Rapid evolution of an adaptive taste polymorphism disrupts courtship behavior

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The evolution of adaptive behavior often requires changes in sensory systems. However, rapid adaptive changes in sensory traits can adversely affect other fitness-related behaviors. In the German cockroach, a gustatory polymorphism, ‘glucose-aversion (GA)’, supports greater survivorship under selection with glucose-containing insecticide baits and promotes the evolution of behavioral resistance. Yet, sugars are prominent components of the male’s nuptial gift and play an essential role in courtship. Behavioral and chemical analyses revealed that the saliva of GA females rapidly degrades nuptial gift sugars into glucose, and the inversion of a tasty nuptial gift to an aversive stimulus often causes GA females to reject courting males. Thus, the rapid emergence of an adaptive change in the gustatory system supports foraging, but it interferes with courtship. The trade-off between natural and sexual selection under human-imposed selection can lead to directional selection on courtship behavior that favors the GA genotype.
Adaptive traits are shaped by environmental pressures that select on natural genetic variation. Generally, traits evolve slowly over a long time1–3, and as natural selection compels evolutionary novelty in the sensory system, sexual selection may constrain these changes, and vice versa4,5. On the other hand, under intense human-imposed selection such as agricultural, aquacultural and pest control interventions, adaptive traits can evolve rapidly and sweep through populations, even if they incur some reproductive costs6. For example, flatwing mutants of the Pacific field cricket (Teleogryllus oceanicus) emerged in males under strong selection by the invasive parasitic fly Ormia ochracea, which locates singing male crickets and lays its eggs on them6,7. The flatwing mutation is adaptive because males evade parasitoids, but the loss of acoustic sexual signaling also reduces their reproductive success. An alternative mating strategy has emerged in flatwing males – they position themselves near singing males and intercept females attracted to the calling males8. The Teleogryllus–Ormia system represents a trade-off between sexual selection (favoring reproductive success) and sexual selection (favoring survival)6,9. Such trade-offs have been documented in visual and acoustic sexual communication, and rapid directional evolution is often imposed by invasive predators or parasitoids. However, such trade-offs between natural and sexual selection, rapid directional changes in sexual communication, and evidence of these processes in ‘real-time’ have not been well documented in reproductive systems that rely heavily on chemoreception.

We employed the German cockroach Blattella germanica as a compelling system in which foraging success and mating success are differentially affected by a recently evolved gustatory polymorphism. Gustatory receptor neurons (GRNs) in the mouthparts of the cockroach detect sugars, such as glucose, maltose and maltotriose, and mediate both foraging and sexual communication10,11. During courtship12, males expose specialized tergal glands13–15 and offer females an oligosaccharide-rich secretion that includes maltose and maltotriose, as well as phospholipids, cholesterol and various amino acids16–23. By attracting the female to his highly palatable secretion, the male places the female in the proper position for copulation and lowers her behavioral threshold to mate, thus gaining competitive advantage in female mate choice10,12. The female must mount the male and feed on the nuptial secretion long enough for the male to extend his abdomen under the female and engage her genitalia; short nuptial feeding results in interrupted and often failed courtship. Mating success is thus maximized through the convergence of the quality of the male’s nuptial secretion and the female’s gustatory sensitivity to it12.

