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Amino acids in nectar enhance longevity of female *Culex quinquefasciatus* mosquitoes

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ABSTRACT

Culex mosquitoes feed on a wide range of nectars consisting of mostly carbohydrates and amino acids, however, little is known about the utilization and effects of these different carbohydrates and their accompanying amino acids on longevity. *Culex quinquefasciatus* larvae were reared on low- and high-quantity food diets to produce adults that were nutritionally representative of wild-caught and laboratory-reared mosquitoes. Emerging adults reared on low- or high-quantity food diets as larvae were then provided *Lantana camara* nectar mimics containing mixtures of carbohydrates and amino acids to evaluate effects of nectar amino acids on longevity. Carbohydrates (with or without amino acids) were a critical component of the adult diet, and in their absence, adult mosquitoes died within 3–5 days. The nectar mimic that contained both carbohydrates and amino acids did not increase adult longevity of males originating from either poorly or well-fed larvae. However, females receiving adult diets containing both carbohydrates and amino acids lived 5% longer than females fed adult diets with only sugar.

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1. Introduction

The discovery that amino acids are the second most abundant component of nectars (Baker and Baker, 1973) and that some insects show a preference for carbohydrate sources containing amino acids (Alm et al., 1990; Mevi-Schutz and Erhardt, 2003b), has led to an increased interest in the role that they play in insect life histories (Baker and Baker, 1973). Some insects rely on nectar as a primary source for amino acids (Baker, 1977), but others feed on fruit, dung, pollen or even blood to obtain supplements (Baker and Baker, 1973; Foster, 1995). Feeding on pollen by female *Aedes aegypti* L. mosquitoes (Eischen and Foster, 1983) or ingestion of amino acids and carbohydrates by various species of male and female butterflies has been reported to enhance longevity, fecundity or both (O'Brien et al., 2003; Mevi-Schutz and Erhardt, 2005; Beck, 2007), although effects are typically not observed in laboratory animals reared from well-fed larvae (Bauerfeind and Fischer, 2005; Mevi-Schutz and Erhardt, 2003a; Hill and Pierce, 1989).

Certain flowers contain high levels of amino acids, and have been studied as potentially important sources for amino acids contributing to insect reproduction (Baker and Baker, 1973). Common lantana (*Lantana camara* L. (Verbenaceae)) is an example of a butterfly flower that contains a high amino acid concentration (16 μmol/ml) as well as the sugars, fructose, glucose and sucrose (Alm et al., 1990). *Lantana camara* is a widely used ornamental and is naturalized in many temperate regions (Morton, 1994). Nectar mimics of *L. camara* have been used in numerous feeding preference studies with butterflies (Mevi-Schutz and Erhardt, 2003b; Alm et al., 1990) and are a known attractant for the tsetse fly (*Glossina* spp.) in Africa (Syed and Guerin, 2004).

The success of mosquitoes as disease vectors relies heavily on prolonged survival, to feed on multiple hosts and incubate pathogen development (Woodring et al., 1996). Feeding on nectar and honeydew enhances mosquito longevity and is also a source for flight energy (Nayar and Sauerman, 1971a, 1971b, 1975; Gary and Foster, 2004). When not feeding on nectar, anautogenous species feed on blood as a source of amino acids to make vitellogenin for egg production (O'Meara, 1987). Feeding pollen to anautogenous *Ae. aegypti* adults increased longevity, egg production in the lab and, if vertebrate hosts are unavailable, pollen feeding may enhance longevity and perhaps fecundity in field mosquitoes (Eischen and Foster, 1983). Adult *Culex nigripalpus* Theobald have been observed feeding on *Lantana* in the field (Haeger, unpublished data, cited in Nayar, 1982), but it remains unclear whether amino acids present in *Lantana* nectar will contribute to increased survival or fecundity. *Culex* mosquitoes are common throughout the United States, and *Culex quinquefasciatus*

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Say are common nuisance species in the Southern U.S. *Culex* species are particularly important as vectors of St. Louis encephalitis virus (SLE) (Jones et al., 2002) and West Nile virus (WNV) (Sardelis et al., 2001; Molaei et al., 2007).

Many physiological attributes, such as body size and nutrient reserves, can affect the longevity of adult mosquitoes when reared under field conditions or in the laboratory. In the field, larvae are exposed to a wide range of nutritional conditions, and exposure to stress factors such as poor habitat quality or high competition, may result in reduced body size of adults (Day and Van Handel, 1986). Field-collected adult mosquitoes contain fewer nutritional reserves than those reared under controlled laboratory conditions (Day and Van Handel, 1986). Adult mosquitoes maintained in the laboratory solely on sucrose were able to store high levels of lipid and glycogen (O'Meara, 1987; Day and Van Handel, 1986), with levels typically greater than field caught individuals (Day and Van Handel, 1986). These data suggest that the condition of laboratory-reared mosquitoes may not necessarily reflect the nutritional state of mosquitoes in field settings.

