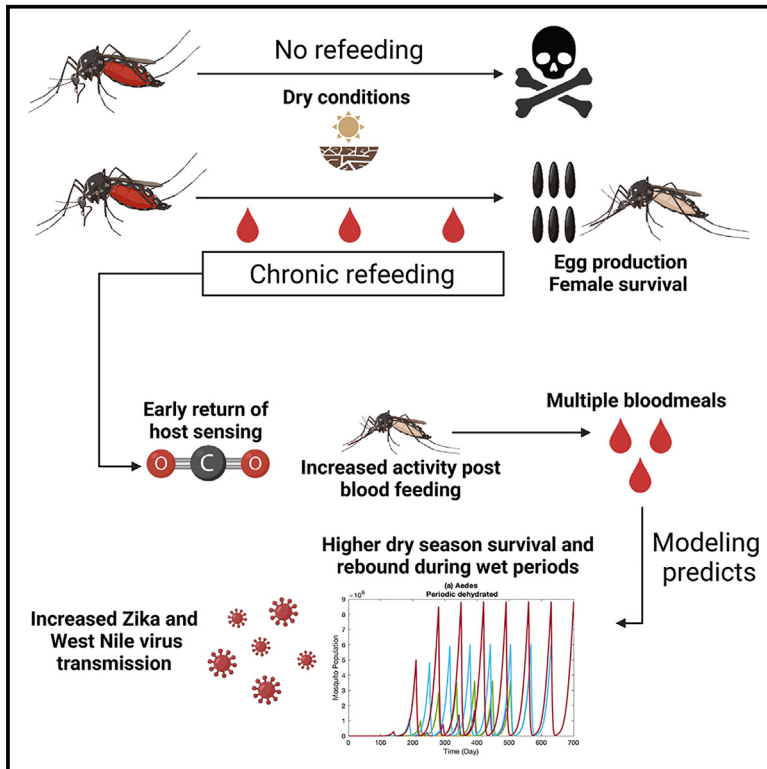


Multiple blood feeding bouts in mosquitoes allow for prolonged survival and are predicted to increase viral transmission during dry periods

Graphical abstract



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In brief

Virology; Environmental health;
Entomology

Highlights

- Dry conditions nearly double mosquito blood feeding rates within a gonotrophic cycle
- Returned CO₂ sensing, increased activity, and refeeding is driven by dry conditions
- In prolonged dry periods, female *Aedes aegypti* mosquitoes can subsist on blood
- Increased refeeding in mosquitoes is predicted to increase pathogen transmission



Article

Multiple blood feeding bouts in mosquitoes allow for prolonged survival and are predicted to increase viral transmission during dry periods

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SUMMARY

Dry conditions increase blood feeding in mosquitoes, but it is unknown if dehydration-induced bloodmeals are increased beyond what is necessary for reproduction. In this study, we investigated the role of dehydration in secondary blood feeding behaviors of mosquitoes. Following an initial bloodmeal, prolonged exposure to dry conditions increased secondary blood feeding in mosquitoes by nearly two-fold, and chronic blood feeding allowed mosquitoes to survive up to 20 days without access to water. Exposure to desiccating conditions following a bloodmeal resulted in increased activity, decreased sleep levels, and prompted a return of CO₂ sensing before egg deposition. Increased blood feeding and higher survival during dry periods are predicted to increase pathogen transmission, allowing for a rapid rebound in mosquito populations when favorable conditions return. Overall, these results solidify our understanding of how dry periods impact mosquito blood feeding and the role that mosquito dehydration contributes to pathogen transmission dynamics.

INTRODUCTION

Blood feeding by female mosquitoes primarily functions as a means for reproduction,¹ resulting in the spread of mosquito-borne pathogens. When an infected female mosquito feeds on a host a transfer of pathogens is possible. While considerable research has been conducted on pathogen transmission in mosquitoes, more research is needed to examine how environmental factors impact mosquito feeding^{2–4} and refeeding in the context of vectorial capacity. Recent ecophysiological studies^{2–7} have increased our understanding of the direct effects of environmental factors on mosquito physiology and behavior. Still, studies on the impact of dry periods on mosquito biology remain limited compared to the focus on thermal effects.^{5–7}

Drought exposure and dehydration significantly impact mosquitoes, shifting population levels, altering feeding propensity, and increasing pathogen transmission.^{5–9} Of interest, dry periods have been associated with increased incidence of viruses transmitted by insects,^{10–13} which is unexpected as periods with reduced water availability have been associated with lower mosquito populations.^{14,15} This increased viral transmission has been linked to specific environmental factors, which

allow for increased mosquito blood feeding and survival rates. For example, culverts become stagnant, altering mosquito-predator interactions and promoting mosquito growth.^{5,6,16} When mosquitoes are blood-fed before dehydration, individuals show an increased survival time compared to their non-blood-fed counterparts, likely due to higher water content at the start of dehydration exposure.⁶ These findings underscore the critical function of blood feeding for the replenishment of water content in dehydrated mosquitoes.^{5,6,17} However, the extent of water replenishment by blood feeding during short bouts of dry conditions (days to weeks), that can extend into a drought (weeks to years), has yet to be examined. Although periods of drought may increase with climate change,¹⁸ a comprehensive understanding of the interactions between temperature, humidity, and mosquitoes as climate change progresses will be critical to address mosquito biology and disease transmission in future scenarios.

Interactions between multiple bloodmeals during a single reproductive cycle and the resulting hydration status of mosquitoes have not been examined. This is surprising as refeeding has been implicated in many other biological changes that occur following dehydration, including fecundity, nutritional reserve



supplementation, and virus dissemination within the mosquito.^{19,20} Notably, a recent study on refeeding in *Aedes aegypti* established higher rates of viral dissemination when a second (uninfected) bloodmeal was allowed within the extrinsic incubation period.¹⁹ These refeeding events produced disturbances and microperforations in the basal lamina of the mosquito, which could increase viral particles escaping from the midgut, resulting in increased viral transmission potential.¹⁹ Combining increased pathogen dissemination following a second bloodmeal¹⁹ with the propensity of mosquitoes to blood feed when dehydrated,^{5,6} mosquitoes exposed to dry conditions may exhibit increased blood feeding and viral transmission rates during periods when refeeding is typically unexpected.

