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# Comparative Physiology of Mosquito Reproductive Systems

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**Abstract** This study systematically analyzed the comparative physiology of mosquito reproductive systems, revealing their impact on reproductive success and disease transmission potential. Analyzing the structure and function of male and female reproductive organs, elucidating the key roles of larval hormone (JH) and 20 hydroxyecdysteroid (20E) in gamete and yolk formation, and demonstrating the ecological adaptability and vector potential diversity brought about by differences in reproductive structures among different mosquito genera. Taking Aedes aegypti as an example, this study explores the delayed egg laying mechanism under drought conditions and the transcriptome changes induced by mating, emphasizing the regulatory role of mating behavior on female reproductive physiology. At the same time, it is pointed out that environmental factors such as temperature, humidity, and nutrition have a significant impact on the reproductive cycle. Although emerging molecular tools such as CRISPR/Cas9 and RNA interference (RNAi) have made progress in studying the functions of reproductive related genes, further understanding of the functions of seminal proteins and long non coding RNAs (lncRNAs) is still needed. The research results provide theoretical support for the development of vector control strategies targeting mosquito reproductive processes, and suggest combining molecular, physiological, and ecological research to optimize the prevention and control of mosquito borne diseases.

**Keywords** Mosquito reproduction; Juvenile hormone; Aedes aegypti; Vector control; Gametogenesis; Molecular tools

## 1 Introduction

Mosquitoes are vectors for some of the most devastating human diseases, including malaria, dengue fever, Zika, and chikungunya. Understanding the reproductive physiology of mosquitoes is crucial for developing effective vector control strategies. For instance, manipulating reproductive processes can help suppress wild populations or replace them with disease-resistant mosquitoes (Degner et al., 2018; Degner et al., 2019). Additionally, the female mosquito's need for blood meals to produce eggs makes her an exceptional disease vector, highlighting the importance of understanding the physiological mechanisms behind egg production and maturation (Riddiford, 2013; Martín, 2021).

Different mosquito species exhibit significant variations in their reproductive systems. For example, Aedes aegypti, the primary vector for dengue and yellow fever, has been extensively studied for its reproductive proteins and genetic architecture (Sirot et al., 2008; Degner et al., 2018; Degner et al., 2019). The reproductive isolation and speciation within mosquito genera, such as Anopheles and Aedes, also play a critical role in their ability to transmit diseases (Zheng, 2020). Moreover, the role of juvenile hormones in regulating reproductive processes, such as egg maturation and vitellogenin synthesis, varies across species (Riddiford, 2013).

This study aims to explore the comparative physiology of the mosquito reproductive system in depth, covering multiple levels such as genetics, proteomics, and physiological characteristics, with the aim of identifying factors that play a key role in reproductive efficiency and disease transmission. Special attention is paid to the role of semen proteins, the regulatory mechanisms of juvenile hormones, and the phenomenon of reproductive isolation, and how these factors affect the effectiveness of vector control strategies. Through such systematic analysis, we hope to provide scientific basis for understanding and intervening in the spread of mosquito borne diseases.

## 2 Morphology of Mosquito Reproductive Organs

### 2.1 Anatomy of male reproductive systems

The male reproductive system in mosquitoes is composed of several key structures that facilitate sperm production, storage, and transfer. The primary organs include the testes, seminal vesicles, and accessory glands. The testes are responsible for the production of sperm cells, which are then stored in the seminal vesicles until copulation. The accessory glands produce various fluids that are essential for the successful transfer of sperm to the female during mating (Horsfall and Ronquillo, 1969; Dottorini et al., 2007).

In Aedes stimulans, the male reproductive organs are derived from one of two sets of primordia provided by the embryo, with the second set capable of producing female reproductive systems under unusual circumstances (Horsfall and Ronquillo, 1969). The male accessory glands in Anopheles gambiae produce proteins that modulate female postmating behavior, enhancing ovulation and reducing mating receptivity (Dottorini et al., 2007). Additionally, the abdominal skeleton and muscles in male mosquitoes, such as Culiseta inornata, are adapted to aid in the rotation of the terminalia, which is crucial for successful copulation (Owen, 1980).

### 2.2 Anatomy of female reproductive systems

The female reproductive system in mosquitoes is equally complex, consisting of the ovaries, oviducts, spermathecae, and accessory glands. The ovaries produce eggs, which travel through the oviducts to be fertilized. The spermathecae are specialized structures that store sperm received from males during copulation, allowing for fertilization of eggs over time (Puniamoorthy et al., 2010; Chiang et al., 2012).