As a perennial household pest with significant public health, social and economic impacts24,25, the German cockroach has been the target of intense human-imposed selection with sugar-containing insecticide baits. In response, multiple cockroach populations rapidly evolved behavioral resistance to these baits in the form of glucose-aversion (GA) — GRNs in the mouthparts detect glucose as a deterrent rather than phagostimulant and drive an avoidance behavior that enables cockroaches to elude the toxic bait26,27,28. The GA trait follows Mendelian-like inheritance patterns27. Because it is highly adaptive in the presence of glucose-containing toxic baits, the GA genotype can rapidly replace glucose-accepting wild-type (WT) cockroaches28. This unique sensory polymorphism is an excellent model of rapid evolution of chemosensory-based behavior in the anthropogenic environment29. Recently, we observed that GA females have lower mating success with WT males than do WT females, but the underlying behavioral mechanisms were unclear30. We also found that salivary glucosidases of both WT and GA females rapidly digest oligosaccharides in food, resulting in lower sugar consumption in GA females but not in WT females31. In this study, we hypothesized that salivary digestion of the nuptial secretion might release glucose, disrupting nuptial feeding of GA females but not WT females, and possibly explaining lower mating success in GA females. To identify behavioral events that mediate successful and failed courtship sequences, we compared seven behavioral parameters in courtship of WT and GA females. By artificially manipulating the nuptial secretion in the male tergal glands, we tested whether the quality of the nuptial secretion intercedes in successful and failed courtship sequences. We also generated recombinant lines of WT and GA cockroaches to confirm an association between the GA trait and rejection of the nuptial secretion. Finally, we tested whether saliva could rapidly digest oligosaccharides in the nuptial secretion, releasing glucose and thus transforming a tasty nuptial gift to an aversive stimulus. Overall, we demonstrate and discuss how glucose-aversion imposes a trade-off by favoring survivorship under natural selection in the presence of toxic baits, but it disrupts courtship under sexual selection. Rapid directional changes in the sexual communication system are expected to correct for the lower reproductive success of GA females.

**Results**

**Interrupted nuptial feeding reduces mating success of GA females.** In choice tests with a GA female and two males, WT males had significantly lower mating success than GA males, whereas both WT and GA males experienced similar mating success with WT females (Fig. 1a left, Supplementary Table 1). In choice assays with a male and two females (Fig. 1a right), there was no significant difference in mating success between GA and WT females when assayed with either a WT or GA male. In no-choice tests (Fig. 1b, Supplementary Table 2), GA females experienced significantly lower mating success than WT females when paired with WT males, but there was no significant difference in mating success in the other three pairings (WT Male/WT Female, GA Male/WT Female, GA Male/GA Female). Because this deficit in GA females was a consequence of behaviors expressed during courtship, we analyzed key behavioral events that correlate with mating success in both strains (Fig. 1c). Comparative behavioral analysis of successful and failed courtship sequences revealed significant differences between WT and GA females in the parameter associated with ‘nuptial feeding’ (Fig. 1d, Supplementary Table 2). In failed courtship sequences, WT females engaged in a single nuptial feeding event of short duration (4.2–4.6 s) (Fig. 1d upper). On the other hand, in successful courtship sequences WT females mounted males 1.4–1.7 times (nuptial feeding events), and the duration of a single successful nuptial feeding event was significantly longer than in failed courtship sequences (5.8–6.9 s; ANOVA, F(3, 167) = 18.97, P < 0.0001).

These findings suggest an association of failure to copulate with short nuptial feeding and that successful copulation requires that females engage in protracted nuptial feeding to enable the male to grasp the female’s genitalia. Additionally, nuptial feeding duration was significantly longer in pairings of WT Male/WT Female than GA Male/WT Female, suggesting that other courtship traits affected the duration of nuptial feeding in WT females. In contrast, GA females had significantly shorter nuptial feeding than WT females in both failed and successful courtship sequences (2.8–3.6 and 3.5–3.8 s, respectively), and no significant difference was found in the durations of failed and successful sequences. Although courtship sequences of GA females with WT males had intermittent nuptial feeding, they significantly increased the number of nuptial feeding events (2.7 events, Supplementary Table 2) and extended the total nuptial feeding...
duration in successful sequences (9.2 s, Supplementary Table 2). In contrast, in failed sequences of GA females the total nuptial feeding duration remained short (2.8 s, Supplementary Table 2). These results reveal that behaviors unique to GA females interrupt nuptial feeding, cause short feeding duration, and result in failure to mate. Additionally, it is important to note that GA females mated successfully with GA males (Fig. 1a, b), without engaging in more nuptial feeding events and despite their relatively short total nuptial feeding duration (4.8 s, Fig. 1d). It appears that the pair of GA cockroaches have a different courtship strategy which does not rely on the females’ GA trait to improve mating success. In this study we focused on the mechanisms that underlie short nuptial feeding of GA females that lead to failure to mate with WT males. Ongoing studies are investigating the mechanisms that enable successful copulation in GA cockroach pairs.