Multiple bloodmeals could be common within mosquito species, where up to 10–40% of mosquitoes will ingest a second bloodmeal before the previous blood has been digested.^{21–26} Ordinarily, a combination of mechanisms, predominantly regulated by hormonal cues, suppress additional blood feeding,^{27,28} but when feeding to repletion is interrupted, the signals that prevent a secondary bloodmeal can be suppressed.^{22,26,29} Another factor is the acquisition of nutrients through a secondary bloodmeal to supplement poor larval nutrition levels, which can be necessary for oogenesis.^{22,26,29} Despite water and sugar availability, medically-important Anopheline and Culicine mosquitoes in natural settings have been shown to take additional bloodmeals within a single gonotrophic cycle.^{20,25,30} Even though multiple blood feedings are observed, a general reduction in blood-fed females in host mimic traps suggests that host attraction is likely to be reduced even if a second meal is possible.^{31–33} This reduction in post-blood feeding attraction is supported by specific molecular changes in the mosquito neurotranscriptome that reduce responsiveness to host cues.³⁴ An overlooked aspect of this interaction is that the bloodmeal can represent a substantial source for hydration,⁶ allowing immediate replenishment of water stores in dehydrated mosquitoes. After the bloodmeal has been processed and excess fluid from the blood has been expelled, a blood-fed female requires 3–4 days to process the blood under preferred conditions.^{28,35} During this time, a female typically resides in an environment that prevents dehydration until egg development is complete.^{36,37} If a suitable refuge is not found, dehydration is likely to occur, and increased water ingestion from blood, nectar, or free water sources will be required.^{5,38,39} Significantly, prolonged dry periods will reduce free water and the water content of nectar sources,^{5,40} suggesting that blood from vertebrates may be a more reliable source of water, especially for anthropophilic and endophilic mosquito species that have extensive interactions with humans.^{5,17,38,39}

Here, we establish the role of dehydrating conditions on the refeeding propensity of mosquitoes. Specifically, we assess the impact of drought-induced refeeding on survival and reproduction, behavioral changes that occur to increase blood feeding, and how these changes can allow for mosquito survival and pathogen transmission during dry periods. Briefly, these studies revealed a drastic increase in refeeding during dry periods associated with a resumption of attraction to CO₂ before the end of a gonotrophic cycle, allowing mosquitoes to survive as adults through periods with low water availability. Transmission and population modeling suggest that this increase in secondary

feeding may underlie mosquito survival through periods of low water availability, and ultimately result in higher drought-induced viral transmission.

RESULTS

Dry conditions result in substantial refeeding that allows for extended survival

Mosquitoes allowed to blood feed and then held under dehydrating conditions (30–40% RH without access to water sources) had substantial increases in blood feeding during digestion and vitellogenesis (Figure 1). Specifically, one day of post-blood feeding dehydration had a minimal impact on refeeding, but 48 h under these dry conditions yielded increased feeding under small, medium, and large cage assays (Figure 1; *Anopheles stephensi* were tested in small cages only). An average of 2.5 feeding events occurred in medium-sized cages, and 2.0 feeding events occurred in large cages, when *Ae. aegypti* were held under dry conditions (Figures 1D, 1E, and S1). Under these conditions, most, if not all, mosquitoes would undergo at least one, if not two, additional feedings before egg deposition in a single gonotrophic cycle (Figure 1). When a recently collected line of *Ae. aegypti* (Thies⁴¹) was tested in medium cages, there were significantly more feeding events under dry conditions (2.4 per 1, dry vs. wet; $N = 3$, t-test, $p < 0.05$), suggesting that prolonged lab-adaptation is not responsible for this behavior. As with sugar feeding, these refeeding events allowed the mosquitoes to survive through periods with no water for up to twenty days (Figure 2B). When allowed to deposit eggs after a prolonged period of chronic blood feeding, there was a reduction in eggs deposited compared to those that were allowed to deposit only four days after a bloodmeal (Figure 2B). This suggests that prolonged retention will allow most eggs to remain viable, a finding that has been previously observed when oviposition sites are not available.⁴² Overall, these studies indicate that dry conditions are likely to result in the ingestion of multiple bloodmeals during each gonotrophic cycle, allowing females to survive for extended dry periods if adequate resting microhabitats or water sources cannot be located.

Increased blood feeding under dry conditions does not increase egg production

As increased feeding is typically associated with a higher egg output, we evaluated if increased blood feeding under dehydrating conditions resulted in the generation of more eggs (Figure 2). When single mosquitoes were evaluated, oocyte numbers were not shifted following an additional bloodmeal (Figure 2E), and in medium cage assays the same number of eggs per mosquito were deposited regardless of exposure to dry conditions (Figure 2C). However, since the number of bloodmeals taken under dry conditions was increased, the number of eggs per bloodmeal were reduced overall (Figure S2B). Oocyte size did not vary based on the number of bloodmeals when individual mosquitoes were examined (Figures S2E and S2F). Even one day after a secondary bloodmeal, the oocyte size and number did not vary compared to the control group (Figures 2E and S2F). These studies suggest that secondary bloodmeals during dry periods

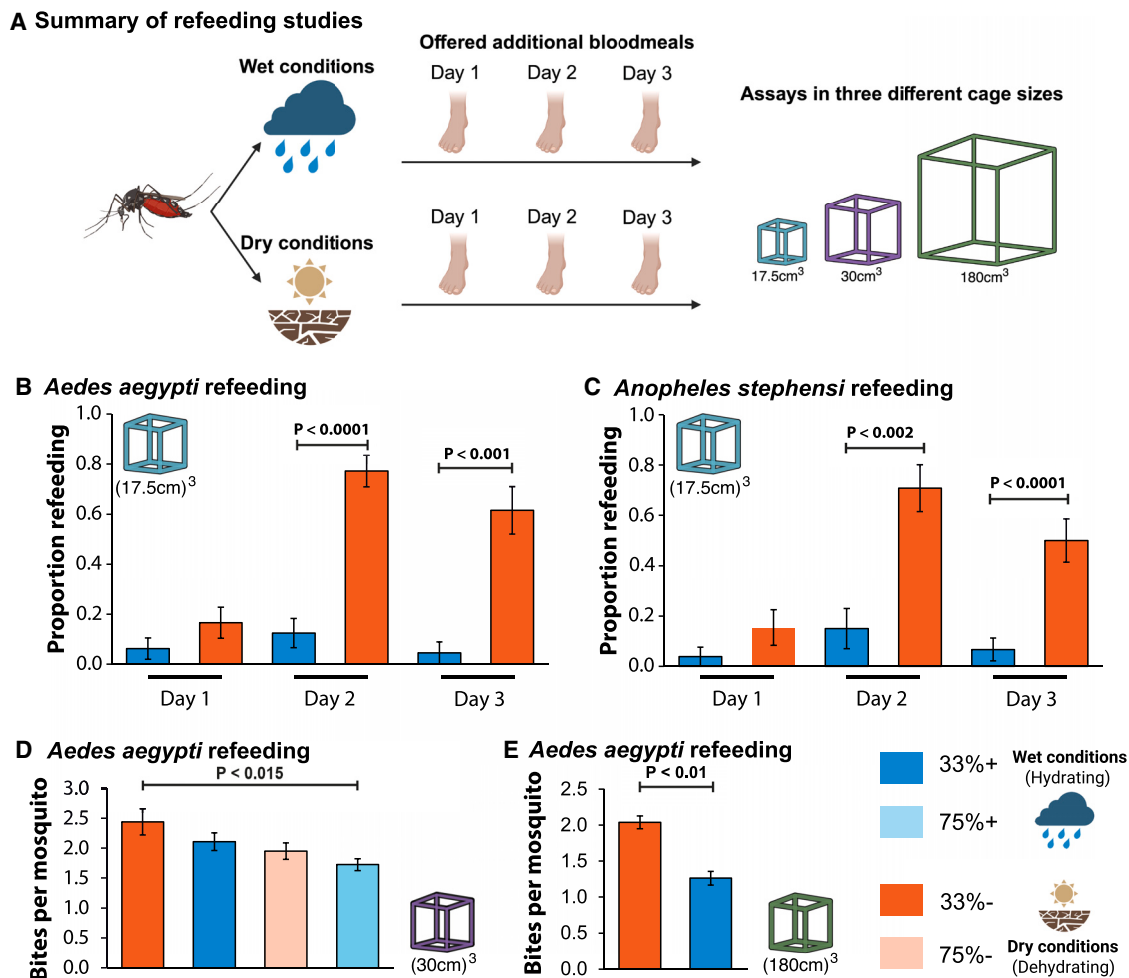


Figure 1. Dehydration induces refeeding in mosquitoes

(A) Summary of the studies with mosquitoes held under wet (hydrating) and dry (dehydrating) conditions. Created in BioRender. Benoit, J. (2025) <https://BioRender.com/y14y842>.

