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Peer reviewed

# Comparing the Individual and Combined Effects of Ant Attendance and Wing Formation on Aphid Body Size and Reproduction

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## Abstract

Species employ multiple strategies to deal with stressful environments, but these strategies often incur costs. Aphids frequently utilize multiple predator avoidance strategies, including attracting mutualist ants for protection and dispersing by producing winged forms. While both strategies can be physiologically costly, the magnitudes of these costs have not been previously compared. In this study, we experimentally manipulated ant attendance in the field and measured the individual and interactive effects of ant attendance and wing formation on body size and reproduction of the ant-tended aphid *Cinara schwarzii* (Wilson) (Hemiptera: Aphididae). Aphid adult body size was smaller in the presence of ants (18%), but controlling for body size, there were no differences in embryo number or size. In contrast, wing formation did not affect adult body size but strongly reduced embryo number (46%) and size (8%). Although ant attendance reduced *C. schwarzii* wing formation, ant attendance and wing formation acted independently on aphid body size and reproduction. For comparison, we confirmed that the manipulation of ant presence had no effect on body size or reproduction of the untended co-existing congener *Cinara solitaria* (Gillette and Palmer) (Hemiptera: Aphididae). Complementing our empirical study, a meta-analysis of 78 responses from 24 publications showed that wing formation consistently and significantly reduces aphid body size and reproduction (37%), while the effects of ant attendance showed a mean positive effect (9%) that did not significantly differ from zero. Together, our empirical study and meta-analysis provide strong evidence for costs of wing formation but not ant attendance for aphids.

**Key words:** ant, aphid, alate, apterous, mutualism

Across ecological and evolutionary timescales, species use multiple strategies to avoid or cope with stressful environments. However, such strategies often incur physiological costs. For example, species can avoid unfavorable conditions by dispersing to new habitats, but dispersal ability trades off with fecundity for many organisms, including insects (Mole and Zera 1993, Guerra 2011) and plants (Leishman et al. 2000, Gómez 2004). Alternatively, species can cope with stressful environments through changes in their traits or interactions with other species. However, while mutualism and facilitation, for example, are often more important in stressful environments (Bertness and Callaway 1994, Maestre et al. 2009) and can expand species' realized niches (Bruno et al. 2003), producing rewards or services for mutualists can be costly (Bronstein 2001). In order to predict when such strategies will thus be favored, it is important to understand their relative costs and benefits.

Aphids are notable for using multiple contrasting strategies to avoid predators, including attracting mutualist ants for protection as well as dispersing by producing winged morphs. In ant–aphid mutualisms, otherwise carnivorous ants tend aphids, collecting sugar-rich excreta—so-called ‘honeydew’—while protecting aphids against predators and parasitoids (Stadler and Dixon 2008). In addition to providing protection, ants benefit aphids by removing honeydew that would otherwise foul (Wood et al. 1988), increasing aphid feeding rates (Banks and Nixon 1958), and removing competing herbivores (Styrsky and Eubanks 2007, Smith et al. 2008). As a result, ant attendance can increase aphid population growth (Addicott 1979, Mooney and Mandal 2010, Nelson et al. 2019), with a parallel benefit to ants (Helms and Vinson 2008).

However, ant attendance also carries costs, likely explaining why only 40% of aphid species are ant tended (Stadler and Dixon 2008). The ecological costs of associating with ants are diverse and

include, for example, the risk of predation by ants when their need for protein outweighs that for carbohydrates (Offenberg 2001, Mooney and Tillberg 2005). Moreover, producing honeydew with ant-attractive constituents is physiologically costly for aphids; ants can induce changes in honeydew chemistry, including shifts from glucose to more complex sugars including sucrose, trehalose, and melezitose as well as a greater diversity and concentration of amino acids (Fischer and Shingleton 2001; Yao and Akimoto 2001, 2002). Studies measuring the effects of ants in the absence of predators have detected multiple direct costs of ant attendance for aphids, including reduced body size, reduced embryo number and size, and increased age at fecundity (e.g., Stadler and Dixon 1998, Yao et al. 2000, Shibao et al. 2009, Yoo and Holway 2011). Yet other studies have demonstrated superior aphid performance under ant attendance in the absence of predators (e.g., Flatt and Weisser 2000, Stadler et al. 2002, Powell and Silverman 2010), warranting a more systematic comparison of the effects of ants on aphid performance across studies.

Alternative to being protected by ants, aphids may behaviorally avoid predation by dispersing (reviewed by Müller et al. 2001, Braendle et al. 2006). From an ecological perspective, aphids are notable for their phenotypic plasticity in wing production, which generally occurs when conditions in the pre- or post-natal environment are poor, such as with crowding, poor host plant quality, or high predation risk (Dixon and Agarwala 1999, Weisser et al. 1999, Müller et al. 2001, Mondor et al. 2005, Purandare et al. 2014). In response to predation, aphids release an alarm pheromone that causes them to walk or jump off of their host plant (Mooney 2006) as well as to produce winged morphs (Kunert et al. 2005), thus serving as an induced defense (Tollrian and Harvell 1999). However, wing formation can carry ecological costs, such as those associated with locating appropriate host plants (Stadler et al. 2001, Poethke et al. 2010), as well as direct physiological costs, such as increased age at fecundity and lower offspring production rates (Braendle et al. 2006).

