LOCUST SWARMING

A chemical defense deters cannibalism in migratory locusts

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Many animals engage in cannibalism to supplement their diets. Among dense populations of migratory locusts, cannibalism is prevalent. We show that under crowded conditions, locusts produce an anticannibalistic pheromone called phenylacetonitrile. Both the degree of cannibalism and the production of phenylacetonitrile are density dependent and covary. We identified the olfactory receptor that detects phenylacetonitrile and used genome editing to make this receptor nonfunctional, thereby abolishing the negative behavioral response. We also inactivated the gene underlying phenylacetonitrile production and show that locusts that lack this compound lose its protection and are more frequently exposed to intraspecific predation. Thus, we reveal an anticannibalistic feature built on a specifically produced odor. The system is very likely to be of major importance in locust population ecology, and our results might therefore provide opportunities in locust management.

Cannibalism is defined as the killing and consumption of all or parts of a conspecific. Although it can sometimes function to eliminate competitors (*1*, *2*) or be the result of interactions connected to mating (*3*), in most cases it is a means of supplementing nutrition (*4*, *5*). When practiced frequently, cannibalism has important ecological consequences for population dynamics and stability, interspecific trophic interactions, and pathogen transmission and epidemiology (*6*–*8*). For predatory animals, cannibalism is a straightforward extension of diet, and many predators include conspecifics among their prey (*9*). A broader range of taxa, including insects, engage in egg cannibalism, which is facilitated by the eggs' small relative size, immobility, and vulnerability (*10*), or in predation on much smaller juvenile conspecifics (*6*). For prey of cannibalism, strong selection pressures favor the development of anticannibalistic strategies to deter potential predators of their own kind (*11*). Among insects, anticannibalistic strategies, although having evolved independently in species across taxa, are mostly reported for egg protection (*12*–*14*). Beyond egg protection, animals can deploy direct defense against conspecifics. For example, in a particular predatory nematode, cannibalism seems to be avoided by a chemically based self-recognition process (*15*). However, anticannibalistic strategies, in general, remain largely unknown.

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Among species of locusts, cannibalism is a common characteristic, especially when they are deficient in protein and carbohydrates (*16*, *17*). Like several other locust behavioral traits, cannibalism shows phase polyphenism in response to changes in local population density. At low densities, locusts live as solitary individuals that avoid physical contact. As the local density increases beyond a critical value, behavioral repulsion declines and the locusts begin to be mutually attracted, thereby increasing the likelihood of encountering conspecifics and engaging in cannibalistic interactions (*16*, *18*). To individuals in a group, cannibalizing vulnerable conspecifics offers the dual benefits of surviving longer and traveling farther than a solitary individual without the opportunity to cannibalize (*18*, *19*). Furthermore, cannibalistic interactions have been suggested as one of the driving forces behind collective mass movement. The threat of cannibalistic attacks, especially from behind, was shown to be one of the factors that potentially underlies swarm behavior and movement in migratory bands of nymphs (*16*). Locusts, in general, are extremely important agricultural pests, with swarms devastating crops valued at billions of dollars. At present, the most serious problems are caused by the desert locust, *Schistocerca gregaria*, but the migratory locust, *Locusta migratoria*, also causes substantial damage in Africa and Asia (*20*–*22*). Our recent study on the migratory locust identified hundreds of volatile compounds emanating from all life stages (*23*). Several of these compounds were shown to be behaviorally repulsive, but their biological functions remain largely unknown. Here, we used *L. migratoria* as a model system, revealing the importance of one specific olfactory cue that is involved in protecting individuals against cannibalism at biological levels, from detection to behavior.

Cannibalism is frequent among crowded gregarious nymphs

Before setting out to study the olfactory background of anticannibalism, we established specific traits of cannibalism in *L. migratoria*. We observed that cannibalism is a characteristic of gregarious locusts at all life stages, even if the insects are supplied with sufficient plant-based food sources. Locusts are hemimetabolous insects, with five juvenile instars before reaching adulthood. In our experiments, the highest rates of cannibalism occurred among nymphs at the fourth-to-fifth instar (fig. S1), when the locusts, in general, are more aggressive toward conspecifics (*24*). We therefore chose this stage to investigate density-dependent variations in the rate of cannibalism. We found that cannibalism among fourth-to-fifth instar nymphs displayed density-dependent changes (Fig. 1). At densities of 5 and 25 individuals per cage (cage volume of 9.4 liters), the behavior was reminiscent of solitary locusts, with no individuals attacking or biting each other. The interactions at these two lower densities were observed visually over the course of 1 hour, and no biting attacks occurred (n = 5 cages). When the density was increased beyond 50 individuals per cage, increasing rates of cannibalism, which leveled out at a density of 250 nymphs per cage, were noted. Nymphs of the migratory locust thus display a clear, density-dependent rate of cannibalism. Our results are consistent with earlier observations in the desert locust (*18*). On the basis of these initial results, a clear selection pressure for anticannibalistic agents and behavior could be expected, and we set out in search of potential olfactory cues.

