For flour beetles, it's better to be a woman in a man's world

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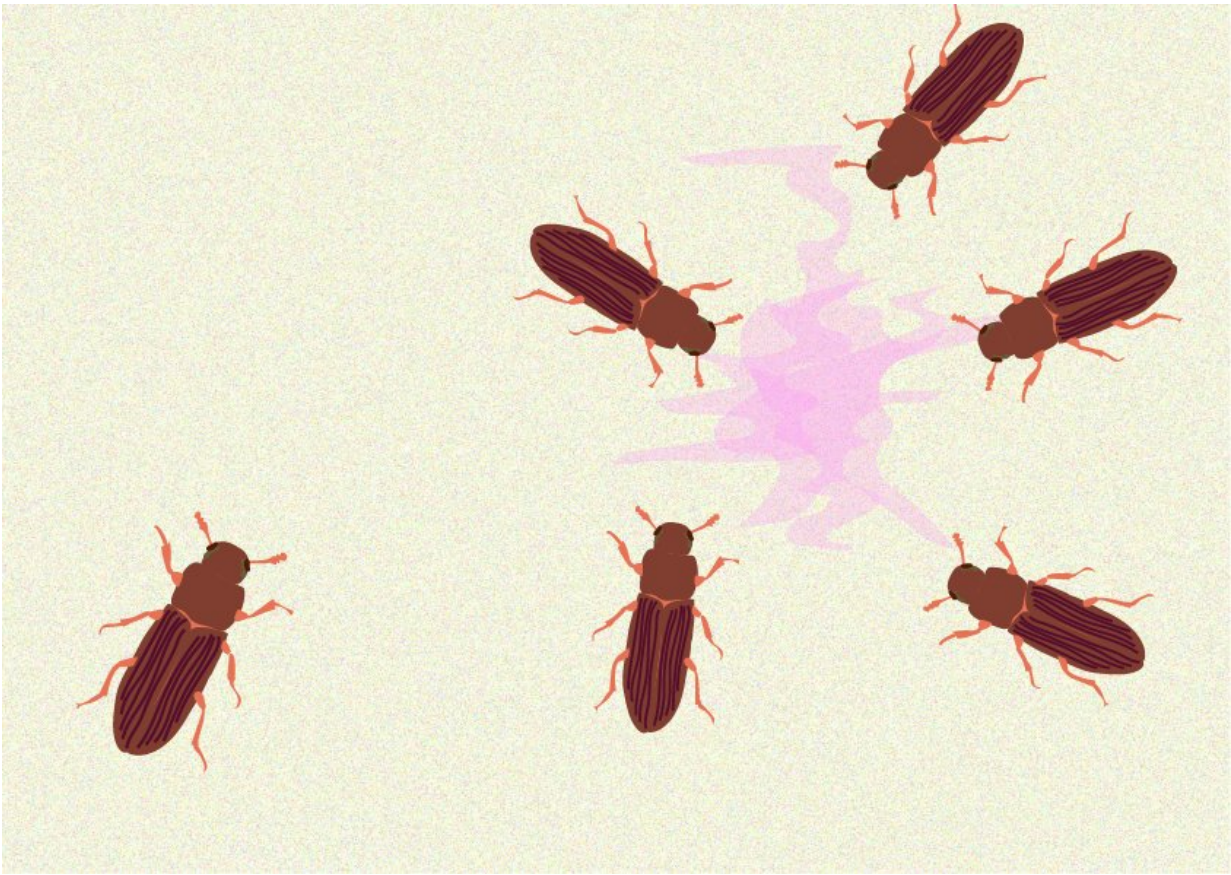

Tribolium castaneum, or the red flour beetle is a worldwide pest. The hardy insect infests stored grains and grain products, and reproduces rapidly. Hardiness and quick reproduction -- traits much deplored in a pest -- however, make the red flour beetle an ideal model organism for ecological, genetic, and reproductive behavior research. Credit: Ipsa Jain

For red flour beetles, being a female in a male world is advantageous. Unlike humans, where this situation traditionally confers a disadvantage, female flour beetles in male-dominated groups seem to reproduce better and live longer than females in groups with equal sex ratios or in female-dominated groups.

