RELATIONSHIP OF FECUNDITY TO THE NUTRITIONAL QUALITY OF LARVAL AND ADULT DIETS OF *WYEOMYIA SMITHII*[1]

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ABSTRACT. The protein content of the larval diet was found to significantly influence oocyte production by autogenous females of the pitcher plant mosquito, *Wyeomyia smithii*. Addition of sucrose to the adult diet of *Wy. smithii* increased adult longevity and, under the conditions of the test, resulted in significantly increased fecundity. There were no significant changes in fecundity when 10% or 25% concentrations of egg albumin were added to the adult diet. Likewise, total amino acid concentrations of 0.37 mg/ml and 1.94 mg/ml in the diets of adult *Wy. smithii* females did not increase fecundity.

INTRODUCTION

Wyeomyia smithii (Coq.) breeds exclusively in pitcher plants of the species *Sarracenia purpurea*. The autogenous (non-bloodfeeding) nature of *Wy. smithii* has been known since the time of Smith's observations in 1902. At adult eclosion, considerable vitellogenesis has already taken place, and eggs mature at the expense of nutrient reserves in the fat body and possibly in larval muscle remnants (Smith and Brust 1971). *Wy. smithii* females can produce eggs without the benefit of any food ingestion whatsoever; however, adult female longevity can be substantially increased by carbohydrate ingestion (Price 1958, Smith and Brust 1971).

Fecundity in *Wy. smithii*, as in most autogenous mosquitoes, is low, ranging from about 30 to slightly more than 100 eggs/female (Istock et al. 1976, Price 1958, Smith and Brust 1971). Smith and Brust (1971) did not detect any significant change in fecundity resulting from honey-feeding by females. However, variations in larval rearing conditions, specifically in temperature, can significantly change fecundity (Bradshaw and Lounibos 1972). Likewise, there is some evidence that the quantity of food available to *Wy. smithii* larvae may change fecundity (Istock et al. 1975, Smith and Brust 1971). Similar differences in autogenous mosquito fecundity related to the larval environment have been noted by Hudson (1970), Lea (1964) and Spielman (1971).

While the fourth instar of *Wy. smithii* appears to be the most important life stage for assimilating nutrients utilized in reproduction (Istock et al. 1975), little attention has been given to the possible contributions that ingestion of plant or other non-host-associated proteins and/or amino acids by adults may make to the reproductive effort of *Wy. smithii*. The most likely sources of protein and/or amino acids for *Wy. smithii* adults are from the pitcher plants. The outside and lip surfaces of *S. purpurea* are dotted with nectar glands (Hepburn et al. 1920, Lloyd 1942). *S. purpurea* nectar is primarily fructose (Hepburn 1927). The nectar, along with volatile olfactory substances and visual stimuli, attracts potential insect prey to the carnivorous plants. Although the amino acid and protein content of pitcher plant nectar has not been investigated, it is quite possible that nitrogenous substances occur in the nectar at nutritionally significant concentrations. Baker and Baker (1975) report that nectar almost invariably contains detectable levels of amino acids. The nutritional benefits of amino acids associated with nectar and/or pollen ingestion have been documented

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in certain *Morpho* and *Heliconius* butterflies (Baker and Baker 1975, Dunlap-Pianka et al. 1977). Likewise, Baker and Baker (1975) examined a number of flowers noted by Hocking (1968) as being routinely visited by arctic mosquitoes. They found that the nectars from these flowers contained rather high concentrations of amino acids, thus opening the possibility that at least some mosquitoes can obtain significant amounts of amino acids from plant sources.

In addition to pitcher plant nectar, the possibility also exists that *Wy. smithii* adults ingest amino acids and proteinaceous materials through imbibing pitcher plant water and fluids associated with decomposing prey. Numerous studies on protein digestion and amino acid absorption have shown that the pitcher plants and indigenous bacteria in the pitcher liquor secrete proteases and that the resulting proteolytic products such as amino acids are absorbed by the plants (Lloyd 1942).

