DIFFERENCES BETWEEN THE NUTRITIONAL RESERVES OF LABORATORY-MAINTAINED AND FIELD-COLLECTED ADULT MOSQUITOES[1]

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ABSTRACT. Individual female mosquitoes from field populations of *Aedes aegypti, Culex nigripalpus* and *Coquillettidia perturbans* were analyzed for sugar, glycogen and lipids. Controls were maintained in the laboratory for 7 to 10 days on 2% and either 10 or 20% sucrose. Mosquitoes held in the laboratory had significantly more glycogen and lipid than field-collected mosquitoes of the same species. Laboratory mosquitoes maintained on 10 or 20% sucrose contained more sugar than did field mosquitoes. Mosquitoes of the above species were collected at field sites in January, April, July and October and nutritional reserves were determined. Seasonal differences in reserves were documented in females of all species. However, even when reserves were at their highest in field-collected mosquitoes they rarely approached the reserves in mosquitoes of the same species maintained in the laboratory on 10 or 20% sucrose. Since laboratory-maintained mosquitoes are nutritionally different from those in the field, results of laboratory studies on flight performance, host attractancy, biting, disease transmission and oviposition behavior may be biased.

INTRODUCTION

Adult mosquitoes require carbohydrates in the form of sugar or glycogen for flight (Nayar and Van Handel 1971) and lipids for long-term maintenance (Van Handel 1984). Mosquitoes in the laboratory are commonly maintained on 10% sucrose (Gerberg 1970) and in nature are known to feed on flower nectars (Bidlingmayer and Hem 1973). Laboratory experiments on attractancy, blood feeding, physiology, flight ability, susceptibility to infection by various pathogens, and field releases of laboratory reared mosquitoes often depend upon mosquitoes maintained on sucrose for as long as 6 weeks (Rowley and Graham 1968, Mitchell et al. 1980, Klowden and Lea 1984).

A number of laboratory studies have been reported which deal with the importance of adult sugar feeding to survival (Nayar and Sauerman 1975). Several reports of the importance of sugar feeding to mosquitoes in the field have also been published (Bidlingmayer and Hem 1973, Magnarelli 1978, Nasci and Edman 1984). All of these studies have depended upon analysis by means of the cold anthrone method (Van Handel 1972). A few studies have been reported which quantify caloric reserves in field-collected mosquitoes (Magnarelli and Andreadis 1984). However, comparisons between the fuel reserves of field and laboratory mosquitoes had not been previously made.

Behavioral differences between colonized material and mosquitoes from the field are often interpreted as resulting from selection pressures. We tested a hypothesis that laboratory-maintained mosquitoes differ nutritionally from field populations. The results suggest that some differences in behavior between colonized and field material could be due to different levels of nutrients and nutrient reserves.

MATERIALS AND METHODS

FIELD-COLLECTED MOSQUITOES. *Aedes aegypti* (Linn.) and *Culex nigripalpus* Theobald were collected in the field with a hand-held aspirator. Resting *Cx. nigripalpus* for the initial field-to-laboratory comparison were collected in a live oak/cabbage palm hammock in Indian River County, Florida on July 6, 20, 27 and 31, 1984. Host-seeking and resting *Ae. aegypti* were collected at a tire dump in Indian River County on August 17 and December 18, 1984. At the completion of each collection the aspirator bags were placed on ice in a cooler and returned to the laboratory. There the mosquitoes were quickly anesthetized with chloroform, separated by species and sex, counted, placed into air-tight vials and frozen at −20°C.

Female *Coquillettidia perturbans* (Walker) were collected in a CDC light trap baited with CO_2 in Orange County, Florida on September 12, 1984. This catch was divided into 4 groups of 100 mosquitoes each. The first was immediately frozen at −20°C and later used for the nutritional analysis. The remaining 3 groups were given 2 or 20% sucrose or water in 4, 50 ml vials placed in 30.5 cm^3 cages in an environmental chamber at 27°C, 85% RH, and a 12:12 light cycle. After 7 days mosquitoes given sucrose were frozen and stored for later nutritional analysis. The mosquitoes given water were checked daily and the dead were collected, fro-

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zen and later analyzed to establish the nutritional base line for this species.

In addition to the above collections, *Ae. aegypti, Cq. perturbans* and *Cx. nigripalpus* were taken from the field and frozen as before in weekly collections throughout 1984. Individuals of each species were analyzed from April (spring), July (summer), October (fall) and January (winter) collections to determine whether there was seasonal variation in available nutrients (sugar) and stored nutrients (glycogen and lipid).

