Trends in Parasitology

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Review

Mosquito Host-Seeking Regulation: Targets for Behavioral Control

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Female *Aedes aegypti* mosquitoes require protein from blood to develop eggs. They have evolved a strong innate drive to find and bite humans and engorge on their blood. Decades of research have revealed that attraction to hosts is suppressed for days after blood-feeding. During this time, females coordinate complex physiological changes, allowing them to utilize blood protein to develop eggs: clearing excess fluid, digesting protein, and egg maturation. How do mechanosensation, nutrient consumption, and reproductive pathways combine to produce the full expression of host-seeking suppression? Understanding mechanisms of endogenous host-seeking suppression may allow them to be 'weaponized' against mosquitoes through exogenous activation and developed as tools for vector control. Recent work allows unprecedented genetic and pharmacological access to characterize and disrupt this behavioral cycle.

Highlights

Female *A. aegypti* mosquitoes bite vertebrate hosts to obtain blood protein necessary to develop their eggs. Following a complete blood meal, female mosquitoes undergo dramatic suppression of their attraction to find and bite human hosts.

Host-seeking suppression lasts for several days, until the female lays her eggs.

Host-seeking suppression requires integration of organismal physiology and is regulated by multiple signaling pathways including: mechanosensation, fluid regulation, nutrient processing, and reproductive pathways.

Recent genetic and pharmacological advances in the study of mosquitoes allow for specific manipulation of these pathways to tease apart their functions.

Regulators of endogenous host-seeking represent potential targets for behavior-based vector-control strategies.

Blood-Feeding and Endogenous Regulation of Mosquito Attraction to Hosts

Mosquitoes are the most deadly animals on the planet, and the vector-borne diseases that they carry pose increasing global public health threats [1]. Some species, including *Aedes aegypti*, a vector for Zika, dengue, chikungunya, and yellow fever, are notoriously dangerous because of their preference for biting humans as opposed to other vertebrate hosts [2–4]. Using host-associated cues, including carbon dioxide [5,6] and volatile odorants [7], mosquitoes search for vertebrate hosts to bite as the source of a blood meal. Although feeding on vertebrate blood is risky, due to host defensive behaviors, **hematophagy** (see Glossary) has evolved multiple times and is thought to be beneficial because blood is a uniquely nutrient-rich source of protein [8]. One common strategy to minimize the risk associated with blood-feeding from a host is to ingest a large amount of protein in a single meal. Blood-feeding is relatively rapid and the mosquito can consume enough blood to double her body weight within several minutes [9]. She then utilizes blood protein to develop 100–150 eggs, which she will be ready to deposit after about 4 days [10]. If there are no available sites for oviposition, she will retain her eggs and maintain host-seeking suppression until she finds an acceptable egg-laying site. In the days that follow a complete meal of blood the female dramatically changes her behavior and almost completely suppresses her drive to bite (Figure 1). This period of host-seeking suppression does not represent a general inhibition of her chemosensory responses [11–13]; instead, she turns her attention to cues, like humidity, associated with finding a favorable site to lay her eggs [14]. After laying her eggs, the cycle starts again as the female returns to the hunt for her next host to provide the blood meal required for her next clutch of eggs. The cycle of blood-feeding and egg-laying, termed the **gonotrophic cycle**, repeats throughout her lifetime.

During the period of suppression, the female undergoes a series of physiological changes, including: extreme abdominal distension, fluid and waste processing, protein digestion and utilization, and egg development (Box 1; Figure 2, Key Figure). Although previous work has described each of these components, the underlying mechanisms are poorly understood. How do the individual elements combine to create the full expression of host-seeking suppression?

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What are the anatomical circuits that underlie this suppression? How are chemosensory circuits modulated to affect behavior? What are the molecular or hormonal pathways that trigger each of these behaviors?

The development of sophisticated genetic tools to study mosquito species allows unprecedented access to investigate the changes in endogenous physiology that regulate host-seeking and to manipulate the underlying molecular and cellular effectors [6,7,15]. These manipulations include targeted mutations that disrupt specific genes as well as access to label and influence defined groups of cells.

