obligatory autogenous species forego blood feeding during their first ovarian cycle even in the presence of hosts and produce their first egg batch utilizing larval nutrient reserves (Van Handel, 1976). Some of these obligatory autogenous species are described as delaying blood feeding although the extent to which such females express their anautogenous phase to produce a second egg batch has been poorly studied. Other obligatory autogenous species forego blood feeding completely and may be able to lay multiple egg clutches provisioned solely by larval reserves (O'Meara, 1985).

Autogeny, whether facultative or obligatory, is genetically determined but its expression can be influenced by certain environmental factors such as larval

* Corresponding author. Tel.: +1-520-621-1772; fax: +1-520-621-9288.

E-mail address: atelang@ag.arizona.edu (A. Telang).

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nutrition, larval density and, during the adult stage, by factors such as host availability, mating and sugar-feeding (Corbet, 1964, 1967; O'Meara, 1979; Russell, 1979a,b; Eberle and Reisen, 1986; Su and Mulla, 1997). For obligatory autogenous species the quantity and quality of larval nourishment have been shown to be crucial factors in determining fecundity levels during the first ovarian cycle (O'Meara and Krasnick, 1970; Kalpage and Brust, 1974). We embarked on this study to take a more detailed and quantitative examination of how important adult features, such as body size, nutrient reserves and fecundity respond to the larval microhabitat condition of food availability. We chose to focus on *Ochlerotatus atropalpus* because it is obligatory autogenous during its first ovarian cycle and may be anautogenous during its second ovarian cycle. It has been previously unknown how larval and adult nutrition influences both ovarian cycles of this obligatory autogenous species.

2. Material and methods

2.1. Animals

Our colony of *Oc. atropalpus* (Bass Rock strain), initiated from eggs obtained from Diana Wheeler's Laboratory (University of Arizona, Department of Entomology), stems from colonies maintained at University of Notre Dame. Mosquitoes were reared in an environmentally controlled insectary maintained at 27 ± 1 °C and 65% relative humidity with a daily photoperiod of 16 h light and 8 h dark. Under both standard and experimental rearing procedures, eggs were hatched overnight in slightly deoxygenated tap water and provisioned with a small quantity of larval food. Larval food consists of 10% solution of bovine liver powder (ICN Biomedicals, Inc.). For standard colony rearing, larvae were raised in plastic trays ($36 \times 21 \times 8$ cm) containing 1.6 l of tap water (resulting depth of water was about 3 cm) and a larval density of 150–160 per pan. Each pan was provided 3.0 ml (300 mg) of food every other day until pupation. Adults were kept in 4.5 l plastic bucket cages with screened lids and were provided cotton pads soaked with 3% sucrose as food. Cups lined with moistened brown paper towels were given to females as oviposition substrate. Our standard blood-feeding system consists of an artificial blood feeder in which porcine blood, supplemented with 100 mM ATP per 1.0 ml blood for phagostimulation, is added to a Parafilm-lined glass vessel. We found that the success of feeding is greatly enhanced if Parafilm is placed in contact with human skin prior to lining glass feeders. Feeding stations are maintained at 37 °C with the aid of a circulating water bath.

2.2. The influence of larval diet on autogeny

The influence of larval nutrition on autogenous egg production was examined experimentally using rearing procedures modified from O'Meara and Krasnick (1970) and from our standard rearing procedures. Specifically, 24-h-old larvae were placed in plastic trays ($27 \times 16 \times 6.5$ cm) containing 1.0 l of tap water (resulting depth of water was about 3 cm) at a larval density of 50 per pan. Experimental larvae were fed 10% bovine liver powder according to the schedule outlined in Table 1. Our experimental design was chosen so that the density of larvae was kept the same but only food quantity was varied. Newly emerged females (0–8 h old) from all three diet regimes were immediately frozen for later analyses of nutrient reserves.

