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Environmental and Genetic Factors Determine Whether the Mosquito *Aedes aegypti* Lays Eggs without a Blood Meal

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Abstract. Some mosquito strains or species are able to lay eggs without taking a blood meal, a trait named autogeny. This may allow populations to persist through times or places where vertebrate hosts are scarce. Autogenous egg production is highly dependent on the environment in some species, but the ideal conditions for its expression in *Aedes aegypti* mosquitoes are unknown. We found that 3.2% of females in a population of *Ae. aegypti* from Kenya were autogenous. Autogeny was strongly influenced by temperature, with many more eggs laid at 28°C compared with 22°C. Good nutrition in larval stages and feeding on higher concentrations of sugar solution during the adult stage both result in more autogenous eggs being produced. The trait also has a genetic basis, as not all *Ae. aegypti* genotypes can lay autogenously. We conclude that *Ae. aegypti* requires a favorable environment and a suitable genotype to be able to lay eggs without a blood meal.

INTRODUCTION

Approximately 14,000 species of insects have evolved to feed on blood,[1] and 300–400 of these species pose a threat to humans.[2] The blood-feeding insects depend on blood in different manners. There are some species, such as the floor maggot, *Auchmeromyia luteola*, that only feed on blood during the larval stage[3]; other species feed exclusively on blood throughout their life cycle, such as tsetse flies and triatomine bugs,[4,5] and a third group relies upon a blood meal only when adults. Among the latter, there are insects that are obligatory blood feeders as adults, other insects that are facultative blood feeders, and finally, those of which the adult male is not hematophagous and only adult females blood feed, such as mosquitoes.[2]

Mosquitoes of the family Culicidae are some of the most important arthropod vectors, transmitting a range of diseases to humans. Blood is the preferred source of nutrients for mosquito females,[6] but females can feed on plant nectar if far away from vertebrate hosts.[7] This may also allow populations to persist through times or places where blood meals are not possible. In some mosquito species, females are able to complete the first gonotrophic cycle without a blood meal, a trait named autogeny, which can be particularly important if hosts are scarce.[2] Autogenous mosquitoes are able to store nutrients carried over from larval stages in the fat body and use these in egg production.[8] Most species will still blood feed if a suitable warm-blooded host is available, which can typically allow them to lay many more eggs with higher viability.[9]

The ability to lay eggs without a blood meal is the ancestral state for mosquitoes as other insect families related to Culicidae are not blood feeders.[10] Within the three subfamilies that comprise the Culicidae, only the Culicinae and Anophelinae feed on blood.[2] Within these groups, autogeny is common in mosquitoes of the genus *Culex*,[11–13] but is rarer in *Anopheles*[14] and *Aedes* mosquitoes.[15–17]

Very little is known about autogeny in *Aedes aegypti,* the primary vector of dengue and yellow fever. Autogeny has been reported in *Ae. aegypti* populations from East Africa, with the highest frequency (34%) recorded in Uganda.[18] The autogeny trait is highly environmentally dependent in other species,[19–21] however the ideal conditions for its expression in *Ae. aegypti* mosquitoes are unknown. In this study, we investigated the factors that can influence autogeny in *Ae. aegypti*. We found that temperature and nutrition during larval and adult stages are the main environmental factors affecting the expression of autogeny. Furthermore, there is genetic variation in the trait as different mosquito lines differ in the expression of autogeny.

MATERIAL AND METHODS

Mosquito strain. We serendipitously found autogenous egg production in a genetically diverse population of *Ae. aegypti* that we collected from Kenya. This population was created in the laboratory using samples of eggs collected from villages around the districts of Kilifi and Malindi, Kenya, in July 2010 (see Osei-Poku and others.[22] for detailed information on sampling site). There are two main genetic forms of *Ae. aegypti* that are found in Africa and across the rest of the tropics.[23] The coastal region of Kenya is the only area where these forms coexist. The population that we studied had clear white scaling on the first abdominal tergite, which suggests that they are the subspecies *Ae. aegypti aegypti.* However, it should be cautioned that genetic studies have questioned whether this trait can distinguish the genetic forms.[23]

In the laboratory, we separately reared eggs collected from either the same oviposition traps or from a pool of oviposition traps from the same area; only four egg collections successfully survived until F2. We crossed females and males from the four egg collections in every possible direction. Equal numbers of the progeny of each cross were then combined to create three cages of an outcrossed population, each with at least 300 mosquitoes. These were maintained for 11 generations.