Moreover, understanding endogenous regulation will identify new molecular targets to directly influence mosquito behavior leading to new approaches for vector control. Recent work has generated comprehensive maps of active **neuropeptide** ligands and their cognate receptors as well as small molecules that can interfere with host-seeking pathways [10,16–19]. This progress in mosquito physiology is a first step towards the development of novel vector-control methods. Equipped with these new biological tools, the field is primed to discover mechanisms that reliably and flexibly change mosquito attraction to humans and to find new ways to disrupt these pathways. Here, we review recent studies of mosquito host-seeking regulation and identify potential targets for triggering host-seeking suppression by artificial means.

Abdominal Distension and Mechanosensation Suppress Host-Seeking after Engorgement

When a female *A. aegypti* mosquito is allowed to blood-feed to repletion she will reliably consume enough blood to double her body weight [20]. Stretch receptors in the abdomen that sense distension likely provide the signal for the female to terminate her meal; after severing the ventral nerve cord females imbibed larger volumes of blood, and the more anterior the cut the larger the volume consumed [21]. Classic work has shown that foregut distension terminates feeding in other species, including the blowfly [22]. Abdominal distention serves as a signal to terminate feeding as well as a mediator of short-term host-seeking suppression. Females who consumed large (>3 μl) meals suppress their attraction to hosts independently of the protein content of the meal, and even air inflation temporarily suppresses host-seeking [23] (Figure 1). The anterior abdominal segments are also likely to mediate the host-seeking suppression signal as well. When females were allowed to feed to repletion, after being fitted with wax girdles to prevent distension, only those females who experienced distension on the anterior portion of the abdomen suppressed their attraction to hosts [23]. These findings indicate that receptors within the first three anterior abdominal segments mediate short-term host-seeking suppression through **mechanosensation**.

These results suggest that stretch receptors, either in the gut or along the abdominal wall, mediate mechanosensitive components of host-seeking suppression. Because short-term suppression can be induced by either air or saline-induced distension, neither of which contain the protein required for egg development, it does not require either protein sensing or egg development signaling.

What are the potential insect mechanoreceptors that could mediate this stretch response? The recent publication of an improved *A. aegypti* reference genome provides comprehensive access to identify candidate mechanoreceptors [24]. There are mosquito orthologs of sensors that have been implicated in mechanosensation including **piezo-like channels** [25,26] and **transient receptor potential (TRP) channels** [27]. Neurotranscriptome data report that mechanosensors, including **pickpocket channels** (which are the insect family of DEG/ENaC channels) [28], are

Glossary

Allatostatins: a group of neuropeptides found in insects that have been shown to suppress food intake.
Arboviruses: viruses that are transmitted to humans by the bites of mosquitoes, ticks, or other arthropods.
Diuresis: urine production, the process by which unwanted substances are excreted via fluid waste.
Fat body: the main organ of intermediary metabolism that serves the storage of fat, glycogen, and protein.
Gonotrophic cycle: a life cycle of feeding and laying eggs; in mosquitoes this refers to the sequence of host-seeking, blood-feeding, egg development, and oviposition.
Hematophagy: the practice of feeding upon blood.
Hemolymph: fluid equivalent to blood in invertebrates that circulates throughout the organism.
Malpighian tubules (MTs): excretory and osmoregulatory system in insects, similar to the kidneys in mammals.
Mechanosensation: the transduction of mechanical force, including stretch and touch, into neuronal activity.
Natriuretic: peptides that promote the process of salt excretion in urine.
Neuropeptides: small amino-acid-based signaling molecules that are produced and released by neurons through the regulated secretory route and act on neural substrates.
Neuropeptide Y: a neuropeptide signaling pathway that plays conserved roles in motivated feeding and satiety behavior.
Pickpocket channels: a family of nonvoltage-gated, amiloride-sensitive cation channels that play diverse roles in salt processing, fluid regulation, mechanosensation, and chemosensation.
Piezo-like channels: a family of excitatory ion channels that are gated by mechanical force.
Postprandial: occurring after a meal.
Transient receptor potential (TRP) channels: a group of ion channels that transduce a variety of sensations such as taste, pain, temperature, mechanosensation, and vision.
Vitellogenesis: the process of yolk formation through nutrient delivery to a developing oocyte.

found in many tissues (although the gut was not sampled in these experiments). Although the identity of the relevant receptor(s), and whether the same receptors regulate meal termination and short-term host-seeking suppression, remains unknown, with recent advances in genetic manipulations of *A. aegypti* mosquitoes the field is now primed to perform small-scale genetic screens to identify the biologically relevant mechanoreceptors.