In Anopheles species, the female reproductive system exhibits rapid evolution, particularly in genes associated with reproductive functions. This rapid evolution is likely driven by the unique life history challenges faced by females, such as blood feeding and the need to store sperm for extended periods (Papa et al., 2016). The internal female genitalia in Sepsidae, another dipteran family, show significant morphological diversity, with structures like the ventral receptacle and dorsal sclerite evolving rapidly, likely due to post-copulatory sexual selection (Puniamoorthy et al., 2010).

### 2.3 Variations in reproductive anatomy among mosquito genera

There are notable variations in the reproductive anatomy among different mosquito genera. For instance, the male reproductive systems of various species within the Anopheles genus show differences in the number and types of accessory gland proteins produced, which can influence female postmating behavior and reproductive success (Dottorini et al., 2007). In contrast, the female reproductive systems of Aedes and Anopheles mosquitoes differ in the structure and function of the spermathecae and accessory glands, reflecting adaptations to their specific reproductive strategies and ecological niches (Horsfall and Ronquillo, 1969; Papa et al., 2016).

The morphology of the reproductive systems in other insect vectors, such as blood-feeding Hemiptera, also shows genus-specific variations. For example, the spermathecae in different genera of Reduviidae are morphologically distinct, and the presence or absence of posterior accessory glands correlates with variations in oviposition behavior (Chiang et al., 2012). These differences highlight the importance of reproductive anatomy in the evolutionary success and ecological adaptation of mosquito species.

## 3. Gamete Development and Maturation

### 3.1 Spermatogenesis in male mosquitoes

Spermatogenesis in male mosquitoes involves a series of complex developmental stages, starting from the production of primary spermatogonia by stem-cell division. This process includes the differentiation of secondary spermatogonia, meiotic divisions, and the morphological transformation of spermatids into mature spermatozoa. The entire process is intricate and involves several stages of cell division and differentiation (Dumser, 1980). In Culex pipiens, the transformation of labelled spermatogonia and primary spermatocytes into mature sperm takes approximately 10 and 9 days, respectively. This includes 3-4 days for the formation of spermatocytes, less than a day for meiosis, and 5 days for the transformation into mature sperm (Sharma et al., 1970).

### 3.2 Oogenesis and follicular development in females

Oogenesis in female mosquitoes is a highly regulated process that involves the development of oocytes within the ovaries. This process is divided into pre-vitellogenic and vitellogenic stages. In Aedes aegypti, the pre-vitellogenic stage occurs before blood feeding, during which primary egg chambers remain developmentally arrested. After blood feeding, the vitellogenic stage is activated, leading to the completion of oogenesis and the formation of mature eggs (Valzania et al., 2019). The process is regulated by various signaling pathways, including insulin-like peptides and ovary ecdysteroidogenic hormone, which stimulate the exit from pre-vitellogenic arrest and promote follicle cell proliferation and oocyte development (Valzania et al., 2019; Phipps et al., 2023).

### 3.3 Hormonal regulation of gamete maturation

Hormonal regulation plays a crucial role in the maturation of gametes in mosquitoes. In female mosquitoes, juvenile hormone (JH) is essential for reproductive maturation. JH levels rise shortly after adult emergence, facilitating previtellogenic development. Nutritional status significantly influences JH-mediated follicular resorption during the previtellogenic resting stage, with higher nutrient availability reducing resorption rates (Zhu et al., 2010; Clifton and Noriega, 2011). Additionally, 20-hydroxyecdysone (20E) is a major hormone regulating egg maturation. It is involved in the synthesis of yolk proteins and the progression of the reproductive cycle, with its action being modulated by insulin-like peptides and other factors (Robinson, 2013; Roy et al., 2016). In Anopheles gambiae, male mosquitoes transfer 20E to females during mating, which, in conjunction with the female protein MISO, promotes the expression of genes necessary for oogenesis and lipid accumulation in oocytes (Robinson, 2013).

## 4 Mating Behavior and Copulatory Mechanisms

### 4.1 Courtship behavior in different mosquito species

Courtship behavior in mosquitoes varies significantly across species. In Aedes aegypti, males transfer juvenile hormone III (JH III) during copulation, which influences the female's reproductive physiology by reducing the rate of previtellogenic resorption and increasing stored ovarian lipids, thereby enhancing reproductive output (Clifton et al., 2014). In Anopheles gambiae, the courtship process involves the transfer of seminal fluid proteins and a mating plug, which contains the steroid hormone 20-hydroxyecdysone (20E). This hormone plays a crucial role in activating vitellogenesis for egg production and induces significant changes in female behavior and physiology (Dahalan et al., 2019; Bascuñán et al., 2020).