Rearing conditions. We reared mosquitoes in a controlled temperature room at 28°C (±1.5°C), 75% (±5%) relative humidity and a photoperiod of 12:12 (light: dark) hours. Eggs used in each experiment were never more than 10 days old. The conditions in which we reared larvae varied slightly in

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each experiment (and are detailed in the following sections), but the standard procedures were as follows. To hatch eggs, we submerged filter papers containing eggs in deoxygenated water and then we density controlled first instar larvae to 8 larvae/100 mL of water and fed with desiccated liver powder (Oxoio). We either used 600 mL plastic containers with 48 larvae, or 1,500 mL with 120 larvae. We added 350 mg of liver powder to the smaller containers and 750 mg to the larger containers. Food was given every other day until pupation and water was changed whenever needed. We maintained a constant replicate number of 70 for the smaller containers, but the number of replicates of the larger containers varied according to each experiment. When adults emerged the standard procedure was to place 20 females and 20 males from each of the 600 mL containers into a small cage (17.5 cm × 17.5 cm × 17.5 cm, BugDorm, Taichung, Taiwan). See each experiment description for the procedure used with adults from the 1500 mL containers. We placed a 50 mL plastic cup containing hay infusion and a strip of unbleached coffee filter paper in each cage to be used by females as an oviposition site. We did some preliminary tests and mosquitoes showed a high preference for oviposition sites containing water that was infused with hay for a day instead of tap water. We placed cotton wool pads soaked in 10% w/v fructose solution (Sigma-Aldrich, St. Louis, MO) on top of each cage, for adult mosquitoes to feed *ad libitum*.

Frequency of autogeny in the outcrossed population and body size. To estimate the frequency of autogeny in the outcrossed population from Kenya, we reared larvae in 13 large (1,500 mL) containers, following the standard rearing procedures previously described. Once emerged, 468 single pairings of male and female were placed into 300 mL-paper cups. We checked if eggs were laid in each cup until 10 days after adult emergence (2 days after the last egg batch was seen). We counted the number of eggs laid in each cup and collected the adult females.

To test if body size correlated with the autogeny trait in *Ae. aegypti,* we measured the wing size (a proxy for body size) of each autogenous female and twice as many anautogenous females from the previous experiment. We carefully removed the right wing of each individual using dissection tweezers and placed them on a microscope slide with a cover slip. A photograph of each wing was taken. We measured each wing from the apical notch to the tip, without the fringe scales,[24] using the program ImageJ.[25]

Genetic variation in autogeny. To examine whether there was genetic variation affecting autogenous egg laying, we tested if females reared from autogenous eggs were more likely to lay eggs without a blood meal than control females from a laboratory stock. We used eggs that were autogenously laid by females from the outcrossed population and eggs from Liverpool (LVP), a laboratory strain obtained from the Malaria Research and Reference Reagent Resource Center (MR4, ATCC, Manassas, VA). We reared larvae from each strain in six 1,500 mL containers, which were placed in three cages following the standard procedures until adult emergence. We recorded the number of eggs laid autogenously in each cage.

We also checked whether the autogeny trait would respond to short-term selection. We maintained a selected treatment where females were never blood fed (eggs were always laid autogenously) and an unselected treatment, where females were allowed to lay eggs autogenously but were subsequently given a blood meal as well. We had three replicate cages for each treatment. We used ~1,300 eggs that were laid either autogenously or anautogenously by females from the outcrossed population to establish the selected and the unselected treatment, respectively. Seventy-three percent of the autogenous eggs hatched, indicating that autogenous eggs are viable. We reared larvae using 1,500 mL rearing containers. Once females emerged, they were counted and the same number of individuals was kept in all cages. Usually the number of anautogenous mosquitoes was greater than the autogenous; the surplus of the first was discarded to ensure the density of individuals in all cages was identical. We blood fed females from the control cages 8 days post adult emergence to allow autogenous egg laying before the blood meal.

The effect of temperature. To test if temperature affects the autogeny trait we had two treatments, low temperature (22°C) and standard temperature (28°C). All rearing conditions, from hatching to adult emergence, followed the standard procedures, with 35 replicates at 22°C and 35 replicates at 28°C. We ran both treatments at the same time but in two different controlled temperature rooms. We checked the oviposition sites daily and recorded the number of eggs laid. We also measured wing size of females of both treatments to test if a longer developmental time would result in larger females.

Larval nutrition and competition. To investigate if larval nutrition affected the autogeny trait, we reared larvae from the outcrossed population (from eggs laid anautogenously) at low and high nutritional conditions. We provided the same amount of food (liver powder) to larvae of both treatments but changed the density of larvae in each condition. For the low nutrition treatment, we had 48 larvae per 100 mL of water and for the high nutrition we used a density of 8 larvae per 100 mL of water. The former treatment generated a larval competition environment, where larvae had lower amounts of food available per individual. We used 600 mL plastic pots as rearing containers and the standard adult rearing procedures with 70 cages—35 cages per treatment (20 adult females and 20 adult males in each cage). We checked daily and collected eggs until 10 days after emergence.