Processing the Blood Meal: Clearing Excess Fluid and Processing Toxic Compounds

After a blood meal, abdominal distention dissipates over the course of about 1 day [10,29] as the female clears excess fluid, salt, and waste. Diuretic hormones and serotonin mediate fluid processing that allows the female to clear excess liquid [30]. Blood-feeding imposes unique physiological constraints for rapid elimination due to the large size of the meal and the toxic compounds, including heme and ammonia, found in blood [31,32]. The excretory system of mosquitoes consists of the **Malpighian tubules** (MTs) and the hindgut, and is controlled by neuroendocrine factors. **Postprandial diuresis** requires dynamic changes in the function of the MTs and hindgut. Within the first 2 h after feeding, about 40% of the fluid from the blood meal is eliminated [33].

Similar to many other organisms, mosquitoes use a variety of neuropeptides and hormones to regulate fluid and salt excretion. Mosquito **natriuretic** peptide acts via cAMP to stimulate secretion of sodium-rich urine [34], and additional diuretic hormone (DH) signaling ligands and receptors have been implicated in blood-meal processing. Levels of DH44 receptor transcript fluctuate in the MTs during periods that correlate with physiological demand for urination [35]. The leucokinin receptor is also found in digestion- and fluid-processing-relevant tissues, and RNAi-based knockdown of the leucokinin receptor reduces excretion rates [36]. The mechanism of diuresis action is through ion channels; Kir potassium channels are required for one of the major routes of potassium ion uptake. These ion channels are targets for vector control, and recent work shows that a small-molecule inhibitor of *A. aegypti* Kir1, VU573, disrupts fluid processing and leads to renal failure and death [16].

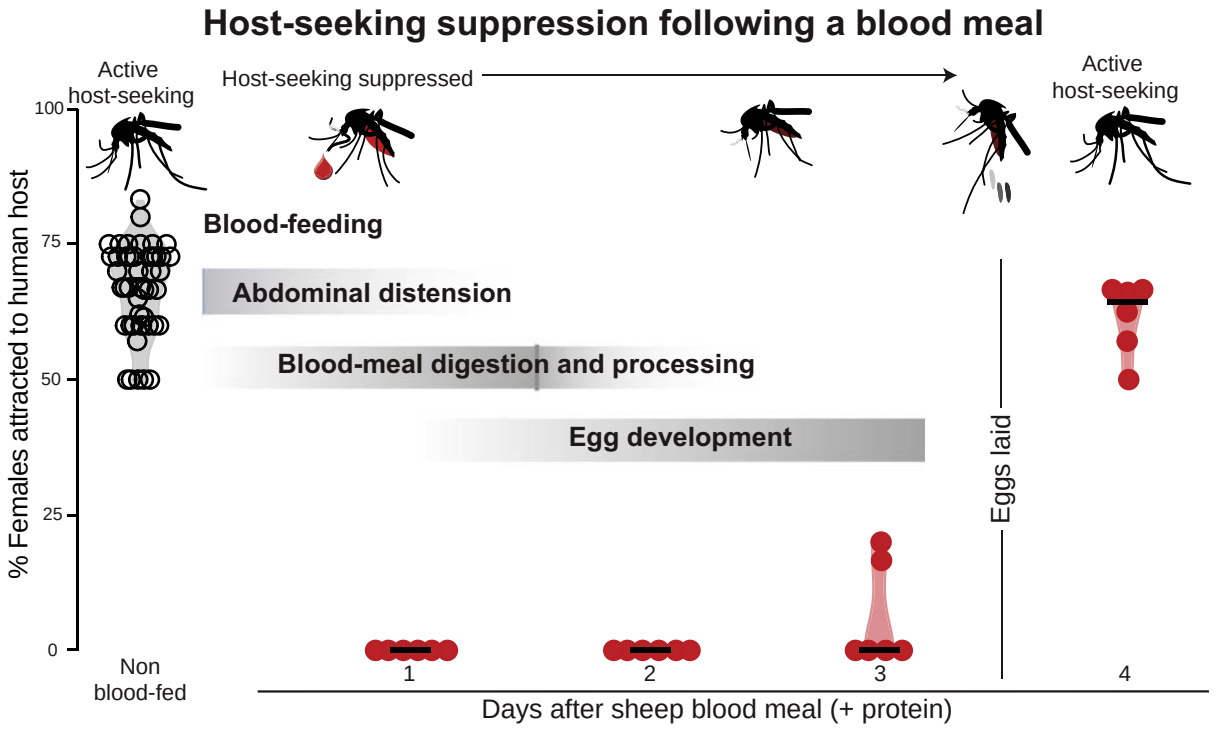
Diuretic hormones clearly play a key role in fluid processing but their mechanisms of action and the extent to which they interact with reproductive pathways remain unclear. Ovarian ecdysteroidogenic hormone (OEH) influences excretion – OEH affects excretion processes 26–48 h after it is released [37]. It is likely that the demands for urination and waste processing change during different stages of blood-meal processing and that distinct signaling pathways mediate early- and late-stage excretion.