**Short nuptial feeding duration is mediated by the quality of the nuptial secretion.** To understand what causes the short nuptial feeding of GA females, we performed bioassays that measured the female’s initial instantaneous response to tastants (Acceptance-Rejection assay)31. Both WT and GA females were equally stimulated to initiate nuptial feeding by the tergal gland secretions of WT males (Fig. 2a, Supplementary Table 3). However, in Consumption assays31, which measured the total amount eaten in a single feeding bout, GA females ingested significantly less nuptial secretion than WT females (Fig. 2b, Supplementary Table 3). These results suggested the presence of a mismatch between the composition of the nuptial secretion and the taste preferences of GA females, and that feeding by GA females was suspended after they initially accepted the nuptial secretion, resulting in failure to mate.

In addition, because GA females also consume less maltose and maltotriose than WT females31, we tested whether longer nuptial feeding and successful mating of GA females could be restored by increasing the quality of the tergal secretion of WT males with fructose, which is not aversive to GA cockroaches (Fig. 2c, Supplementary Table 4)11,26,31. Mating success was low in unmanipulated pairs, with short nuptial feeding by GA females (Chi-square test, \( \chi^2(2) = 19.57, P < 0.0001 \) (upper); \( F_{(3, 222)} = 3.69, P = 0.01 \) (lower)). However, the addition of fructose to the tergal secretion of WT males increased the nuptial feeding duration of GA females, resulting in significantly higher mating success. These results confirm that longer nuptial feeding leads to successful mating, and it can be restored in GA females by modifying the composition of the male’s tergal secretion. They further indicate that components of the nuptial secretion disrupt nuptial feeding of GA females and that the GA trait features prominently in this maladaptive outcome.

**Genetic association of the GA trait with short nuptial feeding.** To determine if the GA trait intercedes in the short nuptial feeding, we generated a recombinant line of WT and GA cockroaches to homogenize their genetic backgrounds and thus created three genotypes: WT_aa, GA_AA and GA_Aa (Fig. 3a). Dose-response curves using the Acceptance-Rejection assay showed that WT_aa females accepted glucose in a dose-dependent manner, whereas GA_AA and GA_Aa females rejected glucose; the effective glucose concentration that elicited aversion in 50% (EC50) of GA_AA females was lower than in GA_Aa females (Fig. 3b, Supplementary Table 5). This result is consistent with our previous findings27 that feeding responses of
GA females may be mediated by not only Mendelian inheritance but also by unknown factors that are either genetically linked with and/or unrelated to the GA trait. To test the association of sensitivity to glucose with courtship parameters, we separated the GA_Aa females into two groups: GA_Aa_low females displayed low sensitivity to glucose and rejected only high concentrations of glucose (>1000 mmol L\(^{-1}\)), while GA_Aa_high females were highly sensitive to glucose and rejected it at lower concentrations (100–300 mmol L\(^{-1}\)) (Fig. 3a, b). In no-choice mating assays with WT males GA_AA and GA_Aa_high females had significantly lower mating success than GA_Aa_low and WT_aa females (Fig. 3c). Although each nuptial feeding event was of short duration, these females extended their total nuptial feeding duration with more nuptial feeding events, while GA_Aa_low females behaved similarly to WT_aa females (Supplementary Table 6). These results indicate that the heritable GA trait is associated with interrupted nuptial feeding in GA females.

Mechanisms responsible for short nuptial feeding in GA females. Previous studies using dissected tergites that included whole tergal glands documented the presence of oligosaccharides composed of glucose, including maltose and maltotriose, but there was no indication that free glucose was a component of the secretion\(^{18-23}\). This is consistent with other studies, showing that GA females and males accept various oligosaccharides, including maltose and maltotriose\(^{26,31}\). Thus, it was unclear how GA females instantaneously perceive and reject the nuptial secretion. In a previous study, we documented that salivary alpha-glucosidases in both WT and GA females hydrolyze dietary oligosaccharides to glucose, decreasing the acceptance of oligosaccharides in GA females, but not in WT females\(^{31}\). Therefore, we suspected that the same mechanism might result in rejection of the nuptial secretion by GA females. We collected the secretion directly from the tergal gland reservoirs of WT males and added either WT or GA female saliva to it, as well as to maltose or maltotriose. All three mixes were accepted by GA females, but at a significantly lower rate than by WT females (Fig. 4a Supplementary Table 7). Co-incubation of saliva with acarbose, an inhibitor of glycoside hydrolase enzymes in cockroach saliva\(^{31}\), confirmed that salivary alpha-glucosidases were highly effective at reducing acceptance by GA females of maltose, maltotriose and male nuptial secretion (Fig. 4b, Supplementary Table 8).