Adult nutrition. To assess if adult nutrition would have an effect on the ability of females to lay eggs autogenously, we varied the fructose concentration given to adults, but larvae received the same amount of food. We reared larvae from the outcrossed population stock using eggs that were laid after a blood meal. Adult rearing followed the standard procedures as described previously, with 70 of the 600 mL plastic containers. After adult emergence, each cage was assigned a specific concentration of fructose solution. Cotton wool pads soaked in the corresponding solution were placed on top of each cage. We used 43 different concentrations of fructose solution (28 concentrations with two replicates and 15 concentrations with one replicate), ranging from 0% to 17.5%. From 0% up to 7%, concentrations increased approximately every 0.3% (i.e. 0%, 0.3%, 0.6%, 1%, etc.) with two replicates (two cages per concentration). From 7.5% to 10%, concentrations raised every 0.5% also with two replicates. From 10.5% to 17.5%, concentrations also raised every 0.5% but with no replicates. This design was to ensure we would

be able to detect any fine differences in the autogeny trait at low concentrations of fructose. Every day we checked if eggs were laid and counted mosquito mortality. Cotton wool pads were re-soaked in the respective fructose solution daily. The experiment was stopped when no eggs were laid for 2 consecutive days.

Statistical analysis. We used R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) to perform all tests and the package ggplot2[26] to plot most graphs. There were many zeros in our data set caused by lack of autogenous egg laying in some cages. To account for that, we fitted a zero-inflated Poisson regression model using the package pscl.[27] We tested if a zero-inflated Poisson was the best model by comparing it to a Poisson generalized linear model (GLM) using a Vuong Statistic for non-nested models. In all cases a zero-inflated Poisson was preferred. We fitted a zero-inflated Poisson model to test whether there was a main effect of temperature, larval nutrition, fructose concentration, and adult age on the number of eggs laid autogenously.

RESULTS

***Aedes aegypti* can lay eggs autogenously at a low frequency.** In a genetically diverse population that originated from *Ae. aegypti* from Kenya, we found that 3.2% ($N = 468$) of females were able to lay eggs autogenously. The mean number of eggs laid by the autogenous females was 18.2 (SD 5.7; Figure 1A). In other species, the body size of mosquito females has been previously correlated with the autogeny trait.[15] Using wing size as a proxy for body size, we found a trend for autogenous females to be larger, however this was not statistically significant (analysis of variance [ANOVA], $F_{1,\ 44} = 2.27$, $P = 0.13$; Figure 1B).

Genetic variation in the expression of autogeny. To examine whether the autogeny trait is genetically inherited, we compared autogeny expression between our Kenyan population and a laboratory line that originated from West Africa. We found that the genotypes differed in their abilities to reproduce autogenously, with the Kenyan mosquitoes laying 511 autogenous eggs and the laboratory stock producing no eggs (three cages of Kenyan mosquitoes produced 78, 249, and 184 eggs, and none of the three cages of the laboratory stock laid eggs).

We next examined whether the variation in autogeny expression that we see within our Kenyan population is genetically determined. To do this, we tested whether the progeny of autogenous mosquitoes were more likely to reproduce autogenously than the rest of the population. We compared the number of autogenous eggs laid in a selected treatment, which laid eggs autogenously and was never fed on blood, to controls that were fed on blood. The latter was allowed to lay eggs autogenously before receiving a blood meal. Across two generations of selection there was a trend for the selected population to produce more autogenous eggs, but this was not statistically significant (ANOVA, effect of selection regimen: $F_{(1–9)} = 3.68$, $P = 0.087$; Figure 1C). To check if autogeny would increase in response to longer-term selection treatment, we maintained the cages for another three generations. Only a small number of mosquitoes remained in a single selected and control cage by this stage, and the number of eggs laid autogenously was the same between treatments (13 and 14 eggs, respectively; Figure 1C). Therefore, although there is likely genetic variation within this population, the autogeny trait does not rapidly change in response to selection.

Females lay more eggs autogenously when kept at 28°C. Temperature had a strong effect on autogenous egg laying. Females reared at 28°C laid a mean of 10.4 eggs in each cage, compared with 0.2 eggs at 22°C (zero-inflated Poisson GLM: $df = 4$, $P < 0.0001$, Figure 2A). At lower temperature, we found eggs in only one cage ($N = 35$), although at higher temperature we encountered eggs in 16 cages ($N = 35$) (Fisher's exact test, $P < 0.001$). This pattern cannot be explained by changes in body size as the adults were larger at the lower temperature (ANOVA, $F_{(1–153)} = 119.3$, $R^2 = 0.43$, $P < 0.0001$; Figure 2B). This increase in size may result from a longer development time between hatching and adult emergence, which took 13 days at 22°C—5 days longer than at 28°C. Because of this extended developmental time at lower temperature, we maintained the cages for 20 days after adult emergence to ensure that a delayed egg laying was not affecting our results.