The purpose of the following experiments was to determine if *Wy. smithii* fecundity could be significantly increased by providing larvae with a high protein diet and/or providing adults with artificial diets containing varying concentrations of protein or amino acids.

METHODS AND MATERIALS

The *Wy. smithii* colony used to provide material for this study originated from larvae collected from *S. purpurea* growing in a bog near Massillon, Ohio in July 1974. All the tests described in this paper were conducted at my residence in San Antonio, Texas.

Larvae were reared in covered aluminum pans 33 × 25 × 5 cm containing 1 liter of tap water. Two hundred larvae were reared in each pan. Larvae were fed either a diet of finely-ground rabbit chow which contained about 16% protein (low protein diet) or a diet of a 1:1:1 pulverized mixture of lactalbumin, brewer's yeast, and standard laboratory chow[3] containing approximately 50% protein (high protein diet).

The larval feeding schedule was the same for both larval foods. Two hundred milligrams of food were initially added to each pan of larvae. After 5 days another 300 mg of food were added. Subsequently, 100 mg of food were added every 2 days until pupation began 18–21 days after egg hatching.

Because of limited facilities, the first experiment, which was performed during the summer, was run at 27–29°C. Although this temperature range is tolerated by *Wy. smithii* and is often encountered by them in nature (Istock et al. 1975), it is somewhat detrimental to adult survival and fecundity (Bradshaw and Lounibos 1972). Subsequent experiments were conducted at 24–27°C. All mosquitoes were reared and maintained under a 16/8 hour light-dark cycle.

In all experiments, 5 females and 10 males, 0–24 hr old, were placed in a 2 liter cardboard carton with a screened top. The lip of a paper cup was inserted through a hole in the bottom of each carton. The cups contained approximately 50 ml of tap water, and they served as the oviposition sites. Vials with cotton wicks, soaked with the experimental adult diets, were inserted through the sides of the cardboard cages. Adult insects had continuous access to these cotton wicks.

Cups were removed daily from the bottom of each cage and the number of eggs on the water surface and on the sides of the cup were counted. Numbers of dead adult mosquitoes of each sex were also counted, and female ovaries were dissected to determine the number of retained stage III, IV and V oocytes (Clements 1963). The contents and the wicks of each food vial were replaced daily.

RESULTS AND DISCUSSION

EXPERIMENT 1. Larvae were fed either the low protein or high protein diet. Adults were subsequently given access to:

[3] Ralston-Purina Co., St. Louis MO.

[4] ICN Life Sciences Group, Cleveland OH.

1) water only, 2) 10% egg albumin[4] in water, 3) 10% sucrose in water, or 4) 10% sucrose and 10% albumin in water. Each treatment was replicated 4 times.

The effects of various combinations of larval and adult diet regimens on fecundity are summarized in Table 1. The interactions of larval and adult diets were analyzed using a 3-factor analysis of variance procedure.

Overall lifetime egg production, including retained stage III, IV and V oocytes was 63.5 eggs/female for adults fed high protein larval diets and 56.4 eggs/female for adults fed low protein larval diets ($P<.10$). Thus, there was some indication that if adults had been reared as larvae under lower temperature conditions, as suggested by Bradshaw and Lounibos (1972), they would have shown significant differences in fecundity attributable to the protein quality of the larval diet.

The addition of albumin to the adult diet of *Wy. smithii* had no apparent affect on fecundity. Neither did albumin appreciably change the longevity of adults having access to it in their diets (Table 1).

When averaged over both low and high protein larval diets, sucrose-fed females developed significantly more oocytes and eggs than did water-fed females ($P<.01$). However, individual means comparisons using t-tests did not usually reflect this overall tendency (Table 1). Since egg production of sucrose-fed females and of water-fed females was essentially the same the first several days after adult eclosion (Figure 1), this increase in fecundity among sucrose-fed females appeared to be only a reflection of the extended period of adult longevity made possible by carbohydrate in the adult diet (Table 1). The longer lifespan presumably enabled sucrose-fed females to utilize more fully their nitrogenous nutrient reserves accumulated during their larval lives.

