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Behavior of Thoracic Spiracles of Aedes Mosquitoes in Controlled Relative Humidities

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tive, or both. A comparison of Table 1 with Fig. 2 and 3 indicates that the value of the higher alternative is the most important factor in determining response intensity. However, the reduced intensity of the response to the alternatives 0-100 and 0-80% RH suggests that the value of the lower alternative is also of some importance, at least when it lies near the lower extreme of the RH scale.

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REFERENCES CITED

- Gunn, D. L., and C. A. Cosway. 1938. The temperature and humidity relations of the cockroach. V. Humidity preference. *J. Exp. Biol.* 15: 555-63.
- Gunn, D. L., and J. S. Kennedy. 1936. Apparatus for investigating the reactions of land arthropods to humidity. *Ibid.* 13: 450-9.
- Madge, D. S. 1961. The control of relative humidity with aqueous solutions of sodium hydroxide. *Entomol. Exp. Appl.* 4: 143-7.
- Perttunen, V. 1953. Reactions of diplopods to the relative humidity of the air. Investigations on *Orthomorpha gracilis*, *Iulus terrestris*, and *Shizophyllum sabulosum*. *Suomalaisen eläin-ja kasvit. Seur. van Julk.* 16(1): 1-69.
- Pielou, D. P., and D. L. Gunn. 1940. The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. I. The reaction to differences of humidity. *J. Exp. Biol.* 17: 286-94.
- Smereka, E. P., and A. C. Hodson. 1959. Some humidity and light reactions of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Can. Entomol.* 91: 784-97.
- Surtees, G. 1965. Laboratory studies on dispersion behavior of adult beetles in grain. X. Reaction of saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), to isolated pockets of damp and mouldy wheat. *J. Appl. Ecol.* 2: 71-80.
- Syrjämäki, J. 1962. Humidity perception in *Drosophila melanogaster*. *Suomalaisen eläin-ja kasvit. Seur. van. Julk.* 23(3): 1-74.
- Willis, E. R., and L. M. Roth. 1950. Humidity reactions of *Tribolium castaneum* (Herbst). *J. Exp. Zool.* 115: 561-87.

Behavior of Thoracic Spiracles of *Aedes* Mosquitoes¹ in Controlled Relative Humidities²

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ABSTRACT

Observations were made on the behavior of the thoracic spiracles of *A. triseriatus* (Say) and *A. aegypti* (L.) in high (90±5%) and low (<5%) relative humidities. Following a 5-minute exposure to low relative humidity, spiracular responses to 0.5% carbon dioxide were reduced

in both species while only *A. triseriatus* showed attenuated spiracular behavior after a 2-minute exposure. Conservative spiracular opening was observed in both unfed and fed mosquitoes. It is postulated that the spiracular response to low relative humidity is a sensory one.

Regulatory valves in the tracheal system of insects presumably developed to facilitate water conservation while meeting respiratory requirements. Although Hazelhoff (1927) was apparently the first to suggest that most water loss occurred through transpiration from the tracheae, Mellanby (1934) experimentally confirmed the hypothesis by showing that factors causing permanent spiracular opening in *Tenebrio* larvae greatly accelerated water loss. Bursell (1957) demonstrated that water loss from *Glossina* was a linear function of humidity when spiracles were kept constantly open with 10%CO₂; however, in the absence of this gas, rates of water loss varied little with respect to humidity. Moreover, spiracular control of water was markedly more efficient in flies with depleted water reserves. Adult *Phormia regina* (Meigen) lost more weight in a low relative humidity than in a high relative humidity. Further, total oxygen consumption was reduced by dry conditions (Buck and Keister 1949); those

authors suggested that the effects of low humidity resulted from spiracular transpiration. Bursell (1963) considered the effects of a xeric environment on water balance in *Glossina* and concluded that the highly developed power of water retention in these forms resulted largely from spiracular control of transpiration. As in *Glossina*, humidity and water balance must play an important though ill-defined role in survival and longevity of mosquito populations (Clements 1963, Hylton 1967). Available evidence points to an intrinsic mechanism of spiracular control of transpiration rather than an extrinsic, sensory one (see also Bursell 1964; Miller 1964a, b; Hoyle 1961).