Well-nourished larvae laid more eggs autogenously. High larval nutrition increases the ability of females to lay eggs autogenously. We reared larvae in high and low densities with the same amount of food, therefore the low

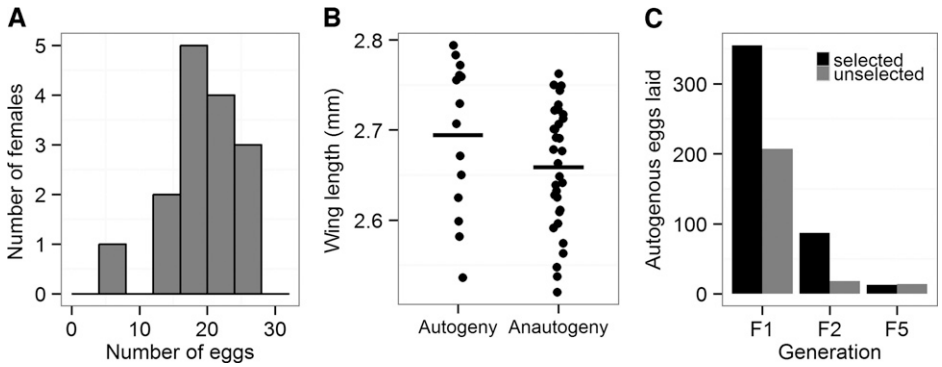

FIGURE 1. Frequency of autogeny, the effects of body size and short-term selection on the autogeny trait in an *Aedes aegypti* population from Kenya. (**A**) Number of eggs laid autogenously by individual autogenous females. Females that did not lay autogenously are not shown. (**B**) Wing size, as a proxy for body size, of autogenous and anautogenous females. (**C**) The number of autogenous eggs laid by mosquitoes selected for the autogeny trait compared with an unselected control. The number of mosquitoes in each generation declined, explaining the overall decline in the number of eggs laid.