### 4.2 Mechanisms of copulation and sperm transfer

The mechanisms of copulation and sperm transfer in mosquitoes are complex and involve multiple physiological and molecular processes. In Anopheles gambiae, copulation results in the transfer of a mating plug that contains 20E and other seminal secretions, which are crucial for modulating female post-mating behavior and physiology (Shaw et al., 2014; Bascuñán et al., 2020) (Figure 1). The mating plug also prevents further insemination by other males, ensuring the male's reproductive success (Mitchell et al., 2015). In Aedes aegypti, seminal fluid proteins transferred during mating induce broad transcriptome changes in the female reproductive tract, priming her for subsequent processes such as blood feeding, egg development, and immune defense (Alfonso-Parra et al., 2016).

### 4.3 Post-mating changes in females

Post-mating changes in female mosquitoes are profound and involve both physical and molecular alterations. In Anopheles gambiae, the mating plug not only prevents further mating but also delivers 20E, which regulates the expression of genes involved in sperm storage and long-term fertility (Shaw et al., 2014; Bascuñán et al., 2020) (Figure 2). The heme peroxidase HPX15, activated by 20E, is essential for maintaining sperm viability in the spermatheca, ensuring the female's fertility over multiple gonotrophic cycles (Shaw et al., 2014). Additionally, mating induces significant transcriptional changes in the female's midgut and reproductive tract, affecting her susceptibility to malaria parasites and overall reproductive success (Dottorini et al., 2007; Dahalan et al., 2019).

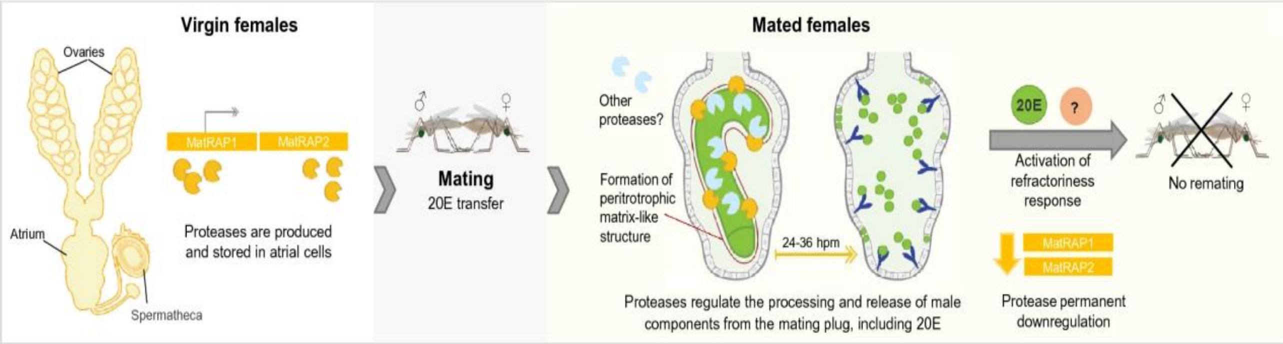


Figure 1 Schematic diagram of the function of atrial proteases in plug digestion and mating refractoriness. Females serine proteases MatRAP1 and 2 are produced and stored in the atrial cells of virgin females. During mating, the male ecdysteroid hormone 20E becomes packaged together with other seminal secretions into a gelatinous mating plug which is transferred into the female atrium and processed within 24–36 h postmating (hpm). MatRAP1 regulates the processing and release of these male components, and is potentially involved in the formation of a peritrophic matrix-like structure that surrounds the mating plug and may protect it from other female (or male) proteases. The correct processing of the mating plug, and the timely release of 20E trigger the refractoriness of females to further mating. Male transferred 20E also permanently downregulates the expression of MatRap1 and 2 after mating (Adopted from Bascuñán et al., 2020)

