

Aggressive mimicry coexists with mutualism in an aphid

Adrián Salazar^a, Benjamin Fürstenau^b, Carmen Quero^c, Nicolás Pérez-Hidalgo^d, Pau Carazo^{a,e}, Enrique Font^a, and David Martínez-Torres^{a,1}

^aInstitut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Apartado de Correos 22085, 46071 Valencia, Spain; ^bInstitute of Biology, Applied Zoology/Animal Ecology, Freie Universität Berlin, 12163 Berlin, Germany; ^cInstitute of Advanced Chemistry of Catalonia, Consejo Superior de Investigaciones Científicas, 08034 Barcelona, Spain; ^dDepartamento de Biodiversidad y Gestión Ambiental, Universidad de León, 24071 León, Spain; and ^eEdward Grey Institute, Department of Zoology, University of Oxford, Oxford OX1 3PS, United Kingdom

Edited by Nancy A. Moran, University of Texas at Austin, Austin, TX, and approved December 3, 2014 (received for review July 23, 2014)

Understanding the evolutionary transition from interspecific exploitation to cooperation is a major challenge in evolutionary biology. Ant–aphid relationships represent an ideal system to this end because they encompass a coevolutionary continuum of interactions ranging from mutualism to antagonism. In this study, we report an unprecedented interaction along this continuum: aggressive mimicry in aphids. We show that two morphs clonally produced by the aphid *Paracletus cimiciformis* during its root-dwelling phase establish relationships with ants at opposite sides of the mutualism–antagonism continuum. Although one of these morphs exhibits the conventional trophobiotic (mutualistic) relationship with ants of the genus *Tetramorium*, aphids of the alternative morph are transported by the ants to their brood chamber and cared for as if they were true ant larvae. Gas chromatography–mass spectrometry analyses reveal that the innate cuticular hydrocarbon profile of the mimic morph resembles the profile of ant larvae more than that of the alternative, genetically identical nonmimic morph. Furthermore, we show that, once in the brood chamber, mimic aphids suck on ant larva hemolymph. These results not only add aphids to the limited list of arthropods known to biosynthesize the cuticular chemicals of their deceived hosts to exploit their resources but describe a remarkable case of plastic aggressive mimicry. The present work adds a previously unidentified dimension to the classical textbook paradigm of aphid–ant relationships by showcasing a complex system at the evolutionary interface between cooperation and exploitation.

aggressive mimicry | aphids | ants | mutualism | polyphenism

A major challenge in evolutionary biology is to understand the factors governing the evolutionary transitions between interspecific exploitation and cooperation. Interactions between ants and aphids can be ranked along a continuum from mutualism to antagonism (1, 2), thus providing an excellent system to address this issue. In the best known relationship between ants and aphids, ants eat the sugar-rich honeydew excreted by the aphids and, in return, provide them with protection and hygienic services (2, 3). This kind of interaction is termed trophobiosis and is considered to be mutualistic (3). Although most ant–aphid trophobiotic associations are facultative, some ant species bring aphids or even aphid eggs into their nests in winter, ensuring the continuity of the relationship (4–6). Here, we report an unprecedented ant–aphid relationship at the evolutionary interface between cooperation and exploitation involving the coexistence of two aphid clonal morphs: a trophobiotic morph and an alternative morph that acts as an aggressive mimic to infiltrate ants' brood chamber and suck on their larvae.

The aphid species participating in this interaction is *Paracletus cimiciformis*, a member of the tribe Fordini in the gall-inducing aphid subfamily Eriosomatinae. *P. cimiciformis* is widely distributed across Europe and has been recorded in Asia and North Africa (7). As is generally true for aphids, but even more so for gall-inducing aphids (8), the life cycle of *P. cimiciformis* is highly plastic, exhibiting numerous polyphenisms (alternative phenotypes

produced by the same genome) (Fig. 1). Like other members of this tribe, *P. cimiciformis* alternates not only between oviparous sexual reproduction and viviparous parthenogenesis, but also between two alternative hosts, and exhibits both wingless and winged morphs. Most unusually, during its root-dwelling phase (Fig. 1), *P. cimiciformis* has two morphologically distinct wingless morphs where other closely related Fordini species have just one (9). Until recently, the only known wingless root-dwelling morph in this species was a flat yellowish-white aphid (hereafter “flat morph”) usually reported (as in other Fordini species) to be ant-attended (7, 10, 11). However, Ortiz-Rivas et al. (9) recently demonstrated the existence of an alternative round, olive-green wingless morph (hereafter “round morph”) resembling the single root-dwelling wingless morph occurring in related Fordini species. Importantly, each of these two morphs of *P. cimiciformis* is able to produce both morphs through parthenogenesis (9) although the factors triggering the production of one morph or the other remain unknown and their phenology is incompletely understood (*SI Text*).

The aim of this study was to explore the potential functional significance of this polyphenism occurring during the root-dwelling phase of the *P. cimiciformis* life cycle. We characterized the nature of each aphid morph's interactions with ants of the genus *Tetramorium*, their main tending ants (7, 10, 11), and particularly with *Tetramorium semilaeve*, one of the most

Significance

The best known relationship between ants and aphids consists in aphids providing ants with honeydew while receiving hygienic services and protection in return. We report an unprecedented aphid–ant interaction in which one of the two clonally produced root-dwelling morphs of the aphid *Paracletus cimiciformis* imitates the cuticular hydrocarbons of *Tetramorium* ant larvae, inducing ants to transport the aphids to their brood chamber, where they suck on ant larva hemolymph. To our knowledge, this strategy constitutes the first known case of aggressive mimicry in aphids. Moreover, because the alternative morph maintains a “conventional” relationship with ants, our findings are unusual in that they report, within the same species (and within a single clone), the coexistence of two evolutionary strategies at disparate points in the mutualism–antagonism continuum.