(B and C) Small cages (10 × 10 × 10 cm, single mosquitoes, $N = 15\text{--}25$) for *Aedes aegypti* and *Anopheles stephensi*, (D) Medium cages with *Ae. aegypti* (30 × 30 × 30 cm, 10 mosquitoes, $N = 4$), and (E). Large cages with *Ae. aegypti* (1.8 × 1.8 × 1.8 m, $N = 8$, ten mosquitoes per cage). Dry conditions increased refeeding for both *Ae. aegypti* and *An. stephensi* (Pairwise Chi-Squared test, ANOVA with Tukey HSD, and Student's t test, $p < 0.05$) under small, medium, and large cage sizes, respectively. Data are represented as mean \pm standard error. Complementary results are included in Figure S1.

are primarily used to increase water content and survival in lieu of reproductive output.

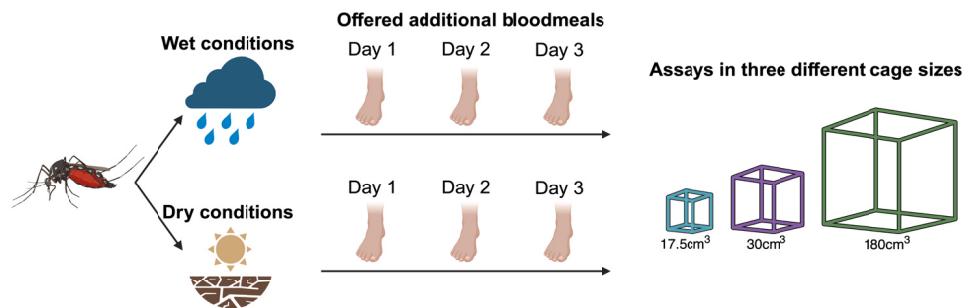
To assess the replenishment of water stores, we examined mass changes following a bloodmeal (Figure 2D). A significant loss of mass occurred when mosquitoes were held under dry conditions, featuring a 30–40% decline in mass following a bloodmeal after two days for *Ae. aegypti* (Figure 2). When held under stable conditions, a decline was noted, but mosquitoes retained nearly 0.8 mg more mass compared to the dehydrated controls after two days under dehydration. Ingestion of a second bloodmeal allowed the mosquitoes to immediately replenish mass up to the amount after the first bloodmeal. As these changes are predominantly due to water loss, prolonged exposure to dry conditions following a bloodmeal will yield mosquitoes that lose nearly half of their water content within two days if unable to rest in stable, relatively humid areas after a blood-

meal. The water content can be replenished if a mosquito obtains a second bloodmeal. Each period of bloodmeal-associated hydration allows for another two days of survival under dry conditions until a water source can be located for water ingestion and egg deposition. Along with these studies on *Ae. aegypti*, similar effects were noted for *An. stephensi* (Figures S2C–S2E).

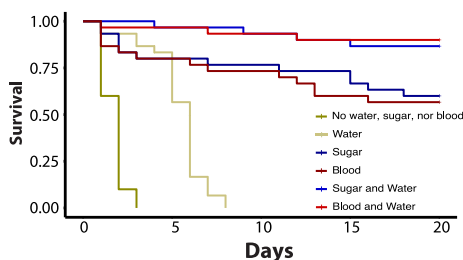
Dry conditions increase activity and prompt an early return of carbon dioxide sensing

As dry conditions prompted a substantial increase in secondary blood feeding, we examined how the activity and behavior of *Ae. aegypti* shifted following a bloodmeal with and without access to water sources (Figure 3). When activity levels were assessed, a general suppression of activity occurred until three days after a bloodmeal (Figures 3B and 3C). During the third day after a bloodmeal, there was a significant increase in activity of

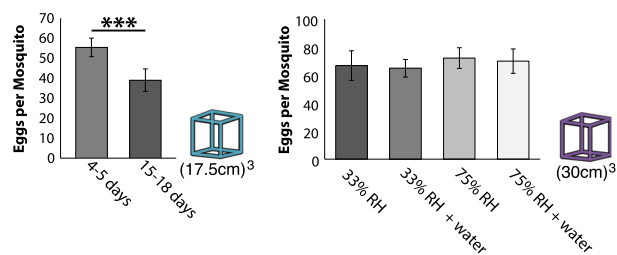
A Survival and mass changes description



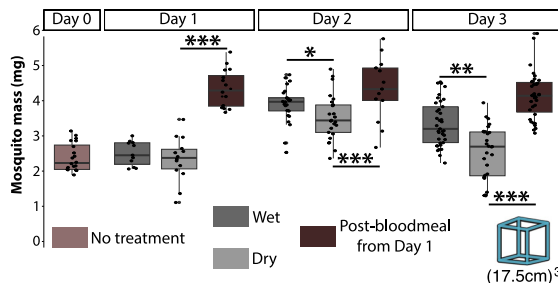
B Prolonged blood feeding



C Egg production



D Mass changes



E Ovariole numbers

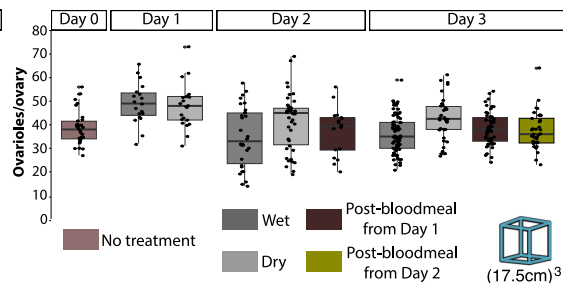


Figure 2. Mosquito mass change and reproductive output following additional bloodmeals

(A) Summary of the studies with mosquitoes held under wet (hydrating) and dry (dehydrating) conditions. Created in BioRender. Benoit, J. (2025) <https://BioRender.com/d82f070>.

(B) Left - Survival of *Aedes aegypti* under dry and wet conditions with and without access to water, sucrose solution, and blood (30 × 30 × 30 cm, 10 mosquitoes, $N = 3$, Cox Proportional Hazards Model), Right - Egg production following prolonged periods of refeeding (30 × 30 × 30 cm, 10 mosquitoes, $N = 3$).

(C) Egg production by *Ae. aegypti* in medium cages (30 × 30 × 30 cm, 10 mosquitoes, $N = 4$).