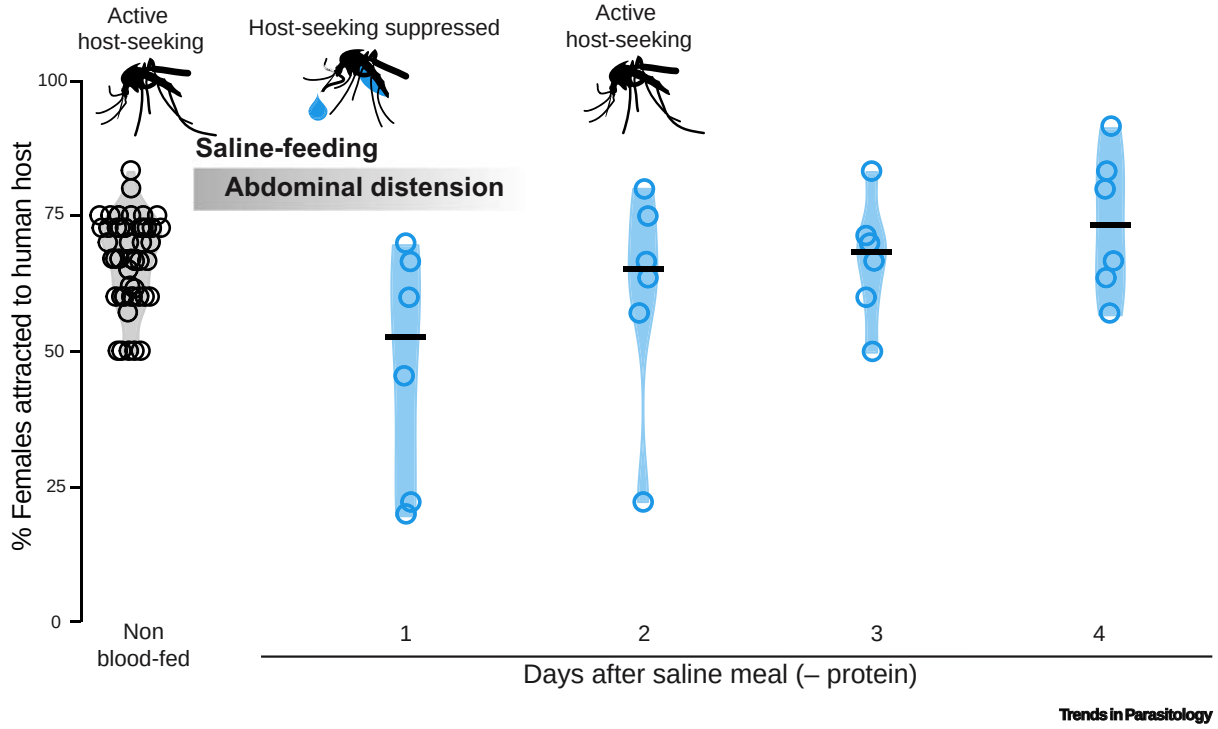
The receptors that mediate fluid processing and diuresis could also be exploited to prevent the spread of disease. Females unable to process the blood meal will fail to develop eggs, and delayed blood-meal processing will likely maintain host-seeking suppression for a longer duration, although this will require direct experimental validation.

Satiety-Related Signaling: Protein and Nutrient Sensing

Components of host-seeking suppression are related to conserved neuropeptide pathways that regulate feeding and satiety. Females utilize the protein and other nutrients in blood for many physiological processes, including egg development [38]. Protein detection and processing influence host-seeking behavior, and artificial meals that consist of only three human blood proteins in saline are sufficient for egg production and host-seeking suppression [10].

