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Histamine excretion in common indoor and hematophagous arthropods

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Histamine is a biogenic amine that regulates multiple physiological functions in diverse organisms, specifically playing a central role in the mammalian immune response. The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), excretes histamine in large amounts in its feces as a component of its aggregation pheromone, which contaminates homes. The potential health risks associated with the presence of indoor histamine are unclear, but to predict future exposure risks, it is critical that we understand if other arthropods excrete histamine in any discernible phylogenetic pattern. In the present study, we evaluated histamine excretion by various arthropods; specifically those commonly found in large numbers indoors, other hematophagous species, and other species in the order Hemiptera. To evaluate arthropods for histamine excretion, rearing containers for each arthropod were swabbed and/or the harborage substrates were collected. Samples were then analyzed for the presence of histamine using gas chromatography–mass spectrometry. For those arthropods where histamine was present above the method detection limit, total histamine excretion was quantified over a period of 2 wk. Our results indicate that histamine excretion is limited to hematophagous hemipterans (bed bugs, bat bugs, tropical bed bugs, and kissing bugs), suggesting that indoor histamine contamination in the United States can be primarily linked to bed bugs.

Key words: bed bug, histamine, hemipteran, feces, indoor environment

Introduction

It is common to find insects, mites, and other arthropods indoors, with a recent study estimating homes can possess over 200 distinct species (Bertone et al. 2016). Often these arthropods are harmless, occasional invaders, but some are uniquely adapted to the indoor environment and can affect human health. For example, cockroaches and their associated allergens have been identified as one of the most important causes of asthma morbidity in sensitized individuals, particularly in urban environments (Rosenstreich et al. 1997, Akerman et al. 2003, Arbes et al. 2005, Pawankar et al. 2012, Do et al. 2016). House dust mites (HDM) are another major source of indoor airborne allergens present in households worldwide (Miller 2019). Asthma and dermatitis have also been attributed to mites of the families Glycyphagidae and Acaridae (Gafvelin et al. 2001), and recently, the fall home invader *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) was suggested as a possible source of indoor aeroallergens (Mertz et al. 2012). Given these health risks, it is important to understand what contaminants arthropods found indoors in large numbers produce and what health risks these contaminants may pose to humans.

In the early 2000s, bed bugs resurged worldwide and became ubiquitous indoor pests (Zorrilla-Vaca et al. 2015). Although considered primarily a nuisance pest due to their bites (Goddard and deShazo 2009), bed bugs were also shown to cause psychological problems, stress, and anxiety mainly due to infestations that persist for a long time because of the difficulty in eradicating them (Goddard and deShazo 2012). Bed bugs were also recently found to contaminate homes with histamine excreted in their feces (DeVries

et al. 2018), with concentration found highest in close proximity to the bed/sleeping surface (Gordon et al. 2023).

Histamine is a biogenic amine that serves as a neurotransmitter in multiple organisms and modulates the mammalian immune response (Pearce 1991, Jutel et al. 2002, Branco et al. 2018, Shulpekova et al. 2021). In bed bugs, histamine is a key component of the aggregation pheromone, serving as an arrestant (Gries et al. 2015). Bed bug histamine is released in the feces, with a recent study showing all feeding life stages capable of doing so, with the greatest amounts released within 4 days of taking a blood meal and continuing up to 2 wk after feeding (Gaire et al. 2022). Histamine has also been found in the excreta of another Hemipteran, *Rhodnius prolixus* (Stål) (Hemiptera: Reduviidae) (Harington 1956), but it is unclear if histamine serves as an arrestant like in bed bugs, or if histamine in the feces has other roles. Histamine has also been found in insect venom, including that from some species of stinging caterpillars, likely exacerbating allergic reactions (Karlsson and Einarsson 1982, Seldeslachts et al. 2022).

Although bed bugs appear uniquely capable of contaminating the indoor environment with histamine, it remains unclear if other species can excrete histamine in such large amounts. Arthropod-excreted histamine can be considered a contaminant given the large amount that is released and the potential health risks from indoor exposure (Siegel et al. 1991, 1992, Kullman et al. 1998). Therefore, it is important to determine if other arthropods can excrete histamine and how histamine excretion evolved, so that future predictions can be made on potential exposure risks. Therefore, we designed a study to determine (i) whether other common indoor arthropods excrete histamine, (ii) whether histamine excretion is related to hematophagy, and (iii) whether other species with a close phylogenetic relationship to bed bugs (e.g., "True bugs," belonging to the order of Hemiptera) excrete histamine.