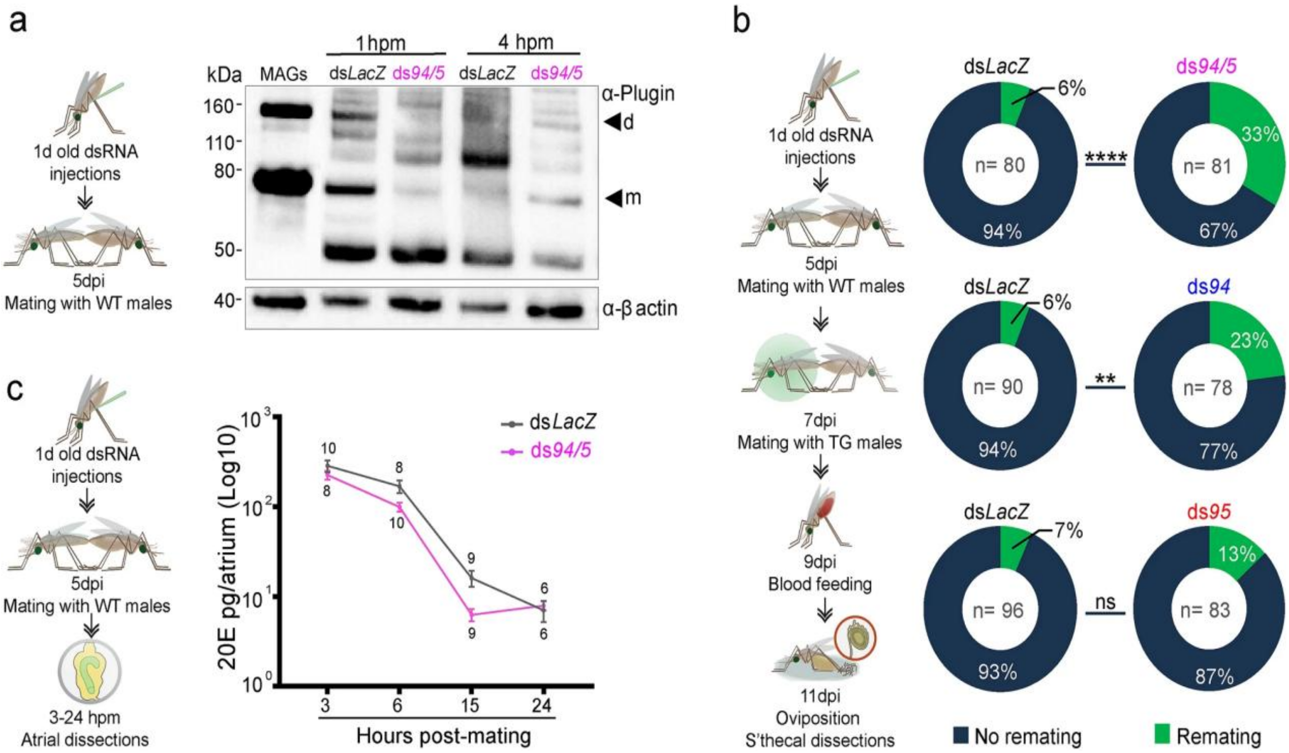


Figure 2 Atrial proteases affect the timely digestion of the mating plug and female refractoriness to further mating

Image caption: (a) Processing of the abundant mating plug protein Plugin in the female atrium is accelerated over the first hours after copulation in proteases-depleted females (ds94/5) compared to a control group (dsLacZ). Male accessory gland (MAGs) extracts were used as controls for unprocessed Plugin. Arrowheads indicate the Plugin monomer (m, 80 kD) and dimer (d, 160 kD). Full-length blots are presented in Supplementary Fig. 5. (b) Remating rates are significantly increased in ds94/5-injected females after exposure to a second male compared to females from the control group (dsLacZ) (top pie charts). This effect appears to be primarily driven by the depletion of AGAP005194 (middle pie charts) rather than AGAP005195 (bottom pie charts). (c) Time-course analysis of the levels of sexually transferred steroid hormone 20-hydroxyecdysone (20E) in ds94/5-injected females reveals a faster decrease in 20E levels in the atrium than the control group (dsLacZ) at earlier time points after mating. Each point represents the mean (± SEM) levels of 20E in pools of three atria collected from 2 to 3 biological replicates. Numbers represent pools per treatment per time point. A schematic diagram of the experiments is provided in all panels. dpi days post-injection (Adopted from Bascuñán et al., 2020)

## 5 Fertilization and Egg-Laying Processes

### 5.1 Mechanisms of fertilization in mosquitoes

Fertilization in mosquitoes involves the storage and maintenance of sperm within specialized organs. In Anopheles gambiae, a key vector for malaria, females mate only once and store sperm in the spermatheca, a specialized organ that preserves sperm viability for extended periods. The heme peroxidase HPX15, activated by mating, plays a crucial role in maintaining sperm functionality, ensuring long-term fertility. This process is regulated by the steroid hormone 20-hydroxy-ecdysone (20E), transferred during copulation, which induces the expression of HPX15 via its nuclear receptor (Shaw et al., 2014).

### 5.2 Factors influencing oviposition site selection

Mosquitoes exhibit complex behaviors when selecting oviposition sites, influenced by various environmental and biological factors. The presence of conspecific larvae can both attract and deter oviposition, depending on their density and developmental stage. For instance, Aedes albopictus shows a hump-shaped density-dependent relationship where low densities of conspecific larvae attract more oviposition, while high densities deter it due to increased competition (Yoshioka et al., 2012; Wasserberg et al., 2014). Additionally, the presence of specific bacterial volatiles, such as those emitted by Klebsiella sp., can attract gravid Aedes aegypti females to lay eggs, indicating the importance of microbial cues in site selection (Mosquera et al., 2022).