Blood-meal digestion consists of early and late phases [39] that are differentially controlled [40]. Early digestion occurs within hours after feeding and is initiated after amino acids are detected





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Box 1. Physiological Changes after Blood Feeding

- Abdominal distension from a large volume of ingested blood.
- Early digestion is initiated when amino acids are detected in the midgut.
- Excess fluids are excreted and toxins are removed via Malpighian tubules through the actions of diuretic hormones and kinins.
- Protein is detected and activates satiety-related neuropeptide pathways that suppress host-seeking as well as mTOR pathways that promote egg development.
- ILPs are secreted from the brain to promote late-stage digestion.
- OEH is released from the brain and signals to the ovaries to release ecdysone.
- Fat body transforms ecdysone into 20E which is released into the hemolymph to signal to the ovaries.
- Juvenile hormone levels are suppressed after blood feeding but later reactivated and signal to the ovaries to complete vitellogenesis.
- Yolk precursor proteins are transported from the fat body to the ovaries.
- After about 4 days, the female lays her eggs, re-enters the next gonotrophic cycle, and searches for her next host as the cycle starts again.

in the midgut [41]. Late-stage processing occurs between 12 and 30 h after feeding; most nutrients are released from the gut during this later period [42–44]. Late-stage digestion requires insulin-like peptides (ILPs) to be released from the brain [39].

The **fat body**, which is the main organ of intermediate metabolism and is similar to the mammalian liver, acts directly as a nutrient sensor [45]. Fat body cells not only control the synthesis and utilization of energy reserves – fat and glycogen – but also synthesize most of the **hemolymph** proteins and circulating metabolites. Vitellogenins for egg maturation are secreted by the fat body and are transported to the ovaries [46].

Previous studies showed that injection of hemolymph from a suppressed female (2 days after blood-feeding) is capable of suppressing host attraction in nonblood-fed females who normally show high levels of attraction to hosts [47]. These findings suggested the presence of a circulating factor that is elevated 48 h after a blood meal and suppresses host-seeking. Subsequent studies have shown that direct injection of specific signaling peptides can also suppress host-seeking in nonblood-fed females. These peptides include Head Peptide-I, **allatostatin**-5, short neuropeptide F2, and short neuropeptide F3, although millimolar concentrations are required for this behavioral effect, suggesting that these effects may be nonspecific, and the site and timing of endogenous release remain unknown [48,49]. All of these peptides activate receptors that belong to the family of **neuropeptide Y**-related receptors. Neuropeptide Y signaling plays deeply conserved roles in motivated feeding behavior and satiety in animals ranging from nematodes to humans [50–57].

A. aegypti NPY-like receptor 7 (NPYLR7) is a key mediator of host-seeking suppression after a blood meal in mosquitoes. *In vitro,* NPYLR7 responds to nine *A. aegypti* neuropeptides, including many of the peptides shown to suppress host-seeking when directly injected [48,58,59]; but the identity of the physiologically relevant signaling peptide(s) and their time course of activity remains unknown.

NPYLR7 mutant females retain some residual host-seeking suppression after a blood meal, suggesting that other receptors or pathways play a role in the full expression of host-seeking suppression. Interestingly, *NPYLR7* mutants show deficits in late-stage blood-meal digestion,

Figure 1. Temporal Measurements of Host Attraction Following Blood or Saline Meals. Mosquito attraction to host cues measured in a uniport olfactometer after protein-rich blood meals that are capable of supporting egg production (top) and protein-free saline meals that are not (bottom) (adapted from Duvall *et al*. [10]). Although protein-free saline meals induce abdominal distension and short-term suppression, only protein-rich meals induce the full expression of host-seeking suppression. Median with range: n = 6–48, 15–25 females/trial.

Key Figure

Tissues and Signaling Pathways Implicated in Host-Seeking Suppression Following a Blood Meal