GC-MS analysis revealed that the tergal secretion contained maltose, maltotriose, and small amounts of glucose (Fig. 4c, Supplementary Table 9). Addition of saliva to either maltose or maltotriose produced glucose, and more glucose was released with longer incubation times (Fig. 4d, e, Supplementary Table 10). In incubations of maltose with 1 µl of GA female saliva, glucose increased 2-fold within 5 s, and another 10-fold after 300 s (Fig. 4d). In contrast, only 3-fold more glucose and 5-fold more maltose were released from maltotriose after a 300 s incubation, indicating that saliva hydrolyzes maltose more efficiently than maltotriose (Fig. 4e). When WT tergal secretion was incubated with GA female saliva, glucose increased significantly between 5 and 10 s of incubation (Fig. 4c), which is a critical period of nuptial feeding that correlates with successful copulation (Fig. 1d). The amounts of maltose and maltotriose decreased during the 300 s incubation with saliva. Notably, the nuptial secretion contains various oligosaccharides composed of glucose\(^{18-23}\), so glucose was likely released from other sugars as well. These results indicate that tergal secretion is rapidly hydrolyzed by female saliva during nuptial feeding, resulting in the deterioration of its taste quality for GA females. Thus, the short nuptial feeding and interrupted courtship of GA females are mediated by distasteful glucose that was released via salivary hydrolysis of the nuptial secretion.

Discussion

We demonstrated that a change in taste valence is adaptive in foraging but interferes with courtship. New phenotypes that rapidly evolve under strong selection can create mismatches and conflicts in their adaptive values in different behavioral contexts\(^{32}\). For example, in Drosophila melanogaster, the DDT resistance allele is associated with phenotypes that have lower male mating success, smaller body size and lower aggressive performance\(^{33}\). As already discussed in the Introduction, the flatwing mutation generates trade-offs between survival and mating success in male crickets\(^{5-8}\).

Using the German cockroach as a model system, we tested whether similar tradeoffs may be evident with gustatory traits – did the rapid evolution of glucose-aversion adversely affect the gustation-dependent courtship system? Fine-scale analysis of the courtship sequences of WT, GA and recombinant lines of cockroaches demonstrated that nuptial feeding in GA females is interrupted into short feeding bouts, resulting in frequent failures of courting males to grasp the female genitalia and mate. Chemical analysis of male tergal secretions and quantitative bioassays of feeding by females revealed that the taste quality of nuptial...
secretion components conflict with the emergent gustatory preferences of GA females. The nuptial gifting strategy likely evolved in the context of male exploitation of the female’s gustatory food preferences and her motivation to feed as her oocytes mature10,34,35. Therefore, the rapid transformation of glucose from appetitive to aversive stimulus resulted in an acute mismatch between the quality of the male’s nuptial gift and taste preferences of GA females. The mechanism that underlies this mismatch is the rapid processing of the male’s nuptial secretion by the female’s saliva. We found that the concentration of glucose in nuptial secretion (4.7 mmol l⁻¹ in WT males, Supplementary Table 9) is not sufficient to stimulate its rejection by GA females, especially considering that glucose is embedded in a mix of phagostimulatory amino acids and lipids16–23. Indeed, GA females initially accepted the nuptial secretion, as did WT females (Fig. 2a). However, as previously shown31, saliva of WT and GA cockroaches contain alpha-glucosidases that hydrolyze oligosaccharides into alpha-glucose. In this study, as we predicted, salivary glucosidases rapidly released glucose during nuptial feeding, interrupting the feeding of GA females but not WT females. The released glucose activates deterrent-sensitive GRNs and suppresses the activation of sugar-sensitive GRNs in GA females11. Fig. 5 summarizes how this mechanism mediates short feeding durations and low consumption in GA females but not in WT females.