We measured specific variables regarding successful autogenous egg production in response to the quantity of larval food in *Oc. atropalpus*. Two replicates of this experiment were conducted. Wing length was used to assess body size and was measured from the point of attachment to the wing tip, not including the fringe. Both the expression and extent of autogenous egg production were measured in 72-h-old, unmated and sugar-fed females. Females were classified as autogenous if primary follicles had advanced beyond Christopher's stage II, similar to Clements' stage 2b (Christophers, 1911; Clements, 1992). We dissected out ovaries and counted the number of primary follicles matured by females as a measure of potential autogenous egg batch. In an earlier experiment, we determined that there was no significant difference between the number of mature follicles dissected and the number of eggs oviposited (data not shown). An individual female was used to measure all three variables: wing length, autogenous egg production and potential egg batch size.

2.3. Storage nutrient analyses

In order to isolate the major nutrient classes and quantify the amount of glycogen, storage lipids and proteins in mosquitoes, we modified the microseparation procedure first described by Van Handel (1965).

Table 1
Schedule for larval food study

Days after hatch	Amount of food given per diet level (mg)		
	A	B	C
1	100	100	100
3	100		
6	100	50	
10	100	100	100
	Larval food = 10% bovine liver powder		

Full details of our microseparation procedure were previously described for *Aedes aegypti* (Zhou et al., 2004) except for the following changes. For this study, we used 9–10 newly emerged mosquitoes for the extraction, and, because we were not interested in quantifying sugars, such relevant parts of the procedure were omitted. After the separation, tubes containing glycogen, lipid and protein were frozen until nutrients could be quantified using colorimetric-based assays. Three replicates of this experiment were conducted.

The amount of storage lipid, triacylglycerol, is determined by a modified Vanillin reagent assay (Van Handel, 1985b). Specific modifications include use of triolein (ICN Biochemicals) as our standard made up to a 1.0 mg/ml concentration from which 0–75 μg quantities served as our calibration series. Frozen lipid fractions, previously dried down by removing chloroform under N_2 gas, were resuspended in 2 ml of fresh chloroform. Sample aliquots were dispensed into glass tubes and chloroform was removed under nitrogen gas; dried lipids were dissolved in 100 μl of 95–98% sulfuric acid and heated at 100 °C for 10 min. After samples have cooled to room temperature, 2.4 ml vanillin reagent is added and mixed. Standards and samples are incubated in the dark for 15 min. and optical densities are read at 525 nm against a reagent blank.

Total amount of glycogen was determined using a modified anthrone-based assay (Van Handel, 1985a). A stock of 1.0 mg/ml glycogen (ICN Biochemicals) served as our standard from which 0–100 μg quantities served as our calibration series. At the end of the microseparation sample glycogen is in pellet form and is resuspended in 2.0 ml water prior to assay. Glycogen aliquots and calibration series are placed in glass tubes and 2.4 ml of anthrone reagent is added to each sample. Samples are heated at 90 °C for 17 min while protected from light, cooled immediately in an ice-bath for 2 min while protected from light and optical densities are read at 625 nm against a reagent blank.

Protein was quantified using the BCA protein assay reagent kit (Pierce). A 1.0 mg/ml solution of bovine serum albumin served as the standard from which 0–80 μg quantities served as our calibration series. At the end of the microseparation sample protein is in aqueous form and assay aliquots are taken directly from this fraction. Both sample aliquots and calibration points are treated according to the test tube standard protocol suggested by Pierce but color development occurred at 60 °C for 30 min (their enhanced incubation protocol). All samples are cooled to room temperature and optical densities are read at 562 nm against a reagent blank, not against water. For all three colorimetric-based assays, a standard curve was generated from calibration series (absorbance values vs. micrograms of each calibration point) from which one calculates the amount of each sample nutrient compensating for both aliquot and original fraction volumes. All nutrients are reported on a microgram per individual mosquito basis.