Several lines of evidence thus suggest that spiracles are instrumental in water conservation while still responding to the often conflicting demands of respiration. On the other hand, no evaluation of spiracular response to humidity by direct observation exists beyond the efforts of Geigy and Huber (1952) to correlate the degree of spiracular opening with ambient humidity. The object of the present communication is therefore to survey the immediate sensory effects of "high" and "low" relative humidity upon spiracular behavior in adult female *Aedes* (Finlaya)

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triseriatus (Say) and *A. (Stegomyia) aegypti* (L.). The intrinsic influence of water balance on spiracular behavior will be explored in a subsequent communication.

METHODS AND MATERIAL

Equipment and methods for observing and recording behavior of the spiracular valves have been described in detail elsewhere (Krafsur et al. 1970) and will be noted only briefly here. Thoracic spiracles of adult mosquitoes were observed at a magnification of 97.5 \times with 2 horizontally mounted microscopes using incident illumination. Each insect was suspended by its mesonotum with a vacuum (18–22 psi) maintained through a blunted 22-gauge hypodermic needle; the needle was mounted onto a mechanical stage capable of orienting the mosquito in 3 dimensions. A microclimate of known composition and relative humidity was provided by shunting suitable amounts of dry, compressed air from steel cylinders through flowmeters and a water tank to a gas delivery tube. The tube directed gas flow over the insect from both the right and left anterolateral positions. Gas flow was standardized at 5000 cc/min and temperature was maintained at ca. 21.1°C. Relative humidity was measured with Leeds and Northrup electronic sensing elements accurate to $\pm 1.5\%$ RH. Oscillograms of spiracular behavior were obtained with the use of a 2-channel pen recorder activated by pushbutton controls directed through an amplifier. This mechanism allowed an observer 3 arbitrary choices in translating spiracular behavior into a permanent record for later analysis. A closed spiracle and 2 magnitudes of opening were recognized. Opening of the 1st magnitude was arbitrarily defined as partial separation of the spiracular lips for commonly $\frac{1}{8}$ – $\frac{1}{4}$ of their length. This amount of separation was, in fact, the extent of "normal" opening in mosquitoes at rest in air. Spiracular opening of the 2nd magnitude was defined as complete separation of the spiracular lips and included opening to the widest possible amplitude. Hypoxia, CO₂, or spontaneous locomotor activity (i.e., momentary struggling and flight) was associated with spiracular opening of the 2nd magnitude.

For the purposes of describing spiracular behavior in qualitative and quantitative terms, the following indices are used in the tables and text. "Amplitude" of spiracular opening refers to the total number of seconds per minute of observation that spiracles were recorded as being open at the 2nd magnitude. Thus "amplitude" describes in units of time complete separation of the spiracular lips. "Duration" of spiracular opening is the total number of seconds per minute of observation that spiracles were recorded as being open regardless of the magnitude of that opening. To determine the amount of spiracular opening at the 1st magnitude from the tables, simply subtract "amplitude" from "duration." "Frequency" refers to the number of spiracular openings per minute of observation and were scored only when opening was from the completely closed position.

Exclusively female pupae were allowed to emerge and the adults were maintained thereafter in gallon-size cardboard containers at 26.6°C and 80 \pm 5% RH. Cotton pads containing 30 cc of 10% sucrose solution were provided fresh daily for nourishment.

Mosquitoes were prepared for spiracle observation in the following manner: (1) a mosquito was gently aspirated into a plastic shell vial; (2) the vial was placed into a freezer at $-4\pm 3^\circ\text{C}$ for 1½–2 min; (3) the immobilized insect was placed on a flat surface and the right prothoracic leg was amputated to prevent occlusion of the right mesothoracic spiracle; (4) the mosquito was attached by the mesonotum to a blunted hypodermic needle; (5) the insect and needle were mounted on the mechanical stage; (6) following recovery from chilling under room conditions, the mosquito was exposed to an air flow of known relative humidity for a stated period of time; (7) observation and recording of spiracular behavior was then undertaken for a continuous 3-min period. The 1st minute of observation was taken in air alone and the following 2 min were taken in air plus 0.5% CO₂.