Crowded locusts emit phenylacetonitrile

To establish candidate odors that possibly protect against cannibalism, we conducted a chemical analysis of all volatile compounds emitted from the body of solitary and gregarious migratory locusts (Fig. 2A). Among these, we identified 17 compounds that are only emitted by the gregarious phase, during which cannibalism occurs. Four of the compounds identified—4-vinylanisole, 2-methyl-5-isopropylpyrazine, 2,4,6-trimethylpyridine, and phenylacetonitrile (PAN)—had been reported earlier (*23*, *25*, *26*), and 4-vinylanisole, 2-methyl-5-isopropylpyrazine, and 2,4,6-trimethylpyridine were identified as intraspecifically attractive compounds (*23*, *26*); the remaining compounds were shown to be behaviorally neutral in our tests (fig. S2). However, PAN, a compound also identified in a number of other organisms (*27*–*29*), has been shown to be a repellent against bird predators in *L. migratoria* and also a male antiaphrodisiac in the desert locust (*30*, *31*). Furthermore, it has been shown to be an honest signal for toxicity because its breakdown can form the toxic product hydrogen cyanide (*30*). Thus, the distinctive function of PAN as a repellent and

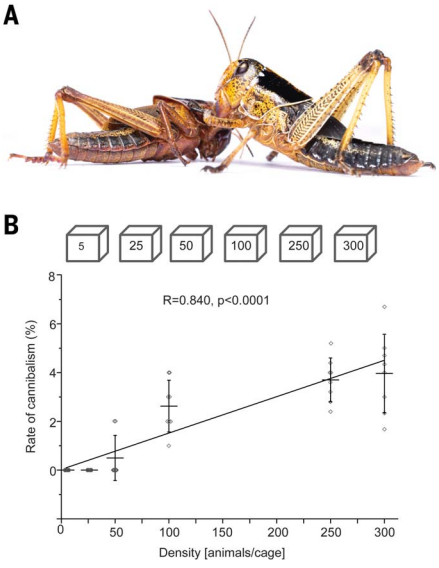

Fig. 1. Cannibalism in *L. migratoria*. (**A**) A fifth-instar migratory locust (right) consuming the body of a conspecific. (**B**) The rate of cannibalism displayed by gregarious fifth-instar nymphs at different population densities. The rate of cannibalism was calculated as the percentage of animals that were completely eaten at each density. Minor attacks also occurred but were impossible to distinguish from other injuries, for example, legs lost from getting stuck in cage ventilation holes. We therefore chose the clearest and most conservative measure, that is, complete consumption. Data are plotted as the mean and standard deviation, with raw data points in the background, and n = 8 replicates per density [displayed Spearman's rank correlation coefficient (R) and p values are based on a Spearman's rank correlation analysis]. The boxes with numbers represent cages and the number of individuals per cage. [Photo credit: B. Fabian]

an honest signal of toxicity in the migratory locust raised our interest in this compound, and we asked if it might indeed be an intraspecific behavioral antagonist, inhibiting cannibalism.

Consistent with earlier studies (*25*), we found that PAN is the dominant volatile component emitted from the body of gregarious nymphs. Moreover, we found that PAN emission by gregarious nymphs covaries with density and thereby parallels the earlier observations of cannibalism rates (Fig. 1B). Nymphs thus begin to release PAN when the population density exceeds 25 individuals per cage (Fig. 2B), whereafter release rates increase gradually with density. Because both PAN release and cannibalism displayed a density-dependent pattern, we hypothesized that PAN could play a role in nymph interactions at high densities.

Locusts exhibit aversion to PAN

Next, we subjected gregarious fourth-to-fifth instar nymphs to dual-choice olfactometer tests to determine the behavioral valence of PAN (Fig. 2C). First, we applied paraffin oil to both sides of the olfactometer as a control and found that nymphs did not show any behavioral preference for a specific side when an olfactory stimulus was absent (Fig. 2D). Applying PAN, we found that the gregarious nymphs displayed significant repulsion at concentrations ranging from 10^{-4} to 10^{-2} dilution (1:10,000 to 1:100 volume:volume dilution). The nymphs significantly preferred to move and remain in the zone suffused with vapor of paraffin oil than the zone smelling of PAN. At a lower concentration of 10^{-5}, the negative effect of PAN was absent (Fig. 2D). We proceeded to evaluate the behavioral performance across developmental stages, phases, and sexes and found that PAN also evoked aversion among adults of both sexes and both phases (Fig. 2E). We thus conclude that PAN is an aversive compound to migratory locusts regardless of age, sex, or phase.

