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Research article

Species introduction shifts a trait's function from mutualism to antagonism: elaiosomes in a myrmecochory cold spot

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Placing traits into novel evolutionary contexts may profoundly alter their functional roles. Here, we investigated whether the elaiosome, a lipid-rich appendage located on seeds, retained its role as a seed dispersal trait promoting mutualisms with insectivorous ants following human-mediated introduction of the elaiosome-bearing *Carduus nutans* into the Argentinean Caldenal. This system is located within the Neotropical region, an alleged myrmecochory cold spot. Specifically, we first tested the assumption that the elaiosome mediates the interaction between *C. nutans* and the native ant *Pheidole bergi*. Then, we explored the hypothesis that, instead of a mutualism, the elaiosome promotes an antagonism between these species because *P. bergi* predates on both insects and seeds. Finally, we assessed the possibility that the elaiosome is rare in our system, as predicted from its location within the Neotropics. By manipulating the presence/absence of *C. nutans*' elaiosomes, we demonstrated that *P. bergi* strongly prefers to collect seeds with versus without *C. nutans*' elaiosomes, indicating that the elaiosome indeed mediates the interaction between these species. While we detected no direct signs of predation on nor alteration of viability in seeds recovered from *P. bergi*'s refuse dumps, 80% of offered *C. nutans* seeds remained inside *P. bergi* colonies, where they are likely consumed by ants, buried too deep for emergence or destroyed by pathogens. Importantly, by quantifying the outcome of the *C. nutans–P. bergi* interaction, we showed that this relationship is strongly antagonistic. Finally, by sampling taxa most likely to have elaiosomes, we identified eight native species with that trait, preliminary confirming that elaiosome-bearing species are uncommon in the Caldenal. Taken together, our findings suggest that the elaiosome promotes an antagonism that deters invasion in a cold spot of myrmecochore diversity. The functions of phenotypic traits can thus vary according to the ecological and evolutionary contexts in which they operate.

Keywords: ant foraging behavior, *Carduus nutans*, Neotropical region, *Pheidole bergi*, seed fate, traits

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Introduction

The biogeographical dispersal of species mediated by humans forces organisms to experience sudden and profound changes in ecological and evolutionary contexts as species are transported rapidly to locations thousands of kilometers from where they originated (Hierro et al. 2005, Latombe et al. 2021). Such dramatic alteration of surrounding biotic and abiotic conditions may place phenotypic traits into contexts for which they did not evolve. Consequently, introducing traits into novel evolutionary contexts may limit the ability of species to establish and increase in abundance in non-native ranges, providing a major source of resistance to species invasion (Pearson et al. 2018a). Yet, that possibility remains largely unexplored. Understanding how species traits match conditions in non-native ranges may advance the predictive power of invasion biology, and thus contribute to the protection and restoration of natural systems.

The elaiosome, a lipid-rich appendage located on seeds and fruits, is a trait widely regarded as an adaptation for seed dispersal by ants (i.e. myrmecochory; Giladi 2006, Dunn et al. 2007). Specifically, myrmecochory is considered as a mutualism because ants obtain food supplies in exchange for providing dispersal services. The nature of the interaction that elaiosome-bearing plants establish with ants may be contingent, however, on ants' diets. That is, this interaction can be mutualistic for insectivorous ants because they consume the elaiosome and disperse the embryo untouched (Hughes et al. 1994), but for omnivorous ants, which are also attracted to elaiosomes, this interaction may differ from mutualistic because they consume not only insects, but also seeds. In line with that possibility, Pearson and collaborators (2014a) found that *Pheidole bergi*, an omnivorous ant (Pirk et al. 2009) native to the semi-arid open forest of central Argentina (Caldenal; Tizón and Quirán 2009), suppressed the emergence of the non-native and elaiosome-bearing *Carduus nutans* more strongly than that of any of the other 17 study species, which lack elaiosomes. Importantly, emergence was negatively correlated with *P. bergi* preference for the seeds of all 18 species. These results suggest that *P. bergi* exerted the largest control on *C. nutans* due to the presence of an elaiosome on the seeds (technically, a fruit called achene) of this plant (Fig. 1). It has been noted, however, that the attraction of ants by this appendage in *Carduus* species still requires demonstration (Pirk and López de Casenave 2017). If the elaiosome indeed mediated the negative effects of *P. bergi* on *C. nutans*, then the elaiosome promoted an antagonism, rather than a mutualism, when the latter met an omnivorous ant with which it did not evolve.

Native myrmecochorous plants are rare in the Neotropical region, where the Caldenal is located (Cabrera 1994), as compared to other biogeographic regions, including the Paleartic, where *C. nutans* originated (Lengyel et al. 2010). Moreover, beyond the description of one native species (*Phacelia artemisioides*, Boraginaceae) as myrmecochorous in a list of 324 native and non-native weeds in the Caldenal (Troiani and Steibel 2008), there is no information about the presence of species with propagules bearing an elaiosome in this system. The myrmecochorous nature of *C. nutans* is, however, not mentioned in that work. Thus, the elaiosome appears to be a rare trait in the Caldenalian community. *Carduus nutans* seems then to have been introduced from a known hotspot for myrmecochore diversity in Eurasia (sensu Lengyel et al. 2010) into a myrmecochore diversity cold spot in the Caldenal. Consequently, the context provided by this non-native region may not be conducive for the elaiosome to play its classic role in seed dispersal.