Our observations suggest that both GA males and GA females might express emergent courtship-related traits that mitigate their lower mating success. Considering GA male adaptations, we found that despite their short nuptial feeding duration, GA males had greater success in mating with GA females than did WT males (Fig. 1b, d). Moreover, GA males that mated with WT females did so despite the preceding nuptial feeding event being significantly shorter than when WT females were courted by WT males (Fig. 1d). These results suggest that elements of the courtship behavior of GA males differ from those WT males. Similar to the emergence of alternative male mate-finding strategies in flatwing crickets6–9, under persistent selection pressure by the gustatory preferences of GA females, GA males might...
express compensatory adaptive courtship traits such as more frequent approaches of females, short latency to copulation attempts, and changes in their nuptial gift components. Considering emergent adaptations of GA females, their saliva had 2-fold less alpha-glucosidase activity than the saliva of WT females. It is possible that lower alpha-glucosidase activity of GA female saliva and the GA trait were jointly selected by sugar-containing toxic baits and the male’s nuptial gift. Our ongoing research is examining the full array of courtship traits of GA cockroaches and whether these traits are genetically linked to the GA trait.

It is imperative that we understand the evolution and spread of insecticide and behavioral resistance to inform ecologically sound pest management strategies. The origin of the GA trait is
unknown, and it is also unclear how it introgresses and is maintained in various field populations. We propose that assortative mating may have far-reaching implications for driving the GA trait into cockroach populations. Our results suggest that in mixed populations of GA and WT cockroaches, GA males may rapidly introgress the GA alleles into the WT population more efficiently than GA females because GA males mate with both genotypes, whereas GA females assortatively mate with GA males. Considering the population-wide changes that followed the emergence of the flatwing mutation in crickets, selection by sugar-containing toxic baits would cause the eradication of the WT and heterozygous GA cockroaches, and their rapid replacement by homozygous GA cockroaches.

In summary, we previously elucidated how an adaptive gustatory trait can rapidly emerge under human-imposed ‘natural’ selection and in this study, we demonstrated that the emergent trait creates mismatches in sexual communication. Overall, pre-oral and oral processing of oligosaccharides by cockroach saliva dramatically extends the phenotypic range of the glucose-aversion genotype in both foraging and sexual contexts. Glucose-aversion thus has created trade-offs among fitness-related behaviors and compelled the rapid evolution of alternative compensatory reproductive tactics.

**Methods**

**Cockroach strains.** All cockroaches were maintained on rodent diet (Purina 5001, PMI Nutrition International, St. Louis, MO) and distilled water at 27 °C, ~40% RH,
and a 12:12 h LD cycle. The WT colony (Orlando Normal) was collected in Florida in 1947 and has served as a standard insecticide-susceptible strain. The GA colony (T-164) was collected in 1989, also in Florida, and shown to be glucose-averse. The F8 generation (free bulk mating without selection), 400 cockroaches were tested to establish the genotypes of the WT and GA strains, two recombinant colonies were initiated in the F1 generation and backcrossed to obtain homozygous glucose-accepting (aa) and glucose-aversive (Aa) lines. Similar results were obtained in both directions of the cross, confirming previous findings of no sex linkage of the GA trait. These two lines were defined as WT_aa (homozygous, glucose-accepting) and GA_Aa (homozygous, glucose-averse). To obtain heterozygous GA cockroaches, GA_Aa, a single intercross group was generated from crosses of 10 pairs of WT_aa × GA_Aa and 10 pairs of GA_AA × WT_aa.

The GA trait follows Mendelian inheritance. Therefore, we used backcrosses, guided by two-choice feeding assays and feeding responses in Acceptance-Rejection assays, to establish the segregation of the GA trait. The WT GA cockroaches (WT_2 × WT_2) produced homozygous F1 cockroaches showing maximal glucose-acceptance. The cross of GA_2 × GA_2 produced homozygous F1 cockroaches showing maximal glucose-aversion. The cross of WT × GA produced F1 heterozygotes with intermediate glucose-aversion. When the F1 heterozygotes were backcrossed with WT cockroaches, they produced F2 cockroaches with a 1:1 ratio of WT and GA genotypes.

