



## Research

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### Author for correspondence:

Wesley Dáttilo

e-mail: [wdattilo@hotmail.com](mailto:wdattilo@hotmail.com)

## Animal behaviour

# Trait-mediated indirect interactions of ant shape on the attack of caterpillars and fruits

Wesley Dáttilo<sup>1</sup>, Armando Aguirre<sup>2</sup>, Pedro Luna De la Torre<sup>1,3</sup>,  
Lucas A. Kaminski<sup>4</sup>, Juan García-Chávez<sup>3</sup> and Víctor Rico-Gray<sup>5</sup>

<sup>1</sup>Red de Ecoetología, and <sup>2</sup>Red de Interacciones Multitróficas, Instituto de Ecología A.C., Xalapa, Veracruz, Mexico

<sup>3</sup>Escuela de Biología, Benemérita Universidad Autónoma de Puebla, Puebla, Pue, Mexico

<sup>4</sup>Departamento de Zoología, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

<sup>5</sup>Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, Mexico

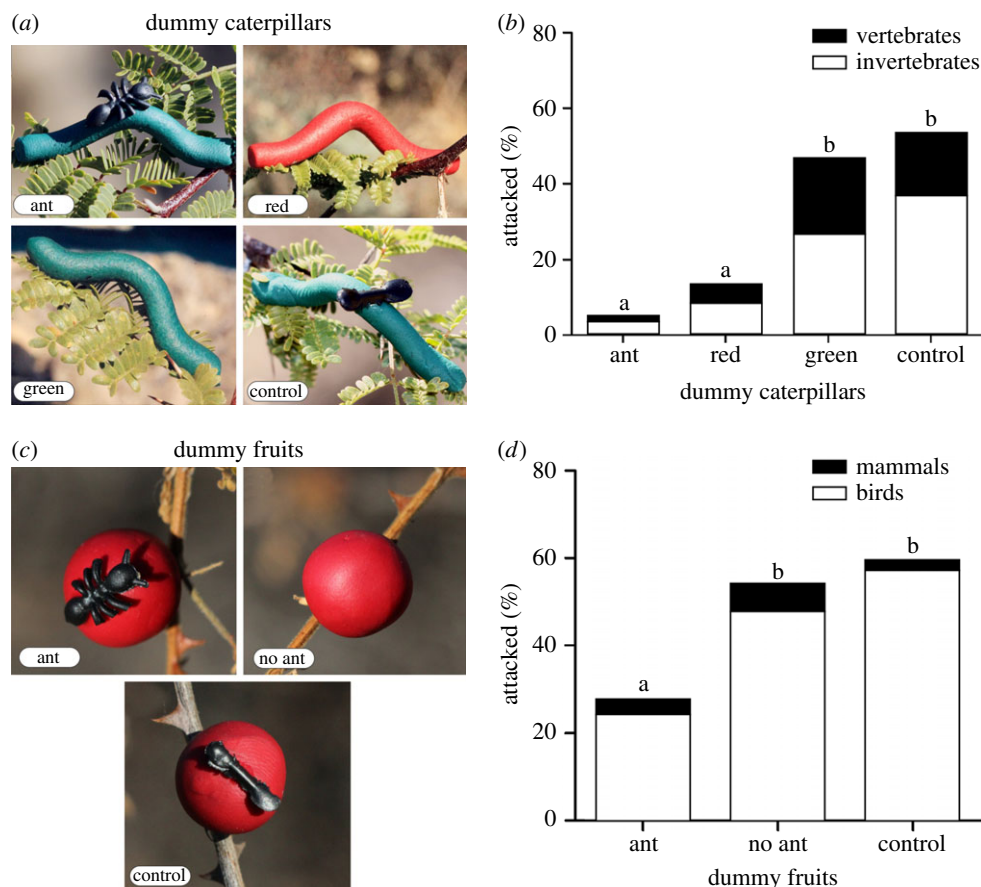
WD, 0000-0002-4758-4379

Mainly owing to their high diversity and abundance, ants are formidable as predators and defenders of foliage. Consequently, ants can exclude both invertebrate and vertebrate activity on plants via direct and indirect interactions as already shown in many previous studies. Here we present empirical evidence that objects resembling ant shape on dummy caterpillars were able to repel visually oriented predators. Moreover, we also show that rubber ants on dummy fruits can repel potential fruit dispersers. Our results have direct implications on the ecological and evolutionary dynamics of interactions in ant-based systems, as ant presence could affect the fitness of its partners. In short, our study highlights the importance of visual cues in interspecific interactions and opens a new way to study the effects of ant presence to test ecological and evolutionary hypotheses.

## 1. Introduction

A fundamental question in ecological studies is to understand the role of species interactions in conservation and maintenance of biological communities [1]. Most studies dealing with species interactions have focused only on pairs of species. However, species are commonly involved in multiple direct and indirect interactions within natural landscapes [2,3]. For instance, a predator can influence the interactions not only through directly eating prey but also indirectly deterring prey from foraging through the potential of being eaten. These trait-mediated indirect interactions (TMII) are diverse and occur when the impacts of one species on another are influenced by one or more intermediate species [4].

