Effects of plant sex on insect abundance across three trophic levels in the perennial shrub *Buddleja cordata*

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Abstract

Although there is a growing interest in the effects of intra-specific plant genetic variation on species interactions, the effects of plant sex, an important axis of genetic variation, have been less studied. In addition, previous work investigating plant sex effects on species interactions has frequently focused on bitrophic interactions (e.g., herbivory), usually ignoring plant sex effects on higher trophic levels (i.e., natural enemies). Here, we investigated the effects of plant sex on herbivore abundance and that of their natural enemies associated with the dioecious shrub *Buddleja cordata* Kunth (Scrophulariaceae). Furthermore, we measured a subset of plant traits frequently involved in herbivore resistance and the potentially underlying plant sex effects. To this end, we recorded the abundances of a specialist leaf-chewing caterpillar [*Acronyctodes mexicanaria* Walker (Lepidoptera: Geometridae)] throughout an entire growing season. We also recorded information about the caterpillar's parasitoids, as well as leaf water content, phenolic compounds, phosphorus, and nitrogen for male and female plants of *B. cordata*. Plant sex did not significantly influence caterpillar abundance but did have an effect on natural enemies, with parasitoid abundance being 2.4-fold greater on female than on male plants. The effect of plant sex on parasitoids remained significant after accounting for caterpillar abundance, suggesting that it was underlain by a trait-mediated (rather than density-mediated) mechanism. Finally, we found that male plants had a higher concentration of phenolic compounds (other traits did not differ between plant sexes). These results provide valuable evidence for the extended effects of plant sex on the third trophic level and point at plant traits potentially mediating such effects.

Introduction

Several studies over the last decade have shown that plant intra-specific genetic variation is a predominant force shaping arthropod communities (Hare, 2002; Hughes et al., 2008; Bailey et al., 2009; Mooney & Singer, 2012).

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Plant genotypes can harbour distinct herbivore communities that systematically differ in arthropod community structure, including differences in density, evenness, and species composition (Johnson & Agrawal, 2005; Wimp et al., 2007; Pratt et al., 2017). Likewise, evidence has mounted for the effects of plant genotypic variation on the outcome of plant–herbivore interactions (Fritz, 1995; Wimp et al., 2007; Abdala-Roberts et al., 2012; Abdala-Roberts & Mooney, 2013), as well as interactions at higher trophic levels, including herbivore–enemy (Gols et al., 2009; Singer et al., 2012; Abdala-Roberts & Mooney, 2013; Poelman & Dicke, 2014) and herbivore–mutualist

interactions (e.g., ant-aphid; Wimp & Whitham, 2001; Mooney & Agrawal, 2008; Abdala-Roberts et al., 2012).

Plant genetic effects on interactions at higher trophic levels can take place via various mechanistic pathways (Hare, 2002; Bailey et al., 2014). For example, plant genetic variation may directly influence the density of herbivores and, indirectly, influence predator and parasitoid abundance (so-called density-mediated indirect effects; Mooney & Singer, 2012). Under this scenario, the ratio of herbivore to natural enemy abundance remains unchanged among plant genotypes, via an 'interaction chain' (sensu Wootton, 1994). However, plant genetic variation may also alter, through changes in plant quality, either herbivore resistance to enemies, due to the sequestration of plant toxins (Singer et al., 2004; Boeckler et al., 2011; Mason et al., 2014) or herbivore susceptibility to enemies due to a reduced growth rate (Singer et al., 2012; Mooney et al., 2012a). This would lead to changes in the ratio of herbivore to natural enemy abundance (so-called trait-mediated indirect effects; Mooney & Singer, 2012). Likewise, plant genotypes may also vary in traits directly affecting natural enemies (attracting or repelling them), with altered enemy behaviour in turn affecting attack rates on herbivores (Gassmann & Hare, 2005; Tamiru et al., 2011; Mooney & Singer, 2012).