Because ant attendance and wing formation are both potentially costly and redundant predator avoidance strategies, they may be negatively correlated or have interactive effects on aphids. Although no previous study has systematically compared the individual and combined effects of ant attendance and wing formation on aphid performance, there is reason to believe that they may have interactive negative effects. For example, myrmecophilous (vs. non-myrmecophilous) aphid species have reduced behavioral and morphological defenses against predators (Mondor et al. 2002, Shingleton et al. 2005, Tokunaga and Suzuki 2008), and they also exhibit reduced defenses in the presence (vs. absence) of ants (Nault et al. 1976, Oliver et al. 2007, Mondor et al. 2008). As a result of such dependence on ant defense, dispersal may be riskier for myrmecophilous species, since they must locate a host plant that is not only high quality but also near attendant ants (Stadler et al. 2001), unless ants assist them with dispersal (e.g., by carrying them to new plants; Collins and Leather 2002). Thus, wing formation could be especially costly for ant-tended aphids. Consistent with this prediction, wing formation can be reduced in myrmecophilous (vs. non-myrmecophilous) aphid species (Stadler et al. 2003) and in the presence (vs. absence) of ants within myrmecophilous species (El-Ziady and Kennedy 1956, Johnson 1959, Tilles and Wood 1982, Mondor et al. 2008). In some cases, ants even exert control over aphid wing formation, reducing dispersal for their own benefit (Johnson 1959, Kleinjan and Mittler 1975, Oliver et al. 2007). Ultimately, predicting the combined effect of ant

attendance and wing formation on aphids is difficult because it depends on the relative costs and benefits of these two strategies, the strength of intraspecific competition and predation, and the risks associated with locating a suitable host plant and attendant ants (Stadler et al. 2001, Poethke et al. 2010).

In this study, we assessed the individual and combined effects of ant attendance and wing formation on the size and reproduction of the ant-tended aphid *Cinara schwarzii* (Wilson) (Hemiptera: Aphididae). We collected winged and unwinged asexual adult *C. schwarzii* aphids (Fig. 1) from *Pinus ponderosa* (Pinales:Pinaceae) Laws. *scopulorum* (Pinaceae) trees, where ants were present or experimentally excluded. For comparison, from the same trees, we also collected co-existing congeneric asexual adult *Cinara solitaria* (Gillette and Palmer) aphids (Fig. 1), which are unwinged and not ant tended, and were thus not expected to be affected by the experimental ant exclusions. To test for the effects of ant attendance and wing formation, nearly 900 winged and unwinged *C. schwarzii* aphids (and *C. solitaria* for comparison) were dissected and measured for size (body, leg, and mouthpart length) and fecundity (embryo number and size). In so doing, we assessed the effects of ant attendance and wing formation on aphid size and reproduction, both within a species and between a tended and an untended species. To complement the findings from this empirical study and evaluate whether similar effects are observed in other aphid species, we conducted a meta-analysis of 78 responses from 24 published studies documenting the individual effects of ant attendance and wing formation on size and reproductive traits within aphid species.



Fig. 1. Dorsal and ventral images of unwinged adult *C. schwarzii* and *C. solitaria* collected from *Pinus ponderosa* at the Manitou Experimental Forest in 2001; 1.0 mm scale bars are included in each image. Whereas *C. schwarzii* is typically cinnamon-brown and 2.2–4.2 mm long, *C. solitaria* is typically yellow-brown and 2.5–3.0 mm long, with short black tibiae and a rounded, tick-like body (Blackman and Eastop 2020) (see online for color version of this figure).

## Materials and Methods

### Natural History

We studied the ant-tended aphid *C. schwarzii* (and the untended aphid *C. solitaria* for comparison) in canopies of *Pinus ponderosa* subsp. *scopulorum* (ponderosa pine) at the Manitou Experimental Forest (39°06′00″N, 105°05′00″W; Woodland Park, CO). The ecology and natural history of this system have previously been described by Mooney and colleagues (Mooney and Tillberg 2005; Mooney 2006, 2007; Mooney and Linhart 2006; Mooney and Mandal 2010). Briefly, two ant-tended (*Cinara arizonica* and *C. schwarzii*) and four untended aphid species (*Cinara glabra*, *C. solitaria*, *Essigella fusca*, and *Schizolachnus piniradiatae*) feed on ponderosa pine in this site, with *C. schwarzii* and *C. solitaria* being the most abundant tended and untended species, respectively. While *C. schwarzii* lives in colonies that include both winged and unwinged asexual aphids during the summer, unwinged asexual *C. solitaria* aphids live singly, with winged forms rarely occurring in this site (Blackman and Eastop 2020, Mooney, personal observation). Because we did not observe sexual morphs during the sampling period and assume that they only become sexual with the onset of fall (Blackman and Eastop 2020), only asexual aphids were sampled in this study. Multiple competing ant species commonly associate with *C. schwarzii* (Mooney and Mandal 2010), but *Formica podzolica* is the most abundant aphid-tending ant in this site. In other *Cinara* species, wing formation has been shown to be induced by crowding and plant shoot growth (Kidd and Tozer 1984, Kairo and Murphy 1999) but to be reduced by ant attendance (Tilles and Wood 1982).