Author contributions: A.S., P.C., E.F., and D.M.-T. designed research; A.S., B.F., C.Q., N.P.-H., and D.M.-T. performed research; B.F., C.Q., and N.P.-H. contributed new reagents/analytic tools; A.S., B.F., C.Q., P.C., E.F., and D.M.-T. analyzed data; and A.S., P.C., E.F., and D.M.-T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. [KM101062–KM101073](https://doi.org/10.1073/pnas.1414061112)).

¹To whom correspondence should be addressed. Email: david.martinez@uv.es.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1414061112/-DCSupplemental.

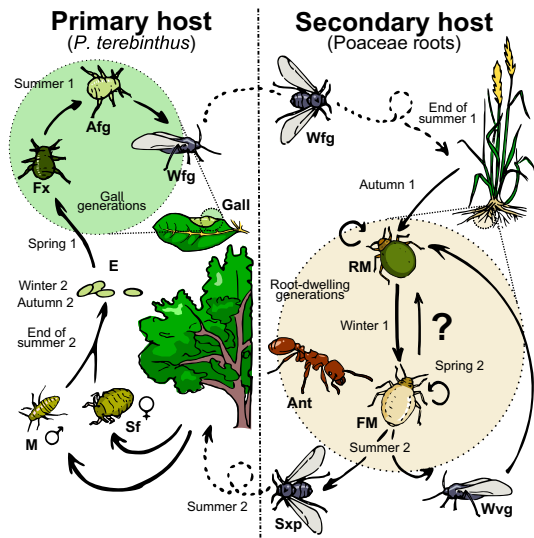


Fig. 1. Simplified diagram of the biannual life cycle of *P. cimiciformis*. Sexual reproduction takes place on *P. terebinthus* trees, its primary host, where up to five different morphs occur. Of these generations, three develop inside distinct galls that they induce in their host's leaves. Toward the end of summer, the last generation born inside the galls consists of winged aphids that fly to the roots of several gramineous species, its secondary host. There, they initiate a succession of root-dwelling wingless parthenogenetic generations consisting of two morphs: the round (RM) and the flat (FM) morphs, respectively. These two morphs participate in mutualistic associations with ants of the genus *Tetramorium*. In summer, two winged morphs may appear. One disperses the clone to new grasses whereas, in regions where *P. terebinthus* is present, a second winged morph will fly back to the primary host to give birth to the sexual morphs. Afg, apterous fundatrigeniae; E, eggs; Fx, fundatrix; M, male; Sf, sexual female; Sxp, sexuparae; Wfg, winged fundatrigeniae; Wwg, winged virginoparae. The question mark denotes unclear phenology details during the root-dwelling phase (SI Text and Fig. S1).

abundant ant species in holm-oak forests and related habitats from the Iberian Peninsula (12). First, we show that ants exhibit markedly different interactions with both morphs. Although the round morph elicits ant behaviors typical of a trophobiotic relationship, aphids of the flat morph are carried by worker ants into their brood chamber, where they treat them like conspecific larvae. Second, we show that cuticular hydrocarbon profiles of *P. cimiciformis* flat morph aphids resemble those of *T. semilaeve* larvae more than those of round morph aphids. Finally, we present evidence that, once in the brood chamber, aphids of the flat morph pierce ant larvae and suck their internal fluids. We conclude that the flat morph of *P. cimiciformis* represents a case of aggressive mimicry in aphids.

Results

Interactions Between Ants and Aphids: Behavioral Observations. Thirteen behaviors performed by worker ants toward aphids and 10 behaviors performed by aphids were identified and recorded (Tables S1 and S2). Interactions between ants and aphids differed according to aphid morph, with some behaviors being observed only in interactions with round or flat aphids, and can be summarized as follows.

Round aphids. In 12 out of 12 staged interactions ants performed antennal waving after contacting a round aphid outside the nest, to which the aphid responded by kicking with its rear legs (rear leg kicking), excreting a honeydew droplet (honeydew excretion) (Fig. 2A), and directing the anus toward the ant (anal pointing, a behavior observed only in this morph) (Movie S1). After contacting the honeydew droplet, ants ceased antennal waving and began antennation (Table S1) of the anal area, feeding on

the honeydew (honeydew consumption) for as long as the flow continued. This sequence was repeated several times (range = 8–28) during the observation periods, but, occasionally (in 3 out of 12 interactions), ants initiated an escalation of aggressive behaviors that ended up with the aphid being killed and consumed. **Flat aphids.** In 12 out of 12 staged interactions, flat aphids responded to being contacted by ants by retracting their limbs and lying motionless. Ants performed antennation, to which aphids responded by excreting honeydew in 5 out of 12 interactions. In sharp contrast to interactions with the round morph, a trophobiotic sequence was never observed with flat morph aphids. Instead, ants always proceeded by picking up the aphid and carrying it into the nest, where it was licked by several workers. During licking, flat aphids occasionally excreted honeydew (4 out of 12 interactions). Finally, flat aphids were transported to the brood chamber, where they were subjected to additional licking and deposited on the pile of larvae (12 out of 12 interactions) (Fig. 2B and Movie S2). Once on the pile of larvae, flat aphids exhibited larva probing (a behavior exclusive to this morph), by which aphids pierced ant larvae with their stylet (3 out of 12 interactions) (Fig. 2C and D and Movie S3).