LABORATORY-MAINTAINED MOSQUITOES. Eggs from laboratory colonies of *Ae. aegypti* (Vero Beach strain, in colony since 1975) and *Cx. nigripalpus* (Vero Beach strain, in colony since 1961) were hatched and larvae reared on a standardized diet (1:1, brewer's yeast:lactalbumin). Adult females were placed 200 per cage into 30.5 cm^3 cages, given either 2 or 10% sucrose as above, and placed in a bioroom. At 10 days, 100 females from each cage were removed, frozen, and stored at −20°C for later analysis. The remaining mosquitoes were given water in place of sucrose and allowed to starve. Dead mosquitoes were collected daily, frozen, and later used to establish nutritional base lines for each species.

ANALYSIS. In each group 20 mosquitoes were individually analyzed for sugar and glycogen (Van Handel 1985a) and 20 for lipid content (Van Handel 1985b). For each species, 40 starved mosquitoes were analyzed similarly to establish base lines. Differences between field-collected and laboratory-maintained groups were evaluated by using the Student *t*-test.

RESULTS

Base line nutrient values for starved individual female mosquitoes of all 3 species appear in Table 1. These values represent carbohydrates and structural lipids upon which the mosquitoes cannot subsist.

The mean nutritional reserves for 10-day old laboratory-maintained mosquitoes given 2, 10 or 20% sucrose and field-collected specimens not provided with sucrose are shown in Table 2. The amount of sugar found in individual mosquitoes depended on the initial concentration and the age of the sugar meal. As a rule, 2% sucrose was absorbed quickly from the crop while 20% sucrose remained detectable in higher concentrations for a longer period of time. The sugar detected in *Ae. aegypti* was greatest in those fed 10% and least in those given 2% sucrose. Field-collected *Cx. nigripalpus* contained as much sugar as laboratory mosquitoes maintained on 2 and 10% sucrose. Field-collected *Cq. perturbans* contained significantly less ($P < 0.01$) sugar than either of the laboratory treatments. More glycogen was stored by *Cx. nigripalpus* and *Cq. perturbans* in the laboratory than by *Ae. aegypti*. Generally, more glycogen was stored by laboratory-maintained mosquitoes than by field-caught members of the same species. The amount of lipid made and stored by individual mosquitoes was directly dependent upon sucrose concentration with significantly more fat stored by mosquitoes maintained on 10 and 20% sucrose.

Field-collected mosquitoes did show seasonal variation in their nutritional composition (Table 3). Sugar was highest in *Ae. aegypti* and *Cx. nigripalpus* in October and in *Cq. perturbans* in July. High glycogen reserves tend to result from increased sugar feeding behavior and were thus greatest in months where sugar feeding predominated for all three species. Lipid reserves were highest in *Ae. aegypti* in October, *Cx. nigripalpus* in April and *Cq. perturbans* in April and July. With all three mosquito species the amount of reserves available to individuals depends directly on the availability of nectar sources to foraging mosquitoes.

Table 1. Mean base line (± standard error) values of sugar, glycogen and lipid for starved colonized and field-collected mosquitoes (n = 40 for each treatment).

Species	μg per mosquito		
	Sugar	Glycogen	Lipid
Aedes aegypti	4.4 ± 0.2	4.4 ± 0.2	26.2 ± 0.9
Culex nigripalpus	4.1 ± 0.3	4.1 ± 0.3	33.0 ± 1.0
Coquillettidia perturbans	0.5 ± 0.01	5.9 ± 0.3	45.8 ± 2.1

Table 2. Mean nutritional reserves in adult, female mosquitoes from different holding and collection treatments.

Mosquito treatments	Nutritional reserves*		
	Sugar	Glycogen	Lipid
Aedes aegypti			
Field-collected	23.2	26.7	86.5
2% sucrose	7.6	46.0	55.2
10% sucrose	63.4	63.8	263.2
Culex nigripalpus			
Field-collected	38.0[a]	95.9[c]	107.0[d]
2% sucrose	25.1[b]	153.0	122.7[d]
10% sucrose	29.9[a, b]	120.9[c]	377.0
Coquillettidia perturbans			
Field-collected	18.8	19.9	96.3
2% sucrose	39.4	189.4	148.8
20% sucrose	329.1	144.5	261.9

* Column means within a group that are not followed by the same letter differ significantly at $P < 0.05$ as tested by the Student's *t*-test.