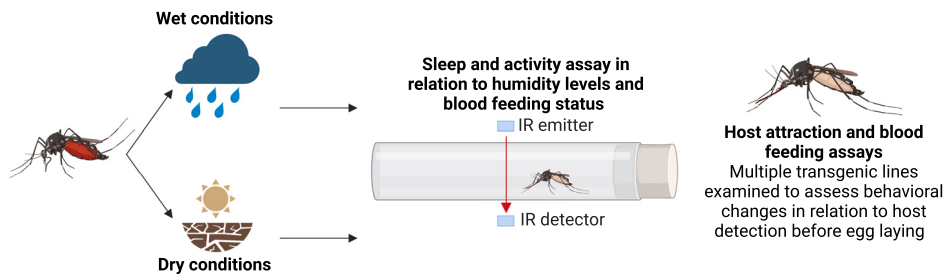
(D and E) Mass and ovariole changes measured in small cages (10 × 10 × 10 cm, single mosquitoes, $N = 15-25$) for *Ae. aegypti*, and each group was held under wet or dry conditions following feeding. Prolonged refeeding reduced the number of eggs oviposited, and bloodmeals resulted in consistently higher masses in *Ae. aegypti* (ANOVA with Tukey HSD, and Student's t test, *, $p < 0.05$; **, $p < 0.01$ ***, $p < 0.001$). Data are represented as mean \pm standard error. Boxplots are represented as median, 25th/75th percentiles, and minimum/maximum values. Complementary results are included in Figure S2.

mosquitoes held under dry conditions (Figures 3B and 3C). However, the movement for both groups was still considerably lower than the non-blood fed group (Figure 3B). As activity increased, we showed that sleep decreased in the dehydrated group (Figure 3D). This suggests an early increase in activity and decrease in sleep after a bloodmeal when female mosquitoes are exposed to dry conditions.

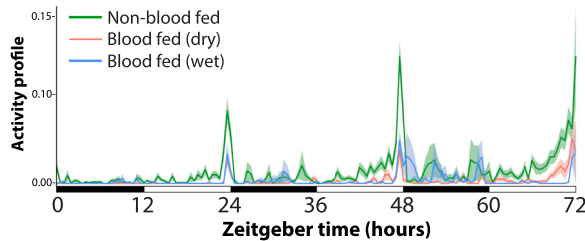
Increases in activity and host feeding suggest there may be a reconstitution of host-sensing, which we examined using mutant mosquitoes with altered host and humidity detection.⁴³⁻⁴⁷ In general, most of the mutant strains showed a similar response to the control lines, both in the time it took the mosquitoes to

feed on the host (Figure S3), and in the number of refeeding events that were increased by dehydration (Figure 3). The lone exception was Gr3 mutants with impaired CO₂ sensing, which did not show an increase in blood feeding during dehydration (Figure 3E). To confirm if increased CO₂ sensing was associated with dehydration, we performed host feeding assays with and without the presence of CO₂, which showed increased refeeding on an artificial host when CO₂ was present (Figure 3F). A secondary experiment was conducted where mosquitoes were exposed to butanal to reduce response to CO₂,⁴⁸ which confirmed that increased CO₂ sensing is a significant factor associated with dehydration-induced refeeding (Figures 3E

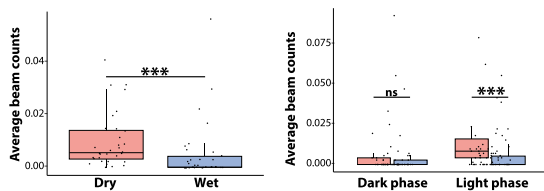
A Summary of activity and host-seeking



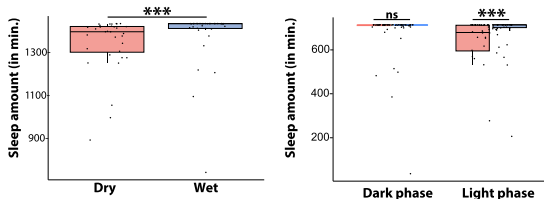
B Activity in relation to feeding and humidity



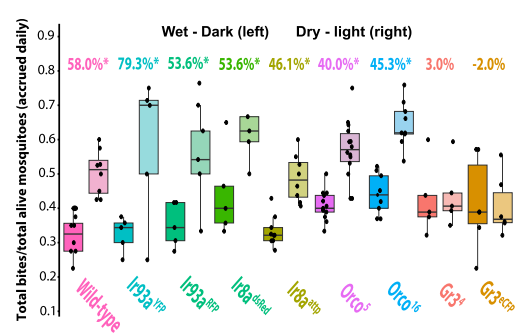
C Activity levels 3 days after bloodmeal



D Sleep levels 3 days after bloodmeal



E Blood feeding rates in mutant lines



F Blood feeding in three box assays

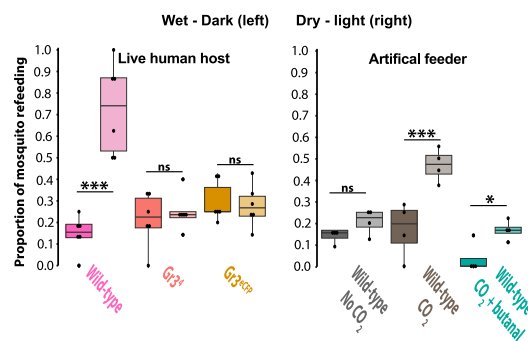


Figure 3. Shifts in activity and impact of impaired host sensing on dehydration-induced refeeding

(A) Summary of the studies with mosquitoes held under wet (hydrating) and dry (dehydrating) conditions. Created in BioRender. Benoit, J. (2025) <https://BioRender.com/u41n605>.

(B–D) Activity and sleep profiles in blood-fed *Aedes aegypti* when held under dehydrating conditions (33% +/- 5% RH) and hydrating conditions (75% +/- 5% RH with access to water) compared to non-blood-fed mosquitoes with access to 10% sucrose (N = 32–48). Dry conditions prompted increased activity and reduced sleep on day three following a bloodmeal, but prior to oviposition (differences driven by light-phase, Wilcoxon rank-sum test, $p < 0.001$).

(E) Use of *Ae. aegypti* mutant lines to assess host feeding (N = 5–14). All mutant lines showed increased blood feeding under dehydration (ANOVA with Tukey HSD, $p < 0.05$), except for mutant lines with defects in CO₂ (both GR3 mutant alleles), percent values indicate mean biting differences between dry and wet conditions of each mosquito line.

(F) Utilization of three-box choice assays to directly examine the role of CO₂ detection on multiple feeding attempts using both a live host and an artificial feeder (Hemotek). A lack of CO₂ with artificial feeding or impaired sensing of CO₂ (Gr3 mutants with a live host or butanal treatment with artificial feeding) leads to a reduction in dehydration-induced refeeding (Pairwise Chi-Squared test, ***, $p < 0.0001$; *, $p < 0.05$). Boxplots are represented as median, 25th/75th percentiles, and minimum/maximum values. Complementary results are included in Figure S3.

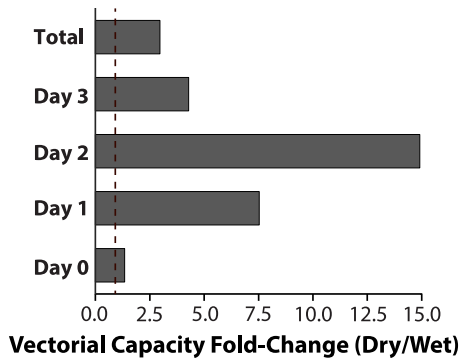
and 3F). These studies highlight that dehydration leads to mosquito sensory and behavioral changes that increase blood feeding, even within a single gonotrophic cycle.