For statistical interpretation of the data, F-tests were used to determine if treatment variances were equal. The appropriate "Student's" t-test was then employed to test equality of treatment means.

RESULTS

The 1st series of observations was directed towards evaluation of spiracular behavior in high (90 \pm 5%) and low (<5%) relative humidities following a 5-min period for acclimatization of the mosquito to the experimental conditions. Two-day-old *A. aegypti* were subjected at random to 1 of the relative humidities; a total of 30 insects was observed in each

Table 1.—Spiracular responses to high (90 \pm 5%) and low (<5%) relative humidity in air and air plus 0.5% CO₂ in *A. aegypti* and *A. triseriatus* following a 5-min accommodation period (30 mosquitoes observed in each treatment).

Treatment	Spiracular opening			
	Frequency ^a	Amplitude ^b \pm SE	Duration ^b \pm SE	Activity ^c (%)
<i>A. aegypti</i> in air				
High RH	3.8	0.	44.4 \pm 2.3	30
Low RH	4.4	0.	39.8 \pm 2.5	30
<i>A. aegypti</i> in air + CO ₂				
High RH	0.5	57.6 \pm 2.2	58.8 \pm 1.5	53
Low RH	1.3 ^d	50.5 \pm 2.7 ^e	53.0 \pm 1.9 ^e	50
<i>A. triseriatus</i> in air				
High RH	4.5	0.	43.4 \pm 2.3	3
Low RH	4.4	0.	40.1 \pm 2.8	13
<i>A. triseriatus</i> in air + CO ₂				
High RH	2.1	50.8 \pm 1.9	54.7 \pm 1.4	7
Low RH	2.6	40.3 \pm 2.5 ^d	48.5 \pm 2.1 ^e	13

^a No. of openings per min of observation.

^b Sec per min of observation.

^c Percent of individuals showing spontaneous locomotor activity.

^d $P < 0.005$.

^e $P < 0.01$.

environment. Two-, 3-, and 4-day-old *A. triseriatus* were similarly tested. In both *A. aegypti* and *A. triseriatus* (Table 1), the average duration of spiracular opening was less in air of low than in air of high humidity; however, the differences were not statistically significant largely because variances were high. On the other hand, the amplitude and duration of spiracular opening in response to CO₂ were significantly less in a low than in a high relative humidity.

Observations were made to determine if spiracular behavior could be influenced by relative humidity after only a 2-min exposure period. *A. aegypti*, 2, 3, 4, and 5 days old were randomly examined in an air stream of 90±5 or <5% RH. The pooled observations (Table 2) indicate that means and variances of the 2 treatments were closely similar. Relative humidity thus appeared to have no immediate effect upon spiracular opening in *A. aegypti*.

Spiracular behavior of *A. triseriatus* in high and low relative humidities following 2-min exposure periods was next measured. The hypothesis that spiracular regulation of water loss occurs only when water reserves are depleted (Bursell 1957) was tested by contrasting behavior of 20 individuals each of 3 successive batches of newly emerged day 1 unfed insects in high and low relative humidities. It was anticipated that the water reserves of such mosquitoes would be minimal and certainly less than in older mosquitoes given access to sucrose solution and maintained at a higher relative humidity (sucrose soaked cotton pads, when placed on a mosquito-holding container, raise the internal container humidity by 10% or more). Near the end of the day 1 (1600 hr), the *A. triseriatus* were allowed constant access to the 10% sucrose solution. They were sampled further for observations on spiracular behavior on days 2 through 4. Thus the experiment

was thrice replicated over a 3-week period and a total of 120 mosquitoes was observed in each humidity.

Examination of the pooled observations (Table 3) suggested that in both the starved and fed mosquitoes, spiracular opening was inhibited to a highly significant extent in air of low relative humidity, i.e., the amplitude and duration of opening was reduced. Further, spiracular opening in the starved insects was considerably less than in the older, fed mosquitoes regardless of humidity. On a daily basis, the average duration of spiracular opening was nearly equal between treatments on only day 2; this equality may well have been the result of a particularly heavy and uniform feeding by formerly starved mosquitoes.

Spiracular response to 0.5% CO₂ was severely attenuated in low humidity (Table 3). This result was particularly obvious in the unfed mosquitoes, for here the duration of spiracular opening in low humidity was 76% of that in the high. In the same insects, amplitude of opening was 50% of that in high humidity.