2.4. Analyses of hexamerins

Newly emerged females (0–8 h old) from all three diet regimes were immediately frozen for storage protein analyses. Individual mosquitoes were homogenized in microcentrifuge tubes with plastic pestles in 200 μl of Tris-buffered saline (20 mM Tris, 150 mM NaCl, 5 mM EDTA, 5 mM Benzamidine, pH 7.5). Samples were centrifuged at 13,000*g* for 10 min at 4 °C and aliquots of the supernatant were diluted 1:5 in Tris-buffered saline. For all samples, 10 μl aliquots of diluted supernatant were mixed with 10 μl sample loading buffer and applied to gels. Samples were run on 10 × 8 cm gel units (Hoefer Mighty Small II). Non-denaturing, native 4–20% gradient gels were run using the Laemmli buffer system (Laemmli, 1970), except that SDS and beta-mercaptoethanol were omitted from both sample and running buffers. To establish a pore-limited state, gels were run for 2700 V h at 4 °C. Standard curves were generated using ferritin of known quantities from 0.25 to 10 μg. Internal standards of 1.0 and 10.0 μg ferritin were included in each gel run for quantitative analysis to correct for gel to gel variation. Gel samples were initially fixed in destain solution (10% methanol, 7% acetic acid) for 30 min, fluorescently stained using SYPRO Ruby (Bio-Rad) for 4 h followed by three changes of destain over a 24 h period. Gels were scanned on Typhoon 9400 (Amersham Biosciences), a variable-mode imager that produces digital images of radioactive, fluorescent or chemiluminescent samples. Gels were scanned using the 610 nm emission filter and the 457 nm blue laser. Putative hexamerin and calibration protein bands were quantified using ImageQuant, area quantitation mode (Amersham Biosciences).

We confirmed the identity of the putative hexamerins using high performance liquid chromatography–tandem mass spectrometry. A non-denaturing, native 4–20% gradient gel was run, using an individual mosquito as the sole sample, and was treated as described above. All conditions for running gels remained the same except that gels were stained with Coomassie Blue and destained as described above. Gel bands were manually excised, destained (Gharahdaghi et al., 1999) and digested in-gel with trypsin (Shevchenko et al., 1996). Peptides were injected into a microbore HPLC system (Surveyor, ThermoFinnigan, San Jose, CA) connected to a ThermoFinnigan LCQ-Deca ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) as previously described (Andon et al., 2002). MS/MS data were analyzed using SEQUEST (Yates et al., 1995) and all spectra were searched against the latest

release of the non-redundant protein databases from NCBI. All sample preparations and HPLC–MS/MS analyses were conducted in the Proteomics Laboratory, University of Arizona.

2.5. Analysis of free amino acids

Newly emerged females (0–8 h old) from all three diet regimes were immediately frozen for whole body free amino acid analyses. Three female mosquitoes were homogenized on ice in 100 μl of phosphate buffered saline, pH 7.2 with plastic pestles. Samples were centrifuged at 13,000*g* for 10 min at 4 °C and 20 μl aliquots of supernatant were mixed with 20 μl water and treated with 2.0 μl of 50% sulfo-salicylic acid to precipitate protein. Samples were mixed well, left on ice for 10 min to further facilitate protein precipitation and centrifuged at 13,000*g* for 10 min at 4 °C. Samples were analyzed at the University of Arizona (Laboratory for Protein Sequencing and Analyses) using a Beckman 7300 (post-column, ninhydrin method) dedicated Amino Acid Analyzer. Tryptophan could not be detected using this method so only 19 of the 20 protein amino acids were used to calculate total free amino acids.