In a second round of observations performed to analyze quantitative differences in particular behaviors (*Materials and Methods*), we found highly significant differences in *T. semilaeve* behavior toward *P. cimiciformis* root-dwelling morphs for three out of four behaviors analyzed (Fig. 3). In the two ant subcolonies used for behavioral assays, ants performed significantly more antennation and licking toward flat than toward round aphids (Mann–Whitney *U* test, $P < 0.001$ for both subcolonies) whereas we found the opposite for antennal waving (Mann–Whitney *U* test, $P < 0.001$ for both subcolonies). We did not find differences in the frequency of ant honeydew consumption (Mann–Whitney *U* test, $P = 0.853$ and $P = 0.796$ for ant subcolonies Ts-Cp2 and Ts-Le2, respectively), but honeydew consumption dropped significantly in postadopted flat aphids (only two events were recorded) (Wilcoxon signed-rank test, $P < 0.01$ for both colonies) (Fig. 3).

Adoption Tests. Ant subcolonies identified as *T. semilaeve* (SI *Materials and Methods*) clearly discriminated between the two aphid morphs independently of their sampling site (Fisher exact test, $P < 0.01$ for all *T. semilaeve* subcolonies) (Fig. 4; see Table S3 for further details). Ants carried 67–100% of the flat aphids

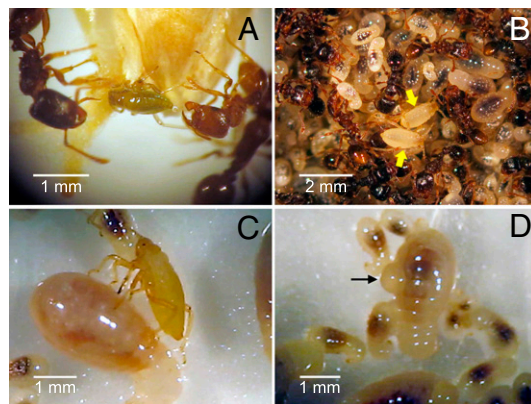


Fig. 2. Pictures representative of the interactions between *T. semilaeve* ants and the two *P. cimiciformis* root-dwelling wingless morphs. (A) *T. semilaeve* ants performing antennal waving (Table S1) on a round aphid, which excretes a honeydew droplet. (B) Flat aphids (arrows) adopted by *T. semilaeve* ants in their brood chamber among the pile of ant larvae. (C) A *P. cimiciformis* flat aphid performing larva probing (Table S2) on a *T. semilaeve* larva. (D) Hemolymph leaking from an ant larva that was subject to larva probing by a flat *P. cimiciformis* aphid (arrow).

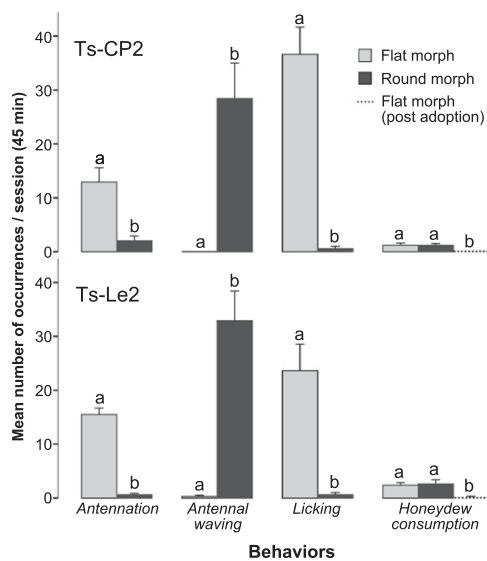


Fig. 3. Comparison of four ant behaviors toward the two root-dwelling *P. cimiciformis* aphid morphs from experiments performed with ant colonies Ts-CP2 (Top) and Ts-Le2 (Bottom) (see *Materials and Methods* and *Table S1* for details). Honeydew consumption in postadopted flat aphids was also analyzed. Bars with different letters are significantly different (Mann–Whitney *U* and Wilcoxon signed-rank tests, $P < 0.01$). Error bars, 1 SEM.

into their brood chambers and kept them among their own larvae until the end of the experiment (5 d). The same behavior was observed for the Tc-Po3 subcolony identified as *Tetramorium caespitum* (Fisher exact test, $P < 0.01$) (Fig. 4 and *Table S3*). This subcolony carried 16 out of 18 (89%) aphids of the flat morph into their brood chamber. All other subcolonies failed to adopt the flat morph or only did so in a minority of cases (0–15%), despite being host to this morph in the field (Fig. 4). In sharp contrast, only 16 out of 192 round aphids (8.3%) were recorded inside the ant nest (but never in the brood chamber), and none survived for more than 48 h (Fig. 4 and *Table S3*).