### Ant Exclusion and Aphid Collection

This work is based on aphids collected during an experiment previously described by Mooney (2006, 2007). During the first week of June 2001, experimental pine trees located within approximately 5 m of *F. podzolica* mounds were selected in 21 blocks of four trees each, for a total of 84 trees. Trees within blocks were less than 8 m apart, and the blocks were distributed over 750 ha. Trees were 4.5–13.5 m (8.6 ± 0.5 m, mean ± SE) in height and 14–32 cm (21.5 ± 8 cm, mean ± SE) in diameter (at a height of 1.4 m). Ants were excluded from two randomly selected trees in each block with sticky paste (Tanglefoot Company, Grand Rapids, MI), with a similar amount of paste applied to half of the trunk diameter of trees with ant attendance. Because previous studies based on data collected from these same trees investigated the effects of insectivorous birds on both ants and aphids (Mooney 2006, 2007; Mooney and Linhart 2006; Mooney and Mandal 2010), three branches on one ant exclusion and one ant attendance tree from each block were enclosed within 2.5-cm opening monofilament netting bags to exclude birds. However, the effects of birds are not the topic of this study and do not influence the overall interpretations of the results presented here.

A total of 252 samples of arthropods were collected in September 2001, June 2002, and July 2002 (84 trees sampled three times each). Two branches were sampled per tree, with one branch sampled both in September 2001 and July 2002, and a different branch sampled in June 2002. For each sample, the branch was beaten repeatedly with a padded bat to dislodge arthropods into a 1.5-m × 1.5-m fabric tub (0.5 m deep), after which all arthropods were preserved in 70% ethanol. This methodology, described in greater detail by Mooney and Tillberg (2005), captures most (>97%) arthropods, including aphids. To confirm the identity of the ants and aphids in this study, voucher specimens were identified by André Francoeur and experts at the Smithsonian Museum of Natural History, respectively.

### Aphid Measurements

We inspected each arthropod sample for winged and unwinged asexual adult *C. schwarzii* and *C. solitaria* aphids in 2010 and 2011 using a dissecting microscope (SteREO Discovery.V8, Zeiss International, Oberkochen, Germany). Of the 252 sampled branches, 205 had one or more adult aphids, including unwinged *C. schwarzii* (134 of the samples), winged *C. schwarzii* (97 of the samples), or unwinged *C. solitaria* (137 of the samples). However, there were no winged *C. solitaria* in any samples. Thus, only three of the four potential aphid classes were characterized in this study (winged and unwinged *C. schwarzii* and unwinged *C. solitaria*). From each sample we counted and identified all aphids and measured up to six randomly selected asexual adult aphids of each type. Adults were distinguished from immature aphids by the presence of a sclerotized ventral plate located just anterior to the genitalia. Overall, a mean of 2.3 ± 1.05 SD aphids per type were measured per sample (unwinged *C. schwarzii*: 2.4 ± 1.10 SD; winged *C. schwarzii*: 1.9 ± 0.96 SD; unwinged *C. solitaria*: 2.4 ± 1.02 SD), totaling 864 aphids (unwinged *C. schwarzii*: 347; winged *C. schwarzii*: 184; unwinged *C. solitaria*: 333).

To determine the effects of ant attendance and wing formation on aphid size, we measured three standard metrics of adult aphid size (e.g., Arakaki 1989, Dixon et al. 1995, Favret and Miller 2012) to the nearest 0.1 mm: body length (anterior of head to anterior of abdomen), mouthpart length (stylet), and the length of one front leg (femur and tibia combined). In addition, we assessed the effects of ant attendance and wing formation on aphid reproduction by dissecting each aphid to count the number of embryos and measure the length of the longest embryo, as has been done in previous studies (Stadler and Dixon 1998). Because immature embryos were sometimes difficult to distinguish from maternal body fat and other organs, we only counted and measured well-developed embryos with pigmented eyespots (Breton and Addicott 1992). While low embryo number or size could be caused by low fecundity, it could also be attributed to differences in individual age or a delay in reproductive maturity that may not necessarily reduce total reproductive output or offspring fitness (Taylor 1975). However, past studies comparing winged and unwinged morphs of other aphid species have shown that the effects of wing formation on embryo size and number can be proportional to the effects on lifetime fecundity (Wratten 1977, Tsuji and Kawada 1987, Newton and Dixon 1990). Here, because mean embryo number and mean embryo size were not significantly correlated ( $F = 2.22$ ;  $df = 1,89$ ;  $P = 0.14$ ;  $R^2 = 0.02$ ), we report on both metrics as potentially relevant but separate metrics of aphid reproductive success.

### Data Analysis

Inter- and intraspecific competition among aphids can influence aphid size and reproduction, and the presence of mutualist ants can influence aphid abundance through predator protection. Accordingly, we documented the effects of ant attendance while including aphid abundance as a covariate. First, we used separate linear models to test whether the abundances of both aphid species (total abundance, with winged and unwinged aphids combined;  $ln+1$  transformed) as well as of winged *C. schwarzii* aphids (including nymphs with wing buds;  $ln+1$  transformed) depended on ant attendance (vs. exclusion) treatment. Then, for each size and reproductive metric, we used a linear model to test for the effects of ant attendance treatment, wing formation, and their interaction, averaging measurements taken from multiple aphids to produce single values for each aphid type (winged or unwinged *C. schwarzii* or unwinged