Materials and Methods

Arthropods Evaluated for Histamine Excretion

A total of 22 arthropod species were investigated for histamine excretion and classified as follows: 13 species of common indoor pests, 3 species of blood feeding arthropods, 5 species of blood feeding hemipterans, and 1 species of phytophagous hemipteran (Table 1). All species were reared under standard laboratory conditions (25 °C, 30%–50% RH, 12:12 L:D cycle) and fed ad libitum, unless otherwise noted. *Blattella germanica* L. (Blattodea: Ectobiidae), *Periplaneta americana* L. (Blattodea: Blattidae), and *Blatta orientalis* L. (Blattodea: Blattidae) were reared in plastic containers with cardboard harborages and provided with water and rat chow (Mazuri Exotic Animal Nutrition, St. Louis, MO, USA). *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) were reared in plastic containers at 25 °C and 80% RH and fed on pine mulch (Walmart, Lexington, KY, USA). *Musca domestica* were purchased as pupae (Josh's Frogs, Owosso, MI, USA), raised to adulthood in plastic containers, and provided with a solution of sugar and water. *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) were reared in plastic vials in an incubator (25°C, 40%–60% RH) on a 12:12 L:D cycle and fed on cornmeal–yeast–molasses media (Genesee, San Diego, CA, USA). *Plodia interpuctella* (Hübner) (Lepidoptera: Pyralidae) were collected from a naturally infested package of pasta (Garofalo, Gragnano, IT) and reared in that package in the laboratory. *Anthrenus verbasci* L. (Coleoptera: Dermestidae) were reared in Petri dishes containing dog chow (Giuntini, Citta' di Castello, IT). *Oligomerus ptilinoides* (Wollaston) (Coleoptera: Ptinidae) were reared in plastic containers on a piece of fir wood as collected in the field. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) were reared in plastic containers on organic golden buffalo flour (Heartland Mills, Marienthal, KS, USA) with 10% dried active yeast added as a supplement. *Dermatophagoides farinae* (Hughes) (Acari: Pyroglyphidae), *Glycyphagus domesticus* (De Geer) (Acari: Glycyphagidae), *Tyrophagus putrescentiae* were reared in plastic containers on dog chow (Giuntini, Citta' di Castello, IT), containing the house dust they were collected in, and maintained in an incubator at 25 °C and 75% RH. *Aedes aegypti* L. (Diptera: Culicidae) were reared in meshed plastic cages and fed on defibrinated sheep blood (HemoStat, Dixon, CA, USA) weekly, through an artificial feeding system (Sri-In et al. 2020). *Amblyomma americanum* L. (Acari: Ixodidae) were kept in plastic vials and were fed once on defibrinated sheep blood (HemoStat) through an artificial feeding system. *Ctenocephalides felis* (Bouche) (Siphonaptera: Pulicidae) were reared in plastic containers and fed on sheep blood (Elward Labs, Soquel, CA) only once, 2 days after adult emergence, through an artificial feeding system (Blakely et al. 2023). *Cimex adjunctus* (Barber) (Hemiptera: Cimicidae) came from a single laboratory population and were kept in plastic jars and fed on rabbit blood (HemoStat) every 2 wk through an artificial feeding system (Gaire et al. 2022). *Cimex hemipterus* F. (Hemiptera: Cimicidae) (Greenlane and Kuala Lampur strains) were reared in plastic jars and fed on defibrinated rabbit blood (HemoStat, Dixon, CA, USA) every 10 days through an artificial feeding system (Dery et al. 2020). *Rhodnius prolixus* (Stål) (Hemiptera: Reduviidae) (single laboratory population established at NMSU) and *Triatoma rubida* (Hemiptera: Reduviidae) (Strain from Anthony, New Mexico) were reared in plastic containers and fed monthly on human blood (BioChemed Services, Winchester, VA, USA) containing the anticoagulant sodium citrate (BioChemed Services), through an artificial feeding system (Montes et al. 2002). *Cimex lectularius* L. (Hemiptera: Cimicidae), Harold Harlan laboratory population, were reared in plastic containers and fed on human blood containing the anticoagulant citrate phosphate dextrose (CPD) solution (Kentucky Blood Center, Lexington, KY, USA) weekly, using an artificial feeding system as described by Gaire et al. (2022). *Halyomorpha halys* (Hemiptera: Pentatomidae) were reared in net cages in a greenhouse (25°C, 80% RH) and fed on peperomia and bean plants ad libitum.

Sample Collection Procedures for Histamine Presence/Absence Evaluation

All species were first screened for histamine. Screening was conducted using swabs of the containers housing each arthropod, or substrate material that was conditioned by each species, or both methods. Multiple methods were employed because some species could only be sampled via one method or the other. Container swabbing and substrate collection were conducted using a standardized collection kit consisting of sterile cotton swabs (Cotton Tipped Wood Applicators, Sterile, Dynarex Corporation, Orangeburg, NY, USA) and plastic vials (5 ml, Sarstedt, Inc., Newton, NC, USA) containing 1 ml of HPLC-grade water (VWR International LLC, Radnor, PA, USA). Swab samples were collected by dipping a sterile cotton swab into the HPLC-grade water contained in the plastic vial, swabbing 25 cm^2 of the rearing container surface, then placing the swab back into the plastic vial containing HPLC-grade water. Substrate collections were performed by scooping ~2 ml of material from the bottom of the rearing containers into the plastic vial (only 250 µl were analyzed for histamine for each sample). It should be noted that substrate material was highly variable among species; however, this collection method was vital because of the different types (e.g., solid, liquid)