### 5.3 Environmental triggers for egg-laying

Environmental conditions play a significant role in triggering egg-laying behaviors in mosquitoes. Factors such as temperature, salinity, pH, and the presence of nutrients are critical in determining suitable oviposition sites. Aedes aegypti, for example, prefers laying eggs in natural depressions or artificial containers that meet these criteria (Day, 2016; Mohammed et al., 2023). Moreover, mosquitoes can adapt to polluted environments, as demonstrated by Aedes aegypti's ability to lay eggs in raw sewage, which contains high levels of nitrogenous compounds and microbial contaminants (Chitolina et al., 2016). This adaptability highlights the resilience of mosquito species in diverse and often suboptimal environments.

## 6 Comparative Physiology Across Mosquito Genera

### 6.1 Differences in reproductive strategies between aedes, anopheles, and culex

The reproductive strategies of Aedes, Anopheles, and Culex mosquitoes exhibit significant differences, particularly in their gonotrophic cycles and host-seeking behaviors. Aedes species, such as Aedes aegypti and Aedes albopictus, typically cease host-seeking behavior during the gonotrophic cycle, focusing on egg maturation after a blood meal. In contrast, Anopheles species, including Anopheles gambiae, Anopheles albimanus, and Anopheles freeborni, continue to seek hosts even as their eggs mature, which has implications for parasite transmission dynamics (Klowden and Briegel, 1994). Additionally, the embryonic development times and desiccation resistance of eggs vary among these genera. For instance, Aedes aegypti eggs can survive in dry conditions for months, whereas Anopheles aquasalis and Culex quinquefasciatus eggs have much shorter desiccation resistance periods, lasting only 24 hours and a few hours, respectively (Vargas et al., 2014; Farnesi et al., 2015).

### 6.2 Adaptations to ecological niches

Mosquito species have adapted to their ecological niches through various physiological and behavioral traits. For example, Culex pipiens complex mosquitoes exhibit unique life strategies such as overwintering diapause and specific bloodmeal preferences, which are adaptations to their respective environments (Kang et al., 2020). Aedes species, particularly Aedes albopictus and Aedes aegypti, have adapted to urban environments, often breeding in artificial containers and showing high resilience to overcrowding conditions (Yadav et al., 2017). Anopheles species, traditionally ground pool breeders, have also adapted to urban settings, utilizing gutters and domestic containers for breeding (Yadav et al., 2017). These adaptations are crucial for their survival and reproductive success in diverse habitats.

### 6.3 Evolutionary insights from comparative studies

Comparative studies of mosquito genera provide valuable evolutionary insights, particularly regarding reproductive isolation and genetic diversity. Cryptic species within the Anopheles gambiae complex exhibit significant prezygotic reproductive isolation, driven by different ecological preferences, while Aedes species like Aedes mariae and Aedes zammitii show postmating reproductive isolation (Zheng, 2020). The genetic diversity and expansion of odorant binding proteins (OBPs) in Aedes and Culex mosquitoes suggest that these gene families have evolved to meet the functional constraints imposed by their environments, aiding in host identification and adaptation (Manoharan et al., 2013). Additionally, the presence of insecticide resistance mutations in Culex pipiens and Anopheles hyrcanus populations highlights the evolutionary pressures exerted by vector control measures and agricultural pesticide use (Fotakis et al., 2017).

## 7 Case Study: Reproductive Adaptations in Disease-Vector Mosquitoes

### 7.1 Focus on aedes aegypti and disease transmission

Aedes aegypti is a primary vector for several significant viral diseases, including dengue, Zika, and chikungunya. The reproductive biology of Ae. aegypti plays a crucial role in its capacity to transmit these pathogens. During mating, males transfer proteins and other molecules to females, which significantly influence female post-mating behaviors and physiology, enhancing their reproductive success and vectorial capacity (Sirot et al., 2008; Villarreal et al., 2018). These male-derived molecules increase female fecundity and blood-feeding frequency, which are critical factors in the transmission potential of vector-borne pathogens (Villarreal et al., 2018).

### 7.2 Unique reproductive adaptations enhancing vector capacity

Ae. aegypti exhibits several unique reproductive adaptations that enhance its capacity as a disease vector. For instance, males transfer seminal fluid proteins (SFPs) during mating, which affect female sexual refractoriness, blood feeding, digestion, flight, ovarian development, and oviposition (Sirot et al., 2008; Degner et al., 2019). Additionally, under drought-like conditions, females can retain mature eggs in their ovaries for extended periods, maintaining egg viability until suitable conditions for laying are met. This adaptation is facilitated by two rapidly evolving genes, tweedledee and tweedledum, which are essential for extended egg retention (Venkataraman et al., 2023) (Figure 3). Furthermore, mating induces broad transcriptome changes in the female reproductive tract, priming females for subsequent processes such as blood feeding, egg development, and immune defense (Alfonso-Parra et al., 2016).