*C. solitaria*) per branch sample. Because we sampled aphids from the same trees on three separate occasions, we averaged all responses measured within the same tree across sampling dates to account for any non-independence due to repeated sampling. Moreover, to account for any potential spatial non-independence, we initially included sampling block as a random effect in each model. However, because some models were over-fitted and incorporating the effect of block did not qualitatively affect any results, we removed it from the final models. To account for any potential effects of the bird exclusion treatment that was used to address a separate set of questions on the same trees (Mooney 2006, 2007; Mooney and Linhart 2006; Mooney and Mandal 2010), we included it as a fixed effect in all statistical analyses (but do not focus on these results because the effect of birds is not the topic of this study). Analyses of wing formation rate and aphid size and reproductive metrics also included total aphid abundance (all aphid species combined;  $\ln+1$  transformed in the wing formation analysis to improve normality of residuals) as a covariate to control for changes in the aphid's competitive environment. Moreover, to test the hypothesis that embryo number and length are constrained by adult aphid size, we included aphid body length as a fixed effect in analyses of these reproductive metrics. In doing so, we test for the association between body size and embryo size and length, while also controlling for the effects on adult aphid size in tests for effects of ant attendance and wing formation on embryo number and length.

Data analysis was conducted in R v3.3.2 (R Core Team 2019). All models were constructed using the 'lm()' function in the 'stats' package (R Core Team 2019). To assess the statistical significance of all results, we conducted *F*-tests with type III sums of squares using the 'Anova()' function in the 'car' package (Fox and Weisberg 2010). When interaction terms were not statistically significant, they were removed from the models to test for the significance of main effects. Least-squares means were calculated using the 'emmeans' function in the 'emmeans' package (Lenth 2020).

## Literature Review

To compare the results from this study with those from past studies, we reviewed and quantitatively summarized studies published from 1955 to 2019 on the individual effects of ant attendance and wing formation on aphid size and reproduction. While there are other anti-predator defenses (e.g., behavioral, chemical, and morphological defenses) (Malcolm 1990, Shingleton and Foster 2001, Dion et al. 2011) that could also have direct effects on aphid size and reproduction, the purpose of this meta-analysis is to complement our empirical data in characterizing the relative effects of ant attendance and wing formation. We identified studies to include in April 2019 by searching Google Scholar (using keywords such as 'aphid', 'ant', 'wing', 'direct', and 'fitness'), the bibliographies of past reviews (e.g., Müller et al. 2001, Braendle et al. 2006, Stadler and Dixon 2008), and the studies citing or cited by known studies. All included studies measured effects of ant attendance or wing formation on one or more metric of aphid size or reproduction. Specifically, metrics measured from individual aphids (aphid size, gonad number, embryo number, embryo size, or time to reach developmental benchmarks) came from both field and laboratory studies, whereas metrics based on changes in the number of aphids (fecundity or population growth rates) only came from studies of aphids under laboratory or field conditions that eliminated interactions with natural enemies and competing herbivores. Similarly, effects on aphid population growth were excluded if ants were reported to prey on aphids (e.g., Oliver et al. 2012). By doing so, we tested for the direct rather than

ecological effects (e.g., defense against natural enemies) of ant attendance on aphid fecundity and population growth. However, because measurements taken from individual aphids came from both field and laboratory studies, they could reflect both direct and indirect ecological effects of ant attendance. While we would have ideally only tested for direct effects, too few studies have measured the direct effect of ant attendance on the size or reproduction of individual aphids in the absence of predators and competitors.

We quantitatively summarized the effects of ant attendance and wing formation on four classes of aphid size and reproductive metrics: (1) adult size, (2) time to reach developmental benchmarks, (3) gonad size, embryo size, or embryo number, and (4) individual fecundity or population growth. Whereas metrics 1–3 are based on measurements of individual aphids, metric 4 is based on changes in the number of aphids in the colony. We calculated effects on each class of size or reproductive metric both as the percentage change (positive or negative) and as the log response ratio (LRR; Hedges et al. 1999), in which the response values with ants or wings present were divided by the values with ants or wings absent and then natural-log transformed. When secondary factors were involved (e.g., varying plant quality; Stadler et al. 2002), effects were calculated separately for all levels of these factors and then averaged together to produce single effect sizes for each group (ants vs. no ants or wings vs. no wings). When repeated measures were involved (e.g., Tsumuki et al. 1990), effects were calculated for when response values were at a maximum.

Effects on development time were multiplied by  $-1$  so that negative and positive LRR values represented negative and positive effects on aphids, making these effects comparable to those from the other three classes of size and reproductive metrics. To calculate means and 95% CIs for the effects of ant attendance and wing formation on each of the four size or reproductive metric classes, multiple LRRs reported within the same class for an aphid species were first averaged to produce a single value ('mean LRR'). To calculate the overall effects of ant attendance and wing formation on aphid traits (across all classes), mean LRRs for each class of size or reproductive metric were averaged for each aphid species. These species-level averages were then used to produce means and 95% CIs for the overall effects. Finally, to calculate the overall effects of ant attendance for aphids that are obligately versus facultatively tended, species were first assigned to these groups based on descriptions provided by study authors. Mean LRRs for each size or reproductive class were then averaged for each of the aphid species for which ant effects were reported. These species-level averages were then used to produce means and 95% CIs for the overall effects of ant attendance for obligately versus facultatively tended species. In all cases, using species-level averages avoids the issue of pseudo-replication inherent to analyzing multiple responses from a single species. Nevertheless, variation among species is likely due to a combination of species traits and differing experimental conditions and protocols, and the data currently available cannot be used to draw conclusions about individual species.