Plant sex is an important source of phenotypic variation in dioecious plants (Ågren et al., 1999; Cornelissen & Stiling, 2005) and is often genetically based (Ming et al., 2011). Several studies have shown that plant sexes vary phenotypically (Barrett & Hough, 2013), including differences in physical and chemical traits putatively associated with resistance to herbivores (Cornelissen & Stiling, 2005). Female plants are expected to invest more resources into reproduction, such that allocation trade-offs are expected to lead to decreased growth and in turn higher defences (and less herbivory) relative to male plants (Eckhart & Seger, 1999; Cepeda-Cornejo & Dirzo, 2010). These predictions have been tested empirically for several plant species, with evidence from recent work being non-supportive – for example, *Valeriana edulis* Nutt. ex Torr. & A Gray (Mooney et al., 2012b; Petry et al., 2013); *Baccharis salicifolia* (Ruiz & Pav.) Pers. (Abdala-Roberts et al., 2016; Nell et al., 2018) – relative to more consistent patterns reported for other species in earlier studies (see revision by Cornelissen & Stiling, 2005). In addition, only a handful of studies have also tested for the multitrophic effects of plant sex (e.g., Mooney et al., 2012b; Petry et al., 2013; Abdala-Roberts et al., 2016; Nell et al., 2018). This knowledge gap is important, as variation in natural enemy top-down pressure can help explain effects of plant sex on herbivores, as well as the community-level consequences of plant sexual dimorphism. Even less work has addressed the plant traits (e.g., defences) underlying plant sex effects on higher trophic levels (but see Nell et al., 2018).

In this study, we investigated the effects of plant sex on the abundance of a specialist leaf-chewing caterpillar, *Acronyctodes mexicanaria* Walker (Lepidoptera: Geometridae), and its parasitoids associated with the dioecious shrub *Buddleja cordata* Kunth (Scrophulariaceae). To this end, we recorded insect abundances throughout an entire growing season and additionally measured a suite of leaf structural (water content), chemical (phenolic compounds), and nutritional (phosphorus and nitrogen) traits potentially associated with effects of plant sex on these insects. Specifically, we sought to answer (1) are there differences between plant sexes in plant traits, and caterpillar and parasitoid abundance? And (2) are the effects of plant sex on parasitoids fully accounted for by differences in caterpillar abundance (i.e., density-mediated indirect effects), or are such plant sex effects on the third trophic level mediated by changes in species traits (e.g., plant sex-driven differences in herbivore resistance or susceptibility to enemies) leading to trait-mediated indirect effects? The answers to these questions may advance our understanding of the multitrophic consequences of plant sex on associated insect communities and provide a general sense for the mechanistic pathways underlying such effects.

Materials and methods

Study area
The study site was located at 'Pedregal de San Angel Ecological Preserve'. This preserve covers approximately 176 244 ha and is located at the main campus of the National University of Mexico, Mexico City (19°19′N, 99°11′W, 2 250 m above sea level). The climate at this site is temperate subhumid with a summer rainy season (mean annual temperature = 15.5 °C, annual precipitation = 870 mm). The vegetation of this field site is xerophytic scrub (Rzedowski, 1978) with a rocky substrate of volcanic origin (Carrillo, 1995).

Study system
Buddleja cordata is a perennial dioecious shrub or small tree (1.5–6 m tall) which is distributed from Mexico to Guatemala. It is commonly found along forest edges and watercourses at elevations of 1 500–3 000 m. At the field site, this species usually flowers from June to February and produces fruits from October to April (J Hernández-Cumplido, pers. obs.) and is heavily attacked by the specialist leaf chewer *A. mexicanaria* (García-García & Cano-Santana, 2015). Larvae of this species cause extensive leaf damage and are found on plants from late June (when trees start to produce flowers) to January (when trees are

producing fruits), with peak abundances occurring in September (García-García & Cano-Santana, 2015). This insect is multivoltine with at least two generations per year. The most common enemies of *A. mexicanaria* at the study site are gregarious parasitic wasps of the genus *Parapenteles* (Hymenoptera: Microgastrinidae), representing 87% of total parasitoid abundance recorded for *A. mexicanaria* during the study. Much less abundant were an unidentified solitary braconid parasitic wasp and an unidentified solitary tachinid fly, representing 8 and 5% of total parasitoid abundance, respectively (J Hernández-Cumplido, pers. obs.). All these parasitoids are koinobionts and attack second and third instars.

Measurements of herbivore and parasitoid abundance

In May 2017, we selected 102 female and 99 male trees similar in size (ca. 2 m tall). The sex of each individual was determined over the previous growing season, based on observations of fruiting. Distance among individuals was at least 3 m to avoid contact between trees. Twice a week, from mid-August to late December 2017, we manually collected all *A. mexicanaria* larvae found on each tree. Other herbivores were uncommon (J Hernández-Cumplido, pers. obs.). Once collected, we placed *A. mexicanaria* larvae in ventilated 500-ml plastic containers and incubated them under controlled laboratory conditions (10 °C minimum temperature at night and 25 °C maximum temperature during daytime, L14:D10, 45% r.h.). Larvae were reared on leaves from the same plant on which they were collected. We inspected each container daily to record parasitoid emergence. The total number of *A. mexicanaria* larvae collected per plant and the total number of parasitoids (species pooled) emerged from caterpillars of each plant were used for statistical analysis.