PAN is mainly detected by one olfactory receptor that is necessary for aversive behavior

We now turned to the olfactory system of *L. migratoria* in search of the olfactory receptors (ORs) involved in the detection of PAN. Initially, we cloned 49 ORs and functionally expressed them in the antennal trichoid 1 empty neuron system of *Drosophila melanogaster*, in which the endogenous receptor OR67d is lacking (*32*). We chose this system because it involves the SNMP1 coreceptor that was earlier shown to be expressed in locust olfactory sensory neurons (*33*). The alternative Δ-halo system lacks SNMP1 (*34*). To establish the molecular specificity of the locust ORs, we performed single-sensillum recording (SSR) measurements in the transgene fly lines while stimulating with PAN. These recordings revealed that the fly line bearing the *L. migratoriaOR70a* (*LmOR70a*) gene displayed the highest response to PAN among all the fly lines tested (Fig. 3A). The PAN response of LmOR70a was almost fourfold and fivefold higher than that of the second- and third-best ORs LmOR20 and LmOR75, respectively (Fig. 3B). We then asked whether LmOR70a was exclusively tuned to PAN and screened 204 locust volatiles reported previously from our laboratory (*23*) (tested at 10^{-1} dilution) in continued recording experiments (table S1). We identified two additional compounds, which are structurally similar to PAN (benzaldehyde and cinnamaldehyde), that activated neurons expressing LmOR70a, though at higher concentrations (Fig. 3, C and D). However, the vapor pressure of the second-best ligand, benzaldehyde, is about 14 times higher than that of PAN, meaning that the dose-response curve should be corrected accordingly (Fig. 3D). The fact that two additional compounds activated LmOR70a, albeit to a much lower degree, prompted us to return to behavioral experiments. We found that none of these odors elicited repulsion or preference (Fig. 3E), suggesting that these two compounds might also be detected by other ORs that modulate the input of LmOR70a-expressing neurons to the locust brain and thereby change the behavioral response. Specific receptors that detect both benzaldehyde and cinnamaldehyde were indeed identified in a parallel study (*23*). Another possible explanation for the specific response to PAN could be the presence of other receptors that are specific to this compound among those receptors that were not tested. However, the fact that all electroantennogram (EAG) responses were abolished in locusts when the LmOR70a receptor had been made nonfunctional (see next paragraph and Fig. 3H) is contrary to this explanation. After testing 49 ORs and more than 200 relevant odors, we thus established LmOR70a as a highly sensitive and specific detector of PAN.

Olfactory sensory neurons that bear different types of ORs are located in olfactory hairs, or sensilla, on the locust antenna. To determine in which type of sensilla the neurons detecting PAN were located, we carried out SSR experiments with two types of locust sensilla (basiconic and trichoid) while stimulating with PAN. These are the sensillum types that house olfactory sensory neurons that express the olfactory coreceptor Orco, which is known to be present in all OR-expressing neurons (*35*, *36*). Basiconic sensilla house 20 to 50 sensory neurons, whereas trichoid sensilla house two or three (*35*). The high number of neurons present in the basiconic sensilla prevented us from discriminating responses from individual neurons on the basis of action potential (spike) amplitudes, and the response was calculated as the total number of spikes elicited from the complete population of neurons housed in the sensillum. We found a strong response from neurons present in basiconic sensilla, whereas no response was observed from neurons in trichoid sensilla (Fig. 3F). We then asked whether it was LmOR70a-expressing neurons that were housed in basiconic sensilla and contributed to the response to PAN. First, we performed RNA in situ hybridization to determine the distribution of neurons expressing LmOR70a in the locust nymph antenna and found that LmOR70a-expressing neurons were indeed housed exclusively in basiconic sensilla (Fig. 3G). Next, we generated a loss-of-function allele of the *LmOR70a* gene using CRISPR-Cas9 genome editing. A single guide RNA (sgRNA), targeting the first exon of the *LmOR70a* gene, introduced an 8–base pair (bp) deletion, resulting in a truncated LmOR70a protein (fig. S3A). Through EAG recordings, we could show that the response to PAN was abolished in the *LmOR70a*$^{-/-}$ line, whereas the response to another odor remained unaffected. (Fig. 3H and fig. S3, B and C). When we then recorded the