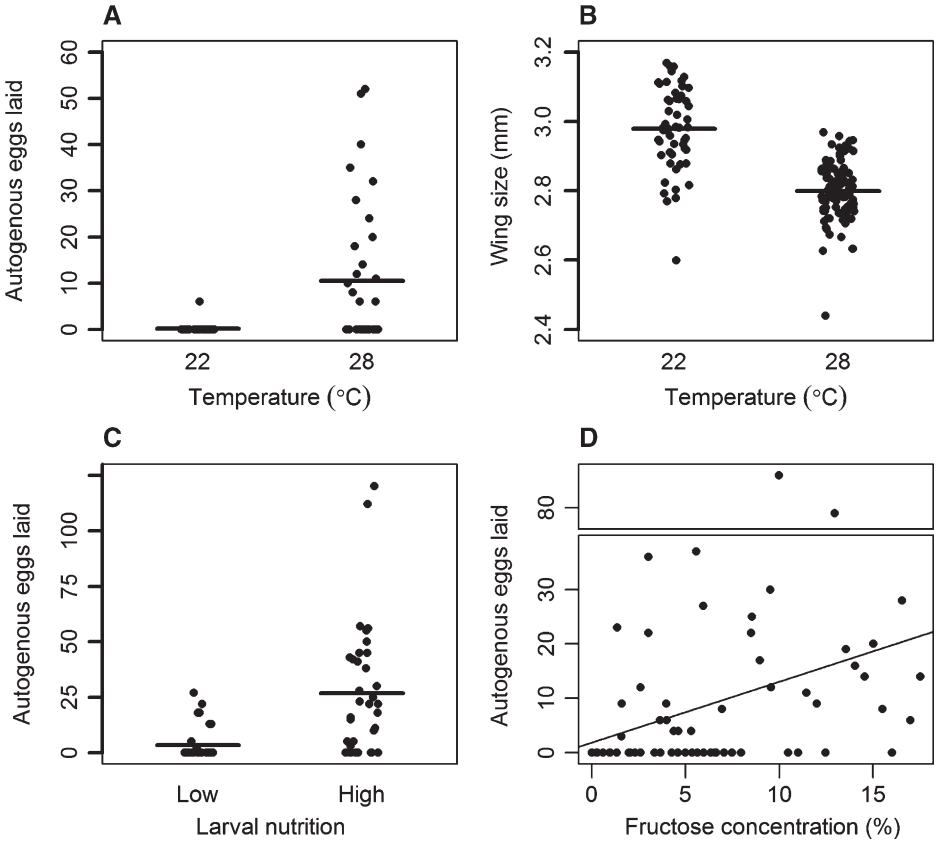

FIGURE 2. The effects of temperature, larval nutrition, and adult nutrition on the autogeny trait in an *Aedes aegypti* population from Kenya. (**A**) Mosquitoes reared at 28°C laid more autogenous eggs than at 22°C. (**B**) Females' body size, measured as wings length, was larger when reared at 22°C compared with 28°C. (**C**) Females that were well nourished during the larval stage laid more autogenous eggs than females that received a poor diet. (**D**) The number of eggs laid autogenously is greater when adult females are fed with higher fructose concentrations.

density larvae received proportionally more food. Females that were well nourished during larval stages laid more eggs (zero-inflated Poisson GLM: $df = 4$, $P < 0.001$, Figure 2C) and more frequently—we found eggs in 27 cages ($N = 35$) of the high nutrition treatment and only eight in low nutrition cages ($N = 35$) (Fisher's exact test, $P < 0.001$).

Sugar feeding greatly increases the number of eggs laid autogenously. The number of eggs laid autogenously was considerably greater when adult females fed on high fructose concentrations (zero-inflated Poisson GLM: $df = 4$, $P = 0.0001$, Figure 2D). No eggs were laid in cages that were given < 1.6% fructose, and the majority of cages with over 8% fructose had eggs. Interestingly, among the cages where eggs were laid there was no strong effect of fructose concentration, suggesting that sugar feeding above a particular threshold (in this case 1.6%) may primarily affect the probability of autogeny rather than number of eggs produced by the autogenous females.

Age influences the ability to lay eggs autogenously. We used the females from the adult nutrition experiment to examine the effect of age on autogenous egg laying. Females started laying eggs autogenously 3 days post-emergence and stopped by the ninth day post-emergence (Figure 3A), with the peak of egg laying on Day 4 post-emergence. We found that younger females laid a greater number of eggs than older females (zero-inflated Poisson GLM, main effect days post emergence: $df = 6$, $P < 0.0001$; Figure 3B).

DISCUSSION

We found that autogenous egg production in *Ae. aegypti* is highly dependent on environmental conditions. High temperatures, good larval nutrition, and adult sugar feeding all greatly increase the ability of females to lay eggs autogenously.

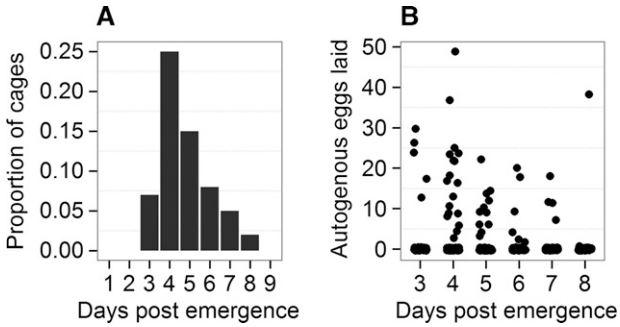

FIGURE 3. Age affects the probability of *Aedes aegypti* females from an outcrossed population from Kenya laying eggs autogenously. The effect of mosquito age on the proportion of cages with autogenous eggs (out of 70 cages) (**A**) and the number of autogenous eggs in each cage (**B**).

Furthermore, the trait varies genetically and only certain genotypes of *Ae. aegypti* are able to reproduce autogenously.

The frequency of autogeny in an outcrossed population of *Ae. aegypti* from Kenya was 3.2%. This suggests that autogeny alone is unlikely to sustain *Ae. aegypti* populations in Kenya. In Uganda, however, the trait was found in 34% of females.[18] In *Aedes albopictus* and *Culex annulirostris,* the trait was detected in 5% to 8% of wild-caught individuals,[28,29] whereas 27% of *Culex salinarius*, 87% of *Culex pipiens f. molestus*, and 100% of *Ochlerotatus atropalpus* were autogenous.[12,30,31] Although it is clear that there are large differences in the importance of autogeny across species, these exact numbers must be treated with care. First, the comparison between studies is difficult as each study rear mosquitoes under different conditions and it is also difficult to extrapolate these estimates to wild populations, where natural environment is dramatically different to laboratory conditions. Furthermore, our population has been reared in the laboratory for 11 generations; therefore, it is possible that selection in the laboratory may have altered the rate of autogeny relative to wild Kenyan populations. In addition, some females may not have laid eggs autogenously simply because they did not mate or lacked the correct cues to oviposit in our rearing cups.