### 7.3 Implications for vector control strategies

Understanding the reproductive adaptations of Ae. aegypti can inform more effective vector control strategies. For example, targeting the male reproductive gland proteins that influence female post-mating behavior could reduce mosquito populations by disrupting their reproductive success (Sirot et al., 2008). Additionally, manipulating the genes involved in egg retention could prevent mosquitoes from exploiting unpredictable habitats, thereby reducing their population in adverse environmental conditions (Venkataraman et al., 2023). Moreover, strategies that interfere with the mating-induced transcriptome changes in females could impair their ability to reproduce and transmit diseases (Alfonso-Parra et al., 2016). Finally, considering the impact of environmental adaptation on mosquito fitness and persistence can help predict disease risk and improve the effectiveness of population control measures (Bennett et al., 2021).

## 8 Impact of Environmental Factors on Reproductive Systems

### 8.1 Effects of temperature and humidity

Temperature and humidity significantly influence the reproductive activity and survival of mosquitoes. For instance, Aedes aegypti females exhibit reduced egg production and altered oviposition patterns with increasing temperatures. At 25°C and 80% relative humidity, females produced 40% more eggs and had double the survival rate compared to those at 35°C and 80% relative humidity. Additionally, at 35°C and 60% relative humidity, oviposition was inhibited in 45% of females, and only 15% laid more than 100 eggs, indicating that the intensity of temperature effects is modulated by humidity levels (Almeida et al, 2010; De Almeida Costa et al., 2010). Similarly, the development and survival of Anopheles mosquitoes are temperature-dependent, with higher temperatures reducing longevity, body size, and fecundity (Agyekum et al., 2021). Humidity also plays a crucial role, often overlooked, in shaping the thermal performance of mosquito-borne pathogen transmission, affecting mosquito fitness and population dynamics (Brown et al., 2023).

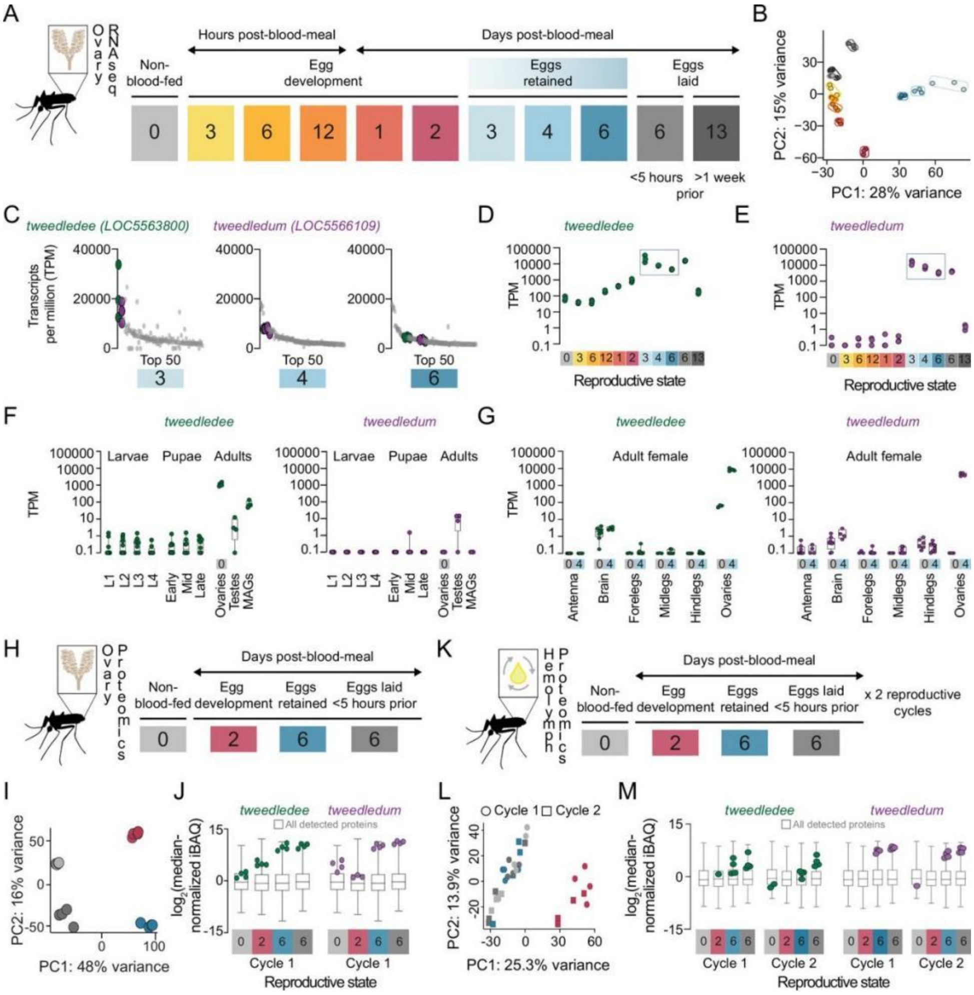

Figure 3 tweedledee and tweedledum are ovary-enriched and strongly upregulated during egg retention (Adopted from Venkataraman et al., 2023)