and size of the feces of these arthropods. For the blood-feeding hemipteran species, the substrate consisted of cardstock paper used as harborage in the rearing containers, conditioned with fecal material (5 cm^2). All materials were stored at –20 °C until histamine analysis. Information on the estimated population size (including life stages present) and estimated time in the container was recorded for each species (Table 1). Three replications of swab and substrate samples were performed for each arthropod species, and each replicate came from a different rearing container. These samples were subjected to histamine analysis by gas chromatography–mass spectrometry (GC–MS), and only those species that excreted histamine above the method detection limit (MDL) were further investigated to quantify histamine excretion.

Sample Collection Procedures for Histamine Quantification From Species With Detectable Levels of Histamine

Multiple individuals (6–10) were tested for each species, with some variability in life stages, either large nymphs or adults, based on species availability: adult male bed bugs (n = 10), adult male bat bugs (n = 10), adult male tropical bed bugs (n = 6), and fifth-instar nymphs for both species of kissing bugs (n = 10 for each species). Arthropods were starved for 2 wk to quantify histamine excretion and then moved individually into 4-ml glass vials (VWR, Radnor, PA, USA) or 20-ml plastic vials (Fisher Scientific, Hampton, NH, USA) depending on the arthropod's size. Arthropods were starved prior to the experiment to make sure that they did not have undigested blood from the previous feeding. A piece of cardstock paper (3 cm^2; Office Depot, Lexington, KY) was placed inside the vials and used as a harborage. Arthropods were weighed on a digital analytical balance (±0.1 mg, MIDUO, Beijing, China) before feeding, after feeding, and 2 wk after feeding to determine meal size and body mass loss over this time of digestion. After 2 wk, arthropods were removed from the vials, and the vials containing the conditioned paper substrate were stored at –20 °C until histamine analysis.

Histamine Analysis

Histamine was analyzed as described by Gaire et al. (2022) with some modifications. Briefly, all samples were spiked with mass-labeled histamine (histamine-α,α,β,β-d4; CDN Isotopes, Quebec, Canada) as an internal standard (in 0.1 M HCl; Macron Fine Chemicals, Radnor, PA) then extracted in HPLC-grade water (VWR International LLC, Radnor, PA, USA). Histamine was extracted by vortexing samples for 30 s, followed by 10 min of rocking, and 3 min of centrifugation at 400*g*. The resulting supernatant was transferred to a new vial to which 1 ml of toluene (VWR Chemicals, Radnor, PA) and 2 ml of alkaline buffer solution (pH 12; Honeywell International Inc., Charlotte, NC) were added. Next, 100 µl of the derivatizing agent isobutyl-chloroformate (Alfa Aesar, Haverhill, MA) was added to each sample, and vials were placed on a rocker for 45 min. Samples were then centrifuged for 3 min at 400*g*. The top organic layer (toluene) was then transferred to a clean 1.5-ml vial, evaporated to dryness under N_2, and resuspended in 1 ml of toluene. Extracted and derivatized samples were then stored at –20 °C until GC–MS analysis.

Histamine quantification was carried out using an Agilent Technologies 8860 GC (Santa Clara, CA) connected to a 5977B MS, operated in pulsed splitless mode (15 psi for 0.5 min, then 6 psi) with an inlet temperature of 280 °C. The GC–MS was outfitted with a 30 m × 0.25 mm × 0.25 µm HP-5MS UI column (Agilent Technologies), and helium was used as the carrier gas at a flow rate of 1.5 ml/min. The oven program was set at a temperature of 100 °C, which increased at a rate of 30 °C/min until it reached 320 °C, then held at this temperature for 5 min. The transfer line temperature was 280 °C, the MS quadrupole temperature was 150 °C, and the MS source temperature was 230 °C. The internal standard (m/z 197) and histamine (m/z 194) retention time was 6.32 min. The internal standard quantification method used a 13-point calibration curve ranging from 0.01 to 100 µg/ml.

MDL was used to determine the threshold under which the GC–MS could not detect histamine. The MDL was generated in accordance with the Environmental Protection Agency (EPA) standard operating procedure (protocol EPA 821-R-16-006), and it was determined by making seven samples of the 0.05 µg/ml histamine spiking level processed in three batches. The standard deviation of the average of all the samples was calculated and multiplied by the t statistic (3.143), which gave an MDL of 0.16 µg/1 ml of toluene. This equates to 0.16 µg/25 cm^2, 0.16 µg/250 µl of substrate volume, 0.16 µg/14-day insect fecal deposition period.

Statistical Analysis

Analysis of variance (ANOVA), followed by Tukey's HSD test (P < 0.05) was used to compare histamine excretion, blood consumption, and normalized histamine excretion (histamine excretion divided by blood consumption) among those species that excreted histamine. Histamine excretion and blood consumption were both log-transformed to achieve normality. Linear regression was used to analyze the relationship between histamine excretion and blood consumption, as well as body mass loss and blood consumption. All statistics were performed using R Studio (Vienna, Austria, Core Team R, 2021).

