Air pollution impairs successful mating of flies, shows study

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Courting chain of four male Drosophila flies. Male flies exhibited unusual courting behavior towards other males that had been exposed to increased ozone levels as they are nowadays often found in cities in the summer. Credit: Benjamin Fabian, Max Planck Institute for Chemical Ecology

Insect sexual communication relies to a significant extent on

pheromones, chemical attractants that specifically allow males and females of a species to mate. Sex pheromones are distinctive to males and females of a species. Even the smallest differences, such as those observed in the formation of new species, ensure that mating no longer takes place, because males and females only find each other through the unmistakable odor of their conspecifics.

Most insect pheromones are odor molecules containing carbon-carbon double bonds. Such double bonds are known to be easily destroyed by ozone. "We already knew that environmental pollutants such as ozone and nitric oxide degrade floral scents, making flowers less attractive to their pollinators. Since compounds with carbon double bonds are particularly sensitive to ozone degradation, and almost all insect sex pheromones carry such double bonds, we wondered whether air pollution also affects how well insect females and males find and identify each other during mating," says Markus Knaden, who heads the Odor-guided Behavior Group in the Department of Evolutionary Neuroethology and is a lead author of the study, published March 14 in *Nature Communications*.

Knaden was also involved in a 2020 study published in the *Journal of Chemical Ecology* on the effects of air pollution on moths.

To study the effects of ozone on the mating behavior of the model fly Drosophila melanogaster, the scientists first developed an ozone exposure system for flies that could mimic ozone levels in the air as they are nowadays often measured in the cities in the summer. To do this, the researchers had to create a continuous airstream with precisely defined ozone levels, which is complicated by the fact that ozone is not a stable chemical compound and decomposes easily. At the same time, flies often carry very small amounts of pheromones even under normal conditions.

"We therefore needed a technique that would allow us to measure even tiny amounts of pheromones on individual flies that had either been exposed to ozone or not prior to the measurements. To do this, we used what is known as a thermal desorption unit coupled to a gas chromatograph/mass spectrometer, which allowed us to measure tiny amounts of odors emitted by individual flies," said first author Nanji Jiang, describing the technical challenges.

Ozone destroys carbon-carbon double bonds in pheromones

In the experiments, male flies were exposed to slightly elevated ozone concentrations. The scientists then measured whether the flies still emitted their pheromone. When the flies were exposed to 100 ppb (parts per billion, corresponding to a concentration of 10–9) of ozone for two hours, the measured pheromone levels decreased significantly compared to a control group that had only been exposed to ambient air.

In addition to males of the model fly Drosophila melanogaster, the researchers also tested male flies of eight related species of the genus Drosophila. In only one species, Drosophila busckii, the release of specific male pheromones remained unaffected after ozone exposure, but these compounds also do not contain carbon-carbon double bonds and thus do not react that easily with ozone.

Ozone drastically corrupts the mating behavior of flies

The researchers then tested the attractiveness of male flies to their conspecifics. The observations they made were disturbing, which may be mainly due to the role of the respective pheromones. These are emitted by the males in Drosophila species and increase their attractiveness to

females. At the same time, males use the odor to distinguish females from other males: While their pheromone attracts females, it repels other males. During mating, males transfer their pheromone to females. Freshly mated females that smell of the pheromone are no longer attractive to other males for the next couple of hours. Consequently, elevated ozone levels not only caused females to be less attracted to males; rather, ozonated males were suddenly interesting to their male counterparts.

"We knew that elevated ozone levels could affect insect mating systems because the breakdown of carbon double bonds, and thus pheromones, by oxidation is not rocket science in chemistry. Nevertheless, we were shocked that even slightly elevated ozone concentrations had such strong effects on fly behavior. Actually, we initially wanted to focus on the interactions between males and females. We could explain that males started courting each other after a short ozone exposure, because they obviously could not distinguish ozonated males from females. However, we had not thought about this before. Therefore, we were quite puzzled by the behavior of the ozone-exposed males, which lined up in long courtship chains," the authors write.

The research team also observed the effects of high ozone levels in the air on the mating behavior of other Drosophila species. Even males of the species Drosophila busckii were less successful at mating after exposure to ozone, although ozone does not alter the pheromone that has been described to be emitted by D. busckii males. However, other so far not identified ozone-sensitive chemical compounds may also play an additional role in their mating behavior. In eight of the other nine species studied, the research team observed unusual courtship behavior by males toward other males exposed to ozone. Interestingly, one species, D. suzukii, which is known to lack pheromones but courts based on visual cues, was not affected at all by increased ozone levels.

Copulation attempt of two Drosophila males in ozone-enriched air. Credit: Benjamin Fabian, Max Planck Institute for Chemical Ecology

Air pollution contributes to global decline in insect populations

Most insect pheromones contain carbon-carbon double bonds. Therefore, ozone is thought to interfere with sexual communication in many insect species. "Insects and their pheromones have evolved over millions of years. In contrast, the concentration of air pollutants has only increased dramatically since industrialization. It is unlikely that the communication systems of insects, which have evolved over the course

of evolution, will be able to adapt to new conditions within a short period of time if pheromones are suddenly no longer there. The only solution to this dilemma is to immediately reduce pollutants in the atmosphere," says Bill Hansson, head of the Evolutionary Neuroethology Department and co-founder of the Max Planck Center next Generation Insect Chemical Ecology (nGICE), which is dedicated to studying the effects of climatic changes and atmospheric pollution on insects and their chemical communication. In particular, research focuses on the effects of anthropogenic climate change on insect ecosystem services, outbreaks of invasive insect species, and the spread of disease vectors in Europe.

The scientists in Jena want to study the effects of ozone on a broader range of insects, including moths that usually follow pheromone plumes over long distances. Sex pheromones are also crucial cues for insects to distinguish between conspecifics and closely related species. "We would like to find out, whether high levels of ozone lead to increased hybridization rates when closely related fly species share their habitat. Finally, chemical communication in insects is not restricted to mating behavior. All social insects like bees, ants, and wasps, use chemical cues to identify members of their colony. We also study whether the social structure within ant colonies is affected, when ants return from their foraging trips during which they became exposed to increased levels of pollutants. You don't even want to envision what happens if social structures in ant colonies or beehives suddenly collapse because pheromone communication no longer works," says Knaden, who also studies the behavior of desert ants.

High ozone levels are not only harmful to human health. The current lifestyle of industrialized nations comes at very high costs to the environment and climate; many indirect effects are not even known. The current study provides an additional explanation why insect populations are declining dramatically worldwide, apart from the application of insecticides and the elimination of habitats. If chemical communication

is disrupted by pollutants in the air, they cannot reproduce at a sufficient rate. This may also affect many pollinators, such as bees and butterflies. The fact that 80% of our crops need to be pollinated by insects makes it clear what scale this problem may take in the future, if we do not succeed in drastically reducing air pollution.

More information: Markus Knaden, Ozone exposure disrupts insect sexual communication, *Nature Communications* (2023). DOI: 10.1038/s41467-023-36534-9.
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