Current Biology Vol 20 No 15
R628

Mammalian herbivore breath alerts aphids to flee host plant

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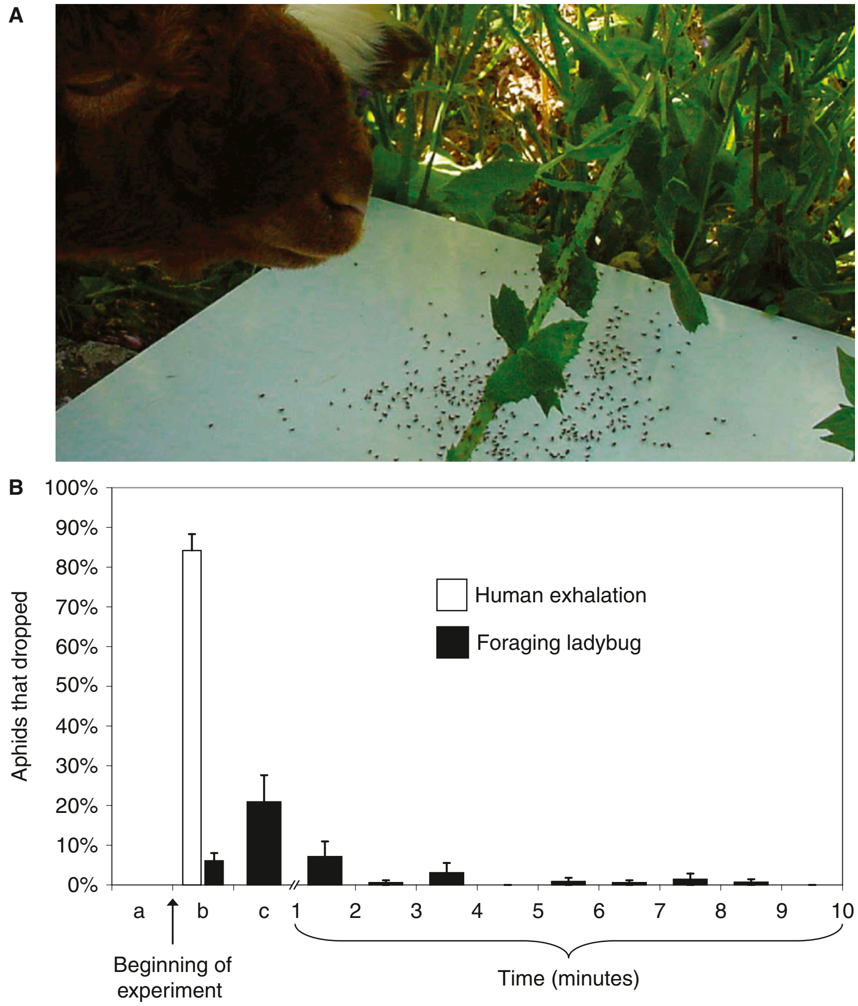
Mammalian herbivores profoundly influence plant-dwelling insects [1]. Most studies have focused on the indirect effect of herbivory on insect populations via damage to the host plant [2,3]. Many insects, however, are in danger of being inadvertently ingested during herbivore feeding. Here, we report that pea aphids (*Acyrthosiphon pisum*) are able to sense the elevated heat and humidity of the breath of an approaching herbivore and thus salvage most of the colony by simultaneously dropping off the plant in large numbers immediately before the plant is eaten. Dropping entails the risk of losing the host plant and becoming desiccated or preyed upon on the ground [4,5], yet pea aphids may sporadically drop when threatened by insect enemies [6]. The immediate mass dropping, however, is an adaptation to the potential destructive impact of mammalian herbivory on the entire aphid colony. The combination of heat and humidity serves as a reliable cue to impending mammalian herbivory, enabling the aphids to avoid unnecessary dropping. No defensive behavior against incidental predation by herbivores has ever been demonstrated. The pea aphids' highly adaptive escape behavior uniquely demonstrates the strength of the selective pressure large mammalian herbivores impose on insect herbivores.

We first noticed the remarkable ability to escape incidental predation in two aphid species living on plants that are highly palatable to herbivores: *Uroleucon sonchi*, which lives on sow thistle (*Sonchus oleraceus*; Figure 1A), and the pea aphid, which lives on alfalfa and other legumes. We designed a set of indoor experiments using pea aphids to quantify the amount of mass dropping and to determine which cues the aphids use to detect the oncoming herbivore. Initially we allowed a goat (*Capra hircus*) to feed on potted alfalfa plants (*Medicago sativa*; *n* = 18 plants) infested with aphids. Strikingly, 65% ± 6% (SEM) of the aphids in the colonies dropped to the ground right before they would have been



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Physical parameters of the airstream	Ambient humidity %(RH)	Airstream humidity %(RH)	Airstream temperature °C	N	Aphid dropping %
Control	70 ± 5	70 ± 5	22 ± 0.2	92	**0**
Warm, ambient humidity	70 ± 5	70 ± 5	35 - 36	48	**4** [c]
Humid, room temperature	70 ± 5	90 - 100	22 ± 0.2	101	**14** [c]
Warm and humid	70 ± 5	90 - 100	35 - 36	87	**38** [b]
Warm and humid	50 ± 1	90 - 100	35 - 36	59	**87** [a]