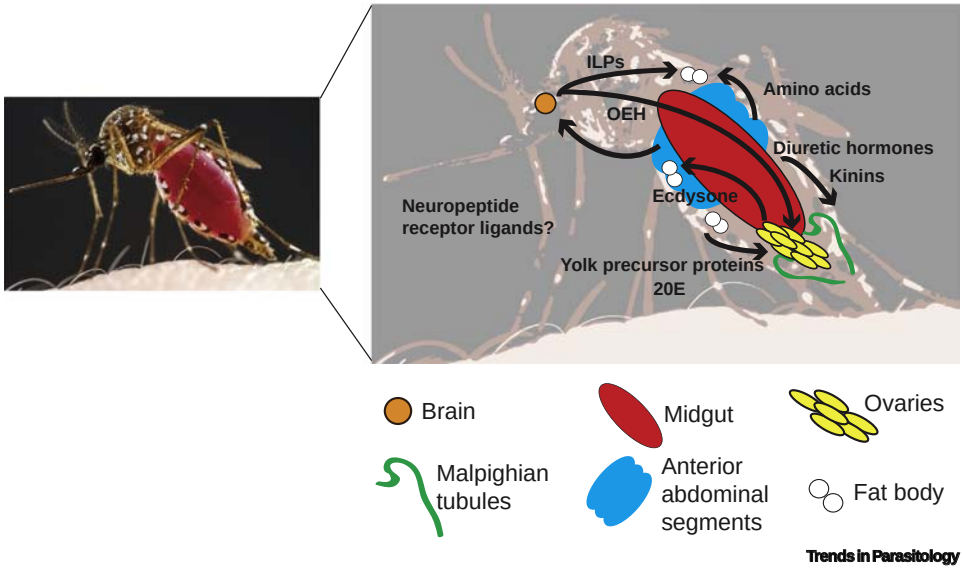

Figure 2. Tissues and organs involved in blood-meal processing and host-seeking suppression after blood feeding (photo credit: Alex Wild). Signaling molecules and key tissues are shown, and arrows indicate transport and cross-tissue signaling. Signals from the gut, brain, fat body, and female reproductive system coordinate to produce host-seeking suppression following a blood meal. Abbreviations: ILPs, insulin-like peptides; OEH, ovarian ecdysteroidogenic hormone.

suggesting that this pathway may overlap with digestion signaling pathways, including the ILP signaling implicated in late-stage digestion [10,39]. Christ *et al.* found that levels of both short neuropeptide F-2 and allatostatin-5 change in the antennal lobe after a blood meal and that, while injection of each peptide individually only partially suppressed host-seeking, coinjection resulted in levels of suppression similar to those seen in blood-fed females [49]. Allatostatin pathways are also implicated in regulation of feeding behavior [60]; in *Drosophila,* activation of allatostatin A-expressing cells suppresses feeding [61]. These findings suggest that multiple satiety-related neuropeptide pathways may coordinate their actions after blood-feeding to suppress host-seeking.

Small-molecule activators of NPYLR7 block host-seeking and biting for up to 2 days *in vivo* in laboratory strains after being delivered via artificial membrane feeder – suggesting that these could be deployed as pharmacological vector-control tools delivered via membrane feeders housed in human-mimic traps [10]. Although these initial studies hold promise, ongoing work to improve the stability and pharmacokinetics of these drugs will be required before they are feasible for field deployment to induce longer-term suppression of host-seeking. Additionally, it is also possible to screen for agonists of other neuropeptide receptors, including the allatostatin receptors, to find additional drugs that could block mosquito attraction to humans. Future work to determine whether or not these drugs bind to the same site as the endogenous ligands will also be required to evaluate whether or not these exogenous activators compete directly with endogenous peptides and if selective pressure will result in mutations of the same receptor site that may allow mosquito populations to develop resistance to these approaches.

Egg Development and Reproductive Signaling

Ostensibly, a primary goal of host-seeking suppression is to redirect the female's attention away from irrelevant host-associated cues so that she can attend to cues, like humidity, that signal the presence of an appropriate site to lay eggs.

The blood obtained from successful host-seeking and subsequent biting is critically important for egg development, and nutrient and reproductive pathways are integrated early: amino acid signaling through the target of rapamycin (TOR) activates egg development, and TOR activity increases dramatically in both the ovary and the fat body after blood-feeding [62,63]. Another key physiological event for reproduction is **vitellogenesis**, the production and secretion of yolk precursor proteins in the fat body and their accumulation by developing oocytes. Juvenile hormone (JH), which plays both developmental and reproductive roles, is a key regulator of ovarian follicle development that undergoes dynamic changes after blood-feeding [64] and incorporates signaling from the previtellogenic nutritional state with the reproductive state [65,66]. After a blood meal, JH synthesis is initially suppressed, but this is followed by a reactivation that is required for the completion of vitellogenesis [67].

Earlier work suggests that both physical and chemical stimuli from blood-feeding are required for the promotion of egg development. Small (1 µl) blood meals did not result in egg development in most females, but if this volume was increased with water, more females developed eggs [29]. Digestion products from the blood meal provide raw materials and serve as the principal stimulus for egg development. These signals may be released from midgut secretory cells in response to either components of the blood meal or from stretching of the gut.