2.6. The influence of adult diet on autogeny

We were also interested in how larval nutrition affected a female's need for adult nourishment. First, do adult *Oc. atropalpus* females need a sugar meal to produce eggs autogenously? Second, upon completing her first egg cycle, do females reared on either a rich or poor larval diet equally feed on blood? And, finally, is a blood meal necessary to produce a second egg batch? Larvae of *Oc. atropalpus* were reared on either the rich or poor diet to obtain adults at extreme levels of nutritional condition. Individual, newly emerged females ($n = 42$ from each larval diet group) were housed in small cages with two males (previously sugar-fed) and were fed either water ($n = 21$) or 3% sucrose ($n = 21$). Plastic 25 ml scintillation vials, cut transversally, were lined with brown paper towel and served as oviposition containers. Egg sheets were checked daily to monitor each female's ovarian cycle. After completing the first egg cycle females within the sugar diet group were further split and either fed sugar ($n = 7$), water ($n = 7$) or were offered blood ($n = 7$). Previous trials indicated that only females that had fed on sugar, regardless of the quality of the larval diet, lived passed their first ovarian cycle. Experimental females were given the opportunity to blood feed for two consecutive days during both the light and dark periods. Feeders were replenished with a fresh blood meal at the start of each feeding period. Females that failed to blood feed during this period were removed from the study. Again, egg sheets were checked daily to monitor each female's second ovarian cycle.

2.7. Data analyses

Both wing length and fecundity was analyzed using analysis of variance with diet quality as the explanatory variable. Total amount of protein, lipid and glycogen was analyzed using both analysis of covariance and analysis of variance; individual dry weight served as the covariate and we included sex (or species), diet and an interaction term in both statistical models. Levels of both hexamerins and free amino acids were analyzed using analysis of variance with diet as the explanatory variable. The influence of larval food on blood feeding and the influence of adult diet on the likelihood of producing a second egg batch were treated as categorical data and were analyzed as two-way contingency tables using the Pearson chi-square (χ^2) statistic. 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.

3. Results

3.1. Body size, fecundity and nutrient storage

Fig. 1 shows the number of primary follicles matured per female *Oc. atropalpus* in relation to her wing length in response to variable food amount. Wing length, used to assess body size, was greatly affected by larval nutrition (Fig. 1). Mean wing length was significantly influenced by food quantity (ANOVA $P < 0.0001$) and was greatest in response to diet A (mean ± s.e. = 3.8 mm ± 0.02) and smallest in diet C (mean ± s.e. = 3.0 mm ± 0.02). Each female was also used to measure potential autogenous egg batch in relation to larval nutrition. Food quantity significantly influenced the number of eggs matured by females (ANOVA $P < 0.0001$). Females produced the largest number of eggs on diet A (mean ± s.e. = 159 ± 3.7) but egg production was reduced by more than half on diet C (mean ± s.e. = 71 ± 3.7). Simple linear regressions conducted separately for each larval diet support this interpretation. Analysis for differences among slopes was conducted using methods described by Zar (1996). We found strong evidence that the slopes differed among the three diets ($F_{2,\,54} = 92$, $P < 0.0005$) and a post-hoc Tukey-test for multiple comparisons found significant differences among all three slopes ($q_{40\ \text{d.f.}} > 5.528$ critical value, $P < 0.001$).

Fig. 2B–D displays differences in storage nutrient levels between male and female *Oc. atropalpus* in