Ants are formidable as predators and defenders on the foliage mainly owing to their high abundance and diversity [5], which could exhibit different examples of TMII on plants. For instance, ants' presence on plants can deter the oviposition of butterflies [6]. On the other hand, plants visited by ants are less attacked by insect herbivores and mutualistic ant–plant interactions are well known and documented [5]. However, in some cases, ants are harmful plant partners; this occurs when ants offer enemy-free space for ant-associated herbivorous (mainly trophobiont insects) or when the ant disrupts the visit by other mutualistic partners, such as pollinators and seed dispersers [6–10]. Ants have become models for a variety of organisms that have evolved to visually mimic them as defence from predation [11]. For instance, the aggressive and efficient anti-herbivore activities of ants makes it highly beneficial for plants to visually mimic ant attendance by dark markings in order to deter herbivores without paying the cost of feeding or housing ants [12]. Therefore, the presence of



**Figure 1.** Dummy caterpillars (a) used in Experiment I, showing the four treatments: model green dummy with a rubber ant on them (upper left); artificial green dummy without rubber (lower left); artificial red dummy without rubber (upper right); artificial green dummy with a rubber piece (same size as the ant) on them (lower right). (b) Attacked caterpillars (%) for each of the four treatments by different predator group (invertebrates and vertebrates). Dummy fruits (c) used in Experiment II, showing the three treatments: fruit with a rubber ant on them; dummy fruit without rubber; dummy fruit with a rubber piece on them. (d) Attacked fruits (%) for each of the three treatments by birds and mammals. Different letters assigned to the columns indicate that the results are statistically different. (Online version in colour.)

ants on plants also can affect directly and indirectly the presence of herbivores, frugivores and pollinators.

In this study, we used artificial dummies to answer a simple, but little explored question, involving the trait-mediated indirect effect of ant shape on the foliage and fruits. Specifically, we addressed the following question. Do visually oriented predators recognize objects that resemble ant shape (myrmecomorphy) and avoid caterpillars and fruits? We postulated that the shape of ants alone is sufficient to prevent beneficial predators that specialize on herbivorous insects and interactions with frugivores mainly because there is empirical evidence in the literature that traits mimicking ants can affect trophic interactions involving plants and their partners.

## 2. Material and methods

### (a) Study area

We conducted our fieldwork during November 2014 in an area close to the 'Helia Bravo Hollis Botanical Garden' (18°20' N, 97°28' W, elevation 1507 m), located in the Tehuacan-Cuicatlan Valley, Puebla, Mexico. The main vegetation type in the area is within the Mexican xerophytic region (for details of study site, see [13]).