When the patterns of oviposition over time were compared (Figure 1), the addition of 10% albumin caused no significant variation. Females feeding on sucrose-containing diets consistently showed mul-

Table 1. Effects of larval and adult diets on *Wyeomyia smithii* fecundity and adult female longevity.

Adult Food	No. Eggs Laid		No. Oocytes Developed Beyond Stage II		Mean Longevity in Days	
	Low Protein[a]	High Protein	Low Protein	High Protein	High Pro-tein	Low Protein
Water	30.68±11.95[b]	41.76±20.47[b]	48.88± 8.09[b]	56.96±13.93[b]	4.1±0.4[b]	4.7±0.3[b]
10% Albumin	31.24±16.66[b]	40.98±12.38[b]	51.26± 7.99[b]	50.30±15.54[b]	4.2±1.0[b]	4.5±1.1[b]
10% Sucrose	50.68±21.71[b]	58.20±18.67[b]	59.15±19.68[b]	78.65±13.84[c]	8.4±0.6[c]	7.8±3.1[c]
10% Sucrose plus 10% Albumin	53.36±25.97[b]	55.65±24.19[b]	65.66±17.24[b]	74.60±16.76[bc]	8.3±1.9[c]	7.1±2.8[c]

[a] Type of larval diet—See text for description.
[b, c] Means and their respective standard errors in a column followed by the same letter are not significantly different at the 5% level.

timodal patterns of egg deposition. The first major ovipositional peak occurred 3–4 days post-emergence with progressively smaller peaks discernable every 3–4 days later until the end of the first 10–12 days. Peaks after 12 days were ill-defined, since so few females survived beyond that time. Although Figure 1 only shows the ovipositional patterns for females fed the high protein larval diet, similar oviposition patterns were found among females reared on the low protein larval diet. The observation that females without a carbohydrate source had a first ovipositional peak identical to that of females having access to sucrose contrasts to the findings of Hudson (1970), who found that sugar feeding by facultatively autogenous *Aedes atropalpus* females delayed egg deposition.

EXPERIMENT 2. In an attempt to clarify whether or not oocyte production could be significantly influenced by larval diet, a second group of larvae was reared on either the high or low protein diet described earlier. However, the rearing temperature was 24–27°C instead of 27–29°C as in the first experiment. Ovaries from 25 one-day-old females in each group were dissected and oocytes in stages III, IV and V were counted. Ovaries from females reared on the high protein larval diet contained an average of 80.4 ± 2.1 SE oocytes/female Ovaries from females reared on low protein larval food contained an average of 66.3 ± 3.7 SE oocytes/female. The difference was significant at $P<0.005$ when the means were compared with a t-test. Therefore, it can be concluded that the quality of larval food can have a significant effect on *Wy. smithii* oocyte production.