## Results

### Effects of Ant Attendance on Aphid Abundance and Wing Formation

Overall, the ant-tended aphid *C. schwarzii* was sixfold more abundant than the untended aphid *C. solitaria*, occurring at densities of  $97 \pm 24$  versus  $16 \pm 2$  (mean  $\pm$  SE) aphids per pine branch, respectively. Across all samples,  $11.7\% \pm 1.5$  SE ( $N = 204$ ) of *C. schwarzii*

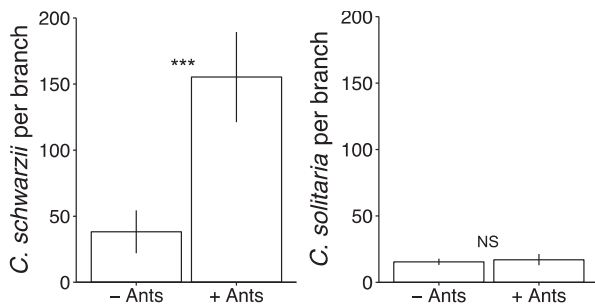
were winged, including both adults and nymphs with developing wing buds. Although ant attendance increased *C. schwarzii* abundance by 306% (Fig. 2; Table 1, A), it significantly reduced the number of winged *C. schwarzii* per pine branch (accounting for the total number of aphids per branch as a covariate) (Fig. 3; Table 1, B). In contrast, ant presence had no detectable effect on the abundance of *C. solitaria* (the untended comparison species) (Fig. 2; Supp Table S1.1, A [online only]), and no winged *C. solitaria* were ever observed.

### Effects of Ant Attendance and Wing Formation on Aphid Size and Reproduction

Ant attendance significantly reduced *C. schwarzii* adult body (18%), leg (34%), and mouthpart lengths (10%) (Fig. 4; Table 1, C–E). Wing formation had more modest effects on *C. schwarzii* body size, having no detectable effects on body or leg length and only marginally significantly increasing mouthpart length by 7% (Fig. 4; Table 1, C–E). Adult body size was significantly positively correlated with both embryo number and embryo length (Table 1, F and G). Accounting for adult body size, ant attendance had no detectable effects on *C. schwarzii* embryo number or length, while wings significantly reduced embryo number (46%) and length (8%) (Fig. 5; Table 1, F and G). There were no significant interactions between the effects of ant attendance and wing formation on any size or reproductive metrics (Table 1, C–G). For *C. solitaria* (the untended comparison species), ant presence had no effect on any of the measurements (Supp Table S1.1, B–F [online only]; Supp Figs. S1.1 and S1.2 [online only]).

### Literature Review

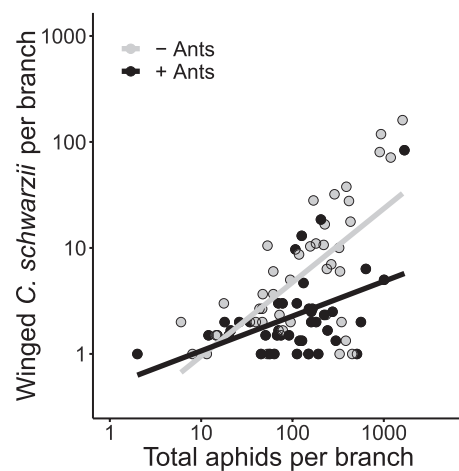
Including the present study, we identified 24 studies that measured size or reproductive traits of 21 aphid species in response to ant attendance (13 studies, 9 aphid species) or wing formation (13 studies, 14 aphid species). Across these studies, 78 separate responses were quantified. These responses included measurements of aphid (1) adult size, (2) time to developmental benchmarks, (3) gonad size, embryo size, or embryo number, and (4) individual fecundity or population growth. Across the 37 responses measuring the effects of ant attendance, the literature reported 12 as showing significantly negative effects on aphids, 12 as not significant, and 10 as significantly positive; the statistical significance of three of the responses was not reported (Supp Table S2.1 [online only]). Overall, ant attendance did not significantly affect aphid size or reproduction (Fig. 6), with a mean LRR of +0.06 (95% CI: -0.06, +0.19), corresponding to a +9% change (95% CI: -7%, +25%). Similarly, ant



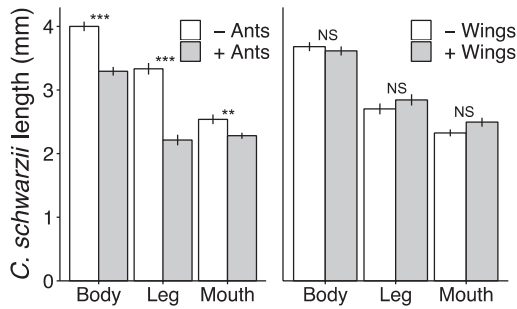
**Fig. 2.** Mean ( $\pm$  SE) number of *C. schwarzii* and *C. solitaria* per branch in ant exclusion and attendance treatments. The statistical significance of the effect of ant attendance is indicated in both panels (NS =  $P > 0.05$ , \*\*\* $P < 0.001$ ).