Increased blood feeding and survival will shift viral transmission and mosquito populations

Previous studies have shown that dry conditions increase the transmission of specific viruses^{10,11} and impact viral dissemina-

tion in mosquitoes.^{7,49} We built upon these observations with our study to model how multiple refeeding events may impact vectorial capacity, mosquito survival, and viral transmission, with details provided in the Supplemental modeling. The multiple feeding events observed under dry conditions are predicted to constitute a 2- to 3-fold increase in vectorial capacity for mosquitoes (Figure 4A). When population growth rates were assessed for *Ae. aegypti* (Figure 4, Supplemental modeling), the

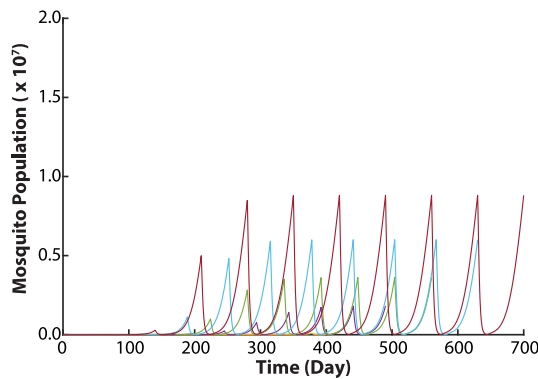
A Vectorial capacity



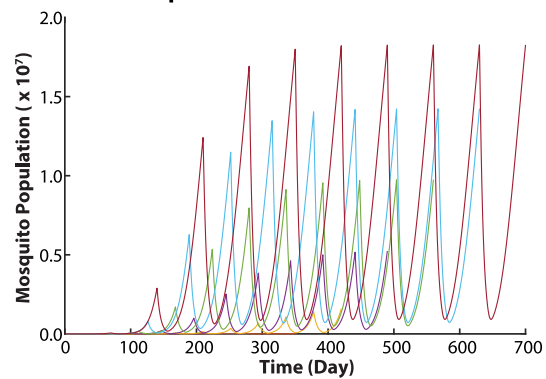
Key for B-E



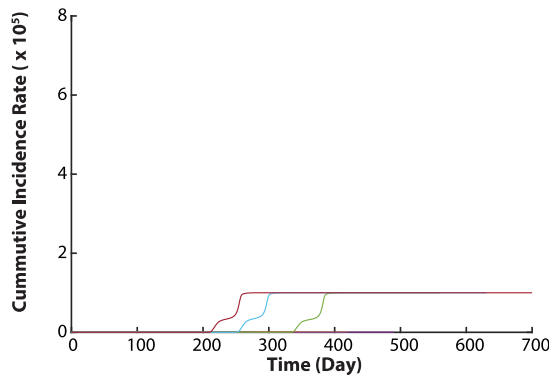
B Periodic dehydration - *Aedes aegypti*



C Periodic dehydration - *Aedes aegypti* with multiple bloodmeals



D Periodic dehydration - Zika transmission



E Periodic dehydration - Zika transmission with multiple bloodmeals

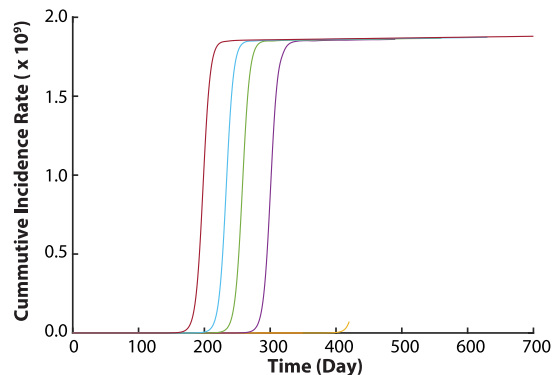


Figure 4. Vectorial capacity and mosquito population growth are increased by multiple bloodmeals

(A) Vectorial capacity was determined according to previous studies^{6,50} with modified parameters based on this study. Each day represents post-blood feeding time, with day 0 indicating the fold-change immediately after the initial bloodfeeding.

(B–E) *Aedes aegypti* population and Zika transmission dynamics in simulated environments, each including dry periods of 14 days with various wet period lengths both without (B) and with access to multiple bloodmeals (C). In (D), cumulative incidence rates for Zika virus per 100,000 humans were determined using population estimates from mosquitoes that did not take multiple bloodmeals (shown in B). In (E), cumulative incidence rates for Zika virus were determined using population estimates from mosquitoes imbibing multiple bloodmeals (shown in C). Details on modeling are provided in the supplemental materials. Associated modeling is included in the supplemental modeling methods.

capability of *Ae. aegypti* females to remain viable for extended periods⁵¹ allowed for populations to rebound after dry periods. With extended viability, *Ae. aegypti* can maintain higher populations during dry periods that can rapidly increase when conditions are more favorable for egg laying and larval growth (Figures 4B and 4C). The increased feeding events, and the resultant increase in survival, during dry periods lead to predicted increases in the transmission of Zika by *Ae. aegypti* (Figures 4D and 4E). Similar results were obtained for *Culex pipiens* in relation to population growth and for West Nile virus transmission (Supplemental modeling).

DISCUSSION

This study established that prolonged exposure to low humidity conditions is a major factor in multiple blood feeding events. Therefore, we expect mosquitoes that cannot find a humid resting site, ample free water, or are otherwise experiencing water loss will frequently refeed to compensate for lost water. If dehydration persists for an extended period, mosquitoes can prolong survival under desiccating conditions by repeatedly refeeding with no apparent detriments to their behavior or physiology, other than reduced egg production. Our findings indicate that this increase in blood feeding is associated with a return of increased activity and CO₂ sensing. Modeling based on increased blood feeding rates for rehydration (this study;⁵), increased viral dissemination from multiple bloodmeals,¹⁹ and prolonged survival by refeeding (this study), suggests that this dehydration-induced refeeding will lead to substantial increases in viral transmission. This provides a key mechanism explaining previous observations that viral transmission increases during dry periods.^{10,11}

With water availability that may be limited during dry periods, as floral nectar and liquid water sources are reduced,^{5,40} we have previously shown that dry periods will increase mosquito blood feeding propensity.^{5,6} We now expand these findings to show substantial increases to blood feeding rates within a single gonotrophic cycle. Multiple blood feeding events have been attributed to an interruption in feeding or a lack of nutrient reserves obtained during larval development.^{21,22} In this study, we establish that exposure to dry conditions can be an underlying factor in the acquisition of secondary bloodmeals. As both *An. stephensi* and *Ae. aegypti* have highly anthropophilic tendencies, there could be specific fitness advantages to taking multiple bloodmeals under dry periods, especially considering that human hosts are readily available in specific environments.^{30,41,52}

A determining factor of dehydration-induced refeeding is the ability of mosquitoes to locate a resting site with relatively high humidity (this study;⁵³) and moderate temperature.⁵⁴ In our studies, humidity exposure was controlled and harborages were eliminated within experimental cages. However, mosquitoes were observed resting on or near the soaked cotton wicks provided under wet conditions in our large cage assays. Mosquitoes likely experienced increased relative humidity during these resting periods, which may be more representative of suitable resting sites exploited by mosquitoes in natural settings. Future studies focusing on varying levels of humidity with and without

suitable resting sites would be valuable to establish more natural scenarios. If unable to locate a suitable resting site, dehydration will be rapid under warm and dry conditions, leading to an increased likelihood of multiple bloodmeals. Mosquito activity, which we observed to increase prematurely after blood feeding in dry conditions, could be increased to alleviate the effects of dehydrating conditions. This could be completed through the location of suitable microclimates or rehydration through the acquisition of free water or from feeding on a living host.