Quantification of plant traits

In September 2017, we collected five fully expanded (mature) leaves with no evidence of herbivore damage from seven randomly chosen trees of each sex to quantify phosphorus and nitrogen. We chose these elements because they represent proxies for leaf nutrient status and are typically found in low concentrations in plant tissues, and can therefore produce nutrient limitation in insect herbivores (Mattson, 1980). After collection, we oven-dried the leaf material for 48 h at 80 °C and ground the leaves with liquid nitrogen. To quantify nitrogen and phosphorus percentage in leaves, we digested approximately 0.3 g of ground dried leaf material in a mixture of selenous sulphuric acid and hydrogen peroxide (Moreira et al., 2018). We then used a colorimetric analysis of diluted aliquots (1:40, vol:vol) of the digestion to quantify nitrogen (indophenol blue method) and phosphorus (molybdenum

blue method) percentage using a Biorad 650 microplate reader (Bio-Rad Laboratories, Philadelphia, PA, USA) at 650 and 700 nm, respectively (Walinga et al., 1995).

Concurrently, we selected another set of 10 plants of each sex and collected two fully expanded (mature) leaves with no herbivory to estimate water content. This trait was chosen because low values of water content are known to result in decreased leaf nutritional quality and palatability for insect herbivores (Marquis et al., 2012). Immediately after leaf collection, we weighed fresh leaves and oven-dried the samples for 72 h at 80 °C until a constant weight was achieved. We then weighed the dry leaves and estimated the percentage of water content as [(fresh weight−dry weight) × 100/fresh weight].

Finally, we also selected another set of eight plants of each sex and collected two fully expanded (mature) leaves with no herbivory to estimate concentration of phenolic compounds. We chose phenolic compounds as defensive traits because they are widely recognized as herbivore feeding deterrents in many plant taxa (Salminen & Karonen, 2011; Mithöfer & Boland, 2012). After collection, we oven-dried the leaf material for 48 h at 40 °C and ground the leaves with liquid nitrogen. We extracted phenolic compounds using 0.5 g of dry plant tissue with 3 ml of 70% methanol in an ultrasonic bath for 15 min. We determined total phenolics colorimetrically by the Folin–Ciocalteu method in a Biorad 650 microplate reader at 740 nm, using gallic acid as standard (Moreira et al., 2014). We expressed concentrations of total phenolics in $\mu g \; g^{-1}$ tissue on a dry weight basis.

Statistical analysis

First, we tested the effects of plant sex (female vs. male, fixed) on herbivore and parasitoid abundance (mean number of individuals per plant). Second, to assess whether effects of plant sex on the third trophic level were density-mediated, we included herbivore abundance as a covariate in the model for parasitoid abundance. If any such effect of plant sex in the initial model became non-significant after including herbivore abundance, then this would indicate that the effect of plant sex on parasitoid abundance is density-mediated (i.e., driven by caterpillar abundance or resource quantity; Moreira & Mooney, 2013; Abdala-Roberts et al., 2016). In contrast, if any such effect of plant sex in the initial model remained significant, this would indicate that the effect of plant sex on parasitoid abundance cannot be explained (at least not largely) by differences in caterpillar density and therefore suggests a trait-mediated indirect effect (i.e., plant sex-mediated differences in herbivore resistance or susceptibility; Moreira & Mooney, 2013; Abdala-Roberts et al., 2016). For all analyses, we used generalized linear models with a Poisson

distribution and log-link function (Proc GLIMMIX in SAS v.9.4; SAS Institute, Cary, NC, USA) (Littell et al., 2006). Finally, we also tested the effects of plant sex (female vs. male, fixed) on leaf traits (nutrients, water, and phenolics) using linear models (Proc GLM in SAS v.9.4; Littell et al., 2006). All plant traits were normally distributed, and we report least square means as descriptive statistics.

Results

Effects of plant sex on insect abundance and caterpillar-parasitoid interactions

Plant sex did not significantly affect caterpillar abundance ($F_{1,199}$ = 3.16, P = 0.076; Figure 1A), but did influence parasitoid abundance ($F_{1,199}$ = 60.64, P<0.001; Figure 1B). Specifically, female plants exhibited a 2.4-fold greater abundance of parasitoids compared with male plants (Figure 1B). Follow-up analyses indicated that the effect of plant sex on parasitoid abundance remained significant after accounting for caterpillar abundance ($F_{1,198}$ = 177.22, P<0.001), suggesting a trait-mediated indirect effect of plant sex on these herbivory–natural enemy interactions.