The frequency of autogeny in a population is likely to be associated with host availability, with the frequency increasing if hosts are scarce.[2] We created the outcrossed population in our laboratory with eggs collected in peri-domestic areas of Kenya. Even though humans are present around the collection sites and it is unlikely that other vertebrates are not available, it is possible that autogeny is used as a reproductive strategy by mosquito females from these areas. Moreover, the precise frequency of autogeny may have changed as a result of inadvertent selection in the laboratory.

Autogeny in *Ae. aegypti* has a genetic basis. We compared a laboratory stock and an outcrossed population from Kenya. Only the latter laid eggs autogenously, suggesting that even if the environmental conditions are favorable, autogeny only occurs in certain genotypes. We also tested if there was heritable variation in autogeny expression in our Kenyan population. Selection on the trait did generate an increase in the number of autogenous eggs, although this did not reach statistical significance. When we maintained the cages for a further three generations we saw a decline in the number of eggs laid autogenously, indicating that the trait does not rapidly respond to selection. Furthermore, the number of eggs laid per female is much lower when laid autogenously,[32,33] which may explain the decline in the number of females across generations. Perhaps the autogeny trait is an adaptation to allow populations to persist in times when hosts are temporarily unavailable, but it is not sufficient to continue the population across multiple generations without blood feeding. The genetic basis of autogeny in *Ae. aegypti* is not known. In *Ae. albopictus,* the trait is dominant and multigenic, with quantitative trait loci affecting autogeny overlapping with loci affecting follicle size (the proxy for autogeny) and body size.[16] In *Oc. atropalpus*, autogeny appears to have a simple genetic basis and is affected by a single dominant autosomal locus, with modifiers affecting the level of fecundity.[34] To our knowledge, it is unknown which genes are responsible for autogeny in any mosquito species.

Temperature had a strong effect on the expression of autogeny in *Ae. aegypti*, with 50 times more autogenous eggs laid at 28°C than 22°C. This is similar to *Cx. salinarius*, which is obligatorily autogenous at warm temperatures, but facultatively autogenous at colder temperatures.[12] In *Culex tarsalis* autogeny is suppressed below 21°C and reaches maximum expression at 32°C.[35] Lower temperatures resulted in a slower developmental time, which was also observed in *Cx. pipiens*.[36] We found that the females' body size was larger when reared at 22°C, which suggests that the effect of temperature on autogenous egg laying is not purely caused by it resulting in more poorly resourced mosquitoes.

Larval nutrition is another important factor affecting autogenous egg laying in *Ae. aegypti.* Females that were well nourished during larval stages laid more eggs autogenously. The importance of larval nutrition in the expression of autogeny has been reported in other mosquito species.[19,31,37] Following blood feeding, amino acid titers increase in the hemolymph triggering a cascade of metabolic events that culminates with eggs production.[38] It is likely that autogenous individuals are able to mobilize enough amino acids stored in the fat body during larval stages to trigger vitellogenesis.[39] The mechanism that facilitates this event is still unknown.

Because larval nutrition largely affects body size and is one of the main factors influencing the autogeny trait, larger females might be expected to be the most likely to reproduce autogenously, as is the case for species like *Aedes bahamensis* and *Oc. atropalpus*.[15,19] However, this is not always the case; there was no correlation between body size and autogeny in a selected strain of *Ae. albopictus* from Japan.[16] Mosquitoes' body size also correlates with protein content, which is generally carried over from larval stages.[24] In *Ae. aegypti,* there was a trend in our data suggesting that larger females lay more autogenous eggs but this was not statistically significant. There are two possible explanations for our results: it may be that our sample size was small and it impaired the detection of the correlation between body size and autogeny expression, or in *Ae. aegypti* nutrition may have a role in autogeny that is independent of body size. Further studies with larger sample sizes are necessary to clearly explain this.

Adult nutrition also affected the autogenous egg laying—females that were offered a higher fructose concentration laid more eggs. There was a threshold fructose concentration below which females did not lay eggs autogenously. *Aedes bahamensis* and *Cx. tarsalis* only laid eggs autogenously if females had access to sugar.[15,21] Caloric intake also determined if *Ae. albopictus* would lay eggs autogenously but it did not affect the number of eggs laid.[40] The concentration of sugar in adult females is responsible for either stimulating or preventing follicular resorption, a strategy used by mosquitoes to reallocate nutrients from reproduction to other physiological activities when the availability of carbohydrates is low.[41,42] Thus, fecundity is greater when females feed on higher sugar concentrations.[41] The peak of egg laying was at Day 4 post adult emergence. *Aedes aegypti* normally lay eggs about 4 days after having a blood meal, when egg maturation is complete. This suggests that in autogenous mosquitoes vitellogenesis may somehow be triggered upon emergence as long as adult females have access to sugar.[13]