Importantly, as conditions within human dwellings are commonly drier or have less access to free water than in outdoor environments,⁵⁵ locating a resting site that suppresses dehydration-induced refeeding may be difficult. In fact, our large cage assays held under household conditions (33 ± 4% RH and 23 ± 2°C), confirmed that nearly 50-60% of *Ae. aegypti* with access to water sources, and 110-120% of *Ae. aegypti* without access to water sources would take a secondary bloodmeal after feeding to repletion in the previous three days. In more confined spaces (medium cage assays), even *Ae. aegypti* held in considerably more favorable conditions (75 ± 4% RH and 26 ± 2°C) saw similar 50-55%, and 100-110% rates of refeeding when held with, and without access to water sources, respectively. Increased feeding in both drier and more confined spaces could explain the high prevalence of multiple bloodmeals when mosquitoes are collected within a household, especially when compared to lower observations of multiple feeding in outdoor biomes.^{21,24,25,28} Interestingly, ecological factors of a similar type, dry season intensity and human population density, were found to be the primary drivers of human odor preference variation in *Ae. aegypti* mosquitoes.⁴¹ It follows then, that if mosquitoes rest in an indoor or outdoor biome, warmer conditions, low humidity, and a lack of water sources will likely lead to substantial refeeding events, yielding two or three bloodmeals during a single gonotrophic cycle. These findings, coupled with intertwined rates of increasing urbanization and predicted mosquito evolution, indicate that mosquitoes will continue to shift toward anthropophilic behaviors.⁴¹ Importantly, our observations of multiple bloodmeals improving survival could extend beyond a single gonotrophic cycle, allowing adult mosquitoes to survive for weeks at a time without access to water.

Our findings on compensatory feeding are consistent with previous research that found small, undernourished female *Ae. aegypti* could initially use a bloodmeal for follicle development and then again for ovary maturation, whereas large, properly nourished females could use a single bloodmeal for ovary maturation.^{22,56} Furthermore, refeeding may be necessary if the first bloodmeal is interrupted. For *Ae. aegypti*, multiple refeeding events are common (10-40% of mosquitoes will ingest a second bloodmeal before the previous blood has been digested²¹⁻²⁶). Our studies add that dehydration is a contributing factor that needs to be assessed when multiple feeding events are observed. The flexibility of these mosquitoes to forgo or delay reproduction in exchange for survival or increased nutritional reserves⁵⁷ permits them to refeed with only minimal physiological impacts.^{22,56,57} Future research on the effects of dehydration would benefit from the inclusion of refeeding propensity after an interrupted feeding event, while also considering mosquito size and nutrition levels. These combined studies would allow

for a comprehensive assessment of mosquito refeeding in relation to all factors that have been suggested to be involved in this process.^{22,28} Previous observations and those in this study of multiple feeding events have shown that the process of oogenesis has commenced before a second or third bloodmeal within the same gonotrophic cycle,^{21,22} indicating that there will be little impact on the number of eggs produced. Here, we confirm little impact on oocyte size or reproductive output in egg number following a second dehydration-induced bloodmeal, so long as mosquitoes can find a location to lay eggs within one week. Even after 20 days of retention the eggs can still be deposited, but there is a reduction in the number produced. Specific genes have been previously linked with egg retention during drought periods in *Ae. aegypti*,⁴² which supports these studies that eggs can be retained, at least in some *Aedes* sp., under sub-optimal conditions until a water source can be located to deposit eggs. Our studies suggest that the second dehydration-induced bloodmeal is not directly required for oogenesis since egg numbers show little change and are only reduced if egg retention is extended⁶; instead, the primary role is to ensure hydration and survival until eggs can be deposited.

Following a bloodmeal, host sensing is suppressed among most mosquito species until eggs are deposited,^{28,58–61} but exceptions are known. For example, *Anopheles* mosquitoes have been shown to have increased behavioral attraction to the host following a bloodmeal.^{28,58} Suppression of blood feeding is due to a combination of three specific factors—mechanosensation of the ingested blood due to abdominal distension, bloodmeal digestion and processing, and egg development.^{28,58} Here, we observe that mosquitoes held under dehydrating conditions resume blood feeding activity and host attraction two-to-three days after an initial bloodmeal. Since this increased blood feeding occurs during oocyte development, the cause is likely associated with improper nutrient-sensing or altered levels of hormones that regulate vitellogenesis and oogenesis processes (see reviews^{22,28}). Another potential mechanism is that factors underlying water-seeking may be altered and increase host detection.^{62,63} Interestingly, a return to blood feeding occurs in mutants with altered humidity detection (*Ir8a* and *Ir93a*,^{43,44,64}), suggesting that our observations are not solely the result of increased water detection and ingestion. Previous studies have shown that dehydrated mosquitoes are remarkably efficient at re-equilibrating hemolymph osmolality following a bloodmeal,⁶⁵ and that dehydration prompts general dysfunction in circulating hemolymph levels through increased osmolarity and altered heart rates,⁶⁶ which likely result in direct impacts to behavioral and physiological processes. As an example, female mosquitoes detect CO₂ with a specific class of olfactory receptor neurons (ORNs) designated cpA that express three conserved members of the gustatory receptor (Gr) gene family associated with CO₂ detection.^{48,67} A possibility is that cpA is reactivated to detect changes in CO₂ during dehydration, allowing mosquitoes to recover sensitivity to this host cue after blood feeding. Additional studies will be required to establish the specific mechanism(s) for how dehydration triggers increased activity and CO₂ sensing in mosquitoes.