Effects of plant sex on leaf traits

There was a significant effect of plant sex on the concentration of total leaf phenolics ($F_{1,14}$ = 20.82, P<0.001), with male plants exhibiting a 37% higher mean value than female plants (Figure 2D). In contrast, there were no effects whatsoever of plant sex on the percentage of leaf nitrogen ($F_{1,13}$ = 4.19, P = 0.06), phosphorus ($F_{1,13}$ = 0.03, P = 0.85), or water content ($F_{1,18}$ = 0.11, P = 0.74; Figure 2A–C).

Discussion

Counter to predictions that female plants should be highly defended and thus exhibit lower herbivore pressure than male plants (Ashman et al., 2004; Cornelissen & Stiling, 2005; Cepeda-Cornejo & Dirzo, 2010), we found that male *B. cordata* plants exhibited a higher concentration of phenolic compounds and there was no detectable difference in caterpillar abundance between plant sexes. In a previous study, Abdala-Roberts et al. (2016) similarly found no effect of plant sex on the abundance of a specialist aphid on *B. salicifolia*, and a subsequent study by Nell et al. (2018) with the same shrub reported no effect of plant sex on secondary metabolites (terpenes) or overall herbivore density. Nonetheless, Abdala-Roberts et al. (2016) reported a significantly greater abundance of another species of generalist aphid on male *B. salicifolia* plants than on female plants. Studies with the herb *V. edulis* have similarly reported inconsistent results, in one instance reporting an effect of plant sex on the abundance of a specialist aphid (greater on female plants; Petry et al., 2013), whereas in another no evidence of sexual dimorphism in aphid abundance was found (Mooney et al., 2012b). Taken together, these results suggest that plant sex-based differences in herbivore pressure might be contingent on herbivore traits, possibly dietary specialization. Although other insect herbivores are relatively rare on *B. cordata*, further work measuring effects of plant sex on other species of moderately abundant generalist insect herbivores would complement our present findings and provide a more complete assessment of effects of plant sex on the insect herbivore fauna associated with this shrub.

Despite the lack of plant sex-based differences in caterpillar abundance, we found a significant difference in parasitoid abundance between male and female plants of *B. cordata*. Females had substantially greater (2.4-fold) parasitoid abundance than males, consistent with previously cited studies reporting greater abundance of ants and other predatory arthropods on female plants in species such as *B. salicifolia* (Nell et al., 2018; but see Abdala-Roberts et al., 2016) and *V. edulis* (Mooney et al., 2012b; Petry et al., 2013). Female bias for higher natural enemy abundances
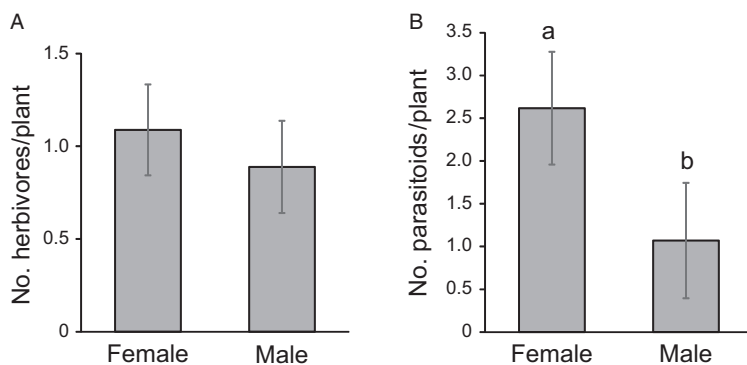
Figure 1 Effects of sex (male vs. female) of the shrub *Buddleja cordata* on the mean (± SEM; n = 99 males and 102 females) number per plant of (A) *Acronyctodes mexicanaria* leaf-chewing caterpillars and (B) parasitoids attacking this herbivore. Means capped with different letters are significantly different (ANOVA: P<0.05).
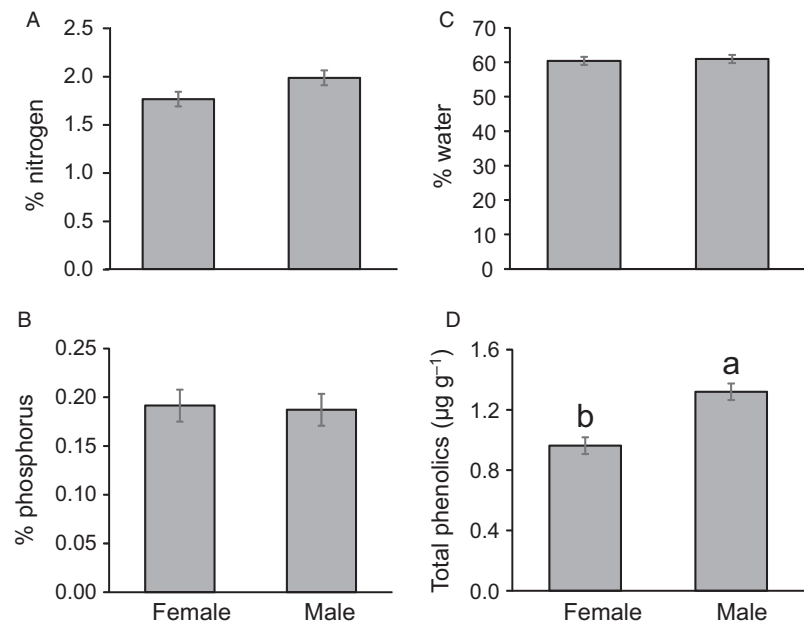
Figure 2 Effects of sex (male vs. female) of the shrub *Buddleja cordata* on the mean (± SEM) percentage of (A) nitrogen (n = 7 males and 7 females), (B) phosphorus (n = 7 males and 7 females), (C) water content (n = 10 males and 10 females), and (D) concentration of total phenolics (n = 8 males and 8 females) in leaves. Means capped with different letters are significantly different (ANOVA: P<0.05).