Blood-feeding initiates the release of OEH [68] and ILPs, including ILP3 and 4, from the brain [69,70]. Within hours of feeding, these signals reach the ovaries to initiate ecdysone secretion. Ecdysone is then processed into the active signaling molecule, 20-hydroxyecdysone (20E), in the fat body (Figure 2). In *A. aegypti* there is a peak of 20E detected in the hemolymph 4–6 h after the blood meal followed by another, larger peak 12–30 h after feeding [71]. However, because the titer of 20E is very low at this point, this implicates another factor that suppresses host-seeking after hour 30 [72].These factors have been hypothesized to be neuropeptide ligand(s) that regulate satiety through NPY-like receptors (see previous section) [73].

Direct injection of 20E can also delay blood meal processing – even in ovarectomized females – suggesting that reproductive and meal processing pathways interact. Recent work suggests that 20E and TOR pathways coregulate miRNA signaling that controls nutrient utilization and egg development via ILPs [74,75]. Although the effects of manipulating ILPs or miRNAs on host-seeking were not assayed in these experiments, in 1979 Beach suggested that direct injection or feeding of 20E can inhibit host-seeking in *Anopheles freeborni* [76]. However, other groups claim that 20E does not specifically affect host-seeking in *A. aegypti* [77] and the observed effects on host-seeking were nonspecific.

The blood meal activates numerous processes and genes in reproductive organs and tissues leading to rapid egg development [28]. These signaling cascades are important for yolk protein synthesis and ovarian development during the period of host-seeking suppression. Exogenous activation of the 20E pathway, using a nonsteroidal agonist in *Anopheles gambiae* mosquitoes, substantially interferes with egg production, mating, *Plasmodium* development, and survival, suggesting that manipulation of 20E signaling can be exploited to disrupt the mosquito behavioral cycle [78]. Recent work suggests that vitellogenin gene expressed in the fat body regulates host-seeking behavior in *Aedes albopictus* [79].

Behavioral Vector Control: Considerations and Potential Field Applications

Although methods of mosquito control that rely on lethal approaches have been successful, populations of mosquitoes have become increasingly resistant to these methods. Resistance emerges in populations of mosquitoes due to strong selective pressures that select for rare individuals that can escape lethality [80]. Nonlethal behavioral approaches represent new approaches that are likely to exert weaker selection pressures and can provide complementary vector-control approaches. Examples of nonlethal approaches include the use of pheromone dispensers to control European grapevine moth populations by disrupting mating [81]. The increasing burden of vector-borne disease highlights the need for novel, complementary approaches to vector control.

Understanding the endogenous pathways that suppress host-seeking after a blood meal will uncover new strategies to exogenously activate them and thereby induce suppression in nonblood-fed animals. Although laboratory studies primarily focus on uniformly reared animals allowed uninterrupted access to a full meal of blood, there is evidence that females in the field feed on multiple hosts within the same egg-laying cycle due to host defensive behaviors, interrupted feeding, or to compensate for poor larval nutrition [82,83]. Using DNA fingerprinting to identify the sources of blood meals taken by mosquitoes in rural Thailand, previous work estimated that 43–46% of females bit more than one human within the same cycle [84]. There is, however, regional and location variation in feeding dynamics depending upon environmental factors and host availability – another study suggests that 10% of blood meals detected in *A. aegypti* females collected in Puerto Rico contained blood from more than one person [85], and multiple feeding rates differ between mosquitoes collected in Puerto Rico versus those collected in Thailand [86]. The variation in blood-meal sizes taken by wild mosquitoes almost certainly leads to variability in amplitude and/or duration of host-seeking suppression [83,87]. Human-mimic traps show high variability in their capability to attract blood-fed females depending on location and species. While some studies find blood-fed females in host-mimic traps [88,89], others find no blood-fed females [90], suggesting that, in the field, blood-fed females still show some level of suppression of their attraction to host-associated cues. Despite the variation on host-seeking, the length of the gonotrophic cycle is 3–4 days in the field in both the rainy and dry seasons, indicating that egg development timing is relatively stable [91].