The two-choice feeding assay assessed whether cockroaches accepted or rejected glucose (binary: yes-no). Insects were held for 24 h without water, or starved without food and water. Either 10 adults or 2-day-old first instar nymphs (30–40 in a Petri dish (90 mm diameter, 15 mm height) with fresh distilled water in a 1.5 mL microcentrifuge tube and a pellet of rodent food, and monitored when they mated. When females formed egg cases, each gravid female was placed individually in a container (95 × 95 × 80 mm) with food and water until the eggs hatched. After removing the female, her offspring were monitored until adult emergence. We recorded the time to egg hatch, the age (in days) at which nymphal stage, and the appearance of adults at the end of adult emergence. The first instar nymphs and adults in each cohort were counted to obtain measures of survivorship. Although there were significant differences in some of these parameters across all four strains, we found no significant differences between the two recombinant lines, except mating success, which was significantly lower in GA_AA than WT_2a.9

Mating bioassays. All mating sequences were recorded using an infra-red-sensitive camera (Polarstar II EQ610, EverFocus Electronics, New Taipei City, Taiwan) coupled to a data acquisition board and analyzed by searchable and frame-by-frame capable software (NV3000, AverMedia Information) at 27 °C, –40% RH and a 12:12 h LD cycle. For behavioral analysis, tested pairs were classified into two groups: one (GA, GA_Aa, WT, and GA_AA) with all four behaviors and the other with no courtship and no mating (nailed). Four distinct behavioral events (Fig. 1c, Contact, Wing raising, Nuptial feeding, and Copulation) were analyzed using seven behavioral parameters as shown in Supplementary Table 2.

We extracted behavioral data from successful courtship sequences, defined as courtship that led to Copulation. For failed courtship sequences, we extracted the behavioral data from the first courtship of both mated and not-mated groups, because most pairs in both groups failed to copulate in their first encounter, and there were no significant differences in behavioral parameters between the two groups. To assay female choice, we conducted two-choice mating assays (Fig. 1a). A single focal WT or GA and two males, one WT and one GA, were placed in a Petri dish (90 mm diameter, 15 mm height) with fresh distilled water in a 1.5 mL microcentrifuge tube and a pellet of rodent food (n = 25 WT and 27 GA). To assay male choice, a single focal WT or GA was given a choice of two females, one WT and one GA, for acceptance-rejection assay (Fig. 1b, c). Experiments were started using 0 day-old sexually unreceptive females and 10–12 days-old sexually mature males. Newly emerged (0 day-old) females were used to avoid the disruption of introducing a sexually mature female into the bioassay. B. germanica females become sexually receptive at 5–7 days of age, so the mating behavior of the focal female was monitored until the 6th day to be sure that the females were evaluated by the number of offspring produced. We assessed the gustatory phenotype of nymphs (either WT-type or GA-type) to determine which of the two adult cockroaches mated with the focal insect. Each gravid female was maintained individually in a container (95 × 95 × 80 mm) with food and water until the eggs hatched. Two day-old females were mixed with one WT or GA nymph and starved without water and food, and then they were tested in Two-choice feeding assays using 1000 mmol L−1 glucose-containing agar with 0.5 mmol L−1 blue food dye vs. plain sugar-free agar with 1 mmol L−1 red food dye. If all the nymphs chose the glucose-containing agar, their parents were considered WT-type. When all the nymphs showed glucose-aversion, they were raised to the adult stage. Newly emerged adults were backcrossed with WT cockroaches, and their offspring were tested in the Two-choice assay. When the parents were both GA, 100% of the offspring exhibited glucose-aversion. When the parents were WT and GA, the offspring showed a 1:1 ratio of glucose-accepting and glucose-aversive behavior.

We conducted no-choice mating assay using the WT and GA strains (Fig. 1b, d). A female and a male were placed in a Petri dish with fresh water and a piece of rodent food and video-recorded for 24 h. The females were 5–7 days-old and males were 10–12 days-old. Four treatment pairs were tested: WT_2 × WT_2 (n = 20, 18 and 14 pairs for 5, 6 and 7 day-old females, respectively); GA_2 × GA_2 (n = 23, 22 and 35 pairs); GA_2 × WT_2 (n = 21, 14 and 17 pairs); and WT_2 × GA_2 (n = 33, 19 and 15 pairs).