In the fed group of mosquitoes exposed to low relative humidity, duration of opening was 88% and amplitude 78% of those exposed to the high humidity. It is therefore apparent that low humidity primarily effects the amplitude of spiracular opening. When noted by age, spiracular opening was most extensive on the day following application of nutrient to the experimental population; beyond day 2, duration and amplitude of spiracular opening progressively declined. Nevertheless, the influence of relative humidity on them was highly significant ($P < 0.005$) each day of sampling.

Immediately following the 2-min exposure to CO₂, occasional mosquitoes were subjected to a reversal in relative humidity; this reversal was easily accomplished by turning a valve and shunting dry air directly over the mosquito for an additional 2 min. Following this procedure, the humidity was again reversed. By then comparing the resulting oscillograms within each 2-min period, it was readily determined that spiracular reaction to CO₂ was indeed modified by relative humidity.

Because there was a minimum difference of 80% RH between treatments it was not altogether surprising that treatment effects were demonstrated in spiracular behavior. The next objective was therefore to contrast spiracular activity in high relative humidity and a more "moderate" 50±6% RH. Day 3, 4, and 5 adult female *A. triseriatus* were randomly exposed to one of the 2 humidities for 2 min prior to recording spiracular activity. The results are shown in Table 2 and strongly suggest marked reduction in spiracular opening may occur even in a moderate humidity.

DISCUSSION

Earlier work has clearly demonstrated that spiracles greatly facilitate water conservation in insects (Mellanby 1934, Buck and Keister 1949, Bursell

Table 2.—Spiracular opening in *A. aegypti* in high (90±5%) and low (<5%), and *A. triseriatus* in high (90±5%) and moderate (50±6%) relative humidities in air and air plus 0.5% CO₂ following a 2-min accommodation period (50 mosquitoes observed in each treatment).

Treatment	Spiracular opening		
	Frequency	Amplitude ±SE	Duration ±SE
<i>A. aegypti</i> in air			
High RH	2.58	0.	50.4±2.0
Low RH	2.68	0.	50.8±1.8
<i>A. aegypti</i> in air + CO ₂			
High RH	0.64	56.0±0.99	58.2±0.57
Low RH	.61	55.6±.86	58.7±.24
<i>A. triseriatus</i> in air			
High RH	3.5	0.	42.4±2.3
Moderate RH	4.0	0.	36.9±2.0 ^a
<i>A. triseriatus</i> in air + CO ₂			
High RH	1.4	47.0±2.0	53.8±1.4
Moderate RH	3.0 ^a	30.0±2.4 ^a	44.4±1.8 ^a

^a $P < 0.005$.

Table 3.—Spiracular behavior of *A. triseriatus* in air and air plus 0.5% CO₂ at high (90±5%) and low (<5%) relative humidities. Observations were made following a 2-min exposure to the desired humidity.

		Spiracular opening in				
		Air		Air plus 0.5% CO ₂		
Treatment	n	Frequency ± SE	Duration ± SE	Frequency ± SE	Amplitude ± SE	Duration ± SE
Day 1, starved						
High RH	30	4.6±0.58	40.9±2.4	1.4±0.34	47.3±1.3	54.8±1.5
Low RH	30	5.1±.52	34.3±2.1 ^a	4.1±.55 ^a	23.8±2.9 ^a	41.8±1.4 ^a
Days 2, 3, 4, fed						
High RH	90	2.8±.20	49.0±1.5	0.9±.16	52.9±1.2	56.4±1.0
Low RH	90	3.6±.27 ^b	42.9±1.6 ^a	2.4±.23 ^a	41.5±1.9 ^a	49.5±1.2 ^a

^a $P < 0.005$.

^b $P < 0.01$.