Based on the encounter between *C. nutans* and *P. bergi* in the Caldenal, we addressed here three hypotheses relevant for understanding shifts in the role of traits in novel evolutionary contexts. First, we explored the hypothesis that the response of *P. bergi* to *C. nutans* seeds is mediated by the elaiosome. Then, we further evaluated the possibility that *P. bergi* negatively affects *C. nutans* seeds because this ant predates on both insects and seeds. Lastly, we assessed the proposition that the elaiosome is a rare trait in the Caldenal, as expected from its location within the Neotropics. To test the first hypothesis, we conducted a field experiment with natural and experimentally manipulated seeds of *C. nutans* and two other plants that bear no elaiosomes, one native and one non-native. For experimental seeds, the elaiosomes of *C. nutans* were removed and added to all three species (for *C. nutans* elaiosomes were naturally attached, removed or artificially reattached). If the elaiosome mediates the response of *P. bergi* to *C. nutans* seeds, then *P. bergi* will respond more strongly to seeds with than without elaiosome in all cases. To investigate effects of *P. bergi* on *C. nutans* seeds, we offered marked *C. nutans* seeds to *P. bergi*, followed their fate, recovered them from refuse dumps to which vertebrate seed predators were precluded access, and assessed seed predation and viability. If *P. bergi* exerts

Figure 1. *Pheidole bergi*, an insectivorous and seed-eating ant that is native to the Caldenal of central Argentina, is strongly attracted to the elaiosome of the non-native *Carduus nutans*. In the picture, *P. bergi* is grabbing a *C. nutans* seed (technically, achene) at the tip where the elaiosome is located before taking the seed to its colony. Credit: Nadia S. Icasatti.

direct negative effects on *C. nutans* seeds, then seeds recovered from protected refuse dumps will show signs of predation and/or seed viability will be reduced in refuse dumps as compared to control groups (i.e. intact seeds). In addition, failure of seeds to emerge from colonies will provide evidence for negative impacts of *P. bergi* on *C. nutans* seeds (Hughes and Westoby 1992, Pearson et al. 2014a). Importantly, we used these seed fate data to determine whether the overall interaction between *C. nutans* and *P. bergi* was mutualistic or antagonistic, following Zwolak and Crone (2012). Finally, to explore the occurrence of the elaiosome in the Caldenal, we sampled the native flora. If the elaiosome is uncommon, then genera known to have species with propagules-bearing elaiosomes (Lengyel et al. 2010) will display few or no native representatives.

Material and methods

Study site

The Caldenal is a district (Cabrera 1994) and vegetation unity (Oyarzabal et al. 2018) in the southernmost portion of the Espinal province within the Neotropical region in Argentina. The dominant tree in that district is *Prosopis caldenia*. Other abundant trees and shrubs in the Caldenal include *P. flexuosa*, *Geoffroea decorticans*, *Condalia microphylla* and *Schinus fasciculata*. The herbaceous layer is dominated by perennial grasses, such as *Piptochaetium napostaense*, *Poa ligularis* and *Jarava ichu* (Cano 1988). Herbs are also common and diverse in the Caldenal, and *Plantago patagonica*, *Solanum elaeagnifolium* and *Daucus pusillus* are among the most abundant ones (Chiuffo et al. 2018). Soils are sandy loam (Cano et al. 1980), the mean annual temperature is 15.4°C (1941–1990, Santa Rosa, La Pampa, 36°37′6.67″S, 64°17′29.31″W, located at the heart of the Caldenal, www.worldclimate.com) and the mean annual precipitation is 648 mm (1911–2018, Santa Rosa, G. Vergara and M. Mendez, Agronomy Dept, UNLPam, unpubl.). Precipitation mainly falls as rain in the spring and summer.

Field assays and sampling

In all field assays, we offered seeds to *P. bergi* near the entrance of their colonies. Although we used both fruits and seeds in our assays, we collectively called them seeds for simplicity. *Pheidole bergi* is common in the Caldenal (Tizón and Quirán 2009). This ant is mainly insectivorous, but it also consumes seeds (Pirk et al. 2009, Pearson et al. 2014a). The genus *Pheidole* is identified as a major harvester of both myrmecochorous and non-myrmecochorous seeds in different systems around the world (Berg 1975, Briese and Macauley 1981, Whitford et al. 1981, Andersen 1982, Hughes and Westoby 1992, Hughes et al. 1994). We are not aware, however, of studies reporting negative effects of *Pheidole* spp. on elaiosome-bearing propagules in Eurasia. We conducted field activities in spring–summer (2017–2019) of the Southern Hemisphere (October–March), in coincidence with seed dispersal of species used in this research and peak activity of *P. bergi*.

Experiment 1

To assess the importance of the elaiosome for the response of *P. bergi* to *C. nutans* seeds, we conducted a seed offering experiment with seeds of *C. nutans*, *Verbesina encelioides*, a native Asteraceae without elaiosome, and *Helianthus petiolaris*, a non-native Asteraceae also with no elaiosome. As for *C. nutans*, seeds in *V. encelioides* and *H. petiolaris* are technically fruits called achenes. In addition to offering seeds collected from natural populations (i.e. natural seeds), in all three species, we offered seeds with the following two treatments: 1) seeds with *C. nutans* elaiosomes glued to them using the glue and 2) seeds with that glue added to the point where seeds attach to the receptacle (i.e. the usual location of elaiosomes). To conduct treatment 1 in *C. nutans*, we glued back the elaiosome to seeds to which the elaiosome had been removed; for treatment 2, we removed the elaiosome and filled the small cavity thus created with glue. Consequently, seed treatments in all three species were composed of: 1) natural seeds, 2) seeds with glued elaiosomes and 3) seeds with glue. We weighed 50 seeds for each of the three species used in this assay to determine mean seed mass per species.

We simultaneously offered 10 seeds representing each treatment (10 seeds × 3 species × 3 treatments = 90 seeds) to *P. bergi* shortly after sunrise, when this ant species is highly active. We offered seeds inside 9 cm diameter plastic dishes placed at 15 cm from the entrance of 21 *P. bergi* colonies, which were distributed within seven haphazardly selected sites (three colonies per site, also haphazardly selected; Supporting information). Sites were separated from each other by a distance varying between 1.5 and 50 km. We placed seeds inside plastic dishes to reduce chances of being removed by wind. To allow ants to exit dishes, we introduced a small caldén twig, which was located at the border and served as a bridge. After arranging seeds near a given colony, we recorded *P. bergi* response for 60 min as 1) removing seeds from the dish and taking them to the colony, 2) removing seeds from the dish and dropping them before reaching the colony or 3) not removing seeds from the dish. We regarded the proportion of seeds taken to ant colonies as a proxy for ant preference. During this assay, we also assessed seed removal by other ant species. We performed seed offerings from 14 Dec 2017 to 19 Jan 2018.