Results

Histamine Presence/Absence Among Various Arthropod Species

Only 5 species of the 22 tested had detectable histamine levels either in swab or substrate samples (Table 1). These species were all hematophagous hemipterans, specifically the common bed bug (*C. lectularius*), the bat bug (*C. adjunctus*), the tropical bed bug (*C. hemipterus*), and 2 species of kissing bugs (*T. rubida* and *R. prolixus*). It should also be noted that histamine was detected above the MDL in both swab and substrate samples, indicating that both methods were appropriate and sufficient to detect histamine (if present). Histamine was not detected in either swab or substrate samples for all other common indoor species tested. Furthermore, histamine was not found in the samples collected from other hematophagous arthropods (*A. aegypti*, *A. americanum*, *C. felis*) nor the phytophagous hemipteran (*H. halys*).

Histamine Quantification From Species With Detectable Levels of Histamine

For those species with histamine detected above the MDL from swab or substrate samples, histamine was further quantified in individuals of each species to get more precise estimates of the amount of histamine they excrete. The amount of histamine excreted ranged from 1.6 to 7.2 µg per insect per day over a period of 2 wk (Fig. 1a). Histamine excretion significantly differed among the species tested (Fig. 1a; $F_{4,41}$ = 6.18, P < 0.001), with *T. rubida* excreting the greatest amount of histamine. Blood consumption also differed significantly among the species with blood intake ranging from 2.5 to 91.5 mg per insect ($F_{4,41}$ = 101.3, P < 0.001) (Fig. 1b), with *C. lectularius*,

Table 1. Histamine detection in swab and substrate samples from various arthropods. For each arthropod tested, the approximate population size, rearing container size, and approximate time in the container are listed. Histamine presence/absence is reported for 3 replicate samples for each arthropod tested. Dashes "–" represent no availability of sample for that collection method for that species

Group	Species	Common name	Approx. population size	Container size	Approx. time in container	Histamine presence	
						Swab[1]	Substrate[2]
Common indoor species	*Blattella germanica*	German cockroach	1000 adults 500 nymphs	3.8 l	2 months	0/3	0/3
	Periplaneta americana	American cockroach	50 adults 20 nymphs	7.6 l	2 months	0/3	0/3
	Blatta orientalis	Oriental cockroach	80 adults 20 nymphs	3.8 l	2 months	0/3	0/3
	Reticulitermes flavipis	Eastern subterranean termite	2400 adults 600 immatures	9 l	19 months	0/3	0/3
	Musca domestica	House fly	800 adults	3 l	10 days	0/3	0/3
	Drosophila melanogaster	Fruit fly	290 adults 400 immatures	61 ml	2 wk	0/3	0/3
	Plodia interpuctella	Indian meal moth	12 immatures	1.5 l	1 month	–	0/3
	Anthrenus verbasci	Varied carpet beetle	100 immatures	64 ml	1 month	–	0/3
	Oligomerus ptilinoides	Borer beetle	10 immatures	100 ml	6 months	–	0/3
	Tribolium castaneum	Red flour beetle	1000 adults	1.8 l	12 months	0/3	0/3
	Dermatophagoides farinae	House dust mite	400 adults 250 immatures	100 ml	6 months	–	0/3
	Tyrophagus putrescentiae	Mold mites	600 adults 450 immatures	100 ml	6 months	–	0/3
	Glycyphagus domesticus	Grocer's mite	70 adults 40 immatures	100 ml	6 months	–	0/3
Blood-feeding species	*Aedes aegypti*	Yellow fever mosquito	150 adults	21.2 l	1 month	0/3	–
	Amblyomma americanum	Lone star tick	110 adults 250 immatures	61 ml	9 months	0/3	–
	Ctenocephalides felis	Cat flea	100 adults	23 ml	1 month	0/3	0/3
Blood-feeding Hemipterans	*Cimex lectularius*	Bed bug	20 adults 100 immatures	137 ml	1 month	3/3	3/3
	Cimex adjunctus	Bat bug	20 adults 75 immatures	98 ml	5 months	3/3	3/3
	Cimex hemipterus	Tropical bed bug	25 adults 113 immatures	148 ml	12 months	3/3	3/3
	Triatoma rubida	Kissing bug	30 immatures	192 ml	1 month	3/3	3/3
	Rhodnius prolixus	Triatomid bug	120 immatures	1.7 l	3 months	3/3	3/3
Phytophagous Hemipterans	*Halyomorpha halys*	Brown marmorated stink bug	150 adults	125 l	1 month	0/3	–

[1]The area swabbed was 25 cm^2 for all arthropods tested (75 cm^2 for *M. domestica*, *C. felis*, *C. hemipterus*, *T. rubida*, *R. prolixus*).