Despite variable blood-feeding and host-seeking suppression observed in the field, exogenous activation of pathways that regulate host-seeking may still suppress biting by hijacking endogenous suppression pathways through pharmacological manipulations. Compounds could be delivered to host-seeking females in saline meals using artificial membrane feeders baited with host-associated cues, or more generally, using attractive sugar baits [92]. Even if these manipulations wear off after several days, this would diminish the vectorial capacity of mosquitoes by reducing the number of potential hosts that they come into contact with by reducing their active biting time. It will be important to model and characterize the efficacy of these interventions in the field where environmental, population dynamics, and mosquito-control techniques affect disease transmission [93].

Recent work has shown that, in *Anopheles* mosquitoes, *Plasmodium falciparum,* the causative agent of malaria, can respond to metabolic changes in the mosquito and that impairing oogenesis by manipulating 20E decreases parasite intensities but increases parasite growth rates, allowing them to become infectious earlier [94]. These findings suggest that disruptions of the metabolic and reproductive pathways in the mosquito may alter pathogen infection. Understanding the interplay between blood-feeding, reproductive physiology, and infectiveness is a key area of future research with important implications for a broad set of mosquito-control strategies.

Concluding Remarks

Host-seeking suppression after a blood meal represents a binary behavioral switch and provides insights into how the nervous system flexibly manipulates innate behavior. During the period of host-seeking suppression females must regulate many components of organismal physiology, including fluid processing, nutrient sensing, and reproduction.

Improved genetic access in mosquitoes now allows for manipulations of specific signaling components to characterize their contributions to the full expression of host-seeking behavior. Using genome-editing tools it is now feasible to perform small-scale genetic screens to identify the biologically relevant receptors and ligands that mediate host-seeking suppression. Are these pathways activated in parallel or are there important interactions between them? Because of the overlapping nature of each of these components it is difficult to distinguish the onset/offset of different components, but new genetic and pharmacological tools allow more precise manipulation of individual targets. Using tools, like optogenetics, to in/activate specific groups of cells with temporal precision, we can now test their contributions to the behavior and determine whether components are necessary or sufficient to produce the full expression of host-seeking suppression.

Anatomical labeling of molecularly defined groups of cells will provide access to the anatomical substrates of host-seeking regulation. It is likely that signals from the gut, ventral nerve cord, brain, fat body, and female reproductive system are required, but further work is needed to understand where the biologically relevant ligands are released and where their receptors are expressed.

Understanding the regulation of host-seeking behavior is increasingly important because both *A. aegypti* and *A. albopictus* are expected to spread throughout temperate regions, including North American and Europe, within the next 5–15 years by expanding into their anthropogenic niche. Predictions now place half of the world's population at risk for an **arbovirus** by 2050 [95]. *A. aegypti* already shows high levels of resistance to insecticides in many parts of the world, highlighting the need for new and complementary strategies for vector control [96,97].

The field of mosquito neurogenetics is also poised to make important discoveries about the chemosensory systems that underlie these dramatic behavioral changes (see Outstanding Questions). How are these pathways regulating the female's behavior? Are they directly acting on peripheral chemosensors or are there central changes that alter the innate drive to blood-feed? Behavioral responses to human host-associated cues are reduced, and previous studies have suggested that peripheral sensitivity to carbon dioxide and lactic acid [12] are reduced but that sensitivity to compounds that may be associated with egg-laying sites is increased [13]. On one extreme, it is possible that peripheral sensitivity is directly downregulated after blood-feeding, and previous studies in mosquitoes suggest that many odorant receptors are transcriptionally downregulated in the antenna after blood-feeding [98,99]. Alternatively, central regulation may change the mosquito's motivation or drive to host-seek versus her drive to attend to cues associated with egg-laying.

Understanding the biology of anthropogenic specialists will provide insight into general mechanisms of behavioral regulation and add to the arsenal of targets for behavior-based vector-control strategies. The best targets will be pharmacologically accessible and will ultimately reduce the vectorial capacity of the female by impairing her drive to find and bite humans.

Outstanding Questions

Host-seeking suppression is coordinated with dramatic changes in organismal physiology, feeding, and reproduction; how do the individual components of host-seeking suppression combine to produce the full expression?

What are the anatomical circuits that suppress host-seeking in the brain, gut, and female reproductive system?

Does host-seeking suppression involve peripheral and central regulation of host-seeking sensory pathways?

Will exogenous activation of these pathways suppress host-seeking in the field where meal size and nutrition are more variable?

Does infection with pathogens change host-seeking regulation?

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