has been attributed, at least partly, to direct effects of female floral traits such as nectar (Petry et al., 2013; Nell et al., 2018), which is an important food source for omnivorous arthropods (Wäckers et al., 2005). Accordingly, *B. cordata* possess nectar-rich flowers which attract birds, bees, and wasps (J Hernández-Cumplido, pers. obs.), and their role in mediating multitrophic interactions warrants further attention.

The fact that parasitoid abundances differed between plant sexes despite having relatively similar herbivore abundances represents a first piece of evidence suggesting that effects of plant sex on the third trophic level were not explained by differences in caterpillar abundance (density-mediated indirect effect). Our follow-up analysis indicated that the effect of plant sex on parasitoid abundance remained significant after including caterpillar abundance, thus corroborating that the indirect effect of plant sex on parasitoids was not density-mediated, but rather occurred through some trait-mediated mechanism such as changes in herbivore susceptibility to enemies or differences between sexes in parasitoid attraction (Petry et al., 2013; Moreira & Mooney, 2013; Abdala-Roberts et al., 2016). To our knowledge, only two previous studies have assessed the mechanisms underlying plant sex effects on natural enemies (Petry et al., 2013; Abdala-Roberts et al., 2016). Contrary to our findings, however, both studies reported density-mediated indirect effects of plant sex on parasitoids (Abdala-Roberts et al., 2016) and ants (Petry et al., 2013), but no evidence of trait-mediated indirect effects. Therefore, whereas our results agree with such studies in that stronger effects of plant sex are found for natural enemies than herbivores, they disagree with respect to the mechanism underlying such effects of plant sex (presumably trait-mediated in our case). More studies are needed to assess the relative importance of these mechanisms in testing the multitrophic consequences of plant sex, as well as to identify ecologically relevant traits (in both plants and insects) explaining trait-mediated effects of plant sex on associated arthropods communities.

There are several non-exclusive explanations for the suggested trait-mediated indirect effect of *B. cordata* sex on parasitoid abundance. One possibility is that female floral traits (e.g., nectar, volatiles) directly influenced parasitoid recruitment (Kessler et al., 2011), resulting in increased parasitoid abundance over and above effects of caterpillar density alone. Another possibility is that higher concentrations of chemical defences (e.g., total phenolics) in males resulted in lower caterpillar quality, either through sequestration of plant compounds or reduced growth, and this then lowered parasitoid abundance (Singer et al., 2004). We ignore whether higher levels of phenolic compounds in male plants influenced the observed pattern by, for example, affecting caterpillar traits which resulted in reduced parasitoid attraction or performance. Other unmeasured physical (e.g., toughness) and chemical (e.g., terpenoids) defences may also explain plant sex effects on caterpillar–parasitoid interactions, and their effects may or may not act in the same manner. For example, unmeasured leaf defences could have instead exhibited lower values in male plants, which would favour faster caterpillar development and in turn explain reduced parasitoid recruitment on males (Moreira et al., 2015;

slow-growth/high-mortality hypothesis; Williams, 1999). Further work involving controlled measurements of herbivore traits on male and female plants (e.g., developmental time, growth), including sequestration of plant compounds, as well as measurements of other plant defensive compounds and floral traits, is necessary to assess the mechanisms driving the effect of *B. cordata* sex on parasitoids and on caterpillar–parasitoid interactions.

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