Dummy Assays. Significant differences were found for the six measured ant responses against dummies impregnated with different extracts (*Table S4*). Worker-ant responses to dummies impregnated with cuticular extracts from ant larvae and flat aphids were indistinguishable (Fig. 5). Both types of dummies provoked antennation (although not significantly different from dummies impregnated with extracts from round aphids) (Fig. 5), and none of them elicited antennal waving by ants (Fig. 5). Moreover, we found significant differences in the number of dummies transported to the ant nest (Fisher–Freeman–Halton test, $P < 0.01$) (*Table S4*). Dummies impregnated with extracts from ant larvae and flat aphids were transported to the nest more frequently (four and five out of eight presented dummies) than dummies impregnated with extracts from round aphids or control dummies (0 in both cases) (Fig. 5). We also found significant differences in the number of dummies transported to the brood chamber (Fisher–Freeman–Halton test, $P < 0.05$) (*Table S4*). Although control dummies or dummies impregnated with extracts from round aphids were never transported to the brood chamber, dummies impregnated with extracts from ant larvae and flat aphids were transported to the brood chamber in three and four occasions, respectively (Fig. 5).

Chemical Analysis of Cuticular Extracts and Electrophysiological Responses of Ants. Representative GC-MS profiles of cuticular hydrocarbons (CHCs) from ant larvae and flat and round *P. cimiciformis* aphids are shown in Fig. 6A. A total of 31

compounds were tentatively identified, and their relative amounts were estimated (*Table S5*). Most of the identified compounds were saturated linear or methyl-branched hydrocarbons showing qualitative and quantitative differences between different extracts. Most compounds were present in all three extracts, albeit in different amounts. However, a few compounds were exclusive to particular extracts or were shared by only two types of extracts (*Table S5*). In particular, compounds 2, 9 and 21 (tricosane, 11-methylpentacosane, and 3-methylheptacosane, respectively) were present only in extracts from ant larvae and flat aphids. A global comparison of the three profiles (*SI Materials and Methods*) revealed that profiles from flat aphids and ant larvae were more similar to each other than profiles of both aphid morphs (Fig. 6B). Of 31 identified compounds, six provoked coupled gas chromatography-electroantennographic detection (GC-EAD) responses on antennae of adult ants (*Table S5*). Interestingly, three of these compounds correspond to peaks that were found only in ant larvae and flat aphids (peaks 2, 9, and 21; see above) whereas the other three correspond to peaks 15, 22, and 27, present in all three extracts.

Detection of Ant DNA Inside Aphids. A *Tetramorium* ant-specific DNA fragment (*SI Materials and Methods*) of the expected length and sequence could be PCR amplified only from DNA samples obtained from *P. cimiciformis* aphids of the flat morph that had previously had access to ant larvae for 24 h. In fact, positive amplifications were restricted to those assays where larva

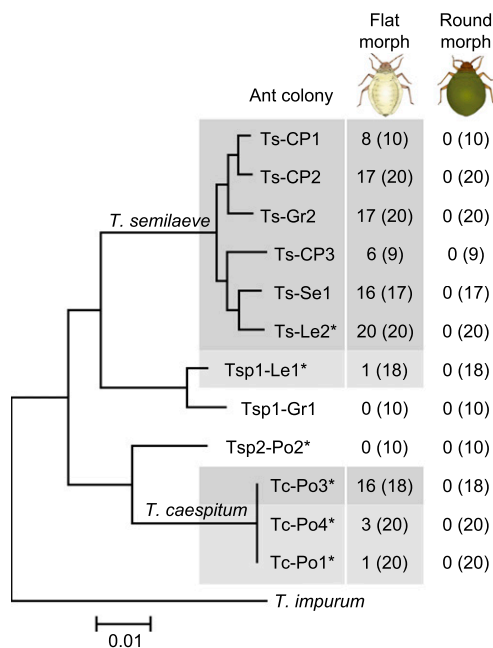


Fig. 4. Phylogenetic relationships of *Tetramorium* ant colonies used in this survey (*Table S6*) along with their response in adoption tests on the two *P. cimiciformis* morphs (*SI Materials and Methods*). The phylogenetic tree was built using COI sequences (*SI Materials and Methods*). The taxonomic status of ant colonies is indicated on the phylogram (Fig. S2). Colonies indicated as Tsp1- and Tsp2- belong to two unidentified species within genus *Tetramorium* (Fig. S2). To root the tree, GenBank sequence EU434801 from *Tetramorium impurum* was used. The bar indicates the number of nucleotide substitutions per site. Asterisks indicate ant colonies found hosting flat aphids when sampled in the field. For each ant colony in the phylogeny, the number of aphids of each morph transported and kept into their brood chamber for at least 5 d is indicated beside the total number of presented aphids (in parentheses). Ant colonies adopted either a relatively high (i.e., >60%, dark gray background), or low proportion of aphids (i.e., <15%, light gray background), or no aphids at all (white background).

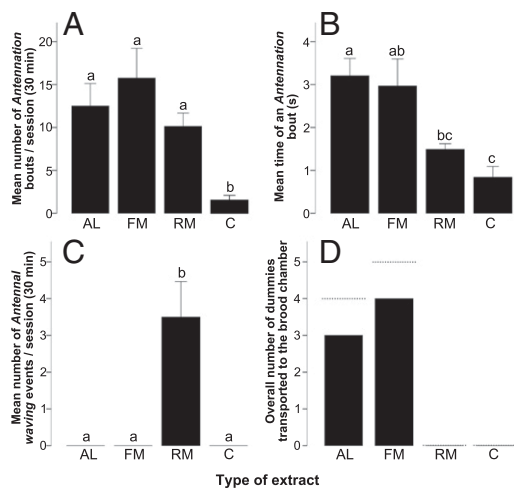


Fig. 5. Ant behaviors toward dummies impregnated with cuticular extracts from ant larvae (AL), flat (FM) and round (RM) aphid morphs, or control hexane solvent (C) (*SI Materials and Methods*). Charts show (A) mean number and (B) mean duration of antennation episodes, (C) mean number of antennal waving events, and (D) number of dummies impregnated with the indicated extracts that were transported into the ant nest (dotted lines) and the brood chamber (bars). Bars with different letters are significantly different (Campbell and Skillings multiple comparison procedure, $P < 0.05$).

probing was observed (6 out of 10). DNA samples extracted from round aphids that had been kept with ant larvae and DNA samples from control aphids (of both morphs) that had never been in contact with ant larvae failed to yield a positive amplification of the ant-specific DNA fragment (Fig. S3).