[2]Substrate material was highly variable among arthropods tested. For common indoor species and blood-feeding species, 0.25 ml of materials found at the bottom of rearing containers was evaluated. For blood-feeding Hemipterans, a piece of paper (5 cm^2) was removed from each container and evaluated.

C. adjunctus, *C. hemipterus* consuming significantly less blood than *R. prolixus* and *T. rubida*. However, when histamine excretion (µg histamine/insect/day) was normalized by blood consumption (µg histamine/insect/day/mg blood consumed), the amount of histamine excreted ranged from 0.12 to 0.92 µg per insect per day over a period of 2 wk. Normalized histamine excretion was found to be significantly different among the species tested ($F_{4,41} = 21.16$, $P < 0.001$) (Fig. 1c), with *C. adjunctus*, *C. hemipterus*, and *C. lectularius* excreting significantly higher amounts of histamine per mg blood ingested compared than *R. prolixus* and *T. rubida*.

Blood Consumption, Body Mass Loss, and Histamine Excretion

Regression analysis confirmed a significant positive relationship between blood consumption and body mass loss for all the arthropods that excreted histamine (Table 2; Figs. 2a–c and 3a–b). Interestingly, significantly positive relationships between blood consumption and histamine excretion were found for all hematophagous hemipterans ($P < 0.05$) (Table 3; Figs. 2d–e and 3c), except for *C. hemipterus* and *T. rubida*, whose data showed no significant relationship ($P > 0.05$; Fig. 2f, Fig. 3d).

Discussion

Our initial screening revealed that only hematophagous insects belonging to the order Hemiptera excreted detectable amounts of histamine. Specifically, bed bugs, bat bugs, tropical bed bugs, and 2 species of kissing bugs excrete quantifiable amounts of histamine that could be detected via both swab and substrate sampling techniques. The lack of detectable histamine from any other arthropod tested suggests that bed bugs are primarily responsible for indoor histamine in the United States. In addition, hemipterans do

not appear to universally excrete histamine based on our failure to detect any histamine from stink bugs (*H. halys*). Testing additional hemipteran species, particularly those closely related to hematophagous hemipterans, would be useful. Furthermore, blood feeding alone does not lead to histamine excretion, as evident by our failure to detect histamine in samples from mosquitoes, fleas, and ticks. While blood feeding on its own is not enough to result in histamine excretion, it does appear to be required given our results with hematophagous and phytophagous hemipterans.

It is possible that the arthropods that did not excrete detectable amounts of histamine may lack the enzyme histidine decarboxylase, which converts the amino acid histidine into the biogenic amine histamine (Moriguchi and Takai 2020), in the digestive/excretory system. Histamine is widely distributed in the nervous system of invertebrates, especially in the optic lobe, where it plays a role as a neurotransmitter (Schmid and Duncker 1993). However, if the histamine excreted in the feces of blood-feeding hemipterans came from biosynthesis in the nervous system cells, we would have likely found histamine in the feces of all arthropods tested. Because only hematophagous hemipterans were found to excrete histamine, the biosynthesis of excreted histamine may occur primarily in the cells of the alimentary system. While this remains to be shown, future studies should explore the role of histidine decarboxylase activity in the alimentary tract in relation to histamine excretion.

Blood is known to contain histidine (Stein and Moore 1954), and blood intake has been shown to play a crucial role in histamine excretion as bugs fed on saline excrete significantly less histamine than those fed on human blood, while showing no difference from those that were not fed at all (starved; Gaire et al. 2022). However, blood does not contain enough histidine to account for the amount of histamine bed bugs excrete. Using mass-labeled histidine, Gries et al. (2018) showed that some bed bug excreted histamine was generated by the decarboxylation of histidine acquired from the blood. However, since 1 ml of blood (~1 g) contains only 11 µg of histidine (Stein and Moore 1954), and bed bugs consume <10 mg of blood, they do not consume enough histidine (<0.11 µg histidine) to account for the amount of histamine we found them capable of excreting following a single blood meal (27.1 µg of histamine per bed bug for 14 days following feeding). Given that histidine decarboxylase is the primary enzyme responsible for histamine biosynthesis, and hematophagous hemipterans lack of a sufficient source of histidine to account for the amount of histamine they produce, they may have another source of histidine. As histidine is an essential amino that can be biosynthesized by bacteria and plants (Kulis-Horn et al. 2014), hematophagous hemipterans may possess microbes capable of producing histidine. In addition, it is also possible that hematophagous hemipterans are synthesizing histidine independently from their microbiome. Alternatively, hematophagous hemipterans might possess a novel enzyme capable of synthesizing histamine independently from histidine, although this has not been investigated.

When we evaluated blood consumption relative to body mass loss, we found a significant correlation in all species (Tables 2 and 3 and Figs. 2 and 3). However, only 3 species out of the 5 had a significant correlation between blood consumption and histamine excretion, possibly due to the higher variability in blood meal size for *R. prolixus* and *C. hemipterus*. Together, these results show a strong relationship between blood consumption, body mass loss (e.g., blood meal processing), and histamine excretion.