Dry periods have been associated with increased viral transmission.^{10,11} This increase was associated with a multitude of

factors, which range from altered mosquito-predator interactions associated with temporal water pools,¹⁶ altered blood feeding propensity,⁵ and altered viral dissemination in the mosquito.^{7,9,49} We expand on these observations to show that dry conditions will increase refeeding, which is commonly observed in field-collected mosquitoes.^{20–22,58} Chronic feeding under dry conditions is predicted to substantially increase mosquito survival, prompting predicted increases in vectorial capacity and viral transmission. Although alternative water sources may reduce chronic blood feeding during dry periods, such as the increased sugar feeding observed in *Ae. albopictus*,³⁹ it is known that standing water sources are limited and the water content of nectar is reduced during drought,^{5,16,40} indicating that increased interactions with a host to blood feed could represent the most reliable means of water acquisition. This could explain why as much as half of field collected mosquitoes had ingested multiple bloodmeals when collected during dry seasons.^{21,25}

Conclusions

Bouts of dehydration yield substantial changes to insects' physiology and behavior. Mosquitoes are exceptionally prone to dehydration due to high water loss rates, but few integrative studies have examined the comprehensive impact of drought conditions on mosquitoes. Here, we demonstrate that dry conditions lead to multiple blood feeding events, allowing mosquitoes to survive drought. This repeated blood feeding is associated with higher activity and an early return of attraction to vertebrate hosts. Increased dry season survival and more frequent blood feeding are predicted to yield higher transmission of mosquito-borne viruses. This suggests that a higher prevalence of drought associated with climate change will have varying impacts on mosquito-borne diseases. Dehydration stress has repeatedly been implicated in water and nutrient depletion, and compensatory mechanisms are known to be utilized to offset the detriments.^{5,6,35} Unfortunately, many of those mechanisms operate through blood feeding, likely resulting in altered disease propagation dynamics within the vector and through host-vector interactions. In addition to postulating the effects on disease transmission, the characterization of refeeding behaviors in dehydrated mosquitoes is essential for determining influences on survival, reproduction, as well as rehydration during and after dehydrating conditions. As climate change is predicted to drive more stark contrasts between wet and dry conditions,¹⁸ understanding how these dynamics alter mosquito biology is critical. This study continues to build on how environmental factors, especially drought-like conditions, alter the behavior and physiology of mosquitoes and ultimately influence disease transmission dynamics.

Limitations of the study

Our results indicate that *Ae. aegypti* and *An. stephensi* mosquitoes can survive through dry periods, for up to weeks at a time, by subsisting solely on blood. This refeeding occurs within the same gonotrophic cycle, which can be extended for weeks if an oviposition site is unavailable. In laboratory settings, refeeding during prolonged dry periods can reduce the number of eggs deposited by mosquitoes, but hydration sources and oviposition sites in natural settings may be more available than

are afforded in laboratory tests. Furthermore, our studies utilize two mosquito species of medical importance, *Ae. aegypti* and *An. stephensi*, but not every experiment was completed for each species and cannot be extrapolated to all mosquito species. Thus, until mesocosm and ecological studies on multiple mosquito species are incorporated, the accuracy and effects of these studies may not be entirely representative of natural mosquito populations.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Joshua B. Benoit (joshua.benoit@uc.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Datasets have been deposited at Zenodo: <https://doi.org/10.5281/zenodo.14210028>, and are publicly available as of the date of publication.
- All original code has been deposited at GitHub and is publicly available through Zenodo: <https://doi.org/10.5281/zenodo.14210017>, as of the date of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.J.H., Y.X., and J.B.B.; Methodology, C.J.H., Y.X., and J.B.B.; Software, C.J.H., and Y.X.; Validation, C.J.H., Y.X., and J.B.B.; Formal analysis, C.J.H., S.C., O.M.A., Y.X., and J.B.B.; Investigation, C.J.H., S.C., O.M.A., M.U., R.F., C.S., E.S., Y.X., and J.B.B.; Resources, C.J.H., S.C., O.M.A., Y.X., and J.B.B.; Data curation, C.J.H., S.C., O.M.A., M.U., R.F., C.S., E.S., Y.X., and J.B.B.; Writing – original draft, C.J.H., Y.X., and J.B.B.; Writing – review and editing, C.J.H., S.C., O.M.A., M.U., R.F., C.S., E.S., S-C.C., J.L.R., M.D., Y.X., and J.B.B.; Visualization, C.J.H., Y.X., and J.B.B.; Supervision, C.J.H., Y.X., and J.B.B.; Project administration, C.J.H., J.L.R., M.D., Y.X., and J.B.B.; Funding acquisition, J.L.R., M.D., Y.X., and J.B.B.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
2-Butanol	Sigma	4435-58-9
D-Sucrose	Fisher Scientific	CAS-57-50-1
Yeast extract	Fisher Scientific	BP1422
Deposited data		
R code	GitHub, Zenodo	https://doi.org/10.5281/zenodo.14210017
Refeeding datasets	Zenodo	https://doi.org/10.5281/zenodo.14210028
Experimental models: Organisms/strains		
<i>Aedes aegypti</i> , Gainesville strain	BEI Resources, NIAID, NIH	MRA-804
<i>Anopheles stephensi</i> , STE2 strain	BEI Resources, NIAID, NIH	MRA-128
<i>Aedes aegypti</i> , Ionotropic receptor 8a mutant line	Raji et al., 2019 ⁴³	https://doi.org/10.1080/19420889.2019.1681063
<i>Aedes aegypti</i> , Gustatory receptor 3 – GR3 mutant line	McMeniman et al., 2014 ⁴⁵	https://doi.org/10.1016/j.cell.2013.12.044
<i>Aedes aegypti</i> , Ir93a mutant line	Laursen et al., 2023 ⁴⁴	https://doi.org/10.1016/j.neuron.2022.12.025
<i>Aedes aegypti</i> , Odorant receptor co-receptor – orco mutant line	DeGennaro et al., 2013 ⁴⁶	https://doi.org/10.1038/nature12206
Software and algorithms		
Adobe Photoshop and Illustrator	Adobe	https://www.adobe.com/
DAMSystem3 Data Collection Software	TriKinetics	https://trikinetics.com/
MATLAB	MathWorks	https://www.mathworks.com/products/matlab.html
R - The R Project for Statistical Computing	R	https://www.r-project.org/
RStudio Desktop	RStudio	https://posit.co/download/rstudio-desktop/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Mosquito husbandry

Standard practices were used for rearing and containment of *Ae. aegypti* and *An. stephensi* mosquitoes. Larvae were fed ground fish food (Tetramin) with the addition of yeast extract (Fisher). Adults were maintained in 30 × 30 × 30 cm cages (BugDorm), under a 16h:8h light:dark cycle, with unlimited access to cotton wicks soaked in deionized water and a 10% sucrose solution, all under insectary conditions at approximately 80% relative humidity (RH) and 27°C (vapor pressure deficit (VPD) = 0.71 kPa), unless otherwise specified.

Mosquitoes

Our studies were conducted on lines of mosquitoes that have been maintained in laboratories for extended periods of time, *Ae. aegypti* (Gainesville strain) and *An. stephensi* (STE) mosquitoes. The maintenance of these lines may have inadvertently selected for traits that are not representative of natural populations, indicating that additional studies with wild-caught mosquitoes are necessary. To alleviate this concern, a secondary refeeding study was conducted with a more recently collected line of *Ae. aegypti* from Africa⁴¹ that confirmed the same behavior was noted. Additionally, refeedings assays were conducted with the following mutant mosquito lines: Ionotropic receptor 8a,⁴³ gustatory receptor 3 - Gr3,⁴⁵ Ir93a,⁴⁴ and odorant receptor co-receptor - Orco.⁴⁶

Human participants: Two White/Caucasian adult male volunteers and one Asian/Pacific Island adult male volunteer, 27- to 40-years-old, offered a leg to cages of mosquitoes in accordance with the approved research protocol, IRB 2021-0971, at the University of Cincinnati. Researcher influence on mosquito feeding preference was incorporated into refeeding time modeling efforts and was not found to influence mosquito feeding results. However, the influence of the researchers on mosquito feeding preference could not be completed for every experiment and many more volunteers would likely be required to determine sufficient influence on the results. Volunteers were randomly assigned to mosquito cages for each of the feeding experiments.