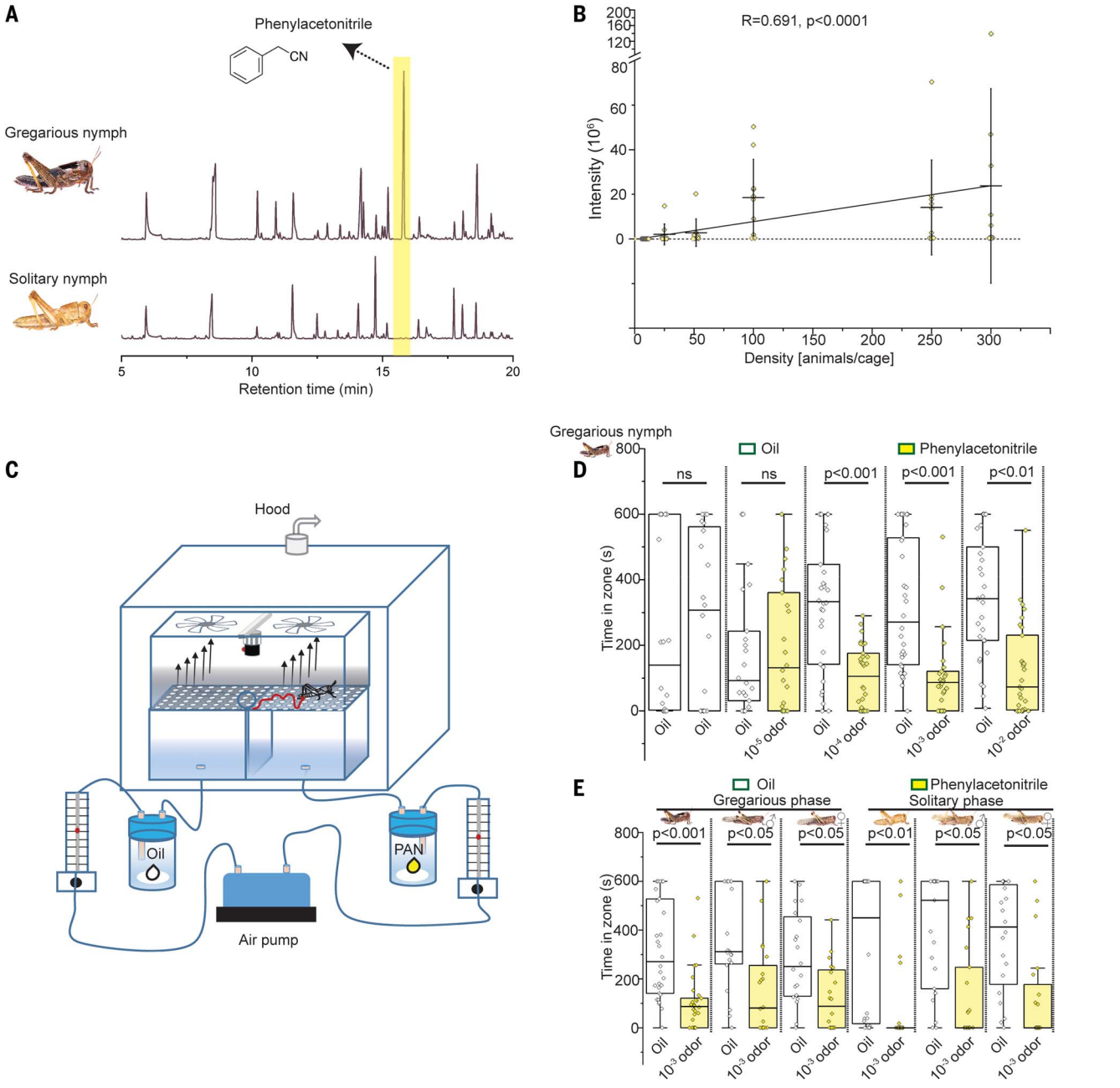

Fig. 2. Gregarious nymphs release PAN to repel others. (**A**) Gas chromatograms of locust body volatiles. PAN is indicated by the yellow bar. (**B**) PAN emitted by gregarious nymphs at different population densities. Data are plotted as the mean and standard deviation, with raw data points in the background, and n = 10 replicates for each population density (displayed R and p values are based on a Spearman's rank correlation analysis). (**C**) Schematic drawing of the dual-choice olfactometer experiment. Details of the experiments are described in the materials and methods. (**D**) Behavioral responses of gregarious nymphs to different dosages of synthetic PAN, with n = 20 to 30 replicates for each concentration. (**E**) Behavioral responses of locusts across phase, sex, and developmental stage to 10^{-3} dilution PAN, with n = 18 to 30 replicates for each type. Data in (D) and (E) are plotted as box and whisker plots (boxes depict median and upper and lower quartiles; whiskers depict quartiles ± 1.5 times the interquartile range). In (D) and (E), p values were determined by a paired-sample Wilcoxon signed-rank test; ns is not significant. [Photo credits: B. Fabian]

SSR response to PAN of neurons housed in basiconic sensilla, we found that in the *LmOR70a*$^{-/-}$ line, the response was close to abolished compared with that of wild-type (WT) nymphs (Fig. 3I). Moreover, we tested gregarious nymphs of the *LmOR70a*$^{-/-}$ line in the dual-choice olfactometer for their behavioral response to PAN and found that the homozygous mutant nymphs had completely lost their aversion to PAN as compared with WT insects (Fig. 3J). We conclude that olfactory sensory neurons that express LmOR70a are present in basiconic sensilla and are responsible for the negative behavioral response to PAN in migratory locusts. This pathway is thus necessary for the aversion to occur.

PAN suppresses cannibalism

When observing locust behavior, we found that cannibalism could be categorized into two forms: one that entails the consumption