When histamine excretion was evaluated per insect per day and compared among the 5 species, *T. rubida* was found to excrete the greatest amount (Fig. 1a). However, when histamine excretion was normalized for blood consumption, tropical bed bugs, bed bugs, and bat bugs were found to excrete significantly more histamine than kissing bugs (Fig. 1c). Although kissing bugs take significantly larger blood meals (Fig. 1b), the concentration of histamine in their feces is much lower than the cimicid species. This uneven scaling suggests that while there may be a shared evolutionary origin for histamine excretion in hematophagous hemipterans, some species may have been selected for higher excretion levels than others. However, in this study, only nymphs of kissing bugs were analyzed for histamine excretion. As bed bug nymphs excrete significant less histamine than adult males (Gaire et al. 2022), it is possible that the same could occur in kissing bugs. Future research should evaluate adults and nymphs of each hematophagous hemipteran evaluated in this study.

Despite different excretion amounts, the reason why only hematophagous hemipterans excrete histamine remains unclear. In bed bugs, histamine has been shown to play a significant role in

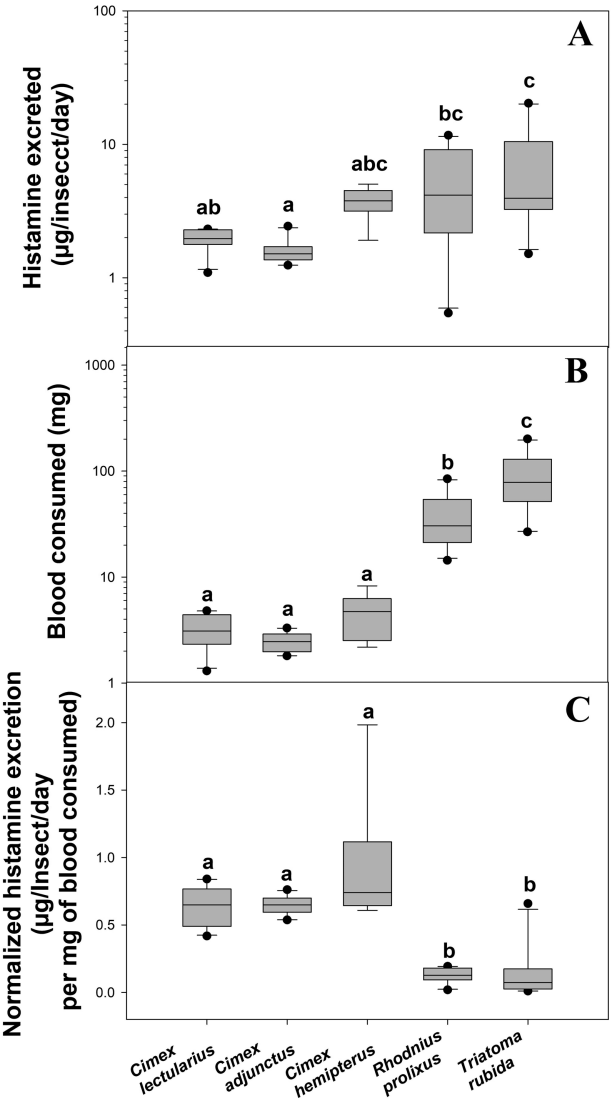

Fig. 1. Histamine excretion and blood consumption among hematophagous hemipterans. a) Histamine excreted per day, per insect; b) blood consumption (mg) after 2 wk of starvation, and c) histamine excretion per day, normalized for blood consumption. Solid lines in the middle of each box represent the mean, and box ends (top, bottom) represent the upper and lower quartiles. Different lowercase letters above bars indicate significant differences among species (ANOVA, followed by Tukey Test; $P < 0.05$).

aggregation, serving as an arrestant (Gries et al. 2015). Whether histamine plays the same role or additional roles in all blood-feeding hemipterans is unknown, but the quantity they excrete may be predictive of function. Kissing bugs have greater mobility and are capable of locating hosts from a greater distance than bed bugs (Guerenstein and Lazzari 2009, Indacochea et al. 2017), and thus arrestants may not be as critical to their survival and fitness.

The ability of multiple species with broad distributions to excrete histamine could raise concerns globally about environmental exposure to histamine. In humans, histamine mediates the immunological responses that can lead to allergic diseases like asthma (Couillin et al. 2004). When exposed to an allergen, either dermally, or through injection, ingestion, or inhalation, our body produces IgE antibodies against that specific allergen. Upon a second exposure to the same allergen, the mast cells of our immune system (primed to respond to that specific allergen) release histamine which spreads to different tissues causing the dilation of blood vessels, gastric acid secretion, airways constrictions, itch, or other symptoms typical in allergic reactions (Beaven 1976). A recent study showed that infested homes had a higher concentration of histamine in house dust than homes that are were not infested (DeVries et al. 2018). A follow-up study revealed that histamine is found throughout homes, being more concentrated in areas where bed bugs are usually located (e.g., sleeping surfaces) (Gordon et al. 2023). Furthermore, Gaire et al. (2022) found that adult female bed bugs could excrete as much as 8 µg of histamine per day. When these data are paired with population estimates, which are often quite high in affordable housing (Cooper et al. 2016), the concentration of histamine in bed bug infested homes could be substantial.