Experiment 2

In a second field experiment, we again offered *C. nutans* seeds to *P. bergi*, but now we followed seed fate to refuse dumps and assessed predation and viability of seeds found there to examine *P. bergi*'s effect on *C. nutans* seeds. Specifically, we offered 10 *C. nutans* seeds directly placed on the ground at 15 cm from the entrance of 30 haphazardly selected colonies located within the campus of the National University of La Pampa (36°33′21.63″S, 64°17′59.03″W; 212 m). Colonies were located within an area of 250 × 250 m and separation between colonies varied from around 50 to 200 m. We used

seeds that looked healthy and filled with an embryo after gently squeezing them. Because *C. nutans* was present in the campus and this assay was conducted at the time this species disperses seeds, we drew a line on each offered seed with a permanent marker to identify it as an experimental seed. Immediately after observing that all seeds had been taken to nests (within 15 min in all cases), we removed refuse dumps located next to colony entrances and placed a wire cage over the entrance to prevent access by small mammals and birds. Cages were constructed of 0.5 × 0.5 cm wire mesh and had a size of 40 × 40 cm wide and 20 cm deep with tops that opened and closed to access the contents. We collected newly formed refuse dumps from each colony for seven consecutive days. We conducted this assay from 5 Jan 2018 to 17 Jan 2018 when campus was unoccupied due to summer break. In the lab, we processed refuse dumps within one month of collection. Processing involved recovering previously offered marked *C. nutans* seeds, exploring them for signs of predation (i.e. seed damage), and assessing their viability with the tetrazolium test (Cottrell 1947). We also measured seed viability in 30 groups of 10 seeds each, belonging to the same seed pool used to conduct this offering experiment and that were kept in the lab. Non-recovered seeds were assumed to remain in the colonies.

We used the data obtained from this experiment to quantify the outcome of the interaction between *C. nutans* and *P. bergi* following the equation proposed by Zwolak and Crone (2012). According to that equation, if seedling emergence in the absence of a granivore (e_s) is lower than in its presence, then the interaction is regarded as mutualistic. On the contrary, if e_s is greater than in the presence of a granivore, then the interaction is antagonistic. Mathematically, the equation is expressed as follows:

$$e_s < P_H P_c e_c + (1 - P_H) e_s = \text{mutualism}$$

$$e_s > P_H P_c e_c + (1 - P_H) e_s = \text{antagonism}$$

where e_s = seedling emergence proportion in a cage without the granivore (obtained from Pearson et al. 2014a), P_H = proportion of seeds removed by the granivore, P_c = proportion of seeds taken and remaining into colonies (i.e. cached seeds in Zwolak and Crone (2012) original presentation), e_c = proportion of seeds that emerge from colonies (i.e. seeds in refuse dumps), $(1 - P_H)$ e_s = proportion of seeds not harvested multiplied by the estimated emergence proportion from the caged seeds.

Experiment 3

In another evaluation of the effect of *P. bergi* on *C. nutans* seeds, we compared seed viability between those found in naturally occurring refuse dumps and seeds collected from plants located next to those dumps. To that end, we collected between one and three refuse dumps and seeds from *C. nutans* plants located within 3 m from refuse dumps in each of eight haphazardly selected sites (Supporting information). Sites were separated by a distance varying from 5 to 130 km. We conducted this sampling in 28 and 29 Nov 2018. Within one month of field sampling, we tested the viability of all *C. nutans* seeds found in refuse dumps and in 30 seeds collected from nearby *C. nutans* plants in the lab.

The occurrence of the elaiosome in the Caldenal

We conducted extensive field sampling to determine the occurrence of the elaiosome in the native flora of the Caldenal. We guided our search by using the biogeographic distribution of taxa bearing elaiosomes published by Lengyel et al. (2010), focusing on genera (e.g. *Bredemeyera*, *Euphorbia*, *Melica*, *Turnera*, *Wedelia*) and families (e.g. Boraginaceae, Polygalaceae) reported to have many members with elaiosomes (see Table 1 in Lengyel et al. 2010). As a consequence, our sampling was not exhaustive, but directed toward the most likely taxa. We searched for target species based on personal knowledge of their locations and information obtained from the SRFA Herbarium (NYBG Steere Herbarium 2022, https://sweetgum.nybg.org/science/ih/). In all cases, seeds were collected from standing individuals within naturally occurring populations. We searched for species during two consecutive seasons, spring and summer 2017–2018 and 2018–2019. Upon collection, we examined seeds under a stereo microscope to visually determine the presence/absence of elaiosomes.

Experiment 4

Because we determined that several native species had seeds seemingly bearing elaiosome, we used seeds of these species, along with those of *C. nutans*, to conduct a final seed offering assay to assess the response of *P. bergi* to all of them. For this field experiment, we used the same methodology as that used to assess the mediation of *C. nutans*' elaiosome in the interaction between seeds and *P. bergi*. Our sample size was, however, larger in this assay as we offered seeds to *P. bergi* at three colonies within each of 10 haphazardly selected sites (Supporting information). Sites were separated from 2 to 120 km. We conducted these offerings between 20 Feb 2019 and 20 Mar 2019. In the lab, we recorded the mass of three seeds for each of the species included in this assay to estimate mean seed mass for each species.