Image caption: (A) Reproductive time-points sampled for bulk ovary RNA-sequencing (RNA-seq), n=3 replicates/group, 11 groups. (B) Principal component analysis (PCA) of DESeq2-normalized, transformed counts from ovary RNA-seq. (C) Top 50 most abundant transcripts ranked by median transcripts per million (TPM) for egg retention groups, 3-, 4-, and 6 days post-blood-meal (total=150 transcripts). Gray dots represent replicates for each transcript in the top 50, green dots indicate tweedledee, and purple dots represent tweedledum. (D-E) Transcript expression pattern in the ovaries of tweedledee (D) and tweedledum (E). The blue rectangle indicates the period of egg retention. (F) TPM values for tweedledee (left) and tweedledum (right) during larval, pupal, and adult stages of development [data from Matthews et al., 2018], MAGs = male accessory glands, n=4-13 replicates/group. (G) TPM values for tweedledee (left) and tweedledum (right) in adult female tissues (data originally from Matthews et al., 2016), reanalyzed in Matthews et al., 2018, n=3-8 replicates/group. (H) Reproductive time-points sampled for ovary proteomics, n=4 replicates/group, 4 groups. (I) PCA of iBAQ values from ovary proteomics. (J) Distribution of iBAQ values as a metric of abundance for all proteins detected per group in ovary proteomics. Overlaid green dots represent individual replicate values for tweedledee and purple dots represent replicates for tweedledum. All values are pre-imputation and represent log2-transformed median iBAQ signals normalized by subtracting the median iBAQ signal for the group. (K) Reproductive time-points sampled for hemolymph proteomics, n=4 replicates/group, 8 groups. (L) PCA of iBAQ values from hemolymph proteomics. (M) Distribution of iBAQ values as a metric of abundance for all proteins detected per group in hemolymph proteomics. Overlaid green dots represent individual replicate values for tweedledee and purple dots represent replicates for tweedledum. All values are pre-imputation and represent log2-transformed median iBAQ signals normalized by subtracting the median iBAQ signal for the group. Box plots in F, G, J, M: median, 1st/3rd quartile, minimum to maximum whiskers (Adopted from Venkataraman et al., 2023)

### 8.2 Influence of nutritional status on reproductive output

Nutritional status, particularly sugar intake, interacts with temperature to influence mosquito reproduction. In Culex pipiens, a diet of 20% sucrose at higher temperatures (30°C) increased egg production, whereas a 2% sucrose diet under the same conditions decreased fecundity. This suggests that higher temperatures necessitate greater nutritional investment for reproductive success (Ferguson et al., 2019). Furthermore, the diet of adult male mosquitoes also impacts reproductive physiology. Males on a low (3%) sucrose diet had significantly smaller male accessory glands and produced fewer larvae when mated with females, compared to those on higher sucrose diets (10% and 20%) (Huck et al., 2021). These findings highlight the critical role of nutrition in mosquito reproductive output and the potential for dietary interventions to control mosquito populations.

### 8.3 Role of seasonal variations in reproductive cycles

Seasonal variations, particularly changes in temperature, significantly affect mosquito reproductive cycles. For example, in temperate regions, seasonal temperature fluctuations influence the population dynamics of mosquitoes, with warmer temperatures leading to increased peak vector abundance and potentially higher disease transmission risks (Ewing et al., 2016). Additionally, the ontogenetic timings and synchrony of pupation in various mosquito species are affected by environmental factors such as food quantity, larval density, and salinity, which vary seasonally. These factors can delay pupation and prolong the pupal stage, ultimately affecting the timing and success of adult emergence (Nayar and Sauerman, 1970). Seasonal changes in temperature also impact the life-history traits of Anopheles mosquitoes, with higher temperatures reducing the length of the gonotrophic cycle and fecundity, thereby influencing seasonal population densities and disease transmission potential (Agyekum et al., 2021).