EXPERIMENT 3. Adults from larvae

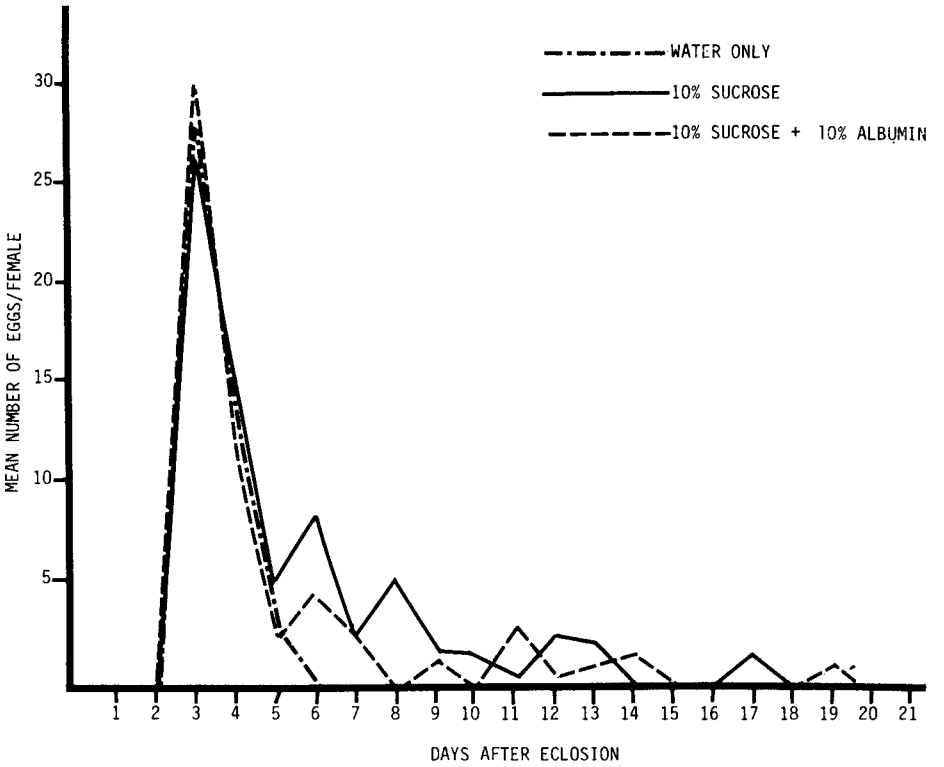

Fig. 1. Ovipositional patterns of *Wy. smithii* females fed the indicated adult diets. See Experiment 1 for rearing details.

reared on high protein food were given access to diets either of 10% sucrose or 10% sucrose plus 25% egg albumin. The treatments were replicated 4 times.

Once again, no differences in fecundity or patterns of egg deposition were evident. Mean total egg production was 78.8 ± 18.9 SE eggs/females for females fed 10% sucrose and 77.8 ± 9.3 SE eggs/female for females having 10% sucrose plus 25% albumin as an adult diet.

EXPERIMENT 4. The possibility that *Wy. smithii* females lack the proper proteases to digest albumin prompted an attempt to determine if amino acid supplements in the adult diet could increase fecundity. Since plant nectars are much more likely to contain significant amounts of amino acids than of protein (Baker and Baker 1975), this approach seemed justified. Without a guide as to the amounts and kinds of amino acids which could possibly influence *Wy. smithii* fecundity, the *l*-amino acid levels found in human plasma (total concentration 0.37 mg/ml) (Altman and Dittmer 1974) were incorporated into 10% sucrose diets. Controls were fed only 10% sucrose. The experiment was replicated 5 times.

The addition of human blood plasma levels of *l*-amino acids to the adult diet of *Wy. smithii* did not significantly affect fecundity or ovipositional pattern. The mean total egg production, including retained oocytes, was 86.2 ± 7.3 SE eggs/female and 92 ± 8.9 SE eggs/female for individuals having access to only sucrose or sucrose plus amino acids, respectively.

EXPERIMENT 5. In this final experiment, the total amino acid concentration was increased to 1.94 mg/ml. This concentration was chosen since it corresponded to a mean "histidine scale score" of 9 which Baker and Baker (1975) assigned to the nectars of flowers pollinated by specialized flies, i.e., carrion and dung-feeding species.

The amino acid ratios were kept the same as those in human plasma. Controls were fed only 10% sucrose solutions. The experiment was replicated 3 times.

The mean lifetime egg production of *Wy. smithii* females having access to the amino acid-sucrose solution was 109.4 ± 3.7 SE eggs/female compared to 105.6 ± 7.8 SE eggs/female for the control group. The means compared by a t-test were not significantly different at the 5% level. The patterns of egg deposition for the 2 groups of females were almost identical, and again, distinct multiple peaks occurred about every 3–4 days (Figure 2).

The reason for the higher lifetime egg production means observed during Experiments 4 and 5 compared to those observed during Experiments 2 and 3 is unknown. However, since the experiments were conducted over a period of several months, it is possible that some type of seasonal cue or freerunning periodicity was responsible for the variation among the separate experiments. In a similar vein, Istock et al. (1976) found unexplainable seasonal periodicity in *Wy. smithii* diapause tendencies under controlled laboratory conditions.