Data analyses

To assess the response of *P. bergi* to seeds in our offering experiments, we analyzed 1) the proportions of seeds taken to colonies, 2) dropped before reaching the colony and 3) left in dishes using generalized linear mixed model (GLMM) with a binomial distribution. In our first offering of natural and experimental seeds, that model considered species, treatments applied to seeds, and their interaction as fixed factors and colony, nested within site, as a random effect. In our offering of *C. nutans* and native species seeds, species was entered in the model as a fixed factor and colony, nested within site, was treated as a random effect. In this assay, no seed was

dropped before reaching ant colonies, so we only analyzed proportions of seeds taken to ant colonies. In the analysis of both seed offering assays, we did not include seed treatments and species that were left in dishes (i.e. treatments and species with zero proportions taken to colonies). We used post hoc tests with Bonferroni correction to conduct comparisons between levels in fixed factors. We also used a GLMM with a binomial distribution to compare the viability of *C. nutans* seeds found in naturally occurring refuse dumps versus those collected from nearby *C. nutans* plants. In that model, seed source was considered as a fixed factor and colony, nested within site, a random effect. In all cases, we tested the significance ($p < 0.05$) of fixed effects from the complete models using a type II, Wald χ^2 test. In addition, we compared the seed mass of *C. nutans*, *V. encelioides* and *H. petiolaris* with a univariate linear model (i.e. ANOVA), followed by the Tukey post hoc test. Finally, we related species seed mass and the proportion of seeds taken to colonies (i.e. *P. bergi* response) in the offering experiment with *C. nutans* and native species' seeds with simple linear regression (SLR). To comply with SLR assumptions of normal distribution and constant variance of the residuals, we transformed proportional data with the *arcsine* function, following Crawley (2005). As above, we did not include species that were not taken to ant colonies and were left in dishes (i.e. species with zero proportions) to reach the required normality of the residuals. We performed GLMMs with R ver. 4.0.3 (10 10 2020) and the packages lme4 (Bates et al. 2015), car (Wald χ^2 test, John and Weisberg 2011) and lsmeans (post hoc tests with Bonferroni correction, Lenth and Herv 2015), the ANOVA with IBM SPSS Statistics 25, and the SLR with SigmaPlot ver. 14.5.

Results

Experiment 1

The proportion of seeds taken to ant colonies in the assay where elaiosomes were experimentally removed and added depended on both species' identity and seed treatment ($\chi^2_{Species} = 122.854$, df = 2, $p < 0.001$; $\chi^2_{Seed\ treatments} = 250.680$, df = 2, $p < 0.001$; $\chi^2_{Species \times Seed\ treatments} = 99.457$, df = 2, $p < 0.001$; Fig. 2a). Importantly, the presence of the elaiosome strongly increased the proportion of seeds carried to colonies in all three study species. For *C. nutans*, ant preference for seeds naturally exhibiting elaiosomes was similar to that for seeds with glued elaiosomes and much greater than for seeds where glue replaced elaiosomes, validating our experimental approach. In addition, preference for seeds of *H. petiolaris* with glued elaiosomes was comparable to that of *C. nutans* seeds with elaiosomes (natural and glued), but removal of seeds with glued elaiosomes of the native *V. encelioides* was lower than that for both *C. nutans* and *H. petiolaris*.

Species and seed treatment also interacted in both the proportion of seeds dropped before reaching ant colonies ($\chi^2_{Species} = 5.693$, df = 2, $p = 0.058$; $\chi^2_{Seed\ treatments} = 19.694$, df = 2, $p < 0.001$; $\chi^2_{Species \times Seed\ treatments} = 38.768$, df = 4,

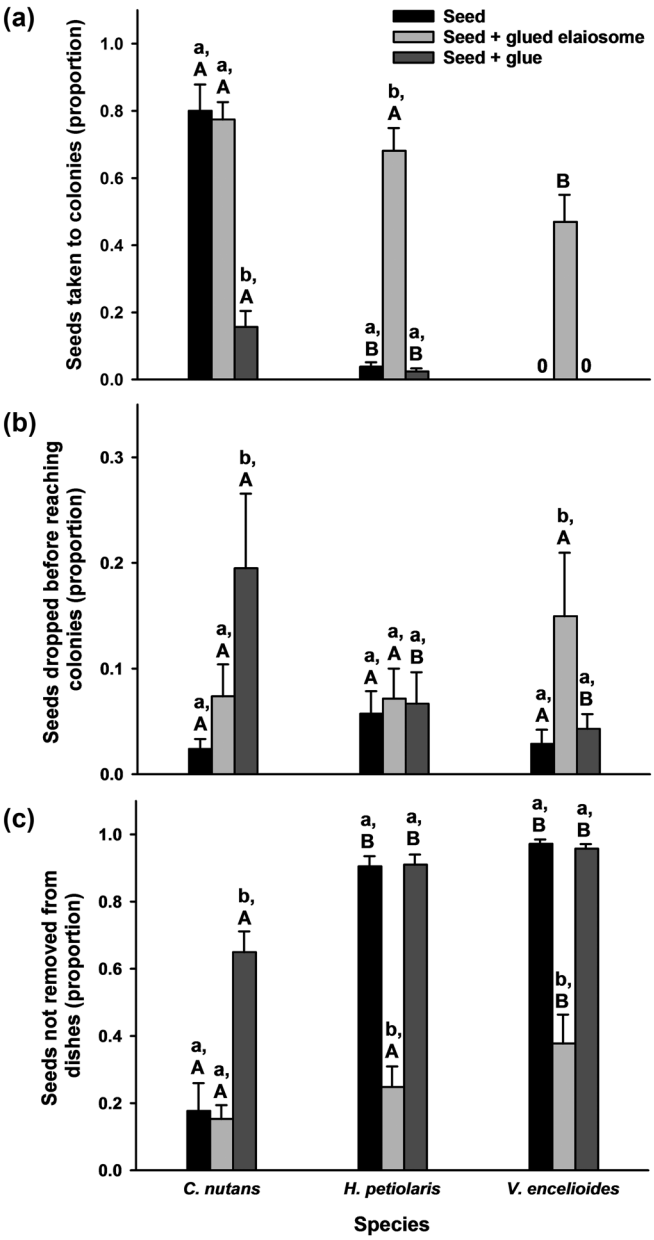

Figure 2. Proportion of *Carduus nutans*, *Helianthus petiolaris* and *Verbesina encelioides* seeds that were taken to colonies of *Pheidole bergi* (a), dropped before reaching colonies (b) or not removed from plastic dishes (c), where they were offered to ants. Offer included natural seeds (seed), wherein *C. nutans* bears elaiosome, seeds to which an elaiosome was experimentally added (seed + glued elaiosome), in *C. nutans*, this treatment involved removing the elaiosome and gluing it back to the seed, and seeds with glue (seed + glue), which in *C. nutans* involved removing the elaiosome and filling with glue the resulting cavity. Bars are means ± SE of seven sites. Different letters above bars indicate significant differences between treatments at $p < 0.05$, with small letters showing differences between seed treatments within species and capital letters those between species within seed treatments. No *V. encelioides* seed without elaiosome was taken to ant colonies.