To confirm that gustatory stimulus guided nuptial feeding, we artifically augmented the male nuptial secretion and assessed whether the duration of nuptial feeding and mating success of GA_2 were affected (Fig. 2c). Before starting the mating assay with 5-day-old GA_2, 10–12 days-old WT_2 were separated into three groups: A control group did not receive any augmentation; A water control group received distilled water with 1 mmol L−1 blue dye (=Blue); A fructose group received 1000 mmol L−1 fructose and 1 mmol L−1 blue dye (=Blue + Fr). Approximately 50 µl of the test solution was placed into the tergal gland reservoirs using a glass micropipette. No-choice mating assays were carried out for 24 h. n = 20–22 pairs for each treatment.

We evaluated the association of short nuptial feeding (Fig. 1a) and the GA trait. We conducted no-choice mating assays using females from the recombinant lines (Fig. 3c). Before starting each mating assay with 4-day-old females from the WT, GA and recombinant lines (WT_2a, GA_AA and GA_Aa), the EC50 for glucose was determined by the instantaneous Acceptance-Rejection assay using 0, 10, 30, 100, 300, 1000 and 3000 mmol L−1 glucose (WT_2 and WT_2a, non-starved; GA_2 and GA_2A, 1-day starved). After the Acceptance-Rejection assay, GA_Aa were separated into two groups according to their sensitivity for rejecting glucose; the GA_2A high sensitivity group rejected glucose at 100 and 300 mmol L−1, whereas the GA_2A low sensitivity group rejected glucose at 1000 and 3000 mmol L−1. We paired these females with 10–12 days-old WT_2a (n = 15 WT_2a, n = 20 GA_AA, n = 20 GA_Aa high and n = 17 GA_Aa low).
The final concentration of the nuptial secretion was 1 male-equivalent/μl in a total volume of 10 μl. This concentration also represented approximately the acceptance EC50 for WT female and GA female (Fig. 2a). The mix of salivary and either sugars or nuptial secretion was incubated for 300 s at 25 °C. Additionally, we tested the effect of only saliva in the Acceptance-Rejection assay. Either 1-day starved or non-starved females were tested with water only and then a 1:1 mixture of saliva and water. Saliva alone did not affect acceptance or rejection of stimuli. n = 20–33 females from each strain.

To evaluate whether salivary enzymes are involved in the hydrolysis of oligosaccharides, the contribution of salivary glucosidases was tested using the glucosidase inhibitor acarbose in the Acceptance-Rejection assay (Fig. 4b), as previously described. The first cruciferol of hufacarbose in HPLC-grade water did not disrupt the acceptance and rejection of tastants. Tests Solutions were prepared as follows: 2 μl of either HPLC-grade water or saliva of GA♀ was mixed with 1 μl of either 250 μmol l⁻¹ of acarbose or HPLC-grade water, then the mixture was added to 1 μl of 400 mmol l⁻¹ of either maltose or maltotriose solution. The total volume was 4 μl, with the final concentration of sugar being 190 μmol l⁻¹. For assays with the nuptial secretion, 1 μl of either HPLC-grade water or saliva from 5 day-old GA♀ was mixed with 0.5 μl of either 250 μmol l⁻¹ of acarbose or HPLC-grade water. This mixture was added to 0.5 μl of 10 male-equivalents of nuptial secretion (i.e., 20 male-equivalents/μl). HPLC-grade water was added for a total volume of 10 μl and a final concentration of 1 male-equivalent/μl. The mix of saliva and either sugars or nuptial secretion was incubated for 5 min at 25 °C. All test solutions contained blue food dye. Test subjects were 5 day-old GA♀ and 20–25 females were tested in each assay.

**Nuptial secretion and saliva collections.** The nuptial secretion of WT♀ was collected by the following method: Five 10–12 days-old males were placed in a container (95 × 95 × 80 mm) with 5 day-old GA♀. After the males displayed wing-raising courtship behavior toward the females, individual males were immediately decapitated and the nuptial secretion in their tergal gland reservoirs was drawn into a calibrated borosilicate glass capillary (76 × 1.5 mm) under the microscope. The nuptial secretions from 30 males were pooled in a capillary and stored at −20 °C until use. Saliva from 5 day-old WT♀ and GA♀ was collected by the following method. The nuptial secretions were briefly anesthetized with carbon dioxide under the microscope and the side of the thorax was gently squeezed. A droplet of saliva that accumulated on the mouthparts was then collected into a microcapillary (10 μl, Kimble Glass). Fresh saliva was immediately used in experiments.