In summary, the presence of protein (albumin) and amino acids in the adult diet of *Wy. smithii* females did not significantly influence fecundity. However, at more optimal larval rearing temperatures, oocyte production differences resulting from differences in the protein quality of the larval diet were found.

The introduction of carbohydrate (sucrose) into the adult diet significantly increased adult longevity. Overall, females given access to sucrose as adults produced significantly more eggs than did females denied sucrose as adults. This difference was apparently the result of more complete utilization of protein and lipid reserves permitted by the extended adult lifespan. This finding contrasts with the conclusion of Smith and Brust (1971) that honey-feeding did not significantly increase *Wy. smithii* fecundity. However, those authors conducted their experiments at 20°C whereas 27–29°C were the temperatures used in this study. Under the cooler temperatures used by Smith and Brust, water-fed females lived an average of 9.1 days which is about 5 days longer than the water-fed females in Ex-

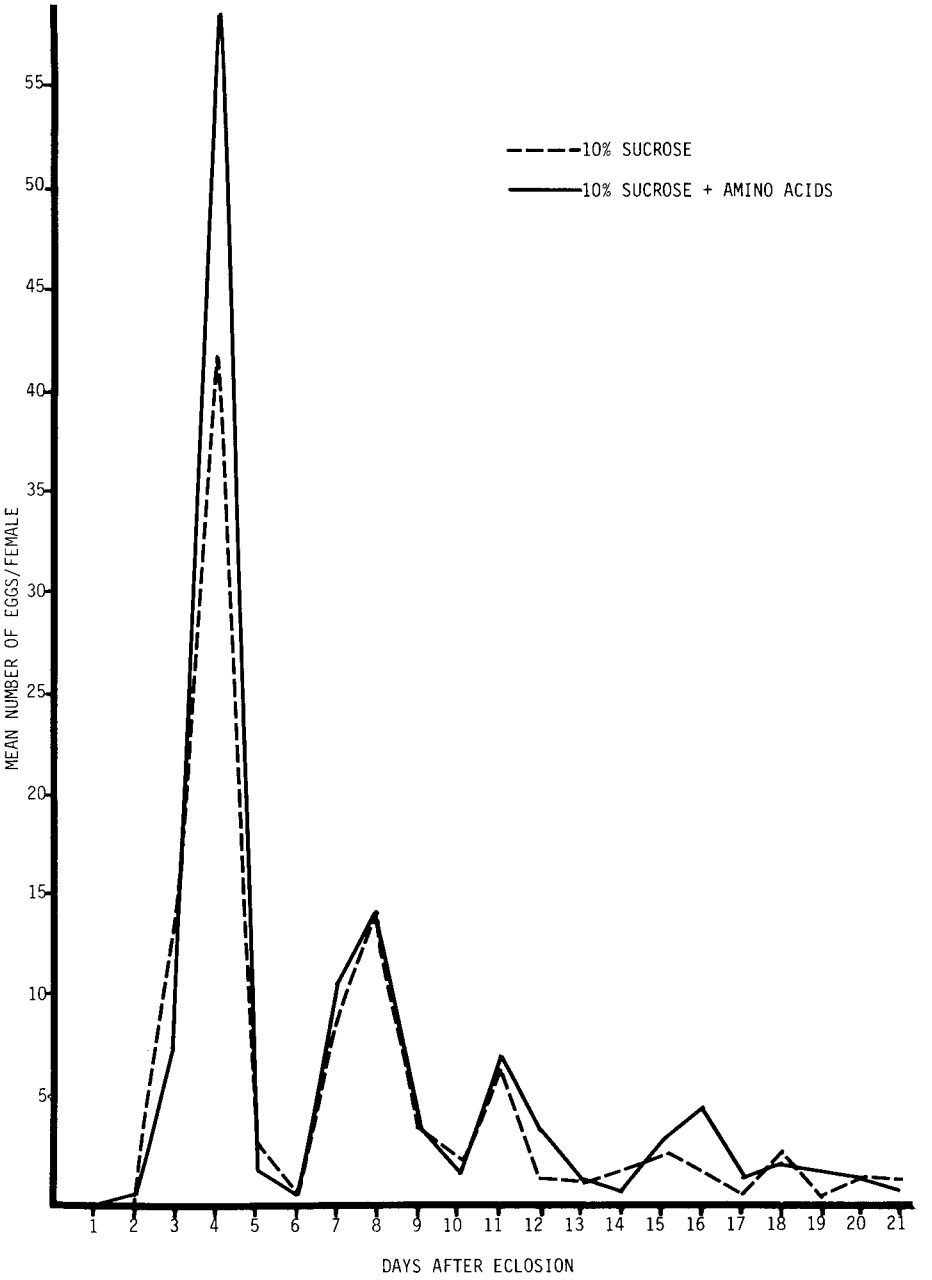

Fig. 2. Ovipositional patterns of *Wy. smithii* females fed the indicated adult diets. See Experiment 5 for rearing details.

periment 1 and 1 to 2 days longer than sucrose-fed females in Experiment 1. Therefore, sugar feeding may only increase *Wy. smithii* female fecundity under temperature conditions elevated somewhat above optimal levels for adult survival.

Based on the results of Smith and Brust (1971), it appears that the reduced metabolic rate in *Wy. smithii* females at lower temperatures (around 20°C) permits nearly complete mobilization of nitrogenous reserves and full development of the oocyte complement in each female before carbohydrate reserves are consumed. Conversely, the higher temperatures used in Experiment 1 of this study evidently increased the catabolism of reserve carbohydrates to the extent that the water-fed females succumbed before they could completely mobilize all of their nitrogenous reserves in the production of eggs.