## 9 Molecular and Hormonal Regulation of Reproduction

### 9.1 Key hormones in mosquito reproductive physiology

Juvenile hormone (JH) and 20-hydroxyecdysone (20E) are pivotal in regulating mosquito reproduction. JH is essential for post-eclosion development and reproductive maturation in female mosquitoes, influencing gene expression in the fat body, an organ analogous to the vertebrate liver (Zhu et al., 2010; Chang et al., 2020). JH, through its receptor Methoprene-tolerant (Met), regulates the expression of numerous genes, including those involved in ribosomal biogenesis and vitellogenesis, which are crucial for egg development (Wang et al., 2017). Additionally, 20E, in conjunction with its receptor EcR, plays a significant role in the reproductive cycle, particularly in the regulation of gene expression during the blood meal digestion and egg development phases (Roy et al., 2015; Ling and Raikhel, 2021).

### 9.2 Gene expression profiles during reproductive stages

Gene expression in mosquitoes is highly dynamic and temporally regulated during the reproductive cycle. In Aedes aegypti, approximately 7,500 transcripts are differentially expressed in four sequential waves during the 72-hour reproductive period post-blood meal. The first wave, regulated by amino acids, occurs between 3 and 12 hours post-blood meal (PBM). The second wave, between 12 and 36 hours PBM, is upregulated by a synergistic action of amino acids, 20E, and EcR. The third wave, occurring between 36 and 48 hours PBM, is primarily regulated by HR3. The final wave, between 48 and 72 hours PBM, is controlled by JH and its receptor Met (Roy et al., 2015). This temporal coordination ensures the proper progression of vitellogenesis and the remodeling of the fat body, essential for successful reproduction (Zou et al., 2013; Saha et al., 2019).

### 9.3 Advances in molecular tools for studying mosquito reproduction

Recent advancements in molecular tools have significantly enhanced our understanding of mosquito reproductive physiology. Techniques such as RNA interference (RNAi), CRISPR gene editing, and transcriptomic analyses have been instrumental in elucidating the roles of key hormones and their receptors. For instance, RNAi has been used to knock down the expression of Met, revealing its critical role in regulating gene expression during post-eclosion development (Zhu et al., 2010; Zou et al., 2013). CRISPR gene-tagging experiments have demonstrated the coordination between JH, 20E, and insulin-like peptides (ILPs) in regulating metabolism during the reproductive cycle (Ling and Raikhel, 2021). Additionally, transcriptomic analyses have identified numerous genes regulated by JH and 20E, providing insights into the complex regulatory networks governing mosquito reproduction (Roy et al., 2015; Wang et al., 2017).

## 10 Conclusion and Future Directions

The comparative study of mosquito reproductive systems has yielded significant insights into the physiological and molecular mechanisms underlying mosquito reproduction. Research has highlighted the variability in male mating success within swarming systems, driven by both scramble competition and female choice, which is crucial for the implementation of reproductive control tools. Additionally, the analysis of ovary-specific genes in both autogenous and anautogenous mosquitoes has provided a deeper understanding of ovarian development and potential intervention points for regulating egg production. The comprehensive cataloging of sperm and seminal fluid proteins in Aedes aegypti has expanded our knowledge of male reproductive biology and identified potential molecular targets for population control. Furthermore, the role of long non-coding RNAs (lncRNAs) in modulating reproductive ability in Aedes albopictus opens new avenues for pest management. Studies on the nutritional impacts on male reproductive physiology have also shown that diet significantly affects male accessory glands and female fecundity.

Despite these advancements, several gaps remain in our understanding of mosquito reproductive biology. The molecular mechanisms of seminal fluid and sperm function are still not fully understood, necessitating further research to identify and characterize the functional roles of these proteins. Additionally, the role of lncRNAs in mosquito reproduction is a relatively new field, and more studies are needed to explore their potential as targets for pest management. The impact of nutrition on male reproductive physiology and its subsequent effects on female fecundity also requires more detailed investigation. Emerging technologies such as RNA interference (RNAi) and CRISPR/Cas9 gene editing hold promise for addressing these gaps by enabling precise manipulation of gene expression and function in mosquitoes.

The insights gained from comparative studies of mosquito reproductive systems can be leveraged to develop innovative mosquito management and control strategies. For instance, understanding the factors influencing male mating success can enhance the effectiveness of sterile insect techniques and Wolbachia-based control methods. The identification of key genes and proteins involved in ovarian development and sperm function provides potential targets for genetic and chemical interventions aimed at reducing mosquito fertility. The discovery of lncRNAs that regulate reproductive ability offers a novel approach for disrupting mosquito reproduction and reducing population sizes. Additionally, exploiting the chemical ecology of mosquito oviposition behavior can lead to the development of more effective surveillance and control tools, such as oviposition traps and infochemical-based attractants and deterrents.

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## Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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