In the United States, environmental histamine can primarily be attributed to the common bed bug, *C. lectularius*, because all other species capable of excreting histamine have not been reliably found capable of establishing indoors. Tropical bed bugs are usually found in the subtropical areas of the world (Lee et al. 2023). In the United States, they have been reported in Florida and Hawaii (Campbell et al. 2016, Lewis et al. 2020), but reports are minimal. Bat bugs are distributed throughout the world, but their high affinity for bats and poor survival on humans limit potential interactions and thus histamine excretion and exposure (Akhoundi et al. 2020). Kissing bugs do not have a widespread distribution worldwide and those with the ability to establish indoors are typically found in rural areas of Latin America (Klotz et al. 2014).

Table 2. Effect of blood consumption (mg) on body mass loss (mg). Regression equations are in the form of: body mass loss (mg) = intercept + slope × blood consumed

	Blood consumed versus Body mass loss					
Species	Slope (±SEM)	Intercept (±SEM)	DF	*F*	R^2	*P*-value
Cimex lectularius	1.07 (±0.17)	0.00 (±0.58)	8	41.2	0.84	<0.001
Cimex adjunctus	1.11 (±0.27)	−0.66 (±0.68)	8	16.6	0.67	0.003
Cimex hemipterus	1.15 (±0.19)	−1.18 (±0.99)	4	34.6	0.89	0.004
Rhodnius prolixus	0.66 (±0.05)	4.38 (±2.41)	8	141.8	0.94	<0.001
Triatoma rubida	0.56 (±0.06)	1.23 (±6.51)	8	81.5	0.91	<0.001

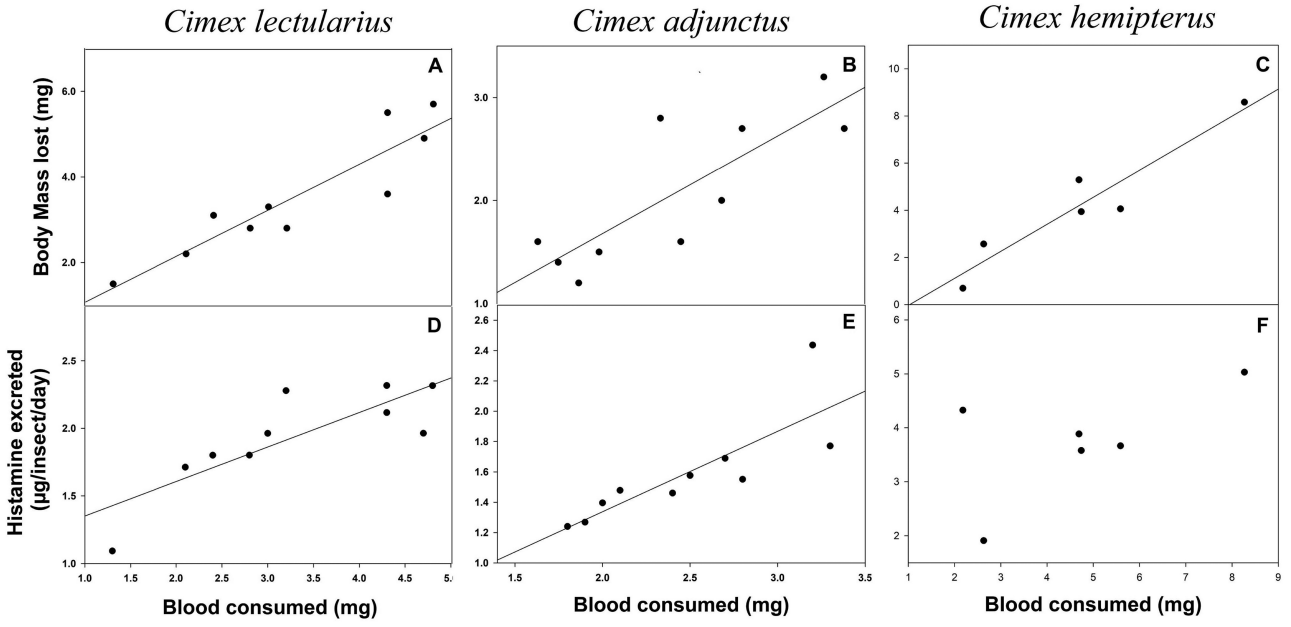

Fig. 2. Relationship between blood consumption (single blood meal) and body mass lost (over 2 wk) for a) *Cimex lectularius,* b) *Cimex adjuncts,* and c) *Cimex hemipterus.* Relationship between blood consumption and histamine excretion for d) *Cimex lectularius,* e) *Cimex adjunctus,* and f) *Cimex hemipterus.* Significant best fit linear regression lines are displayed (see Table 2 for equations).