The patterns of egg deposition during the lifetimes of *Wy. smithii* females were the same regardless of larval or adult diet except that females denied carbohydrates did not manifest the entire oviposition pattern because of their abbreviated lifespans. There appeared to be a definite rhythmicity to egg development and deposition in *Wy. smithii* under the conditions used in this study. The bulk of eggs were deposited in the first week of adult life.

It is particularly interesting that females reared as larvae under suboptimum conditions of high temperature, low protein larval diet, and lack of carbohydrate in the adult diet still laid an average of at least 30 eggs/female. This supports the contention of Istock et al. (1975) that *Wy. smithii* larval cohorts produce adults only when a positive instrinsic rate of increase can be realized and that a fertility rate of at least 25 eggs/female would have to be achieved for an autogenous mosquito population to maintain itself.

It appears that *Wy. smithii* populations in nature could easily maintain themselves in the absence of all adult food. However, when access to carbohydrate is available, adults can increase their longevity, and under stresses of high temperature, can bring more eggs to maturity through a more complete utilization of their nitrogenous nutrient stores. Addition of protein or amino acids to the adult diet apparently cannot significantly influence the genetically-programmed senescence of the ovaries in this autogenous mosquito species.

References Cited

Altman, P. L. and D. S. Dittmer (Eds.) 1974. Biology Data Book, 2nd ed. Vol. III. Federation of Amer. Soc. for Experimental Biology p. 1806–07.

Baker, H. G. and I. Baker 1975. Studies of nectar-constitution and pollinator-plant coevolution p. 100–140. In Coevolution of Animals and Plants. I. E. Gilbert and P. H. Raven (Eds) University of Texas Press.

Bradshaw, W. and L. P. Lounibos 1972. Photoperiodic control of development in the pitcher-plant mosquito *Wyeomyia smithii*. Can. J. Zool. 50(6):713–19.

Clements, A. N. 1963. The Physiology of Mosquitoes. The Macmillan Co., N. Y. 393 p.

Dunlop-Pianka, H., C. L. Boggs and L. E. Gilbert 1977. Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth: Science 197:487–490.