### (b) Effects of artificial ants on dummy caterpillars and fruits

To test the trait-mediated effect of ant shape, we used an experimental approach based on dummies (caterpillars and

fruits) and black rubber ants (1.5 cm length, US Toy Company®). Caterpillar dummies were made of plasticine, oil-based, odourless and non-toxic (3.5 cm length, 0.5 cm width). Fruits consist of red spheres made using 5 g of plasticine (1.5 cm in diameter).

For the experiment with dummy caterpillars, we randomly selected 60 plants (regardless of species) within a linear transect at least 30 m apart. For each plant, we established four treatments (figure 1a): (i) green caterpillar with a rubber ant on them (simulating biotic defence); (ii) red caterpillar (simulating aposematic defence as a null benchmark); (iii) green caterpillar; and (iv) green caterpillars with a rubber piece (similar to ants but without antenna and legs) on them (control treatment). Artificial caterpillars ( $n = 240$ ) and rubbers were fixed with instant glue on mature leaves with no sign of herbivory, distant 30 cm between them at a height of 1 m above the ground.

The experiment with dummy fruits was conducted within a linear transect (different from those used in the first experiment), we randomly selected 94 plants (regardless of species) at least 30 m apart, and on each plant we placed a set with the three treatments distant 30 cm between them at a height of 1 m above the ground ( $n = 282$  artificial fruits). In each experimental plant, we established three treatments (figure 1c): (i) artificial fruit with a rubber ant on them; (ii) artificial fruit without a rubber ant on them; and (iii) artificial fruit with a rubber piece (same size as the ant) on them (control treatment). Fruits and rubbers were fixed on young branches with instant glue. The size and colour of the artificial models are similar to arboreal ants and fruits common in the study area.

Both experiments were checked after 48 h of exposure in the field. We recorded the number of dummies that were or were not

attacked in all treatments. As the plasticine models are malleable, attack types (based on small slits and scrape marks from mandibles, beak and teeth marks, incisor marks and scrapings, and 'crumbs' or rasping marks) can be identified from conspicuous marks left by potential predators (as described in [14]).

### (c) Data analysis

We used maximum-likelihood statistical significance  $G$  tests to compare the frequency of choices between treatments for the two experiments (caterpillars and fruits), testing the null hypothesis that the probability of choice is the same between treatments. Each treatment within an experimental plant was used as independent sample. All *post hoc* pairwise comparisons between treatments were performed using the same  $G$  tests with Bonferroni correction [15]. We used the same  $G$  tests to separately compare the frequency of choices of each group of predators between treatments (i.e. vertebrates versus invertebrates and birds versus mammals).

## 3. Results

We observed that only 71 (29.5%) artificial caterpillars were attacked. Invertebrates and birds were the groups that most attacked the caterpillars,  $n = 35$  (49.3%) and  $n = 18$  (25.3%), respectively. In addition, we observed that there were differences in caterpillar predation rates among the four treatments ( $G = 55.334$ ; d.f. = 3;  $p < 0.001$ ; figure 1*b*). Artificial green caterpillars associated with rubber ants and red caterpillars were the treatments with the lowest and similar predation rates 13.33% ( $n = 8$  caterpillars) and 5% ( $n = 3$  caterpillars), respectively ( $G = 2.6775$ ; d.f. = 1;  $p = 0.1018$ ). While artificial green caterpillars and the control group were the treatments with the highest and similar predation rates, 53.33% ( $n = 32$  caterpillars) and 46.66% ( $n = 28$  caterpillars), respectively ( $G = 0.5337$ ; d.f. = 1;  $p = 0.4651$ ). Caterpillar predation rates by invertebrates ( $G = 14.321$ ; d.f. = 3;  $p = 0.001$ ) and vertebrates ( $G = 50.001$ ; d.f. = 3;  $p = 0.001$ ) differed between the four treatments. In this case, artificial green caterpillars associated with rubber ants and red caterpillars were the treatments with the lowest and similar predation rates for both groups of animals and in the same proportions of the total caterpillars attacked (considering all animal groups together).