Table 3. Mean nutritional reserves in field-collected female mosquitoes taken during different months of the year.

	Mean nutritional reserves*								
	Aedes aegypti			*Culex nigripalpus*			*Coquillettidia perturbans*		
Month	Sugar	Glycogen	Lipid	Sugar	Glycogen	Lipid	Sugar	Glycogen	Lipid
January	28.4[a,b]	16.7[d]	90.6[f]	33.5[g]	19.8[h]	90.2[j]	None collected		
April	11.5[c]	11.8[d]	84.7[f]	20.1	23.7[h]	400.0	43.4	133.5[i]	163.3[m]
July	18.5[a,c]	29.1[e]	58.0	36.4[g]	79.6[i]	111.9[j,k]	80.7	112.3[i]	170.4[m]
October	43.4[b]	29.9[e]	138.7	122.0	67.6[i]	140.0[k]	18.8	19.9	96.3

* Means within a column not followed by the same letter differ significantly at $P < 0.05$ as tested by the Student's *t*-test.

DISCUSSION

Our data show that mosquitoes maintained in the laboratory on sucrose are nutritionally different from their conspecifics in the field. By the time laboratory mosquitoes are 7–10 days-old they have produced and stored from 2 to 10 times the amount of glycogen and from 1 to 3.5 times the amount of lipids found in field-collected mosquitoes. The magnitudes of these differences were both species-specific and dependent upon the sucrose concentration.

The deposition of glycogen reserves was similar in mosquitoes fed 2, 10, or 20% sucrose (Table 2). *Aedes aegypti* stored less glycogen than *Cx. nigripalpus* which stored less than *Cq. perturbans*. Less fat was stored by all species fed 2% than by those fed 10 or 20% sucrose. *Culex nigripalpus* fed 10% sucrose stored more lipid than mosquitoes from any other treatment. Laboratory-maintained *Ae. aegypti* fed 2% sucrose had fewer sugar and lipid reserves than did the field-collected mosquitoes. All 3 species were able to generate and store large amounts of fat in the laboratory. *Culex nigripalpus* and *Cq. perturbans* held on 10 or 20% sucrose produced and stored more glycogen and lipid than *Ae. aegypti* under similar holding conditions. This may represent a basic physiological difference between the relatively long-lived mosquitoes, such as *Culex* spp., which overwinter as adults and the short-lived *Aedes* spp.

Definite seasonal differences in nutrient reserves were observed in field-collected individuals of all 3 species (Table 3). High energy reserves in field mosquitoes would be expected to be associated with prevalent nectar sources and/or low activity levels. The amount of sugar observed in individual mosquitoes is a good indicator of the relative amount of nectar available during different times of the year. Both *Ae. aegypti* and *Cx. nigripalpus* had high levels of sugar during the October sampling period. Likewise, both species had high glycogen content during this period. However, the amount of nutritional reserves only rarely approached that observed in laboratory individuals maintained on high sucrose concentrations.

It is generally assumed that mosquitoes maintained on sucrose in the laboratory and used for behavioral studies are nutritionally similar to field mosquitoes. Here we show that mosquitoes maintained under normal laboratory holding conditions differ significantly from field-collected material. This may have important implications for experiments which depend upon laboratory reared and maintained mosquitoes. We have observed (unpublished observation) that regardless of the sucrose concentration, the major portion of glycogen and lipids was produced and stored by mosquitoes during the first 10 days of adult life and these levels remained high for up to 40 days. Most behavioral experiments (i.e., attractancy, blood feeding, host selection, flight ability) are done with 7 to 10-day old mosquitoes and long-term infectivity studies with viruses or protozoans often require mosquitoes to be maintained for 3–4 weeks. These laboratory mosquitoes are nutritionally different from field mosquitoes and this may bias conclusions derived from mosquitoes maintained in the laboratory. In our experience, 10 to 30-day old field-collected *Cx. nigripalpus* maintained on 10% sucrose are less likely to take blood, seek a host or oviposit than are mosquitoes deprived of sugar 48 hr prior to tests (Day, unpublished). This may be due to artificially enhanced fuel reserves. Klowden (1986) has shown that carbohydrate intake by adult female *Ae. aegypti* is important for both egg development and renewed host-seeking behavior by gravid females. It is now evident that more laboratory studies are necessary to further explore this nutrition-behavior relationship.

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