Hepburn, J. S. 1927. A chemical study of the nectar of the Sarraceniaceae. Trans. Wagner Free Inst. Sci. Philadelphia 11:31–33.

Hepburn, J. S., E. Q. St John and F. M. Jones. 1920. The absorption of nutrients and allied phenomena in the pitchers of the Sarraceniaceae. J. Franklin Inst. 189:147–184.

Hocking, B. 1968. Insect-flower associations in the high artic with special reference to nectar. Oikos 19:359–387.

Hudson, A. 1970. Factors affecting egg maturation and oviposition by autogenous *Aedes atropalpus* (Diptera: Culicidae). Can. Entomol. 102:939–949.

Istock, C. A., S. S. Wasserman and H. Zimmer. 1975. Ecology and evolution of the pitcher-plant mosquito: 1. Population dynamics and laboratory responses to food and population density. Evolution 29:296–312.

Istock, C. A., J. Zisfein and K. J. Vavra. 1976. Ecology and evolution of the pitcher-plant mosquito. 2. The substructure of fitness. Evolution 30:535–47.

Lea, A. O. 1964. Selection for autogeny in

Aedes aegypti (Diptera: Culicidae). Ann. Entomol. Soc. Am. 57(5):656–657.
Lloyd, F. E. 1942. The Carnivorous Plants Chronica. Botanica Co. Waltham, Mass (reprinted 1976 Dover Publications Inc.) 352 p.
Price, R. D. 1958. Notes on the biology and laboratory colonization of *Wyeomyia smithii* (Coquillett) (Diptera: Culicidae). Can. Entomol. 90:473–478.
Smith, J. B. 1902. Life history of *Aedes smithii* Coq. J. New York Entomol. Soc. 10:10–15.
Smith, S. M. and R. S. Brust. 1971. Photoperiodic control of the maintenance termination of larval diapause in *Wyeomyia smithii* Coq. (Diptera: Culicidae) with notes on oogenesis in the adult female. Can. J. Zool. 49:1065–1073.
Spielman, A. 1971. Bionomics of autogenous mosquitoes. Annu. Rev. Entomol. 16:231–48.

COLONY MAINTENANCE OF *ANOPHELES ALBIMANUS* WIEDEMANN BY FEEDING PRESERVED BLOOD THROUGH NATURAL MEMBRANE

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ABSTRACT. Techniques were developed for using natural membranes to successfully feed a colony of *Anopheles albimanus* Wiedemann on bovine blood preserved by defibrination and storage at 5°C. Egg production by mosquitoes fed the preserved blood was 58% lower than production by those fed on live rabbits, but this reduction was not detrimental to colony maintenance and productivity. The advantages associated with membrane feeding make that technique much more desirable than maintaining live animals for that purpose.

INTRODUCTION

In controlling human disease through control of the vectors much research is needed to determine efficacy of control procedures. That research, in turn, often requires maintenance of laboratory colonies of the vectors. This is particularly true in studies of mosquito-borne diseases, and many laboratories around the world now maintain colonies of many species of mosquitoes.

As the necessity for research has increased, the need for more efficient rearing techniques also has increased, so that over the years investigators have experienced increasing efficiency in mosquito rearing. Thus, techniques for feeding blood to colony female mosquitoes, a prerequisite to viable egg production by most species, have undergone several evolutionary changes through the years.

Most early workers in mosquito rearing (Boyd et al. 1935, Rozeboom 1936, Crowell 1940) depended on human blood donors to feed the adult females. In fact some allowed the adult mosquitoes the freedom of the room so they could feed upon the insectary technician (Crowell 1940). Other early workers used animals as sources of blood (Shute 1936), and the majority of current colonies are provided live host animals as a blood source (Gerberg 1970).

There are definite advantages to using human or animal blood donors for mosquito colonies, e.g., the blood is available in a natural condition and is at the correct temperature for good feeding response. There are also obvious disadvantages such as accidental disease transmission from one host to another, and with human donors there is always the danger of hypersensitivity to mosquito bites. In addition, when laboratory animals are used, there is the expense of caging, feed-