1957, Miller 1964a, Kanwisher 1966). Perhaps the role of spiracles in water conservation may best be illustrated in an insect showing discontinuous respiration; in other words, respiration in "slow motion." Saturniid pupae in dry atmospheres lose, through spiracular transpiration, about as much water as metabolically produced CO₂; however, the source of such water is oxidized fat and consequently no net loss of water occurs (Kanwisher 1966). Kanwisher's fantastically sensitive diaferometric techniques indicated that most transpiration occurred during the "burst" period of spiracular opening. Permanently open spiracles allowed transpiration to occur at a 25-fold or even greater rate, ensuring desiccation and ultimate death. In addition to control normally exerted by intratracheal O₂ and CO₂ pressures, spiracular behavior may also be governed to some degree by the water reserves of the insect. There is good evidence that spiracular control of water loss through transpiration rests critically upon water balance in *Glossina* (Bursell 1957) and in dragonflies (Miller 1964a). By measuring the rate of water loss of tsetse flies in varying states of desiccation, Bursell was able to show that spiracular control of transpiration increased as water reserves decreased. It was also demonstrated that transpiration in normal flies was not related to the ambient relative humidity, with the conclusion that decreasing humidity increased the degree of spiracle closure. In dragonflies, desiccation results in a greater concentration of CO₂ necessary to elicit spiracular opening (Miller 1964a); hydration has the opposite effect.

This study demonstrates that, quite apart from the intrinsic effect of water balance upon the degree of spiracular control, relative humidity itself exerts a direct, probably sensory, effect upon the thoracic spiracles of *A. aegypti* and especially *A. triseriatus*. Thus, both spiracular behavior in air and spiracular response to CO₂ become quickly attenuated in low humidity when compared with that in a high humidity. Moreover, this behavior can occur quite independently of whatever state of hydration the mosquito may be in. The principal feature of spiracular behavior in low humidity was a reduction in the ampli-

tude of opening. Secondly, duration of opening was decreased and finally, a slightly increased frequency of opening and closing was usually observed. Attempts by Geigy and Huber (1952) to correlate changes in humidity with the spiracular behavior of tsetse flies failed. Bursell (1957) attributed this failure to the fact that the insects used by Geigy and Huber were given opportunity to feed daily, while his own experiments were conducted with exclusively unfed flies. Thus, Bursell suggested that spiracular control of water loss occurs only when water reserves are somewhat depleted. One-day-old unfed adult *Aedes* quite clearly showed more conservative spiracular opening than did older, fed specimens, and the responses to low humidity were certainly more exaggerated. However, 2-day-old *Aedes* fully replete with 10% sucrose solution also showed marked attenuation of spiracular opening in low humidity. Partly for this reason, I suggest that such behavior in low humidity is a result of a sensory process. Although it can be argued that 1-day-old *Aedes* may inherently show more conservative spiracular behavior, a study of spiracular behavior as a function of age suggested quite the opposite (Krafsur 1971) and it must be concluded that responses to low relative humidity were enhanced by reduced water reserves. The "sensory" effect of relative humidity postulated herein rests also upon the observation that it could be demonstrated within 3 min of exposure and was reversible.

It is interesting to contrast the responses of *A. aegypti* and *A. triseriatus* to relative humidity. In the former species, spontaneous locomotor activity was high and spiracular behavior less conservative than in the much less active *A. triseriatus*. It has been shown that spiracular opening is considerably exaggerated in *A. aegypti*, probably as a result of spontaneous activity (Krafsur et al. 1970); it is suggested that locomotor activity may account for the absence of a significantly different response to low humidity following a 2-min exposure. That is, the respiratory dictates of activity may have overridden the effect of relative humidity upon spiracular behavior in *A. aegypti*.

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REFERENCES CITED

- Buck, J. B., and M. L. Keister. 1949. Respiration and water loss in the adult blowfly, *Phormia regina*, and their relation to the physiological action of DDT. Biol. Bull. 97: 64-81.
- Bursell, E. 1957. Spiracular control of water loss in the tsetse fly. Proc. Roy. Entomol. Soc. London (A) 32: 21-29.
1963. Tsetse-fly physiology. Bull. World Health Organ. 28: 703-9.
1964. Environmental aspects: humidity, p. 323-61. In M. Rockstein [ed.] The Physiology of Insecta. Academic Press, New York.
- Clements, A. N. 1963. The Physiology of Mosquitoes. Pergamon Press Ltd. Oxford. 393 p.
- Geigy, R., and M. Huber. 1952. Untersuchungen über