Table 3. Effect of blood consumption (mg) on histamine excretion (µg/day/insect). Regression equations are in the form of: histamine excretion = intercept + slope × blood consumed

	Blood consumed versus histamine excreted					
Species	Slope (±SEM)	Intercept (±SEM)	DF	*F*	R^2	*P*-value
Cimex lectularius	0.26 (±0.06)	1.09 (±0.22)	8	16.6	0.67	0.003
Cimex adjunctus	0.53 (±0.13)	0.28 (±0.33)	8	16.5	0.67	0.003
Cimex hemipterus[a]	–	–	8	2.2	0.35	0.208
Rhodnius prolixus	0.14 (±0.03)	−0.31 (±1.38)	8	20.6	0.72	0.002
Triatoma rubida[a]	-	-	8	2.3	0.22	0.167

[a]No significant relationship was detected; therefore, slope and intercept estimates are not displayed.

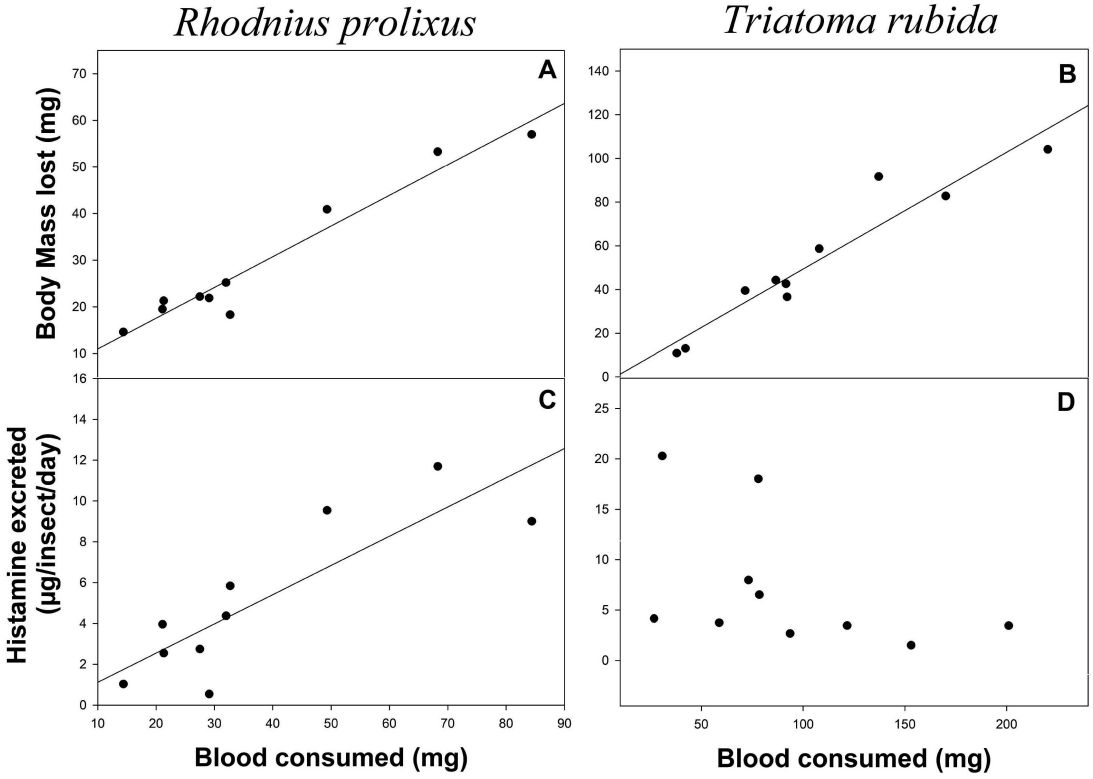

Fig. 3. Relationship between blood consumption (single blood meal) and body mass lost (over 2 wk) for a) *Rhodnius prolixus,* and b) *Triatoma rubida.* Relationship between blood consumption and histamine excretion for c) *Rhodnius prolixus,* and d) *Triatoma rubida.* Significant best fit linear regression lines are displayed (see Table 2 for equations).

Some species, like *T. rubida*, can be found in the United States, but they usually do not establish indoors (Klotz et al. 2014). However, the present results indicate that histamine excreted by other hematophagous hemipterans may be of concern in areas where they are endemic and found in large numbers in human dwellings. This could extend the potential threat of histamine beyond bed bugs when evaluated globally.

Future studies should investigate how to mitigate environmental histamine and how this may impact human health and future bed bug infestations establishment in homes (given histamine's role as an arrestant). In addition, future studies should evaluate the behavioral effects of histamine to determine if it drives arrestment in other hematophagous Hemipterans similar to bed bugs. Ultimately, it is critical to understand if environmental histamine can trigger allergic reactions; this information will allow us to improve human health and recognize the true extent of effects bed bugs have on humans.

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