There are other factors affecting autogeny in other mosquito species that have not been addressed in this study and should be investigated, such as photoperiodicity,[43,44] the effect of mating and the consequent transference of the substance of the male accessory gland to females.[40,45]

We provided data for the understanding of the occurrence of the rare autogeny trait in a highly urban mosquito. We conclude that environmental factors, such as temperature and nutrient availability to both larvae and adults play a major role at the expression of autogeny, but this is not sufficient for autogenous egg production if females lack a genetic predisposition for the trait. The ideal conditions for autogeny expression are very specific, therefore it is unclear how frequently environmental conditions in the wild will allow autogeny.

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REFERENCES

1. Adams TS, 1999. Hematophagy and hormone release. *Ann Entomol Soc Am 92:* 1–13.
2. Lehane MJ, 2005. *The Biology of Blood-Sucking Insects*. Cambridge: Cambridge University Press.
3. Garrett-Jones C, 1951. The Congo Floor Maggot, *Auchmeromyia luteola* (F.), in a laboratory culture. *Bull Entomol Res 41:* 679–708.
4. Leak SGA, 1999. *Tsetse biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomosis.* Wallingford: CABI.
5. Zeledón R, Rabinovich JE, 1981. Chagas' disease: an ecological appraisal with special emphasis on its insect vectors. *Annu Rev Entomol 26:* 101–133.
6. Briegel H, 2003. Physiological bases of mosquito ecology. *J Vector Ecol 28:* 1–11.
7. Scott TW, Amerasinghe PH, Morrison AC, Lorenz LH, Clark GG, Strickman D, Kittayapong P, Edman JD, 2000. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. *J Med Entomol 37:* 89–101.
8. Clements AN, 1992. *The Biology of Mosquitoes: Development, Nutrition, and Reproduction*. London: Chapman & Hall.
9. Junkum A, Choochote W, Jitpakdi A, Leemingsawat S, Komalamisra N, Jariyapan N, Boonyatakorn C, 2003. Comparative studies on the biology and filarial susceptibility of selected blood-feeding and autogenous *Aedes togoi* sub-colonies. *Mem Inst Oswaldo Cruz 98:* 481–485.
10. Pawlowski J, Szadziewski R, Kmieciak D, Fahrni J, Bittar G, 1996. Phylogeny of the infraorder Culicomorpha (Diptera: Nematocera) based on 28S RNA gene sequences. *Syst Entomol 21:* 167–178.
11. Spielman A, 1957. The inheritance of autogeny in the *Culex pipiens* complex of mosquitoes. *Am J Hyg 65:* 404–425.
12. Tveten MS, Meola RW, 1988. Autogeny in *Culex salinarius* from Texas, Florida and New Jersey. *J Am Mosq Control Assoc 4:* 436–441.
13. Provost-Javier KN, Chen S, Rasgon JL, 2010. Vitellogenin gene expression in autogenous *Culex tarsalis*. *Insect Mol Biol 19:* 423–429.
14. Sweeney A, Russell R, 1973. Autogeny in *Anopheles amictus hilli*. *Mosq News 33:* 467–468.
15. O'Meara GF, Larson VL, Mook DH, 1993. Blood feeding and autogeny in the peridomestic mosquito *Aedes bahamensis* (Diptera: Culicidae). *J Med Entomol 30:* 378–383.
16. Mori A, Romero-Severson J, Black WC, Severson DW, 2008. Quantitative trait loci determining autogeny and body size in the Asian tiger mosquito (*Aedes albopictus*). *Heredity 101:* 75–82.
17. Thomas V, Leng YP, 1972. The inheritance of autogeny in *Aedes* (Finlaya) *togoi* (Theobald) from Malaysia and some aspects of its biology. *Southeast Asian J Trop Med Public Health 3:* 163–174.
18. Trpis M, 1977. Autogeny in diverse populations of *Aedes aegypti* from East Africa. *Tropenmed Parasitol 28:* 77–82.
19. Telang A, Li Y, Noriega FG, Brown MR, 2006. Effects of larval nutrition on the endocrinology of mosquito egg development. *J Exp Biol 209:* 645–655.
20. Corbet P, 1967. Facultative autogeny in arctic mosquitoes. *Nature 215:* 662–663.
21. Su T, Mulla MS, 1997. Physiological aspects of autogeny in *Culex tarsalis* (Diptera: Culicidae): influences of sugar-feeding, mating, body weight, and wing length. *J Vector Ecol J Soc Vector Ecol 22:* 115–121.
22. Osei-Poku J, Mbogo CM, Palmer WJ, Jiggins FM, 2012. Deep sequencing reveals extensive variation in the gut microbiota of wild mosquitoes from Kenya. *Mol Ecol 21:* 5138–5150.
23. Brown JE, McBride CS, Johnson P, Ritchie S, Paupy C, Bossin H, Lutomiah J, Fernandez-Salas I, Ponlawat A, Cornel AJ, Black WC, Gorrochotegui-Escalante N, Urdaneta-Marquez L, Sylla M, Slotman M, Murray KO, Walker C, Powell JR, 2011. Worldwide patterns of genetic differentiation imply multiple "domestications" of *Aedes aegypti*, a major vector of human diseases. *Proc Biol Sci 278:* 2446–2454.
24. Van Handel E, Day JF, 1989. Correlation between wing length and protein content of mosquitoes. *J Am Mosq Control Assoc 5:* 180–182.
25. Schneider CA, Rasband WS, Eliceiri KW, 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods 9:* 671–675.
26. Wickham H, 2009. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer.
27. Zeileis A, Kleiber C, Jackman S, 2008. Regression models for count data in R. *J Stat Softw 27:* 1–21.
28. Chambers GM, Klowden MJ, 1994. Nutritional reserves of autogenous and anautogenous selected strains of *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol 31:* 554–560.
29. Kay BH, Edman JD, Mottram P, 1986. Autogeny in *Culex annulirostris* from Australia. *J Am Mosq Control Assoc 2:* 11–13.
30. Vinogradova EB, Karpova SG, 2006. Cultivation of the mosquito *Culex pipiens pipiens f. molestus* (Diptera, Culicidae) without blood feeding. *Parazitologiia 40:* 306–311.
31. Telang A, Wells MA, 2004. The effect of larval and adult nutrition on successful autogenous egg production by a mosquito. *J Insect Physiol 50:* 677–685.
32. O'Meara GF, Evans DG, 1973. Blood-feeding requirements of the mosquito: geographical variation in *Aedes taeniorhynchus*. *Science 180:* 1291–1293.
33. O'Meara GF, Edman JD, 1975. Autogenous egg production in the salt-marsh mosquito, *Aedes taeniorhynchus*. *Biol Bull 149:* 384–396.
34. O'Meara G, Craig G Jr, 1969. Monofactorial inheritance of autogeny in *Aedes atropalpus*. *Mosq News 29:* 14–22.
35. Brust RA, 1991. Environmental regulation of autogeny in *Culex tarsalis* (Diptera: Culicidae) from Manitoba, Canada. *J Med Entomol 28:* 847–853.
36. Strickman D, Fonseca DM, 2012. Autogeny in *Culex pipiens* complex mosquitoes from the San Francisco Bay Area. *Am J Trop Med Hyg 87:* 719–726.
37. Reisen W, Milby M, Bock M, 1984. The effects of immature stress on selected events in the life history of *Culex tarsalis*. *Mosq News 44:* 385–395.
38. Hansen IA, Attardo GM, Park J-H, Peng Q, Raikhel AS, 2004. Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. *Proc Natl Acad Sci USA 101:* 10626–10631.