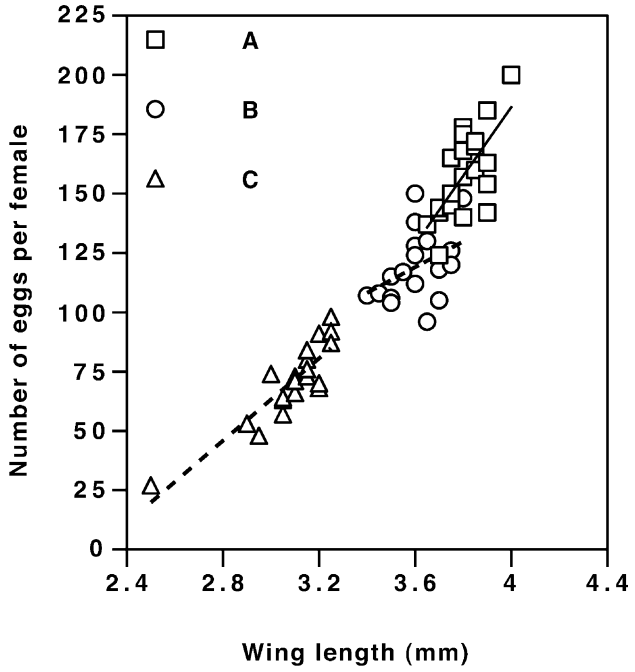

Fig. 1. Relationship between autogenous egg production and wing length in female *Oc. atropalpus* reared on three levels of larval diet. Individual slope lines indicate separate linear regressions on each diet group. Regression equations for each level are given below:

Diet A: $y = 144.6x - 392.1$, $r^2 = 0.47$, $P = 0.0009$

Diet B: $y = 53.9x - 75.2$, $r^2 = 0.17$, $P = 0.0657$

Diet C: $y = 86.6x - 196.8$, $r^2 = 0.78$, $P < 0.0001$.

response to varying levels of nourishment. The quantity of larval nourishment strongly influenced emergent dry weights of both sexes (Fig. 2A) (two-way ANOVA $P < 0.0001$) and we found equally strong evidence for differences between the sexes (two-way ANOVA $P < 0.0001$). However, a significant interaction term in this model (two-way ANOVA $P = 0.002$ for interaction) called for separate analyses that showed that male and female dry weights were similar on the poorest diet only ($P = 0.1295$ from a two-sided t-test). Initially, dry weight was included as a covariate in our ANCOVA model to test all three nutrient stores in response to larval diet and how this may differ between the sexes. However, dry weight explained a substantial portion of the variation in all three ANCOVA models, thus leaving little variation to explain differences between the sexes and the effect of larval nutrition. In our ANOVA model we found that larval diet affected the overall level of nutrients stored by both sexes of *Oc. atropalpus* (Fig. 2B–D). Both total amount of protein and lipid storage differed between the sexes (two-way ANOVA $P < 0.0001$ in both cases) with levels being greater in females on all diets. Larval nutrition affected the level of protein and lipid storage by females (one-way ANOVA $P = 0.0003$ and $P < 0.0001$, respectively) and males (one-way ANOVA $P = 0.0001$ and $P = 0.0003$,