For experimental fruits, we observed that 133 (47.16%) were attacked. Birds were responsible for the largest fruit predation rates,  $n = 122$  fruits (91.72%) compared with mammals,  $n = 11$  fruits (8.28%). In addition, we observed that there were differences in fruit predation rates among the three treatments ( $G = 22.6879$ ; d.f. = 2;  $p < 0.001$ ; figure 1*d*). Fruits without a rubber ant and the control group were the treatments with the highest and similar predation rates 59.57% ( $n = 56$  fruits) and 54.25% ( $n = 51$  fruits), respectively ( $G = 0.5426$ ; d.f. = 1;  $p = 0.461$ ). While only 26 fruits (27.65%) with ant presence were attacked and this value differs from those found for the treatment without ant presence ( $G = 19.8423$ ; d.f. = 1;  $p < 0.001$ ) and the control group ( $G = 13.945$ ; d.f. = 1;  $p < 0.001$ ), indicating a possible negative effect of ant presence on fruit dispersal. Only the fruit predation rates by birds differed between the three treatments ( $G = 23.41$ ; d.f. = 2;  $p < 0.001$ ), where fruits without a rubber ant and the control group were the treatments with the highest and similar predation rates, and in the same proportions of the total fruits attacked (considering all animal groups together).

## 4. Discussion

We present empirical evidence that conspicuous ants could repel visually oriented potential predators of caterpillars and fruit dispersers. In other words, predators of caterpillars and fruit dispersers seem to recognize the body-form of ants and avoid them. Our findings would only support ant mimicry systems where the resemblance to ants is highly effective (i.e. complete ant mimic with legs and antennae or aggressive ants). This new perspective enhances our ability to answer claims that have been made for decades about various traits visually mimicking ants.

Changes in colour pattern and foraging activities of caterpillars over evolutionary time have been attributed, in part, to the selective pressure exerted by visually oriented natural enemies (reviewed in [16]). We know that aposematism (warning) signalling is a common defensive mechanism that affects interactions between different trophic levels, as obtained in our red caterpillar treatment. Here we show that ant presence in caterpillars is sufficient to repel predators. In other words, these two evolutionary routes of alternative defences, despite their inherent costs [17,18], seem to be effective against visually oriented predators. Our results have implications on the ecological and evolutionary dynamics of interactions involving caterpillars, ants, birds, various other animals and plants. Accordingly, our results reinforce the role of establishing enemy-free space in the evolution of myrmecophily in Lepidoptera [3,19], because the simple ability to co-occur with ants can be highly adaptive.

Additionally, we know that in the symbiotic interactions between ants and myrmecophytes, ants may have a deleterious effect on fruit dispersal through decreasing the rates of visitation and fruit removal [11]. Here, we reaffirm that ant presence on fruits can repel potential visually oriented fruit dispersers and we expand this vision not only for specialized myrmecophytic species as previously proposed [8]. Some plants that secrete extrafloral nectar on their fruits to attract protective ants decrease nectar secretion at the end of fruit maturation in order to minimize the negative effect of ants on fruit dispersal [20]. However, most plant species do not have extrafloral nectar on their fruits and, therefore, cannot control the presence of ants on this structure. In addition, many fleshy fruits are attractive to many ant species [5]. Therefore, it is expected that these fleshy fruits could be injured by the presence of ants, decreasing the probability of such fruits to be dispersed.

In short, our study highlights the importance of visual cues of body-form of ants in interspecific interactions and opens a new way to study the effects of ant presence on caterpillar predation and fruit dispersal by testing different ecological hypotheses in different ecosystems around the world where ants are remarkable organisms on plants.

**Authors' contributions.** W.D., A.A., P.L.D.L.T. and J.G.-C. designed the study; W.D., P.L.D.L.T. and L.A.K. analysed the data; W.D., A.A., P.L.D.L.T., L.A.K., J.G.C. and V.R.-G. drafted the manuscript, and all authors revised the manuscript and approved of its final form. All authors agree to be held accountable for the content herein.

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