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Figure 1. Aphids dropping off host plant as a defensive response against mammalian herbivores.
(A) Typical mass dropping of an aphid colony in response to a lamb's exhalation, without physical contact with the plant (demonstrated here with the aphid *Uroleucon Sonchi* and the plant *Sonchus oleraceus*). Notice the lamb on the left and the aphids that are scattered on the white plaque inserted under the plant. (B) Dropping of pea aphids in response to human exhalation and to a foraging ladybug (mean ± SEM). The first interval on the x-axis (a) represents the time before the experiment began. The second interval (b) represents the first 2 seconds of the experiment and the third interval (c) represents the following 58 seconds. (C) Dropping response in experiments with artificial breath apparatus. Experiments were conducted at room temperature of 22°C ± 1°C. Different superscript letters in the aphid dropping column (a>b>c) indicate significant difference ($P < 0.0083$, Z-test for two proportions, Bonferroni corrected).

eaten along with the plant. This mass dropping could have been triggered by a number of cues: plant-shaking, sudden shadowing and herbivore exhalation.

Using a leaf-picking device (Supplemental information), we tested for the contribution of plant shaking to the mass colony dropping of aphids from broad bean seedlings (*Vicia faba*). Leaf picking caused only 26% ± 5% (SEM) of colony members to drop. Casting a shadow on the aphids, as an approaching herbivore might, did not induce any dropping. We then allowed a restrained lamb (*Ovis aries*) to approach aphid infested broad bean seedlings to a distance of approximately 5 cm for 10 seconds. On average, 58% ± 7% (SEM) of the colony dropped in response to the lamb's exhalation. It was now obvious that herbivore breath is the key player in conveying to the aphids the message of imminent obliteration.

Pea aphids may drop off plants in response to natural insect enemies [6]; therefore, at this point we compared the dropping patterns in response to a foraging ladybug (*Coccinella septempunctata*) and to mammalian breath. The difference between the two responses was clear: while ladybug-induced dropping is sporadic, mammalian breath causes the immediate simultaneous dropping of a large proportion of the colony (Figure 1B).

Intrigued by the impact of the breath, we began examining the effects of the major attributes of mammalian exhalation on aphid dropping. We constructed an artificial breath apparatus (Supplemental information), which controlled for various parameters of a steady stream of air. The airstream was pointed at individual aphids, placed on broad bean seedlings, from a distance of approximately 1 cm for 2 seconds.

Pea aphids responded similarly to human and herbivore breath; therefore, we tested the effect of compounds that are present in both bovine and human breath. Carbon dioxide at a concentration of 5%, approximately as in mammalian exhalation, had no effect on aphid behavior; neither did five volatile organic compounds, tested individually (acetone, acetic acid, isopropanol, nonanal and decanal). The compound octenol (1-octen-3-ol) was also tested as it is typically found in bovine breath and is a potent attractant for parasitic insects. No response to octenol was observed. A mixture of all the six volatile organic compounds was also tested, with no response. Similarly, the aphids did not drop when the airstream was imbued with the odor of bovine nasal secretions that were added to the artificial breath apparatus (Supplemental information).

It then became clear that the physical parameters of breath constitute the cues that trigger mass dropping: raising airstream temperature alone had a minor effect on dropping. Increasing airstream humidity resulted in a greater but not significantly different effect (Figure 1C). Only when the airstream was both warm and humid were there substantial dropping rates that increased dramatically to 87% when the humidity in the room was low (Figure 1C), suggesting a dependency of the aphid's sensory system on ambient humidity. Heat and humidity clearly act together synergistically, and as ubiquitous characteristics of the breath of warm-blooded animals are reliable indicators to an aphid of impending mammalian herbivory — and possibly insectivorous birds.

Detection of mammalian breath is a capability shared by a number of invertebrate species. Some parasites, such as mosquitoes and ticks, detect CO_2, exhaled organic volatiles, heat and humidity in their search of a blood meal [7,8]. There are also arthropods that respond to parameters of their mammalian predators' breath by secreting noxious chemicals and employing other defensive responses [9]. The only effective response of a colony of tiny aphids to the warmth and humidity of mammalian breath is to flee the host plant.

We conclude that the mass colony dropping, as opposed to the insect predator induced sporadic dropping, is a pre-encounter escape behavior that reduces the chance of being eaten by mammalian herbivores. This remarkable response to mammalian-specific cues, in spite of the inherent cost of an aphid's dropping off the plant [6,10], points to the significance of mammalian herbivory to plant-dwelling insects. We predict that this sort of escape behavior in response to mammalian breath may be found among other invertebrates that live on plants and face the same threat.

Supplemental Information
Supplemental Information includes experimental procedures and can be found with this article online at doi:10.1016/j.cub.2010.06.065.

Acknowledgments
We would like to thank Toby Gish and Marcia Ford for editorial assistance. We also thank Prof. Gad Katzir for critically reading a previous version of the manuscript and Prof. Ido Izhaki for statistical advice.

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