$p < 0.001$; Fig. 2b) and in that of seeds that were not removed from dishes ($\chi^2_{Species} = 233.960$, df = 2, $p < 0.001$; $\chi^2_{Seed\ treatments} = 342.220$, df = 2, $p < 0.001$; $\chi^2_{Species \times Seed\ treatments} = 112.030$, df = 4, $p < 0.001$; Fig. 2c). In *C. nutans*, the proportion of dropped seeds was around ten and three times greater for those without elaiosome than for natural seeds and seeds with elaiosomes glued on, respectively. In *V. encelioides*, seed dropping was around three times greater for seeds with glued elaiosomes than for seeds with the other treatments (Fig. 2b). The proportion of seeds without elaiosomes that were left in dishes was also more than three times greater than that of seeds with any other treatment in *C. nutans* (Fig. 2c). In *H. petiolaris* and *V. encelioides*, the proportion of natural seeds and of seeds with glue left in dishes were around three times greater than that of seeds to which the elaiosome was experimentally added. Only *P. bergi* was observed visiting plastic dishes and removing seeds in this assay.

Study species differed in seed mass ($F_{Species} = 30.942$, df = 2, 147, $p < 0.001$), such that *C. nutans* (3.7 ± 0.5 mg, mean ± SD) displayed greater seed mass than both *H. petiolaris* (3.2 ± 0.4 mg) and *V. encelioides* (2.9 ± 0.5 mg; $p < 0.001$ for both cases), and *H. petiolaris* exhibited greater seed mass than *V. encelioides* ($p = 0.012$).

Experiment 2

The proportion of *C. nutans* seeds that were recovered from refuse dumps in our controlled assessment of seed fate was 0.203 ± 0.263 (mean ± SD). No sign of predation was observed in those seeds, and in all cases, seed viability was 100%. The number of seeds recovered steadily increased up to day four, upon which seeds were no longer observed in refuse dumps (Fig. 3). No seed was observed to be dropped by ants before reaching colonies.

The proportion of *C. nutans* seedlings that emerged in pots that experimentally excluded the presence of *P. bergi* (e_s) in the study of Pearson et al. (2014a) was 0.375. Then, in our current experiment, the proportion of seeds removed by the ant (P_H) was 1, the proportion of seeds remaining into colonies (P_c) was 0.797 (i.e. 1–0.203), the proportion of seeds that emerged from colonies (e_c) was 0.203 and ($1-P_H$) was 0 because all seeds were harvested. Assuming 100% seedling emergence from seeds exposed to *P. bergi*, the emergence of *C. nutans* seedlings in the absence of this ant (0.375) was more than two times larger than that in its presence ($(1 \times 0.797 \times 0.203) + (0 \times 0.375) = 0.162$), making the interaction between these species strongly antagonistic.

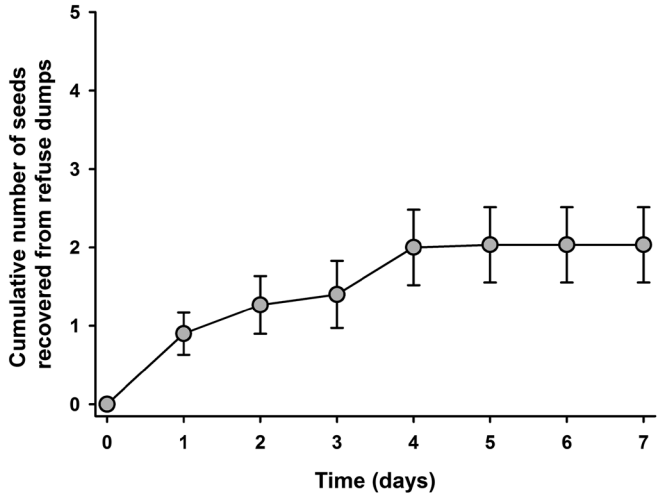

Figure 3. Cumulative number of *Carduus nutans* seeds recovered from refuse dumps of *Pheidole bergi* over time.

Experiment 3

The viability of *C. nutans* seeds collected from naturally occurring refuse dumps (0.985 ± 0.024) was similar to that of seeds collected from nearby *C. nutans* plants (0.999 ± 0.004; $\chi^2_{Seed\ source} = 1.752$, df = 1, $p = 0.186$).

The occurrence of the elaiosome in the Caldenal

The seeds of 24 species native to the Caldenal were examined under a stereo microscope to determine the presence of an elaiosome (Supporting information). Of those 24 species, 14 were preliminarily identified as producing seeds that might have elaiosomes (Table 1). The presence of that trait was, however, not obvious for all of them (Fig. 4).

Experiment 4

The response of *P. bergi* to seeds of those species with putative elaiosomes and *C. nutans* varied from strong to none ($\chi^2_{Species} = 854.170$, df = 12, $p < 0.001$; Fig. 5). *Pheidole bergi* preference for seeds of one of the natives, *Wedelia buphtalmiflora*, was similar to that for seeds of *C. nutans*. In the genus *Melica*, the top flower in the spikelet aborts and transmutes to an elaiosome (Fahn and Werker 1972). That aborted flower was easily identifiable in our *Melica* species; however, the response of *P. bergi* to them was relatively weak. No seed was dropped before reaching ant colonies. Finally, seed mass was not related to ant preference (slope, $t = -0.804$, $p = 0.438$; regression, $F = 0.647$, df = 1, 11, $p = 0.438$, $R^2 = 0.056$), even upon removing the influential data provided by *Hyalis argentea* (slope, $t = 1.138$, $p = 0.282$; regression, $F = 1.295$, df = 1, 10, $p = 0.282$, $R^2 = 0.115$). As before, no ant species other than *P. bergi* was observed visiting plastic dishes and removing seeds in this assay.