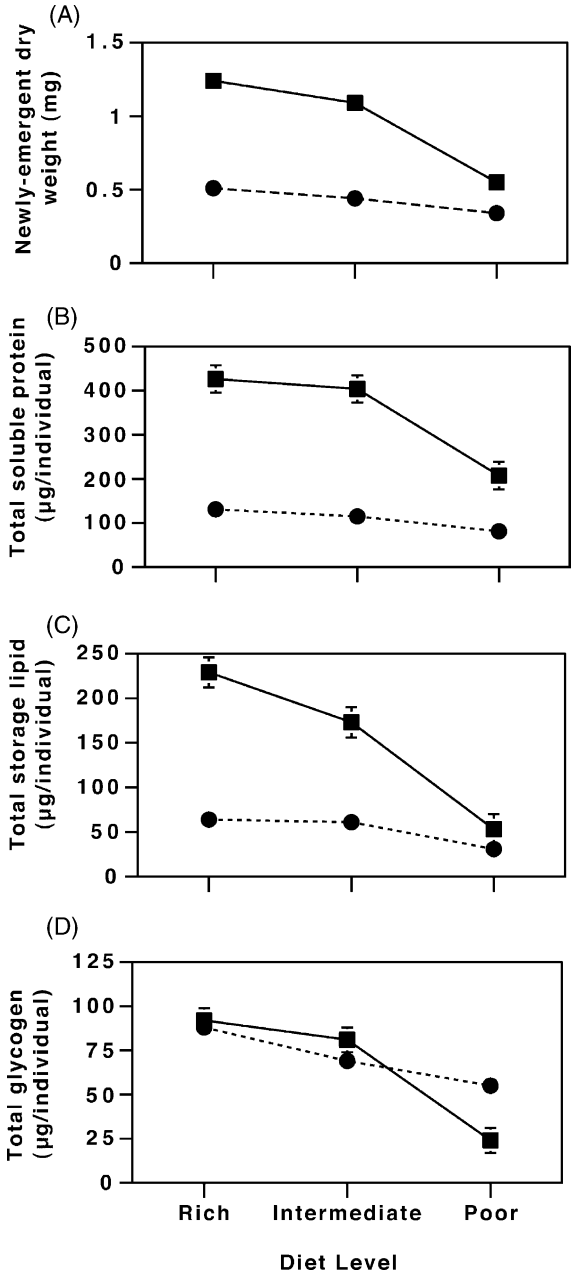

Fig. 2. Effect of larval diet on mosquito dry weight and nutrient storage levels. (A) Comparison of dry weight of newly emerged adults reared on three larval diets, (B) comparison of total soluble protein in newly emerged adults reared on three larval diets, (C) comparison of total storage lipid in newly emerged adults reared on three larval diets, and (D) comparison of total glycogen in newly emerged adults reared on three larval diets in both female (■) and male (●) *Oc. atropalpus*.

respectively). No significant differences were seen between the sexes with respect to glycogen levels (two-way ANOVA $P = 0.329$) but a significant interaction term warranted separate analyses (two-way ANOVA $P = 0.007$ for interaction). Within each sex, larval diet affected levels of glycogen stores (one-way ANOVA $P < 0.0001$ for females and ANOVA $P = 0.0002$ for

males) with levels being roughly equal on all except the poorest diet in which males stocked more glycogen (Fig. 2D).

3.2. Levels of hexamerins and free amino acids

Nutrient analyses clearly showed that the extent of protein and lipid storage by female *Oc. atropalpus* was greatly affected by larval nourishment. In order to better characterize nitrogen storage, we quantified levels of hexamerins and free amino acids in female *Oc. atropalpus* reared on the same three larval diets. Mosquito hexamerins were identified using high performance liquid chromatography–tandem mass spectrometry. In-gel trypsin digestion of bands containing putative hexamerins yielded 19 peptides that, upon being searched against non-redundant protein databases from NCBI, were all identified as *Oc. atropalpus* hexamerin (accession number AF430247). For comparison, Fig. 3 includes the same total soluble protein levels as was shown in Fig. 2B along with levels of hexamerins and free amino acids. The pattern of hexamerin levels closely matched that of total soluble protein levels in response to variable larval nutrition (Fig. 3). The quantity of larval nourishment strongly affected hexamerin levels (one-way ANOVA $P < 0.0001$) but we found only moderate evidence that free amino acid levels were so affected (one-way ANOVA $P = 0.0317$).