Discussion

This study reports, to our knowledge, the first case of aggressive mimicry in aphids, which also constitutes a remarkable example of plastic aggressive mimicry. We show that the two wingless morphs occurring during the root-dwelling phase of the life cycle of the aphid *P. cimiciformis* (Fig. 1) follow distinct strategies that entail disparate relationships with ants. On the one hand, the round morph exhibits the plant sap-sucking feeding behavior characteristic of aphids and establishes a typical mutualistic trophobiotic relationship with ants. On the other hand, aphids of the flat morph, although able to feed on plants (*SI Materials and Methods*), are brought inside the ant brood chamber where they are cared for by the ants. Our results show that the latter strategy

is accomplished by flat morph aphids by mimicking ant larvae chemical signals and that, besides obtaining ant care, aphids in the brood chamber actively suck hemolymph from ant larvae. This strategy fits the definition of aggressive mimicry (13). As both morphs can give birth to the alternative morph, their distinct behavioral strategies may occur simultaneously, but, because aphids of the flat morph are transported by ants into their nests, they usually take place in separate locations (Fig. S1).

Inter-Morph Behavioral Differences in Ant–Aphid Interactions. Behavioral observations revealed striking differences in ant behavior toward the two aphid morphs. The large number of antennal waving bouts and honeydew consumption episodes (Table S1) by ants interacting with the round morph agree with a conventional trophobiotic interaction, as does the observation of anal pointing, a trophobiotic behavior characteristic of myrmecophilous species (Table S2 and Movie S1). Antennal waving, which was more frequent toward round aphids (Fig. 3), is often referred to as “solicitation” (3) because, when ants perform it over aphids, the latter typically lift up their abdomen and excrete honeydew. In contrast, antennation and licking (Table S1) were more frequent toward flat aphids (Fig. 3). Antennation is involved in many aspects of ant social life such as kin recognition, brood discrimination, and enemy specification whereas licking is a grooming behavior performed by ants on their larvae and other nest mates (3). The occurrence of a set of distinctive behaviors typical of ant-larvae care, including antennation, transportation to the brood chamber, and licking (Table S1), along with their high adoption rate, strongly suggests that the flat morph is successfully mimicking ant larvae (Movie S2).

Chemical Mimicry of Ant Larvae CHCs. The main avenue of ant communication is chemical (3, 14), and cuticular hydrocarbons have been shown to be particularly important in mediating nest-mate recognition (15). Several social parasites have developed the capacity to exploit this circumstance, penetrating ant societies by displaying chemicals that mimic those of their hosts. This strategy can be accomplished either by acquiring the host cuticular compounds through direct body contact (chemical camouflage) or by synthesizing them (chemical mimicry) (16, 17). The latter mechanism has been previously invoked to explain aphid egg protection by ants (6) and demonstrated to mediate the avoidance of frequent aphid predation by ants in a specific trophobiotic association (18). Our results show that chemical mimicry is used by flat morph aphids to deceive worker ants and infiltrate their brood chamber. First, results of adoption tests show that color (green vs. whitish for the round and flat morphs,

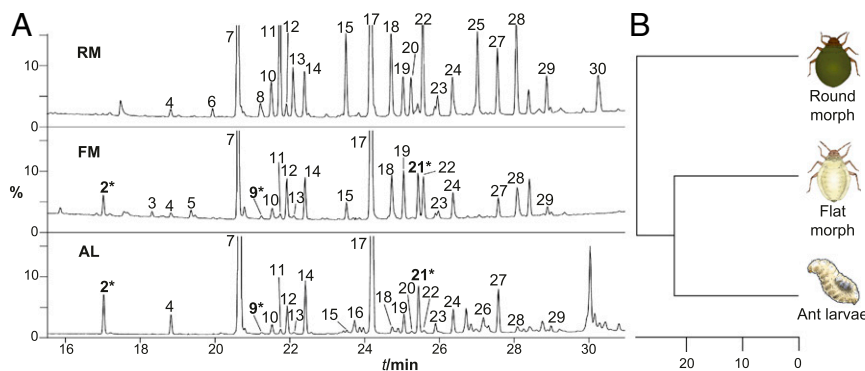


Fig. 6. (A) Representative GC-MS profiles of hexane cuticular extracts from ant larvae (AL) and flat (FM) and round (RM) aphid morphs. Reproducible peaks are indicated by numbers corresponding to compounds listed in Table S5. Asterisks indicate the three compounds exclusively detected in ant larvae and flat aphids. t/min , retention time (min). (B) UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram built using Euclidean distances as a measure of global similarity among CHC profiles (*SI Materials and Methods*).