39. Attardo GM, Hansen IA, Raikhel AS, 2005. Nutritional regulation of vitellogenesis in mosquitoes: implications for anautogeny. *Insect Biochem Mol Biol 35:* 661–675.
40. Chambers GM, Klowden MJ, 1996. Distention and sugar feeding induce autogenous egg development by the Asian tiger mosquito (Diptera:Culicidae). *J Med Entomol 33:* 372–378.
41. Clifton ME, Noriega FG, 2011. Nutrient limitation results in juvenile hormone-mediated resorption of previtellogenic ovarian follicles in mosquitoes. *J Insect Physiol 57:* 1274–1281.
42. Clifton ME, Noriega FG, 2012. The fate of follicles after a blood meal is dependent on previtellogenic nutrition and juvenile hormone in *Aedes aegypti*. *J Insect Physiol 58:* 1007–1019.
43. Sota T, Mogi M, 1994. Seasonal life-cycle and autogeny in the mosquito *Aedes togoi* in northern Kyushu, Japan, with experimental analysis of the effects of temperature, photoperiod and food on life-history traits. *Res Popul Ecol (Kyoto) 36:* 105–114.
44. Sota T, Mogi M, 1995. Geographic variation in the expression of autogeny in *Aedes togoi* (Diptera: Culicidae) under different temperature and photoperiod conditions. *J Med Entomol 32:* 181–189.
45. O'Meara GF, Evans DC, 1977. Autogeny in saltmarsh mosquitoes induced by a substance from the male accessory gland. *Nature 267:* 342–344.