3.3. The influence of adult diet on autogeny

Subsets of females emerging from both the poor and rich diets were fed either water or sugar during the course of their first gonotrophic cycle. Regardless of the quantity of larval food given, and regardless of whether females were fed water or sugar post-emergence, 100% of females produced their first egg batch autogenously. While larval food amount strongly affected total number of eggs laid by females (two-way ANOVA $P < 0.0001$), egg batch size did not depend on whether females were fed sugar or water (two-way ANOVA $P = 0.2349$), regardless of the level of larval nourishment (lack of interaction, $P = 0.493$). Subsets of sugar-fed females, emerging from both poor and rich larval nutrition, were offered blood after completing their first egg cycle ($n = 7$ for each larval diet group). Contingency analysis showed that the likelihood of taking a blood meal depends on the extent of larval nourishment (Pearson Chi-square $P = 0.0002$). Only seven of 14 females offered blood fed on blood; all seven that fed on blood had emerged from a poor larval diet. The other seven females emerging from a rich larval diet failed to blood feed over the two-day period given and died shortly thereafter. We did not observe the presence of secondary follicles upon dissecting these females. Contingency analysis also suggested that the likelihood of producing a second egg batch depends highly on blood feeding after the first egg cycle is complete (Pearson Chi-square $P < 0.0001$). Overall, 35 of 42 females did not produce a second egg batch. Twenty-eight of these females were only given sugar or water during their second gonotrophic cycle thus demonstrating their inability to provision eggs on a non-protein diet; the remaining seven consisted of females emerging from a rich larval diet and were offered blood, but none of these females blood fed and so could not lay a second egg batch. However, of the seven that did lay a second egg batch, all seven females had emerged from a poor larval environment and had taken a blood meal. On average, these females laid 126 eggs (±s.d. of 35 eggs) over the course of both their autogenous and anautogenous ovarian cycles, thereby increasing their overall egg production compared to that of C-diet females producing eggs autogenously (see Fig. 1).

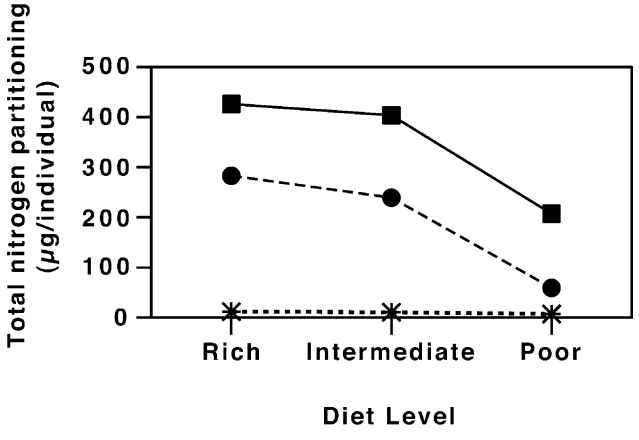

Fig. 3. Effect of larval diet on the partitioning of nitrogen in total soluble protein (■) between total storage hexamerins (●) and total free amino acids (∗) in newly emerged female *Oc. atropalpus*.

4. Discussion

Female reproductive success is affected by both larval and adult nutrition: her reproductive potential is dictated by larval nourishment but her adult nutrition will determine how well that potential is met (Clements, 1992). However, most studies we are aware of use both crowding and food limitation in combination to produce mosquitoes of different size classes. These studies have contributed much to our understanding of how female body size (and her associated nutrient reserves) affect how she utilizes adult diets of sugar and blood and how her fecundity is subsequently affected (Nayar and Sauerman, 1971; Briegel, 1990a,b; Naksathit et al., 1999; Timmermann and Briegel, 1999;

Briegel et al., 2002). Unfortunately, with this approach one cannot make unequivocal conclusions regarding larval nourishment given the adverse effects measured in response to crowding (Barbosa and Peters, 1970; Ikeshoji and Mulla, 1970a,b; Barbosa et al., 1972). The experimental design used in our study, closely following that of O'Meara and Krasnick (1970), specifically tests the effects of larval nourishment on important adult mosquito characteristics.

We first measured particular adult characteristics in relation to a variable larval diet. Female *Oc. atropalpus* emerging from a rich larval diet attained the largest body size, assessed by wing length, and produced the largest number of eggs. When reared on a relatively poor larval diet, females were significantly smaller and less fecund. Linear regression and separate slope analysis suggested that the association between body size and autogenous egg production differed according to the different larval diets. On a poor diet females increased egg production for every unit increase of body size. Whereas, on the intermediate diet, all females attained a similar body size and level of egg production, and, on the richest diet, females allocated resources in excess to size toward egg production. The important role of nourishment in promoting or limiting insect reproduction and fecundity levels is well known and has been adequately reviewed elsewhere (Engelmann, 1970; O'Meara, 1987; Wheeler, 1996).