respectively) is not the main cue triggering adoption because aphids were adopted in complete darkness. Second, experiments using dummies impregnated with cuticular extracts from the two aphid morphs revealed that chemical compounds present in the aphid's cuticle were, by themselves, capable of eliciting ant behaviors similar to those reported above toward live aphids of both morphs (Figs. 3 and 5). Moreover, dummies impregnated with extracts from ant larvae and flat aphids elicited adoption responses in ants. Third, comparisons of global CHC profiles revealed more similarities between *T. semilaeve* ant larvae and flat aphids than between the two genetically identical aphid morphs. Finally, of the six cuticular compounds eliciting electrophysiological responses from ant antennae, three were shared by flat aphids and ant larvae but were absent in round aphids (the other three were shared by both aphid morphs and ant larvae) (Table S5). Because the above results were obtained using aphids that had never been in contact with ants (*Materials and Methods*), our results strongly support adding *P. cimiciformis* to the short list of insects known to biosynthesize mimetic chemicals to break into the brood chamber of their hosts (3, 16, 19).

Although we did not test the extent of aggressive mimicry by aphids across the *Tetramorium* genus, two facts suggest that this phenomenon affects more than one species. First, ant colonies that hosted *P. cimiciformis* in the field were molecularly identified as pertaining to different *Tetramorium* species (Table S6). Second, some of those colonies successfully adopted the mimic aphid morph in the laboratory (Fig. 4). In addition, the fact that some of the ant colonies/species that hosted aphids in the field responded differently in adoption tests performed with alien aphids (not adopting, adopting a small proportion, or adopting a large proportion of aphids) is suggestive of chemical covariation between mimics and/or their models.

On the Nature and Evolution of the Flat Morph Mimicry. Behavioral observations of flat aphids in the brood chamber, along with the recovery of ant DNA from flat aphids kept with ant larvae, indicate that the chemical mimicry described above is of an aggressive nature. We hypothesize that the evolution of the exceptional phenotypic plasticity described here must have been driven by fitness returns other than those obtained from a typical trophobiotic relationship. Conventional trophobiotic relationships may provide protection against fungi, excessive moisture, the low temperatures of winter, or the summer droughts, without the need for aphids to evolve complex adaptations to infiltrate the most heavily defended place of an ant nest (the brood chamber) (3). In fact, trophobiosis is widespread among other Fordini species, in which their single wingless root-dwelling morph has been repeatedly reported to overwinter inside ant nests (4, 5, 20). Furthermore, our findings support a trophobiotic relationship between *T. semilaeve* ants and the *P. cimiciformis* round morph. It thus seems likely that the evolution of the flat morph in *P. cimiciformis* has been fueled by a reward available only in the ants' brood chamber, and that this new interaction evolved from an ancestral exclusively trophobiotic condition.

Former reports (4, 5) (*SI Text*) considered *P. cimiciformis* a social parasite of ants that obtained liquid food by trophallaxis (i.e., regurgitation of alimentary fluids by ants as they feed each other and their own larvae) (3). Trophallaxis has also been reported for other ant social parasites that use mimicry to access ant resources (19). However, based on the structure of its mouthparts, other reports ruled out the possibility that *P. cimiciformis* could perform trophallaxis (21). After many hours of observations of aphid-ant interactions, we never observed anything resembling trophallaxis. In fact, the only aphid behavior we observed inside ants' nests that might be related to feeding was larva probing (Fig. 2C and Table S2), consisting in aphids piercing ant larvae with their stylets (*Movie S3*). Detection of ant-specific DNA inside flat aphids that had access to ant larvae confirmed that flat aphids

are actually sucking hemolymph from ant larvae. Therefore, our results confirm that *P. cimiciformis* is a social parasite of ants (4, 5), but through a mechanism wholly different from trophallaxis. The benefits that aphids obtain by ingesting hemolymph of ant larvae and the plastic physiological mechanisms that allow them to tap into such an unusual resource (given that aphids were considered strictly herbivorous until now) remain to be investigated.

With respect to its role in the aphid life cycle, current evidence suggests (*SI Text* and Fig. S1) that the flat morph is adapted to overcoming harsh environmental conditions. By being transported by the ants deep into their brood chambers, aphids of the flat morph would be safe from temperature extremes experienced by other root-dwelling aphids that stay closer to the roots to feed. We suggest that lack of access to plants in the brood chamber may have driven the evolution of the ability to use a different food resource (i.e., hemolymph of ant larvae), at least temporarily. The fact that flat morph aphids inside ant nests give rise, when harsh conditions are over, to four morphs representing life history strategies adapted to different temporal and spatial uncertainties (Fig. 1 and Fig. S1) suggests that the flat morph is at the center of a diversified strategy for survival and recolonization of the aphid host plants.

That *P. cimiciformis* is capable of such phenotypic plasticity so as to develop into two coexisting discrete phenotypes reflecting distinct strategies in their relationship with ants is remarkable, all the more because these strategies are located at opposite sides of a continuum between cooperation and exploitation. Our findings agree with current knowledge indicating that galling aphids in the subfamily Eriosomatinae (in which the tribe Fordini is included) are especially prone to the evolution of complex polyphenisms, including social behaviors (8). Particularly relevant is the reported presence in several species within this subfamily, including a member of the tribe Fordini (22, 23), of sterile soldiers or other aggressive defending morphs that protect harmless reproductive members of the colony.