- Bau und Funktion der Stigmen bei verschiedenen *Glossina*-Arten und bei *Stomoxys calcitrans*. Acta Trop. 9: 233-63.
- Hazellhoff, E. H. 1927. Die Regulierung der Atmung bei Insekten und Spinne. Z. Vergl. Physiol. 5: 179-90.
- Hoyle, G. 1961. Functional contracture in a spiracular muscle. J. Insect Physiol. 7: 305-14.
- Hylton, A. R. 1967. Low humidity water-retention ability in *Eretmapodites chrysogaster* and *Aedes albopictus*. Ibid. 13: 153-7.
- Kanwisher, J. W. 1966. Tracheal gas dynamics in pupae of the cecropia silkworm. Biol. Bull. 130: 96-105.
- Krafur, E. S. 1971. Influence of age and water balance on spiracular behavior in *Aedes* mosquitoes. Ann. Entomol. Soc. Amer. 64: 97-102.
- Krafur, E. S., J. R. Willman, C. L. Graham, and R. E. Williams. 1970. Observations on spiracular behavior in *Aedes* mosquitoes. Ibid. 63: 684-91.
- Mellanby, K. 1934. The site of water loss from insects. Proc. Roy. Soc. Ser. B. 116: 139-49.
- Miller, P. L. 1964a. Factors altering spiracle control in adult dragonflies: water balance. J. Exp. Biol. 41: 331-43.
- 1964b. Factors altering spiracle control in adult dragonflies: hypoxia and temperature. Ibid. 41: 345-57.

Influence of Age and Water Balance on Spiracular Behavior in *Aedes* Mosquitoes^{1,2}

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ABSTRACT

In female *Aedes triseriatus* (Say) (Diptera: Culicidae), duration of spiracular opening in air declined with sample age; in response to 0.5% CO₂, both amplitude and duration of opening declined. There was no relationship of frequency of spiracular opening to age. Aspects of feeding behavior were observed and related to spiracular behavior in *A. triseriatus* and *A. aegypti* (L.). In the former species there was a diurnal weight loss; in both species inclusion of 0.2M KCl in 0.3M sucrose solution led

to a reduction in the amount of fluid imbibed. Spiracular opening became conservative in *A. triseriatus*, and to a lesser extent in *A. aegypti* when KCl was included in the diet. *A. aegypti* engorged more than *A. triseriatus* and showed looser spiracular control. The course of starvation was more rapid in *A. triseriatus* than in *A. aegypti*; the rate of water loss in the former was 4 times that in the latter. Each species showed increasingly conservative spiracular behavior as starvation proceeded.

Because of the great surface area to volume in insects, water conserving mechanisms are of considerable importance to survival. Occlusile spiracles are one feature whereby water loss through respiratory transpiration is reduced (Edney 1967). Low relative humidities seem to accelerate the mortality rate of caged mosquitoes (Clements 1963, Hylton 1969). Similarly, field populations of *Anopheles gambiae* Giles and *A. funestus* Giles suffer a daily mortality rate in the dry season almost double that in the wet season (Krafur 1970). These observations may suggest that mosquitoes, quite in contrast to *Glossina* (Bursell 1957, 1961), have a poorly developed ability to regulate water loss in a xeric environment.

The work presented here explores some relationships between desiccation and spiracular behavior in *Aedes* (*Finlaya*) *triseriatus* (Say) and *A. (Stego-*

myia) *aegypti* (L.). That the potassium ion might play an important part in the regulation of spiracular behavior as advocated by Hoyle (1961) and Miller (1964) was also briefly investigated. Observations were made on the course of desiccation and on water balance. Because a fluctuation in water balance with age was noted, spiracular behavior was investigated as a function of this variable.

METHODS AND MATERIALS

Techniques and apparatus used for observing and recording spiracular behavior were described in detail elsewhere; procedures in rearing and handling the biological material were also previously outlined (Krafur et al. 1970).

In studying spiracular behavior as a function of age, 500 ♀ adult *A. triseriatus* mosquitoes were maintained in gallon ice cream cartons at ca. 26°C and 80% RH and given constant access to 30 cc of 10% sucrose solution in fresh cotton pads provided each morning. Three such pads were placed on the muslin netting covering each mosquito-holding container.

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