**GC-MS procedures for analysis of sugars.** Standards of D(+)-glucose (Sigma-Aldrich), D(-)-maltose (Fisher Scientific) and maltotriose (Sigma-Aldrich) were diluted in HPLC-grade water (Fisher Scientific) at 10, 50, 100, 500 and 1000 ng/μl to generate calibration curves. Samples were vortexed for 20 s and a 10 μl aliquot of each sample was transferred to a Pyrex reaction vial containing a 10 μl of either HPLC-grade water or saliva from 5 day-old GA♀ was mixed with 0.5 μl of either 250 μmol l⁻¹ of acarbose or HPLC-grade water. This mixture was added to 0.5 μl of 10 male-equivalents of nuptial secretion (i.e., 20 male-equivalents/μl). HPLC-grade water was added for a total volume of 10 μl and a final concentration of 1 male-equivalent/μl. The mix of saliva and either sugars or nuptial secretion was incubated for 5 min at 25 °C. All test solutions contained blue food dye. Test subjects were 5 day-old GA♀ and 20–25 females were tested in each assay.

The results of sugar analysis using GC-MS are reported in Supplementary Figs. 1–4.

**Analysis of nuptial secretions.** We focused the GC-MS analysis on glucose, maltose and maltotriose in WT♀ nuptial secretion (Fig. 4c). To quantify the time-course of saliva-catalyzed hydrolysis of WT♀ nuptial secretion to glucose, 1 μl of GA♀ saliva was mixed with 1 μl of 10 male-equivalents/μl. We incubated the mixtures for 0, 5, 10 and 300 s at 25 °C, and added 4 μl of methanol to stop the enzyme activity (n = 5 each treatment). Each sample contained the nuptial secretions of 5 males to obtain enough detectable amount of sugars. For the statistical analysis, the amounts of sugars were divided by 5 to obtain the amount of sugars in 1 male (1 male-equivalent). These amounts were also used for generating Fig. 4c and Supplementary Table 9. In calculations of the concentration of the three sugars (mmol l⁻¹), the mass and volume of the nuptial secretion were measured using 70–130 male-equivalents of undiluted secretion of each strain (n = 3). The mass and volume of the nuptial secretion/male, including both lipid and aqueous layers, were approximately 30–50 μg and 40–50 nl. Because it was difficult to separate the lipid layer from the water layer at this small scale, we roughly estimated that the tergal reservoirs of the four cockroach lines had 30 nl of aqueous layer that contained sugars.

To quantify the time-course of saliva-catalyzed hydrolysis of maltose and maltotriose to glucose, 1 μl of GA♀ saliva was mixed with 1 μl of 200 mmol l⁻¹ of either maltose or maltotriose (Fig. 4d, e). Incubation time points were 0, 5, 10 and 300 s at 25 °C. and methanol was used to stop the enzyme activity. Controls without saliva were also prepared using HPLC-grade water instead of saliva and 300 s incubations. n = 5 for each treatment.

**Photomicroscopy.** The photographs of the tergal glands and mouthparts (Fig. 5) were obtained using an Olympus Digital camera attached to an Olympus CX41 microscope (Olympus America, Center Valley, PA).

**Statistics and reproducibility.** The sample size and number of replicates for each experiment are noted in the respective section describing the experimental details. In summary, the samples sizes were: Mating bioassays, n = 18–50; Feeding assays, n = 16–65; Sugar analysis, n = 5; Life history parameters, n = 14. All statistical analyses were conducted in R Statistical Software (v4.1.0; R Core Team 2021) and JMP Pro 15.2.2 software (SAS Institute Inc., Carey, NC). For bioassay and sugar analysis data, we and we the means and standard errors, and we used the Chi-square test with Holm’s method for post hoc comparisons, t-test, and ANOVA followed by Tukey’s HSD test (all a = 0.05), as noted in each section describing the experimental details, results, and in Supplementary Tables 1–11.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Data associated with this manuscript have been archived in Dryad Digital Repository (https://datadryad.org/stash/share/d(!gk7WrCVFKAh01ppeiwlOk7Uak5V1ZBo).

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