Discussion

By manipulating the presence versus absence of *C. nutans*' elaiosomes, we demonstrated that *P. bergi* strongly prefers to collect seeds with versus without *C. nutans*' elaiosomes, indicating that the elaiosome indeed mediates the interaction between *C. nutans* and *P. bergi*. While we detected no signs of predation on nor reduced viability of *C. nutans* recovered from *P. bergi* refuse dumps, we found that 80% of offered elaiosome-bearing *C. nutans* seeds remained inside *P. bergi*

Table 1. Species used to assess the response of *P. bergi* to *C. nutans* and native species seeds. Except for *C. nutans*, all species are native to the Caldenal, our study system. The question mark after yes in the With elaiosome column indicates doubts about the presence of the elaiosome in *Melica argyria* and *M. macra* because although the aborted flower that presumably transmutes to an elaiosome in *Melica* (Fahn and Werker 1972) was observed in seeds of those species, responses of *P. bergi* to them were ones of the lowest among study species. The endemic nature of species follows Instituto de Botánica Darwinion (www2.darwin.edu.ar). A dash under the Neotropical endemism column indicates that no information for the species was provided in that database. Values under the Seed mass column are means ± SDs.

Species	Family	Life form	With elaiosome	Seed mass (mg)	Neotropical endemism
Carduus nutans	Asteraceae	annual herb	yes	3.7 ± 0.5	no
Cyclospermum leptophyllum	Apiaceae	annual herb	yes	2.0 ± 0.4	–
Euphorbia dentata	Euphorbiaceae	annual herb	yes	6.2 ± 0.2	–
Heliotropium curassavicum	Boraginaceae	perennial herb	yes	1.8 ± 0.4	–
Hyalis argentea	Asteraceae	perennial herb	no	32.3 ± 2.0	yes
Melica argyrea	Poaceae	perennial grass	yes?	1.0 ± 0.06	yes
Melica bonariensis	Poaceae	perennial grass	no	1.1 ± 0.06	yes
Melica macra	Poaceae	perennial grass	yes?	1.4 ± 0.2	yes
Polygala aspalatha	Polygalaceae	perennial herb	yes	0.9 ± 0.3	–
Polygala stenophylla	Polygalaceae	perennial herb	yes	0.8 ± 0.2	yes
Rhamphopetalum microphyllum	Polygalaceae	perennial shrub	no	5.6 ± 2.1	yes
Sesuvium sp.	Aizoaceae	perennial herb	yes	0.2 ± 0.05	–
Sphaeralcea crispa	Malvaceae	perennial herb	no	1.5 ± 3E-16	yes
Turnera sidoides	Turneraceae	perennial herb	yes	1.7 ± 0.4	–
Wedelia buphtalmiflora	Asteraceae	perennial herb	yes	5.4 ± 1.00	yes

colonies. Most of these seeds were likely consumed by *P. bergi*, which is a known seed predator that gathers seeds from many species lacking elaiosomes (Pirk et al. 2009, Pearson et al. 2014a). Even if not consumed, viable seeds left within the colony are likely buried too deep for emergence or destroyed by pathogens. Importantly, our quantitative analysis showed that *C. nutans* and *P. bergi* engage in a strongly antagonistic interaction. Our estimation of the proportion of seeds left inside colonies was remarkably similar to that reported by Pearson and colleagues (2014a), even when we took the extra care of protecting and daily collecting refuse dumps. Protecting cages did not prevent, however, access of ants to refuse dumps, opening the possibility that our approach could have overestimated seed losses. Finally, our efforts to identify native species with elaiosomes in the Caldenal produced only eight species. Although our sampling was not exhaustive, and hence cannot reveal the relative abundance of elaiosome-bearing plants in the Caldenal, our data appear to confirm the rarity of the elaiosome in this system. Taken together, our findings suggest that the elaiosome promotes an antagonism that deters invasion in a cold spot of myrmecochore diversity, providing support to the idea that placing traits into novel evolutionary contexts may profoundly alter their functional roles.

Recent work has noted that the attractiveness of *Carduus* species' seeds to ants has not been definitively linked to the elaiosome (Pirk and López de Casenave 2017). Our study offers strong experimental evidence for the key role played by the elaiosome in the response of *P. bergi* to *C. nutans* seeds. Our study also suggests that the presence of the elaiosome overrides *P. bergi* preference for smaller seeds (Pearson et al. 2014a) because the species with larger seeds (*H. petiolaris*) was preferred over that with smaller seeds (*V. encelioides*) when the elaiosome was glued to them. Preference was also independent of seed mass in our seed offering experiment conducted with elaiosome-bearing propagules of several species.

The classical services that insectivorous ants are proposed to provide for plants with elaiosomes include reduction of intraspecific competition, deposition of seeds in favorable microsites (i.e. directed dispersal), random dispersal (i.e. accidental seed dropping), and escape from seed predators, including fire (Giladi 2006, Lengyel et al. 2010, Farji-Brener and Werenkraut 2017, Willis and Landis 2018), a common natural disturbance in the Caldenal (Medina 2007). If those benefits are larger than the costs of seed damage, then *P. bergi* would have a net positive effect on *C. nutans*. Our quantification of the outcome of the *C. nutans*–*P. bergi* interaction shows, however, that *P. bergi* removal of *C. nutans* seeds is antagonistic in the Caldenal. Additionally, other sources of evidence point to the same conclusion. First, *P. bergi* is extremely efficient at removing *C. nutans* seeds to the point of greatly reducing seedling recruitment (Pearson et al. 2014a). Second, we found that most *C. nutans* seeds appear to enter the colonies (i.e. few are dropped in transport) and 80% of those entering the colonies remain there, where they likely die. Attesting to this likelihood, we have observed no *C. nutans* recruitment/establishment on or nearby *P. bergi* colonies at any of our 17 sites used in seed offering assays nor at the ten sites included in Pearson et al. (2014a; only one of these sites overlapped). Finally, extensive surveys indicate that the relative abundance of *C. nutans* is low and its distribution narrow in the Caldenal. According to these surveys, the mean cover of *C. nutans* in sites altered by fire, grazing and maintenance of roadsides (i.e. the main disturbances in the Caldenal) was 5, 3 and 5%, respectively (Chiuffo et al. 2018). Also, *C. nutans* occupied the position 30 (out of 78), 52 (out of 82) and 53 (out of 97) in the rank abundance of species recorded in those sites, respectively, and it was found in only 3% of study plots. In addition, the relative abundance of *C. nutans* is lower in the Caldenal than in its native range of Anatolia (Pearson et al. 2018b).