**Table 1.** Results from statistical analyses of the effects of ant attendance and wing formation (as well as bird exclusion treatment and aphid number as covariates) on *C. schwarzii* abundance and size and reproductive traits

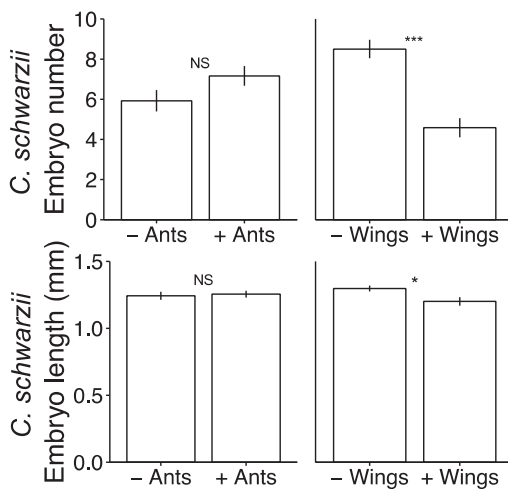
Variable	Effect	df	F	P
A. Aphid number	Ant	1,81	12.66	<0.001
	Bird	1,81	13.43	<0.001
B. Wing formation	Ant	1,79	1.26	0.266
	Aphid number	1,79	30.11	<0.001
	Ant* Aphid number	1,79	4.86	0.030
	Bird	1,79	3.56	0.063
C. Body length	Ant	1,125	48.25	<0.001
	Wing	1,125	0.44	0.507
	Ant* Wing	1,124	0.89	0.348
	Bird	1,125	0.13	0.723
D. Leg length	Aphid number	1,125	3.31	0.071
	Ant	1,127	81.66	<0.001
	Wing	1,127	1.33	0.251
	Ant* Wing	1,126	0.01	0.904
E. Mouth length	Bird	1,127	0.94	0.333
	Aphid number	1,127	2.84	0.094
	Ant	1,127	8.88	0.003
	Wing	1,127	3.88	0.051
F. Embryo number	Ant* Wing	1,126	0.04	0.833
	Bird	1,127	0.96	0.330
	Aphid number	1,127	1.65	0.201
	Ant	1,124	2.54	0.114
G. Embryo length	Wing	1,124	35.17	<0.001
	Ant* Wing	1,123	1.07	0.303
	Bird	1,124	0.11	0.737
	Aphid number	1,124	4.45	0.037
Body size	1,124	8.32	0.005	
	Ant	1,84	0.09	0.760
	Wing	1,84	6.19	0.015
	Ant* Wing	1,83	0.24	0.626
Bird	1,84	0.68	0.411	
	Aphid number	1,84	4.06	0.047
	Body size	1,84	43.71	<0.001



**Fig. 3.** Number of winged *C. schwarzii* per branch depending on the total number of aphids (all species) per branch in ant exclusion and attendance treatments. The ant attendance treatment  $\times$  total aphid number interaction and the main effect of total aphid number are statistically significant ( $P < 0.05$  for both effects), but the main effect of ant attendance is not ( $P = 0.266$ ).



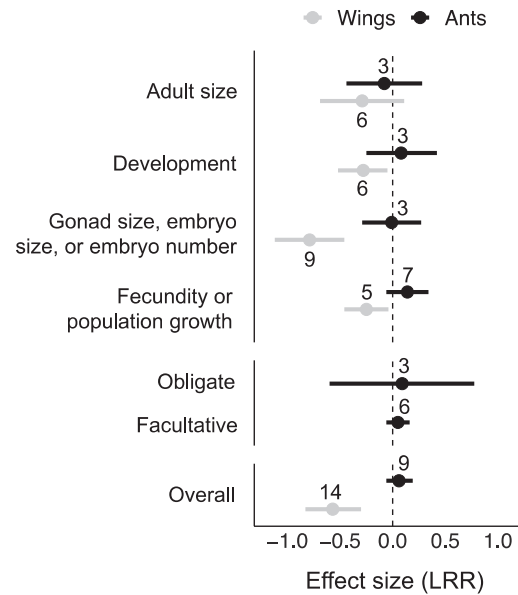
**Fig. 4.** Least-squares mean ( $\pm$  SE) adult winged and unwinged *C. schwarzii* body size measurements in ant exclusion and attendance treatments. The effects of ant attendance and wing formation were statistically independent for each variable ( $P > 0.05$ ). The statistical significance of the main effects of ant attendance and wing formation is indicated in each panel (NS =  $P > 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ).



**Fig. 5.** Least-squares mean ( $\pm$  SE) aphid embryo number and length, controlling for aphid abundance. Values are presented for adult winged and unwinged *C. schwarzii* in ant exclusion and attendance treatments. The effects of ant attendance and wing formation were statistically independent for each variable ( $P > 0.05$ ). The statistical significance of the main effects of ant attendance and wing formation is indicated in each panel (NS =  $P > 0.05$ , \*  $P < 0.05$ , \*\*\*  $P < 0.001$ ).

attendance did not significantly affect any of the individual classes of aphid size or reproductive metrics, with a mean LRR of  $-0.08$  (95% CI:  $-0.44, +0.28$ ) and  $-6\%$  effect (95% CI:  $-40\%, +28\%$ ) on adult size, a mean LRR of  $+0.08$  (95% CI:  $-0.25, +0.42$ ) and  $+11\%$  effect (95% CI:  $-32\%, +53\%$ ) on time to developmental benchmarks, a mean LRR of  $-0.01$  (95% CI:  $-0.29, +0.27$ ) and  $-0.5\%$  effect (95% CI:  $-29\%, +28\%$ ) on gonad size, embryo size, and embryo number, and a mean LRR of  $+0.14$  (95% CI:  $-0.06, +0.34$ ) and  $+18\%$  effect (95% CI:  $-10\%, +46\%$ ) on individual fecundity and population growth (Fig. 6). Of the nine aphid species for which ant effects were assessed, study authors reported three to be obligately tended and six to be facultatively tended. The overall effects of ant attendance were similar between these two groups (not significant).