Conclusions

The results of this study show that the flat morph in *P. cimiciformis* mimics the CHC profile of *T. semilaeve* ant larvae to gain access to its host's resources in the form of hemolymph of ant larvae. Such a strategy fits the description of aggressive mimicry. This strategy likely evolved from a preexisting trophobiotic relationship that has been retained in the alternative round morph. This evolutionary process could have been favored both by a prolonged close association of aphids and their future prey (as observed in other Fordini species spending the winter in ant nests), and by a morphological preadaptation for piercing and sucking shared by all aphids, which would have been exapted to feed on larvae.

Many questions remain to be elucidated, such as the costs and benefits incurred by the three intervening actors (the two aphid morphs and the ants). The dual strategy developed by the aphid *P. cimiciformis* outlines a complex evolutionary scenario. On the one hand, the round morph and the ants, engaged in a trophobiotic relationship, should be subjected to the conflicts of interest typical of mutualism, with selection driving each partner to maximize its benefit by giving the least of its own energy and resources. On the other hand, the flat morph and the ants can be expected to be engaged in an arms race, with selection favoring improved deceiving abilities in the aphid and increasingly finer discrimination abilities to detect noncolony members in the ants. However, it is also possible that deception by aphids is not so costly for ants. Nondestructive larval hemolymph feeding has been reported for queens of some ant species (24). Hosting flat aphids may ensure, at an affordable price for ants, future supplies of sugar-rich honeydew by the round morph. Future research should address these theoretical scenarios. Another step in this endeavor will be to survey the extent of the geographical

and taxonomical variation in the response of *Tetramorium* ants toward sympatric and allopatric aphids and its covariation with the cuticle composition of ant larvae and aphids. Similarly important will be to investigate the signature of frequency-dependent selection (25) and the proximate factors underlying the regulation of the relative frequencies of each root-dwelling morph, such as the presence and relative abundance of ants, the availability of traditional food sources, and other ecological or biogeographical parameters. We believe that, beyond providing an unusual case of a “wolf in sheep’s clothing,” this system opens up a host of interesting and potentially novel questions about the evolution of cooperation and exploitation.

Materials and Methods

Ant and Aphid Samples. We used a total of 12 ant colonies of different *Tetramorium* species collected from different locations (Table S6). Sequences of a fragment of the cytochrome c oxidase subunit 1 gene (COI) were used for species identification. Six colonies were assigned to *T. semilaeve* (Ts), three to *T. caespitum* (Tc), and three to two unknown *Tetramorium* species (Tsp1 and Tsp2, respectively) (Table S6 and Fig. S2). Six of these colonies were established from field colonies in which *P. cimiciformis* (flat morph) was present (Table S6). Colonies containing hundreds of worker ants and larvae were established in acrylic formicaries at least 14 d before the beginning of the experiments. Behavioral experiments were carried out using ant subcolonies consisting of 30–50 workers and a minimum of 15 larvae taken from the main colonies.

Two clonal colonies of *P. cimiciformis* aphids were established from aphids sampled in two Spanish locations ~700 km apart. Pc-CP aphids, from Cortes de Pallás, descended from winged clonal aphids obtained from a *Pistacia terebinthus* gall (9) located within 1 km from tested Ts ants from the same location (Table S6). Pc-Le aphids, from León, descended from an aphid found in the Ts-Le2 ant nest (Table S6). Both aphid colonies were maintained on shoots of wheat. Aphids used in all our experimental assays were from long-established parthenogenetic laboratory cultures. In this way, we made sure that chemical signals likely involved in the studied interactions (see below) were innate rather than acquired from any external environmental source. This methodology was especially relevant for Pc-Le aphids because they were derived from aphids collected from an ant nest. Further details on ant species identification and aphid handling can be found in *SI Materials and Methods*.

Interactions Between Ants and Aphids: Behavioral Observations. For general behavioral observations, including compilation of a catalog of behaviors

occurring during ant–aphid interactions, ant subcolonies Ts-CP2, Ts-Le2, and Tsp1-Le1 were used (Table S6) along with the two aphid clones described above. Aphids were singly presented on a shoot of wheat in a plastic container connected to each artificial ant nest. The response of ants to 18 flat and 18 round aphids was recorded in the course of 36 1-h observation periods (6 for each combination of ant subcolony and aphid clone). The interactions were filmed on video, and films from a subset of 24 interactions (12 with flat and 12 with round aphids) involving *T. semilaeve* subcolonies Ts-CP2 and Ts-Le2 were further analyzed to extract information on the behavioral stages involved in each type of interaction. These colonies were selected because they were found to consistently adopt flat aphids (other ant colonies behaved aggressively toward both aphid morphs and their rate of adoption was much lower than in *T. semilaeve*).

To analyze behavioral differences in ant responses to each aphid morph in further detail, we conducted a second set of behavioral assays using ant subcolonies Ts-CP2 and Ts-Le2 (Table S6). Aphids used in these assays were from the same locality as the ant colonies (i.e., Pc-CP and Pc-Le, respectively) and were obtained as described above. At the beginning of each experiment, a subcolony was connected to a plastic container bearing a single aphid on a shoot of wheat. A total of 10 aphids of each morph and location were tested. We scored the following ant behaviors: antennation, antennal waving, licking, and honeydew consumption. Data were obtained using instantaneous sampling (sampling interval = 30 s) by recording for 45 min the number of ants performing each of the selected behaviors toward the focal aphid (26). Similarly, we recorded honeydew consumption from postadopted flat aphids (24 h after the initial session). Nonparametric Mann–Whitney *U* or Wilcoxon signed-rank tests were used to compare ant behaviors toward the two aphid morphs.