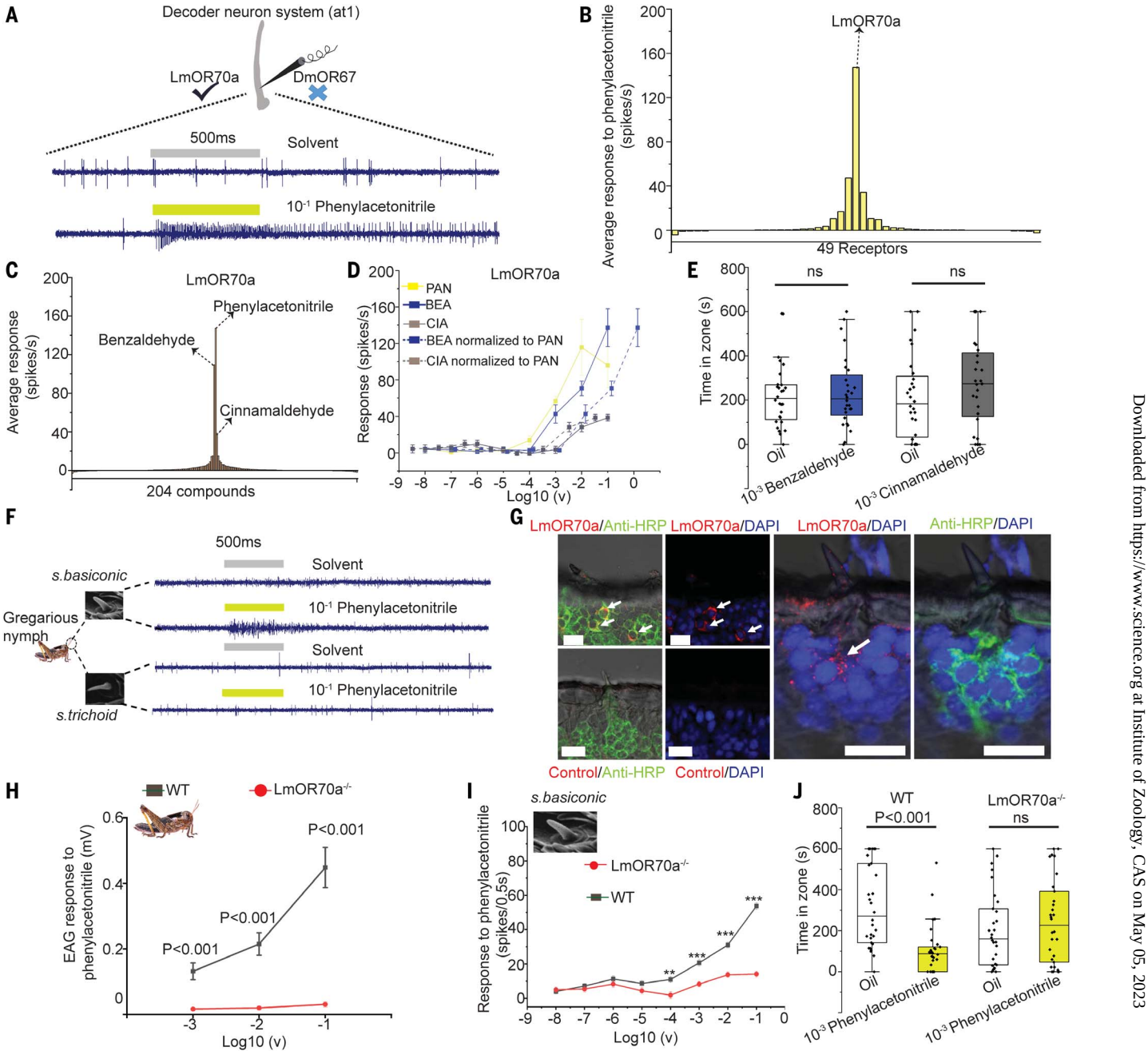

Fig. 3. LmOR70a detects PAN and mediates aversiveness in locusts. (**A**) Representative SSR traces of LmOR70a-expressing *Drosophila* neurons to 10^{-1} dilution PAN. DmOR67 is absent in the at1 neurons of these flies. (**B**) Response to PAN from *Drosophila* decoder neurons expressing 49 different LmORs, with $n = 5$ or 6 recordings for each receptor (*41*, *42*). (**C**) Response of *Drosophila* decoder neurons expressing LmOR70a to 204 odorants (each odor was applied at 10^{-1} dilution), with $n = 6$ recordings for each odorant. (**D**) Dose-response curves of *Drosophila* decoder neurons expressing LmOR70a to the three most active odor molecules, with $n = 6$ recordings for each compound. Data points are mean ± SEM. Dashed curves indicate responses to the two less active compounds after correction according to vapor pressure relative to PAN [the vapor pressure at 25°C of PAN is 0.089 mmHg, of benzaldehyde (BEA) is 1.27 mmHg, and of cinnamaldehyde (CIA) is 0.0289 mmHg]. (**E**) Behavioral responses of gregarious nymphs to benzaldehyde and cinnamaldehyde, with $n = 27$ or 28 biological replicates for each odor. (**F**) Representative spike traces from SSR recordings from locust basiconic and trichoid sensilla in response to 10^{-1} dilution PAN. Also shown are scanning electron micrographs of a basiconic and a trichoid sensillum from the locust antenna. (**G**) In situ fluorescence hybridization revealing that LmOR70a-expressing olfactory sensory neurons are housed in basiconic sensilla. White arrows in all images indicate LmOR70a-positive cells, and the white scale bars represent 20 μm. DAPI, 4′,6-diamidino-2-phenylindole; HRP, horseradish peroxidase. (**H**) EAG dose-response curves to PAN at different concentrations recorded in WT and *LmOr70a*$^{-/-}$ locusts, with $n = 10$ to 12 recordings for each locust type. Data points are mean ± SEM. v, dilution. (**I**) SSR dose-response curves in WT and *LmOr70a*$^{-/-}$ locusts, with $n = 35$ (WT) and 27 (*LmOr70a*$^{-/-}$) sensilla. Data points are mean ± SEM. The inset shows a scanning electron micrograph of a basiconic sensillum. (**J**) Behavioral responses to PAN in WT and *LmOr70a*$^{-/-}$ locusts, with $n = 29$ or 30 replicates for each assay. Data in (E) and (J) are plotted as box and whisker plots (boxes depict median and upper and lower quartiles; whiskers depict quartiles ± 1.5 times the interquartile range). In (E) and (J), p values were determined by a paired-sample Wilcoxon signed-rank test, whereas p values in (H) and (I) were determined by a two-tailed unpaired Student's t test. ***$p < 0.001$; **$p < 0.01$; ns is not significant. [Photo credits: B. Fabian]