Work from Deepa Agashe's group at the National Centre for Biological Sciences (NCBS), Bangalore, demonstrates that this effect is mediated chemically by compounds from the beetles' stink glands. With help from Radhika Venkatesan's team, also from NCBS, the researchers have discovered that the compounds responsible for this phenomenon are ethyl benzoquinone and methyl benzoquinone, the weapons of choice for female flour beetles, used to engage in chemical warfare to reduce each other's reproduction.

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Although theory holds a 1:1 sex ratio of adult males to females is optimal for most species, variable sex ratios do exist in certain populations. The evolutionary consequences of such conditions have been extensively studied; for example, females benefit from a male-biased sex ratio if they can choose and mate with a high-quality male, or if she can mate with several males to ensure high genetic diversity in her brood. Alternatively, under such conditions, forced mating by desperate males could reduce a female's life and her reproductive success. Most of these studies, however, focus mainly on male behavior, and little is known about how female behavior, especially non-sexual competition amongst females, affects the impact of sex ratio on reproductive success.

"We decided to test how female-female interactions could affect reproductive success under different sex ratios using T. castaneum," says Deepa Agashe. "We set up three types of groups—male-biased groups, with one female and three males, female-biased groups with one male and three females, and unbiased groups, with three males and three females," she adds. The beetles were provided with an ample amount of flour, and females were periodically isolated from their groups for a day to lay eggs in isolation, after which, they were returned to their groups.

From these experiments, Agashe's team found that females in the male-biased groups consistently laid more eggs, and had more surviving offspring than females from either of the other two groups.

"The results we got were so stark and clear," states Imroze Khan, the lead author, from Agashe's group. "And then, when I was handling the beetles one day, I wondered if the pungent smell from the boxes that housed the beetles had any connection to the effects we were seeing in our experiments," he adds.

The'pungent odor Khan mentions is a characteristic smell associated with flour beetle infestations. As pheromones and other beetle-secreted chemicals accumulate, they condition the flour, giving it a characteristic aroma. When Khan, teaming up with Arun Prakash, exposed freshly mated young female beetles to flour conditioned by female-biased groups, he found that their egg-laying was reduced. However, this reduction in egg-laying did not occur when conditioned flour from male-biased groups was used.

Female beetles seemed to be secreting something into the flour, that could suppress other females' reproduction.

Agashe's group hypothesized that the weapons of choice in this reproductive war were compounds called benzoquinones, which female

beetle stink glands produced in fairly large amounts. However, the identities of the benzoquinones involved in reducing egg-laying were unknown. At this point, Agashe and Khan reached out to Radhika Venkatesan for help.

"Since my group specializes in identifying natural chemical compounds, we teamed up with Deepa's lab, and IDed methyl benzoquinone and ethyl benzoquinone as candidate compounds that could affect egg-laying," says Venkatesan.

Now, Agashe's group began planning experiments to test if the pure compounds could reduce egg-laying just like flour conditioned by female-biased groups could. Here, however, the researchers ran into a problem.

"Although methyl benzoquinone is readily available commercially, ethyl benzoquinone is very unstable, and so cannot be bought. This is where our collaboration with Radhika was even more fortuitous; she synthesized ethyl benzoquinone for us in her lab," says Agashe.

When the experiments were finally performed, the results were most satisfying. Adding ethyl and methyl bezoquinone to stink gland extracts in increasing dosages increasingly suppress egg-laying in female beetles.

"Finding a chemical cause for a fundamental biological phenomenon is pretty rare, but we have done it with this work. Now, we're looking forward to addressing a whole new set of questions on how these chemicals affect the beetles physiologically" says Agashe.

More information: Female Density-Dependent Chemical Warfare Underlies Fitness Effects of Group Sex Ratio in Flour Beetles, *American Naturalist*, DOI: 10.1086/695806 , www.journals.uchicago.edu/doi/suppl/10.1086/695806