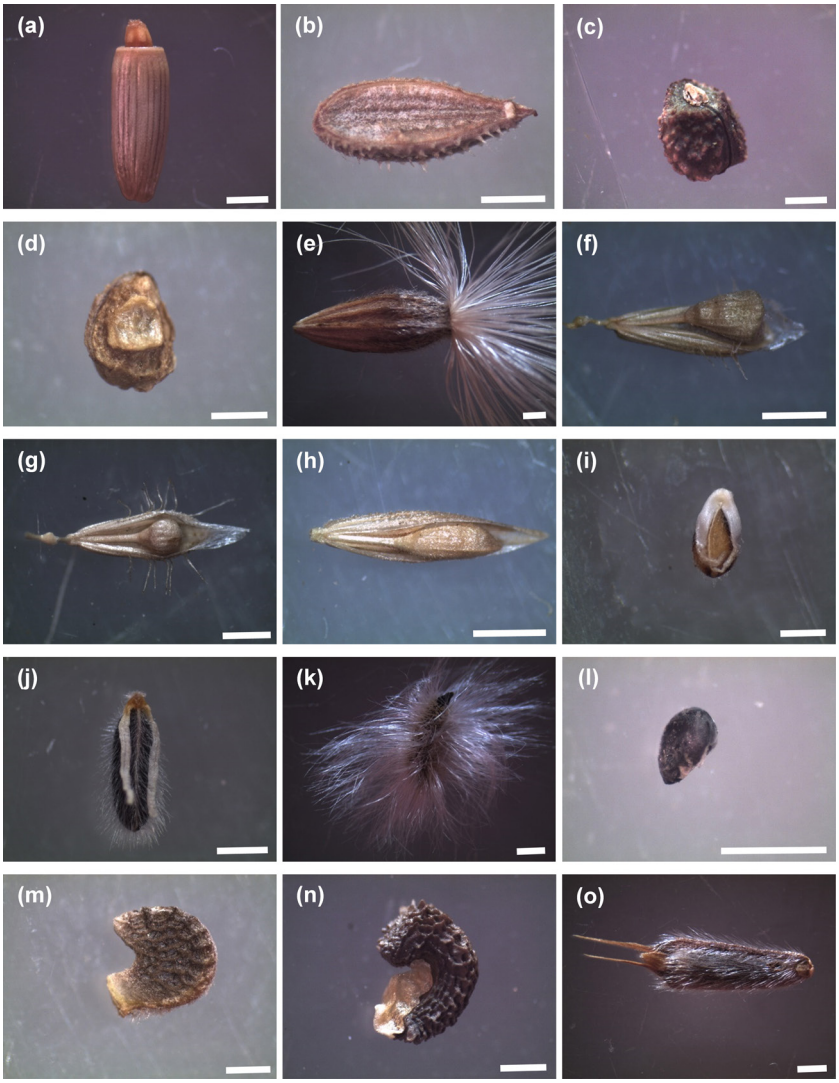

Figure 4. Propagules of the 15 species used to assess the response of *Pheidole bergi* to *Carduus nutans* and native species seeds. *Carduus nutans* (a), *Cyclospermum leptophyllum* (b), *Euphorbia dentata* (c), *Heliotropium curassavicum* (d), *Hyalis argentea* (e), *Melica argyrea* (f), *Melica bonariensis* (g), *Melica macra* (h), *Polygala aspalatha* (i), *Polygala stenophylla* (j), *Rhamphopetalum microphyllum* (syn. *Bredemeyera microphylla*; k), *Sesuvium* sp. (l), *Sphaeralcea crispa* (m), *Turnera sidoides* (n), *Wedelia buphtalmiflora* (o). In all cases, white lines represent 1 mm scale. Elaiosomes do not seem to be present in *H. argentea*, *M. bonariensis*, *R. microphyllum* and *S. crispa*, whereas its presence is unclear in *M. argyrea* and *M. macra*. Coincidentally, *P. bergi* displayed the lowest preference for seeds of those species. Species nomenclature follows Instituto de Botánica Darwinion (www2.darwin.edu.ar).

All five natives most preferred by *P. bergi* are also rare in the Caldenal (Chiuffo et al. 2018; J. L. Hierro and W. A. Muiño pers. obs.). Future research directions should include conducting a full sampling of the Caldenal flora to establish the proportion of species with elaiosome in this system, as well as their relative abundances. Also, because we only visually determined the presence of elaiosomes by placing seeds under a scope and assessing the response of ants to seeds, chemical analyses are needed to definitively establish the presence of the elaiosome in those species (Hughes et al. 1994). Our current information suggests, however, that the presence of elaiosome-bearing plants seems to be rare in our study system. Uncommon and novel traits have been associated to mechanisms through which non-native plants dominate natural communities (Callaway and Aschehoug 2000, Callaway and Ridenour 2004, MacDougall et al. 2009); in contrast, our work indicates that those traits can also mediate biotic resistance to invasion.