Detailed information on protocols used to perform adoption tests, dummy assays, chemical analysis of cuticular extracts, and detection of ant DNA inside aphids can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank J. M. Collantes for drawings. We thank A. Peiró and P. Del Campo for help, A. Guerrero for assistance in the identification of cuticular compounds, and Lukasz Depa for providing ants and aphids from Poland. We thank the Servei Central de Suport a la Investigació Experimental (Universitat de València) for collaboration. This work was supported by project CGL2011-27404 from the Spanish Ministerio de Economía y Competitividad. A.S. was supported by an “Atracció de Talent” fellowship from the Universitat de València. P.C. was supported by 7th Framework Programme Marie Curie Intra-European Fellowship PIEF-GA-2010-273010 from the European Union.

- Ewald PW (1987) Transmission modes and evolution of the parasitism-mutualism continuum. *Ann N Y Acad Sci* 503:295–306.
- Stadler B, Dixon AFG (2008) *Mutualism: Ants and Their Insect Partners* (Cambridge Univ Press, New York).
- Hölldobler B, Wilson EO (1990) *The Ants* (Harvard Univ Press, Cambridge, MA).
- Zwölfer H (1958) Zur Systematik, Biologie und Ökologie unterirdisch lebender Aphiden (Homoptera, Aphidoidea) (Anoeciinae, Tetraneurini, Pemphigini und Fordinae): Teil III (Fordinae). *Zeitschrift für Angew Entomol* 42:129–172.
- Zwölfer H (1958) Zur Systematik, Biologie und Ökologie unterirdisch lebender Aphiden (Homoptera, Aphidoidea) (Anoeciinae, Tetraneurini, Pemphigini und Fordinae): Teil IV (Ökologische und systematische Erörterungen). *Zeitschrift für Angew Entomol* 43:1–52.
- Matsuura K, Yashiro T (2006) Aphid egg protection by ants: A novel aspect of the mutualism between the tree-feeding aphid *Stomaphis hirukawai* and its attendant ant *Lasius productus*. *Naturwissenschaften* 93(10):506–510.
- Nieto Nafria JM, Mier Durante MP, Binazzi A, Pérez Hidalgo N (2002) *Fauna Ibérica, Vol 19. Hemiptera, Aphididae II* (Museo Nacional de Ciencias Naturales-CSIC, Madrid).
- Wool D (2004) Gall-forming aphids: Specialization, biological complexity, and variation. *Annu Rev Entomol* 49:175–192.
- Ortiz-Rivas B, Martínez-Torres D, Pérez Hidalgo N (2009) Molecular phylogeny of Iberian Fordini (Aphididae: Eriosomatinae): Implications for the taxonomy of genera *Forda* and *Paracletus*. *Syst Entomol* 34:293–306.
- Blackman RL, Eastop VF (2006) *Aphids on the World's Herbaceous Plants and Shrubs* (Wiley, Chichester, UK).
- Heie OE (1980) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. I General Part. The Families Mindaridae, Hormaphididae, Thelaxidae, Anoeciidae, and Pemphigidae. *Fauna Entomol Scand* 9:1–236.
- Acosta Salmerón FJ, Martínez Ibáñez MD, Serrano Talavera JM (1983) Contribución al conocimiento de la mirmecofauna del encinar peninsular. II: Principales pautas autecológicas. *Bol Asoc Esp Entomol* 7:297–306.
- Ruxton GD, Sherratt TN, Speed MP (2004) *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals, and Mimicry* (Oxford Univ Press, New York).
- Wilson EO (1965) Chemical communication in the social insects. *Science* 149(3688): 1064–1071.
- Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu Rev Entomol* 50:371–393.
- Howard RW (1993) *Insect Lipids: Chemistry, Biochemistry, and Biology*, eds Stanley-Samuelson DW, Nelson DR (Univ of Nebraska Press, Lincoln, NE), pp 179–226.
- Akino T (2008) Chemical strategies to deal with ants: A review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods. *Myrmecological News* 11:173–181.
- Endo S, Itino T (2013) Myrmecophilous aphids produce cuticular hydrocarbons that resemble those of their tending ants. *Popul Ecol* 55:27–34.
- Akino T, Knapp JJ, Thomas JA, Elmes GW (1999) Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc Biol Sci* 266:1419–1426.
- Kunkel H (1973) Die Kotabgabe bei Aphiden (Aphidina, Hemiptera) unter Einfluss von Ameisen. *Bonn Zool Beitr* 24:105–121.
- Schmidt H (1952) [*Paracletus cimiciformis* v. d. Heyd., and its parasitic adaptation to the ant host]. *Z Parasitenkd* 15(4):283–289.
- Stern DL, Foster WA (1996) The evolution of soldiers in aphids. *Biol Rev Camb Philos Soc* 71(1):27–79.
- Inbar M (1998) Competition, territoriality and maternal defense in a gall-forming aphid. *Ethol Ecol Evol* 10:159–170.
- Masuko K (1986) Larval hemolymph feeding: A nondestructive parental cannibalism in the primitive ant *Amblyopone silvestrii* Wheeler (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 19:249–255.
- Cheney KL, Côté IM (2005) Frequency-dependent success of aggressive mimics in a cleaning symbiosis. *Proc Biol Sci* 272(1581):2635–2639.
- Martin P, Bateson P (2007) *Measuring Behaviour: An Introductory Guide* (Cambridge Univ Press, Cambridge, UK), 3rd Ed.