In contrast, across the 41 variables measured in response to wing formation, the literature reported 24 to show significantly negative effects on aphids, 7 to be non-significant, and none to be significantly positive; the statistical significance of 10 of the responses was not reported (Supp Table S2.1 [online only]). Overall, wing formation had a significant negative effect on aphid size and reproductive metrics



**Fig. 6.** Mean effect sizes (LRR)  $\pm$  95% CIs for the effects of wing formation and ant attendance, presented both separately for four classes of aphid size and reproductive metrics as well as for all classes combined. Effects of ant attendance are also presented separately for aphids that are obligately vs. facultatively tended. In each case, multiple effect sizes were first averaged to produce a single value for each aphid species. Numbers next to each 95% CI bar indicate final sample sizes (after averaging by aphid species). Effect sizes are significant if the 95% CI does not include zero, with a positive effect indicating a benefit to aphids and a negative effect indicating a cost to aphids.

(Fig. 6), with a mean LRR of  $-0.57$  (95% CI:  $-0.83, -0.30$ ), corresponding to a  $-37\%$  effect (95% CI:  $-51\%, -23\%$ ). There were significantly negative effects on three of the four individual size and reproductive metric classes, with a mean LRR of  $-0.28$  (95% CI:  $-0.52, -0.05$ ) and  $-23\%$  effect (95% CI:  $-39\%, -7\%$ ) on time to developmental benchmarks, a mean LRR of  $-0.79$  (95% CI:  $-1.12, -0.46$ ) and  $-50\%$  effect (95% CI:  $-66\%, -34\%$ ) on gonad size, embryo size, and embryo number, and a mean LRR of  $-0.25$  (95% CI:  $-0.46, -0.04$ ) and  $-21\%$  effect (95% CI:  $-38\%, -4\%$ ) on individual fecundity and population growth (Fig. 6). However, the effect of wing formation was not significant for adult body size, with a mean LRR of  $-0.29$  (95% CI:  $-0.69, +0.11$ ) and  $-21\%$  effect (95% CI:  $-47\%, 5\%$ ) (Fig. 6).

## Discussion

While attracting mutualist ants for protection and dispersing by producing winged forms have both been thought to impose direct costs for aphid size and reproduction, no previous study has assessed the existence and compared the magnitudes of such costs. In this study, ant attendance reduced *C. schwarzii* adult body size, but it did not influence embryo number or embryo size when variation in adult body size was accounted for. At the same time, ant presence had no effect on any size or reproductive metric of the untended congener *C. solitaria* (used as a comparison). In contrast, wing formation did not affect *C. schwarzii* adult body size but strongly reduced embryo number and embryo size, suggesting that wing formation is more costly than ant attendance for aphids in terms of reproduction. Although ant attendance increased the abundance but reduced the rate of wing formation of the tended aphid species *C. schwarzii*, ant attendance and wing formation acted independently on each

*C. schwarzii* size and reproductive metric. Overall, the results from this study corroborate a pattern in the published literature of strong negative effects of wing formation but not ant attendance on all metrics of aphid size and reproduction, except aphid body size. However, additional studies are needed to determine how such effects on individual aphids scale up to influence the fitness of the aphid clonal genotype as a whole.

Although ant attendance was correlated with reduced adult body size for *C. schwarzii*, ant attendance had no detectable effects on embryo number or embryo size, which have been demonstrated to be reliable correlates of lifetime fecundity (Wratten 1977, Tsuji and Kawada 1987, Newton and Dixon 1990). Several past studies have measured the effects of ant attendance on aphid size and reproduction, and although sample sizes were low for some classes of metrics, the overall effects of ant attendance were not significant. Nonetheless, these studies variously demonstrated costs, no detectable effects, and positive effects of ant attendance on aphids (Supp Table S2.1 [online only]; Fig. 6), with the latter demonstrating a benefit of ant attendance other than predator protection (e.g., Banks and Nixon 1958, Mooney and Agrawal 2008, Rice and Eubanks 2013). Such wide variation in responses is likely due to differences in one or more factors that may mediate ant effects, including host plant quality (Cushman 1991, Breton and Addicott 1992, Stadler et al. 2002, Mooney and Agrawal 2008), ant colony nutritional status (Offenberg 2001, Oliver et al. 2012, Petry et al. 2012), ant tending intensity (Yoo and Holway 2011), or whether aphids are facultatively or obligately ant tended. For example, we might expect that aphid species obligately associated with ants would evolve the means to minimize and tolerate any potential size or reproductive costs of ant attendance, while facultative species may bear comparatively greater costs. However, the current literature on this topic is too sparse to rigorously test this hypothesis, and the few studies to date show that the effects of ants are similar in magnitude between obligately and facultatively tended aphid species (Supp Table S2.1 [online only]; Fig. 6). Overall, these findings are consistent with the more broadly observed pattern of context dependence in mutualisms (Bronstein 1994).