Amino acids in nectar may provide an important metabolic substrate with the potential to enhance mosquito survival and vector capability. We evaluated whether amino acid supplementation in artificial nectar increased mosquito longevity. Effects of adult-acquired amino acids may only be apparent under nutritionally-stressed conditions. For example, in map butterflies, *Araschnia levana* L., the fecundity-enhancing effects of nectar amino acids were only detectable in adults that were poorly-fed as larvae (Mevi-Schutz and Erhardt, 2005) and preference for carbohydrates containing amino acids was only detected in adults that were poorly-fed as larvae (Mevi-Schutz and Erhardt, 2003b). Therefore, we reared mosquito larvae on high- or low-quantity food diets to mimic the range of nutritional conditions mosquitoes may encounter in the field. These high- and low-food larval treatments produced males and females that differed in body size and nutritional reserves at emergence. Nectar sugars are known to enhance longevity, so we assessed the effects of amino acids on adult longevity both in the presence of sugars and in the absence of sugars. Furthermore, because mating is known to enhance longevity ion some mosquitoes (Liles and DeLong, 1960; Briegel and Kaiser, 1973), we tested whether larval nutrition had any effect on mating propensity.

2. Materials and methods

2.1. Mosquito rearing

A colony of *Cx. quinquefasciatus* established in Gainesville, FL in 1995 was used in this study. Larvae were reared in 2.5 L of water at a density of about 500/rearing container (35.5 cm × 48.3 cm × 6.4 cm) (28.0 ± 1.0 °C, 81.2 ± 0.1% RH, 14 L:10 D photoperiod). Diets were chosen to maximize differences in size without disrupting development time (Telang and Wells, 2004) (Table 1). Rearing larvae at a fixed density with varying amounts of food allowed us to evaluate effects of nutrition on larvae and subsequent effects on size and nutritional state of adults. The number of days from egg to pupation for the larvae reared on low-food diets was equal to that of the larvae reared on high-food diets.

2.2. Winglength and dry weight measurements

The effect of the larval rearing regimes on the size of resulting adults was initially determined by measuring winglength and dry weight. From each diet regime, samples of 10 newly emerged males and females were frozen (−20 °C). For each individual, wings were removed, mounted on glass slides, viewed at 10× magnification with a dissection scope and photographed using a digital eyepiece camera with ScopePhoto 1.0 (Scopetek, Hangzhou). Measurements were made using a calibrated ocular micrometer from the alular notch to the distal end of the R_2 wingvein, fringehairs excluded (SigmaScan Pro 5.0, SPSS, Inc., Chicago, IL) and average winglength was determined.

Table 1
Feeding schedule of larval *Cx. quinquefasciatus* under low- or high-food diets. Larval food = 3% bovine liver powder (LP): 2% Brewer's yeast (BY) (30 g bovine liver powder and 20 g Brewer's yeast in 1 L of water), 2% hogchow (36 g finely ground hog chow in 1800 ml of water).

Days after hatch	Amount of food given per diet level (ml)	
	Low	High
1	50 ml; 3% LP; 2% BY	50 ml; 3% LP; 2% BY
3		50 ml; 2% hogchow
4	25 ml; 2% hogchow	50 ml; 2% hogchow
5		50 ml; 2% hogchow

Dry weights were determined from the average of 5 replicate samples of 10 male and 10 female mosquitoes from each larval feeding regime. Samples were frozen at −20 °C just after emergence, frozen, freeze dried to constant mass and weighed.

2.3. Glycogen and lipid analyses

Initial nutritional reserves of the mosquitoes immediately after adult emergence were determined by measuring glycogen and triglyceride content. Samples that were previously freeze dried and weighed (see Section 2.2) were analyzed for glycogen and lipid using the hot anthrone assay for glycogen (Van Handel, 1985) or sulphosphovanillin assays for lipids (Van Handel, 1985, as modified by Hahn, 2005). In preparation for the assays, freeze-dried mosquitoes were homogenized in microcentrifuge tubes with 100 μl of saturated sodium sulfate, 200 μl of methanol, 100 μl of ultrapure water and 500 μl of 1:1 chloroform: methanol. Lipid solutions were washed through glass pipette columns of 0.2 g silicic acid with four rinses of 1 ml of chloroform to extract only neutral lipids (Hahn, 2005). Five replicate samples were completed for each sex and larval rearing regime.

2.4. Mating assay

Upon adult emergence, groups of 35 males and 35 females were placed together in separate 0.47 L paper containers (Solo Cup Company, Highland Park, IL), each representing one replicate. The mosquitoes were held for 15 days to ensure mating. The containers were modified with fabric screening to allow viewing through the top, and a 1.5 ml microcentrifuge tube was inserted into the side, to dispense treatment solutions *via* a piece of a saturated cotton dental wick (1 cm × 4.5 cm) (Richmond Dental, Charlotte, NC). This assay was conducted separately from the longevity assay (see Section 2.5), however, because the aim was simply to confirm that mating occurred and use that data to make inferences about the survival of mosquitoes in the longevity assay, the mosquitoes were maintained only on a 5% sucrose solution. Mosquitoes were maintained at 28.1 ± 1.0 °C, 81.2 ± 0.1% relative humidity and 14 L:10 D photoperiod. Five replicates were completed using mosquitoes fed a high-food diet as larvae and five replicates with those fed a low-food diet as larvae. Ten randomly chosen mosquitoes from each replicate were collected and kept at −20 °C until spermathaecal dissections were performed. Upon dissection, the number of adult females from high-food larval diets or low-food larval diets containing sperm was counted to assess female mating.