The quantity of larval diet affected both male and female *Oc. atropalpus* emergent dry weights and the extent of nutrient stores they emerged with. Both male and female dry weights and levels of nutrient reserves decreased in response to poor larval nourishment, but females were far more sensitive to the quantity of larval nutrition. Female *Oc. atropalpus* accumulated more protein and lipid compared to males at all levels of larval nourishment but similar or less levels of glycogen. This is not surprising given the importance of both protein and lipid as yolk ingredients in mosquito and other insect eggs (Clements, 1992; Chapman, 1998). Past work by Van Handel (1976) described *Oc. atropalpus* as a closed system by nicely demonstrating that the amount of both protein and lipid found in mature ovaries was entirely accounted for by the disappearance of these macronutrients from the abdomen, presumably from the fatbody. It was also not surprising that larval nourishment affected male nutrient composition. It is becoming increasingly clear that males contribute a great deal more toward female reproduction in addition to sperm (Klowden and Chambers, 1991; Fernandez and Klowden, 1995; Klowden, 1999; Gillott, 2003), however, we currently have no such evidence for male *Oc. atropalpus* transferring additional substances to females.

Oc. atropalpus females attained large protein and lipid reserves on a rich larval diet. Given that these particular nutrient stores will serve predominantly as yolk precursors for autogenous egg production, we further characterized nitrogen storage by measuring whole-body levels of hexamerins and free amino acids. Hexamerin storage proteins in *Oc. atropalpus* were previously characterized and found to consist of subunits of three different sizes with females containing all three but males containing only two (Wheeler and Buck, 1996). Larval and pupal expression of the gene encoding for the female-specific hexamerin protein has also been recently characterized (Zakharkin et al., 2001). Our study showed that the pattern of hexamerin accumulation matched levels of total soluble protein in response to variable larval nourishment but we found only suggestive evidence that free amino acid levels were so affected. This is not the first report of mosquito hexamerin quantification. Wheeler and Buck (1996), using *Oc. atropalpus* females emerging from a general colony rearing condition, quantified hexamerin subunits separately using SDS-PAGE. Their reported level of total hexamerins in newly emerged females, when subunits are added together, closely matches our reported levels of hexamerins found in females emerging from poor larval nutrition (Fig. 3). The less than optimal colony rearing condition used in that earlier study (Wheeler and Buck, 1996) (Norman A. Buck, personal communication) was not intended to examine storage protein levels in response to optimal rearing conditions but to autogenous egg production in general.

Female *Oc. atropalpus* are 100% autogenous during their first ovarian cycle, regardless of their larval diet. However, earlier studies reported that these females might take a blood meal after laying their first egg batch (O'Meara, 1985). We found that only females emerging from a poor larval diet had the propensity to blood feed, after laying their first egg batch autogenously, and were the only ones to produce a second egg batch. Females emerging from a rich larval diet showed no such inclination to blood feed after completing their first ovarian cycle. However, detailed behavioral observations were not conducted, so we do not know if these females alighted and probed the blood-feeding surface before refusing to feed. It is also possible that these females would respond differently if offered a live animal host. These and other important conditions that may affect expression of the secondary ovarian cycle in this species await further analysis. Regardless of larval diet, females given access to water or sugar only after their first egg cycle were unable to lay a second egg batch, thus indicating the need for an adult diet of protein for oogenic cycles subsequent to the first autogenous egg batch. The lifetime fecundity of a female is a function of her total number of ovarian cycles and egg batch size (Clements, 1992). Female *Oc. atropalpus* emerging with small nutrient reserves will

suffer low fecundity levels if she only displays her autogenous phase. However, if she secures a blood meal, she expresses her anautogenous ovarian phase and increases her overall reproductive capacity. Our study of autogenous mosquitoes, those that provision eggs using larval-derived nutrients and either delay or completely forego blood feeding, offers comparative insights into the nutritional ecology of mosquito reproduction.

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