While ant attendance was not associated with reduced *C. schwarzii* embryo number or size after controlling for the reduction in adult body size, a smaller body might still impose ecological costs, especially since adult body size was positively correlated with embryo number and embryo size. The fact that *C. schwarzii* adult body size was reduced under ant attendance, while *C. solitaria* (the untended comparison species) adult body size was not suggests that such effects were due to the effects of ant attendance, rather than simply the manipulation of ant presence on the plant. It is possible that if smaller aphids are more susceptible to natural enemies, investment in a large body size may trade off with attracting mutualist ants as opposing predator avoidance strategies, causing aphids to be smaller under ant attendance. *Cinara schwarzii* aphids could also have smaller body sizes under ant attendance if it causes them to accelerate their development time or invest more energy in producing honeydew containing ant-attractive constituents (Fischer and Shingleton 2001; Yao and Akimoto 2001, 2002; Vantaux et al. 2015). However, our meta-analysis did not detect any significant effects of ant attendance on aphid body size or development time metrics. Alternatively, aphids could have been larger without ant attendance if predators preferentially consumed smaller aphids and were more abundant on ant-free trees. Additional studies that exclude predators but not ants are needed to understand whether ants reduce *C. schwarzii* body size directly or indirectly, through changes in the effects of predators. Nonetheless, our meta-analysis

failed to find a significant effect of ant attendance on aphid adult body size (Fig. 6).

In contrast to ant attendance, wing formation carried significant costs for *C. schwarzii* in terms of reproduction; while wing formation had no detectable effects on body length, it was associated with large reductions in embryo number (46%) and size (8%). It is possible that wing formation is negatively correlated with these reproductive metrics due to some other underlying factor (e.g., aphids on poor quality host plants may be more likely to both produce winged morphs and have reduced fecundity), but these results match those from other studies conducted under more controlled lab conditions (e.g., Wratten 1977, Newton and Dixon 1990). Past studies measuring the effects of wing formation on aphid reproduction directly comparable to those measured here have documented similarly strong costs of wings (Supp Table S2.1 [online only]; Fig. 6). These results thus contribute to a large body of evidence for dispersal-fecundity trade-offs in insects (Roff and Fairbairn 1991, Bonte et al. 2012). Nonetheless, despite its direct physiological costs for individual aphids, wing formation likely often increases the fitness of the aphid clonal genotype as a whole by contributing to dispersal.

We had predicted that ant attendance and wing formation could have interactive effects on aphid size and reproduction or be negatively correlated as potentially redundant and costly forms of predator defense. Although ant attendance and wing formation acted independently on all size and reproductive metrics measured, winged *C. schwarzii* production increased with total aphid abundance but occurred at a reduced rate under ant attendance. Many past studies have similarly found aphid wing formation to increase with aphid abundance due to crowding or an associated increase in natural enemy abundance (Müller et al. 2001, Purandare et al. 2014) but to decrease with ant attendance, including in other *Cinara* species (El-Ziady and Kennedy 1956, Johnson 1959, Tilles and Wood 1982, Mondor et al. 2008, Tegelaar and Leimar 2014). However, it is still unclear whether and when this reduction in wing formation in response to ant attendance is beneficial or costly to aphids. While it is possible that ants reduce the need for aphids to disperse by protecting them against predators, ants may also actively limit aphid dispersal for their own benefit by chemically inhibiting wing production or physically removing wings from aphids (Oliver et al. 2007). Moreover, aphid genotypes that produce greater concentrations of ant-attractive sugars (e.g., melezitose) in their honeydew can have reduced rates of alate production even in the absence of ants, suggesting a physiological trade-off (Vantaux et al. 2015). Additional work is needed to determine whether the decline in the rate of winged aphid production imposes a net cost to aphid clonal genotypes by reducing their ability to spread on the landscape. Nonetheless, because ant attendance increases aphid colony size, it is also possible that ant-tended aphid colonies often produce an overall greater number of winged aphids (despite producing fewer winged aphids proportionally).

In summary, this study adds to an emerging pattern of direct costs of wing formation but not ant attendance for aphids. No significant effects on aphid size or reproduction were detected for ant attendance in this meta-analysis, although ant-tended *C. schwarzii* had reduced body sizes, and past studies are equally divided between showing costs and benefits of ant attendance for aphids. In contrast, this and all previous studies have demonstrated sizeable size and reproductive costs of wing formation for individual aphids. Notably, the published literature shows the effects of ant attendance to range from weakly negative effects to strongly positive effects as great in magnitude—but in the opposing direction—to those of wing formation (Supp Table S2.1 [online only]; Fig. 6). So while the effects of ant



attendance are highly context dependent, the potential for strongly positive effects is clear. Finally, the findings of consistent costs of wing formation but not ant attendance, as well as a reduction in wing formation under ant attendance, suggest that ants may have compounding positive effects on aphids by inhibiting winged aphid production and its associated costs. However, additional studies are needed to determine when, and in which contexts, the benefits of dispersal for the aphid clonal genotype as a whole outweigh the costs of wing production for the individual.

## Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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## Data Availability Statement

Data from this study are available from the Dryad Digital Repository: <https://doi.org/10.7280/D1M107> (Nelson and Mooney 2020).

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