METHOD DETAILS

Mosquito refeeding

Resting conditions between bloodmeals were used to represent conditions that would be common in human dwellings.^{21,23,25} Re-feeding was tested under three scenarios - in small cages with single mosquitoes, and in both medium and large cages with ten mosquitoes per cage. For small cages, individual mosquitoes were placed in 17.5 × 17.5 × 17.5 cm cages (BugDorm) held at 33 ± 4% RH and 26 ± 2°C (low humidity; VPD = 2.34 kPa) and two at 75 ± 4% RH and 26 ± 2°C (high humidity; VPD = 0.87 kPa) with access to water. For medium cages, 10 adult mosquitoes (7–14 days old) were placed into four 30 × 30 × 30 cm cages (BugDorm), two with access to DI water and 10% sucrose solution *ad libitum* and two without (+/-), before the cages were placed into separate humidity-controlled 60-quart plastic containers with lids. Four plastic containers were used, two held at low and high humidity. This design yielded four experimental conditions: Two cages of 10 mosquitoes at 33% RH, one with access to water and sucrose solutions *ad libitum* (33+) and one without (33-), and two cages at 75% RH, one with water and sucrose solutions (75+), and one without (75-). Mosquitoes were placed into these experimental containers for 18 h before being provided the opportunity to blood feed on a live human host for approximately 10 min (27-year-old male, leg inside the cage; IRB 2021-0971, University of Cincinnati). A feeding opportunity was presented every 12 h after the initial feeding for a total of three days. After the 72-h feeding period, all experimental cages remained within their respective humidified chambers but were supplemented with water, 10% sucrose solution, and oviposition dishes. Mosquitoes were permitted to lay eggs for one week before total egg counts were determined. In addition, in the small cage assays, mosquitoes were dissected, and the ovariole number was assessed to ensure that there were no changes in the number of progeny generated due to multiple feeding events in our experiments.

During the prolonged exposure experiment, mosquitoes were held under dry conditions (75% RH, no access to water) with or without access to water and sugar and allowed to blood feed every 48 h as survival was assessed. After 20 days, all experimental cages were provided access to water, 10% sucrose solution, and oviposition dishes. The mosquitoes were permitted to lay eggs for one week before total egg counts were determined.

Groups of 10 adult mosquitoes were collected into two 1.8 × 1.8 × 1.8 m cages (BioQuip) kept at 33 ± 4% RH and 23 ± 2°C (VPD = 1.99 kPa), one with access to DI water and 10% sucrose solution *ad libitum* and one without (+/-). Mosquitoes were placed into these experimental cages for 18 h before being provided the opportunity to bloodfeed on a live human host for approximately 15 min (three 27-to 40-year-old male volunteers, leg inside the cage; IRB 2021-0971, University of Cincinnati). A feeding opportunity was presented every 24 h after the initial feeding for a total of three days. These assays were repeated with the use of mutant mosquito lines to assess refeeding potential, which included the following mutants: Ionotropic receptor 8a,⁴³ gustatory receptor 3 - Gr3,⁴⁵ Ir93a,⁴⁴ and odorant receptor co-receptor - Orco⁴⁶ to assess host cue detection. The time until each mosquito bite was recorded to determine the time until initial feeding (bites on day 1) and time until refeeding (days 2–4). Proximity in the large cages allowed for mosquitoes to be at a maximum of over two meters from the host, while the two sets of smaller cages only permitted host-sensing to occur at a maximum of about 10 or 30 cm. Although our studies likely indicate what may be observed within a small room or inside a small domicile, the responses of mosquitoes in much larger outdoor environments will require additional research.

Activity and sleep measurements after blood feeding

The rest-activity rhythms of the mosquitoes were quantified with the aid of a Locomotor Activity Monitor 25 (LAM25) system (TriKinetics Inc., Waltham, MA, USA) and the DAMSystem3 Data Collection Software (TriKinetics) based on methods developed for mosquitoes.^{68–70} Individuals were blood-fed (27-year-old, male volunteer, leg inside the cage; IRB 2021-0971, University of Cincinnati) before being transferred to glass tubes simulating wet or dry conditions, with sponges soaked in DI water or with dry sponges, respectively. The glass tubes were then positioned horizontally in the LAM25 system, allowing the simultaneous recording of 32 individuals in an “8 × 4” horizontal by vertical matrix. Replicates were intermixed between trials to allow for randomization. The entire set-up was placed in a secluded incubator at 24°C, 70–75% RH, under a 12h:12 h L/D cycle. After 3–6 h (coinciding with the start of the night phase), activity level was measured in 1 min bins (the number of times an individual crosses an infrared beam) for three days. Sleep (period of inactivity lasting 120 min or longer) and activity levels were assessed as previously described in mosquitoes.^{68–70} For comparison with unfed mosquitoes, some individuals were included in the set-up as described above, and data was also retrieved from a previous study.⁶⁸ Data collected with the DAMSystem3 was processed using the Rethomics platform in R with associated packages, including *behavr*, *ggetho*, *damr*, and *sleepr*.⁷¹

Uniport assays for blood feeding assessment

A modified uniport assay was developed based on Castillo et al.⁷² Briefly, three mosquito cages (30 × 30 × 30 cm, BugDorm) were connected by a 10 cm i.d. acrylic tube (10 cm in length with removable covers to prevent mosquito movement). Mosquitoes were initially confined to the first cage, provided a bloodmeal, and held under conditions that allowed for hydration (30–40% RH with access to multiple water sources) or promoted dehydration (30–40% RH without access to water sources). After two days, mosquitoes were allowed access to all three chambers, and a human host (three 27-to 40-year-old male volunteers, leg inside the cage; IRB 2021-0971, University of Cincinnati) or host mimic (Hemotek) was added to the third chamber. After 5, 10, and 30 min, the number of mosquitoes that had successfully started blood feeding was assessed. Application of CO₂ to wildtype and butanal-treated

mosquitoes was added during the process of artificial feeding. This was accomplished by providing a steady flow of CO₂ (50-60 ml of CO₂ per minute) through a vinyl plastic tubing placed adjacent to the Hemotek feeder.

Vectorial capacity, disease transmission modeling analyses, and statistical analyses

As in a previous study,⁶ vectorial capacity was calculated to determine fold-changes between wet and dry conditions over the 3-day testing period. Egg production was determined from our prolonged survival data, and the overall biting rate per day was determined from our large-cage refeeding data. Disease and population growth modeling was determined based on methods previously developed for mosquito survival and viral transmission in relation to drought stress⁵ and completed in MATLAB (MathWorks). A description of the modeling is provided in the supplement (Supplemental materials, [Figure S4–S13](#); [Tables S1–S6](#)).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis details including test, sample sizes, exclusion criteria, replication details, and significance are included in the appropriate figure legends. A concise table including statistical tests, the values of n, and what n represents for all figures, including supplementary figures has been added to the supplement ([Table S6](#)). Graphical abstract and experimental design figures were generated with <https://BioRender.com>.