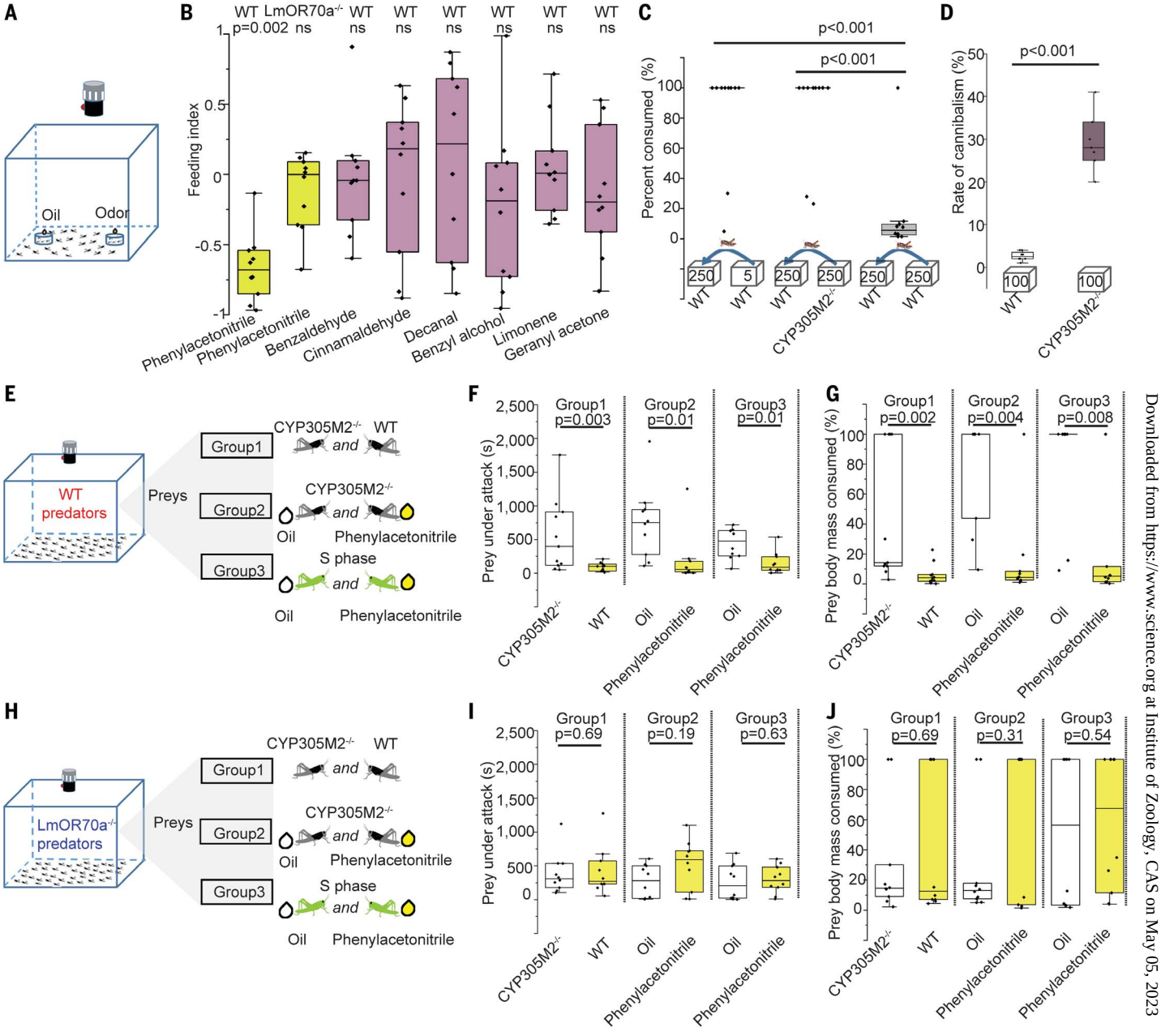

Fig. 4. PAN deters locust cannibalism. (**A**) Schematic of the dual-choice predation experiments using nymph corpses. (**B**) Results of the dual-choice experiments in which starved nymphs chose between corpses with odor and corpses without odor, with n = 10 biological replicates for each assay. The feeding index was calculated as (O − C)/(O + C), where O is the consumed mass of corpses perfumed with odor and C is the consumed mass of corpses perfumed with paraffin oil as a control. (**C**) The rate of cannibalism on living individual nymphs transferred from low-density to high-density cages and from high-density to high-density cages and on *CYP305M2*$^{-/-}$ mutants transferred from high-density to high-density cages. The rate of cannibalism as a percentage was calculated as (B − A)/B, where B is the mass of the prey before the assay and A is the mass of the prey after the assay, with n = 10 biological replicates for each assay. The boxes with numbers represent cages and the number of individuals per cage. (**D**) The rate of cannibalism displayed by WT and *CYP305M2*$^{-/-}$ mutants at a density of 100 individuals per cage. The rate of cannibalism was calculated as the percentage of animals that were completely eaten, with n = 8 (WT) and 7 (*CYP305M2*$^{-/-}$). (**E**) Schematic of dual-choice cannibalism experiments on three pairs of nymphs that were introduced to 50 WT starved nymphs: group 1, a *CYP305M2*$^{-/-}$ individual (no PAN) and a WT individual (producing PAN); group 2, a *CYP305M2*$^{-/-}$ individual (no PAN) and a *CYP305M2*$^{-/-}$ individual perfumed with PAN; and group 3, a solitary-phase individual (no PAN) and a solitary-phase individual perfumed with PAN. (**F** and **G**) Quantification of the time that prey nymphs were under attack (F) and percentage of the prey body that was consumed (G) when prey nymphs were introduced to WT nymphs. (**H**) Schematic of dual-choice cannibalism experiments on three pairs of living gregarious *CYP305M2*$^{-/-}$ or solitary nymphs treated with paraffin oil (control) or with PAN and then introduced to 50 *Lm0r70a*$^{-/-}$ starved nymphs. (**I** and **J**) Same as for (F) and (G) but for the experiment shown in (H) with *Lm0r70a*$^{-/-}$ nymphs as potential predators. In (F), (G), (I), and (J), n = 10 or 11 replicates for each assay. In (B), (F), (G), (I), and (J), p values were determined by a paired-sample Wilcoxon signed-rank test; in (C) and (D), p values were determined by a two-tailed unpaired Student's t test. All data were plotted as box and whisker plots (boxes depict median and upper and lower quartiles; whiskers depict quartiles ± 1.5 times the interquartile range). ns, not significant.