Pheidole sp. can have largely destructive effects on elaiosome-bearing seeds in Australia, where this ant readily predates on seeds or consumes them upon carrying the seeds to the interior of their colonies (Hughes and Westoby 1992). Estimates of the proportion of seeds eaten by *Pheidole* sp. inside colonies after seven days in that region (61% in collar protected colonies and 66% in unprotected colonies; Hughes and Westoby 1992) are, however, lower than our estimate of the proportion of *C. nutans* seeds left inside *P. bergi* colonies after the same time period. Regardless of differences in the

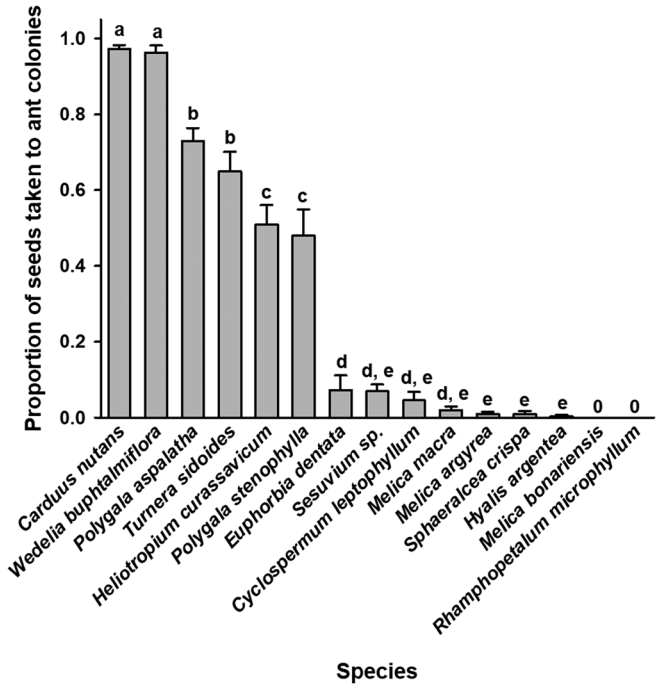

Figure 5. Proportion of seeds of *Carduus nutans* and 14 species native to the Caldenal that were taken by *Pheidole bergi* to their colonies. Bars are means ± SE of 10 sites. Different letters above bars indicate significant differences between species at $p < 0.05$.

strength of the impact of *Pheidole* species on seeds between systems, negative effects of *Pheidole* sp. on species with elaiosome in Australia (Hughes and Westoby 1992) indicate that antagonistic interactions between ants and elaiosome-bearing plants can also occur within hotspots of myrmecochore diversity as Australia (Lengyel et al. 2010). Although some ants, like *Pheidole* sp., predate on seeds with elaiosomes, other ant species within those hotspots can provide the services typically associated with myrmecochory (Hughes and Westoby 1992). Our work suggests that in systems where the required mutualistic partners are absent or rare, the elaiosome may promote an antagonism with local omnivorous ants.

The interactions that plants establish with ants have traditionally been deemed as generalized (Mittelbach and Gross 1984, MacMahon et al. 2000, Bronstein et al. 2006). Accordingly, the elaiosome is proposed to promote community invasion through generalized mutualisms with local ants around the world (Pemberton and Irving 1990, Jensen and Six 2006, Alba-Lynn and Henk 2010, Ortiz et al. 2021). In some cases, this view rests on observing the presence of an elaiosome on the propagules of a given non-native plant; in others, on the response of native ants to elaiosome-bearing propagules. In all cases, however, those observations are assumed to translate into myrmecochory without quantifying seed fate. In evaluating seed fates in our system, we found that the elaiosome likely deters plant invasion via an antagonistic interaction with a dominant native ant. Our study, thus, adds to the notable biotic resistance to invasion reported for natural communities in the Caldenal (Hierro et al. 2006, 2011, Pearson et al. 2014a, b, 2018c, Chiuffo et al. 2018). More generally, our work has major implications for plant invasion because it indicates that the assumed mutualistic association between non-native plants with elaiosome and native ants may not universally hold. Moreover, we predict that the nature of this relationship will depend on the relative abundance of ant feeding behaviors within the recipient community. That is, if the dominant ants of the recipient community are strongly insectivorous, then introduced elaiosomes should retain their mutualist roles and facilitate plant invasion, but if the dominant ants are omnivorous, then the elaiosome will generate antagonistic interactions that may mediate biotic resistance. The functions of phenotypic traits would thus not be fixed, but would vary according to the ecological and evolutionary contexts in which they operate.

Speculations

Our work seems to provide a rare example of shifting the nature of species interactions from mutualistic in the native range to antagonistic in the non-native range. Previous studies have shown that changes in the strength and outcome of species interactions between those ranges benefit non-natives (Callaway and Aschehoug 2000, Reinhart et al. 2003, 2010, Callaway et al. 2004, 2011, Hierro et al. 2017). Our study suggests, however, that shifts in species interactions between distributional ranges may not only facilitate, but also resist invasion. In addition, our study raises the possibility that myrmecochory is conditioned by the coevolutionary history of the species engaged in the interaction (Hierro and Callaway 2021). As for shifts in interaction outcome, sharing no evolutionary history with members of the recipient community has been proposed as a mechanism through which non-natives dominate those communities (Rabotnov 1974, Callaway and Ridenour 2004). According to our findings, lacking coevolved interactions may also explain the control of non-native by native species, bringing new light into the consequences that coevolutionary conditioned interactions may have for invasion.

Alternative viewpoints

Our conclusion that the function of the elaiosome shifted from mediating a mutualism in the native range to mediating an antagonism in a non-native region rests on two assumptions. Firstly, we assumed that all seeds left inside *P. bergi* colonies (80% of offered seeds) translated into plant mortality. Secondly, we assumed that applying Zwolak and Crone (2012)'s equation to the interaction between *C. nutans* and ants in the native range would result in a mutualistic outcome. Sampling colonies and associated refuse dumps over a time longer than that in our work and estimating the proportion of seeds eaten by ants inside colonies along the lines proposed by Hughes and Westoby (1992) can improve the quality of our inference. Similarly, assessing the outcome of

plant–ant interactions in native and non-native ranges has the potential to advance ecological understanding.

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Author contributions

Jose L. Hierro: Conceptualization (lead); Formal analysis (lead); Funding acquisition (lead); Methodology (lead); Project administration (lead); Writing – original draft (lead). **Walter A. Muiño**: Methodology (supporting); Writing – review and editing (supporting). **Alejandro Farji-Brener**: Funding acquisition (supporting); Writing – review and editing (supporting). **Marina C. Cock**: Formal analysis (equal); Writing – review and editing (supporting). **Dean E. Pearson**: Conceptualization (supporting); Funding acquisition (supporting); Methodology (supporting); Writing – review and editing (equal).

Data availability statement

Data are available from the Github Digital Repository: https://github.com/JoseHierro/Data-for-Oikos-2022.git (Hierro et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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