of an already dead body and one that involves more active predation on juvenile conspecifics while still alive. Given this, we first wanted to know whether the presence of PAN influences cannibalistic feeding on a dead nymph body. We chose the corpses of fifth-instar gregarious nymphs as potential food sources and noted that these corpses completely stopped releasing PAN 24 hours after death (fig. S4A). We then compared the consumption of natural corpses, only scented with paraffin oil, with those perfumed with PAN in a group of 20 starved, healthy, fifth-instar gregarious nymphs (Fig. 4A). The results showed that the tested insects refused to approach and feed on PAN-scented corpses and instead feasted on the oil-treated ones (Fig. 4B). Weight-wise, the amount of PAN-perfumed corpses that were consumed by feeding nymphs was five times lower than the amount of oil-treated corpses that were consumed. Next, we repeated these experiments but replaced the WT feeding nymphs with *LmOR70a*$^{-/-}$ insects. The *LmOR70a*$^{-/-}$ nymphs did not show any biting or feeding preference between the corpses loaded with oil or PAN (Fig. 4B). We thus conclude that PAN deters locust nymphs from feeding on dead bodies of conspecifics and that LmOR70a-expressing sensory neurons are responsible for this distinction.

To test the distinctive anticannibalistic role of PAN, we tested another four locust volatiles that were previously reported as aversive to gregarious nymphs (*23*) for their effects on preventing feeding on conspecific corpses. None of these compounds reduced feeding on dead nymph bodies, suggesting that PAN might be the only compound that specifically suppresses locust cannibalism (Fig. 4B). The two compounds that activated LmOR70a-expressing neurons to a lower extent than PAN did not reduce feeding on dead nymph bodies either, pointing again at the possibility that the specific ORs involved in the detection of these odors (*23*) modulate their importance.

Next, we wanted to know whether the emission of PAN by living gregarious locust nymphs protects them from conspecific predation. The ideal test would be to have a living gregarious locust devoid of PAN but otherwise intact in every way. Taking advantage of our earlier results, which showed that nymphs from low-density cages produced no PAN, whereas those bred in high-density cages produced high levels, we performed a first test. Nymphs transferred from low-density to high-density cages were directly and completely cannibalized, whereas those transferred from high-density to high-density cages were left untouched (Fig. 4C).

To further investigate the effect of PAN production, we took advantage of the findings from earlier investigations that showed that one member of the cytochrome P450 gene family called CYP305M2 is crucial for PAN biosynthesis in *L. migratoria* (*30*). Building on these insights, we generated a loss-of-function allele of *CYP305M2* using CRISPR-Cas9 genome editing. A sgRNA targeting the second exon of the *CYP305M2* gene introduced a 26-bp deletion, resulting in a truncated CYP305M2 protein (fig. S4B). As shown earlier, PAN biosynthesis covaries with population density. Therefore, to eliminate density effects, we raised *CYP305M2*$^{-/-}$ locusts and WT locusts under identical conditions. Chemical analysis of the emissions of *CYP305M2*$^{-/-}$ and WT locusts showed that the mutant locusts were unable to produce PAN (fig. S4A), whereas the release levels of other major compounds, body color, and mobility characteristics remained unchanged as compared with WT insects. An interesting characteristic of these mutant locusts was that they exhibited such a high degree of cannibalism that production in high numbers became extremely hard. However, because we had access to nymphs that did not produce PAN, we repeated the experiment shown in Fig. 1B but with *CYP305M2*$^{-/-}$ locusts at the lowest density at which significant degrees of cannibalism were shown among the WT locusts (100 individuals per cage). The rate of cannibalism among the *CYP305M2*$^{-/-}$ locusts was found to be almost 10 times higher than that among WT animals (Fig. 4D). We then performed a series of dual-choice predation experiments by introducing a pair of nymphs, one either emitting or perfumed with PAN and the other devoid of the smell of PAN, into an observation chamber containing 50 starved gregarious nymphs (Fig. 4E). First, we examined the predation choice between *CYP305M2*$^{-/-}$ and WT nymphs and found that *CYP305M2*$^{-/-}$ nymphs were frequently attacked and preferentially consumed over WT nymphs. Both the time being under attack and the amount of the body that was consumed were five- and sevenfold higher in *CYP305M2*$^{-/-}$ nymphs than in WT insects, respectively (Fig. 4, F and G). Second, we used *CYP305M2*$^{-/-}$ locusts scented with PAN or paraffin oil to test predation choice. *CYP305M2*$^{-/-}$ locusts were each perfumed with 300 ng PAN, an amount similar to the biosynthesis level in WT nymphs (fig. S4C) (*30*). In predation experiments as described above, oil-treated *CYP305M2*$^{-/-}$ locusts were, in most cases, completely consumed by starved conspecifics, whereas PAN-treated *CYP305M2*$^{-/-}$ nymphs were seldom injured or consumed (Fig. 4, F and G). In a final experiment, we replaced the *CYP305M2*$^{-/-}$ nymphs with WT solitary ones because solitary nymphs are also unable to produce PAN. We found that starved nymphs also refused to attack and feed on PAN-treated individuals in this setup, whereas they very frequently fed on the nonscented, solitary locusts (Fig. 4, F and G). To determine whether LmOR70a was also required for these PAN-dependent interactions, we used *LmOR70a*$^{-/-}$ locusts as starved predators and repeated the choice experiments, including both *CYP305M2*$^{-/-}$ and solitary locusts (Fig. 4H). We found that *LmOR70a*$^{-/-}$ locusts displayed no predation preference for prey individuals with or without PAN (Fig. 4, I and J). These results together confirm that PAN produced by gregarious nymphs deters intraspecific predation and that the detection of PAN by LmOR70a-expressing neurons governs the suppression of cannibalism among gregarious locust nymphs.

Discussion

Locusts are infamous for their habit of forming giant swarms, causing severe damage to many crops (*17*, *37*). Such enormous densities of individuals raise questions regarding the interactions that occur within the swarm. In our study, we reveal an interesting impact of the compound PAN. The production of PAN in different locust species was reported decades ago, and several hypotheses have been postulated regarding its function (*30*, *31*, *38*, *39*). In the desert locust, it has been proposed to be both an aggregation pheromone and a male courtship inhibitor (*31*, *38*, *39*). In another exhaustive study, Wei *et al*. described that in *L. migratoria*, PAN acts as an olfactory aposematic signal in defense against general predators, for example, birds (*30*), and that it is an honest signal of toxicity. However, insects living in dense groups have been shown to be less vulnerable to predation than solitary individuals (*37*, *40*) but still face a substantial risk of being eaten by conspecifics. Cannibalism has been hypothesized as being one of the factors that underlies swarm dynamics (*16*). Irrespective of the specific swarm situation, cannibalism occurs frequently among crowded locust nymphs, and avoiding it should be beneficial. Here, we report that PAN has a clear anticannibalistic function among locust nymphs. Taking all results under consideration, we suggest that this might be the original function of the compound, because a more widespread and less density-dependent occurrence might be expected in a purely antipredatory compound. Indeed, only crowded gregarious nymphs of the migratory locust release high amounts of PAN, whereas solitary individuals do not. This dichotomy further emphasizes that the production of PAN comes with specific costs and has a specific function that seems to be restricted to crowded conditions. Under these conditions, the level of PAN production varies between individual nymphs, which might explain why some degree of cannibalism remains. However, when PAN production was abolished in mutant locusts, cannibalism increased manifold, revealing that cannibalism is strongly suppressed in WT animals.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
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A chemical defense deters cannibalism in migratory locusts

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Editor's summary
Locusts spend much of their lives living as peaceful herbivores. However, in response to various cues, they change morphologies, aggregate, and create huge swarms. Swarming locusts look different and behave differently, including engaging in cannibalism. Chang *et al*. identified a mechanism though which young locusts can limit their potential to be preyed upon by conspecifics (see the Perspective by Couzin and Couzin-Fuchs). A specific compound, phenylacetonitrile, is produced by locust nymphs raised in crowded conditions and protects the animals from their cannibalistic relatives. Characterization of this mechanism could contribute to an improved understanding of locust swarming behavior. —Sacha Vignieri