

SHORT COMMUNICATION

Consumption of lipid-rich seed arils improves larval development in a Neotropical primarily carnivorous ant, *Odontomachus chelifer* (Ponerinae)

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Abstract: *Odontomachus chelifer* ants collect fallen arillate seeds of *Cabralea canjerana* (Meliaceae), a bird-dispersed tree of the Atlantic rain forest. In the nest the larvae are fed with the lipid-rich aril, and the viable seed is discarded. Benefits from secondary seed dispersal by ants are well documented for tropical plants, but benefits to ants from consuming vertebrate-dispersed diaspores are uncertain. Twelve captive colonies of *O. chelifer* were used to investigate the effect of aril consumption on larval development. Treatment colonies were supplemented with 1 g of *C. canjerana* arils, whereas control colonies were supplemented with 1 g of synthetic diet. Egg and larval production did not differ between experimental colonies after 5 mo. Aril-fed larvae, however, grew 3.5 times larger than those in control colonies. Essential fatty acids in the arils possibly account for improved larval development. Consumption of lipid-rich arils may be critical under scarcity of arthropod prey. Improved larval development through aril consumption confirms that this ant-seed interaction is facultatively mutualistic. This result is meaningful given the predominantly carnivorous diet of *O. chelifer*, and the generalized nature of ant-seed/fruit interactions in tropical forests. Whether or not benefits to larvae translate into significant gains for ant colonies is uncertain.

Key Words: ant-plant interaction, Atlantic rain forest, *Cabralea canjerana*, Formicidae, frugivory, Meliaceae, mutualism, seed dispersal

Mutualism is defined as an association between organisms of two species in which both species benefit by a fitness increase (Boucher *et al.* 1982). Most studies on mutualistic associations, however, have not demonstrated mutual benefits between participant species (Bronstein 1994). Although studies of seed dispersal of true myrmecochores (i.e. plants adapted for dispersal by ants) suggest reciprocal benefits to dispersing ants, only a few investigated the positive effects of the ant-seed interaction for ant colonies. For instance, colonies of *Aphaenogaster rudis* (Myrmicinae) fed with elaiosomes (lipid-rich seed appendage) produced higher numbers of reproductive females than colonies reared without this food resource (Morales & Heithaus 1998).

Unspecialized interactions between ants and fallen fleshy seeds/fruits of non-myrmecochorous plants (i.e. not adapted for dispersal by ants) can involve dozens of plant and ant species on the ground in tropical forests. Although primarily carnivorous, ants in the subfamily Ponerinae commonly search for nutritious seed arils and fruit pulp as secondary food sources on the forest floor, and may positively affect seed germination and seedling recruitment (Christianini & Oliveira 2010, Fourcassié & Oliveira 2002, Passos & Oliveira 2002).

In the Brazilian Atlantic rain forest, the ponerine ant *Odontomachus chelifer* (Latreille) acts as secondary disperser of the lipid-rich arillate seeds of *Cabralea canjerana* (Meliaceae) (Pizo & Oliveira 1998; Figure 1a). The seed aril of *C. canjerana* has one of the highest lipid concentrations (70.8% of dry mass) yet reported (Jordano 1993). The tree is primarily dispersed by birds, which drop hundreds of intact or partially eaten seeds below parent plants where seed predation is severe (Pizo 1997). Large *O. chelifer* foragers (size \approx 1.8 cm) carry the seeds

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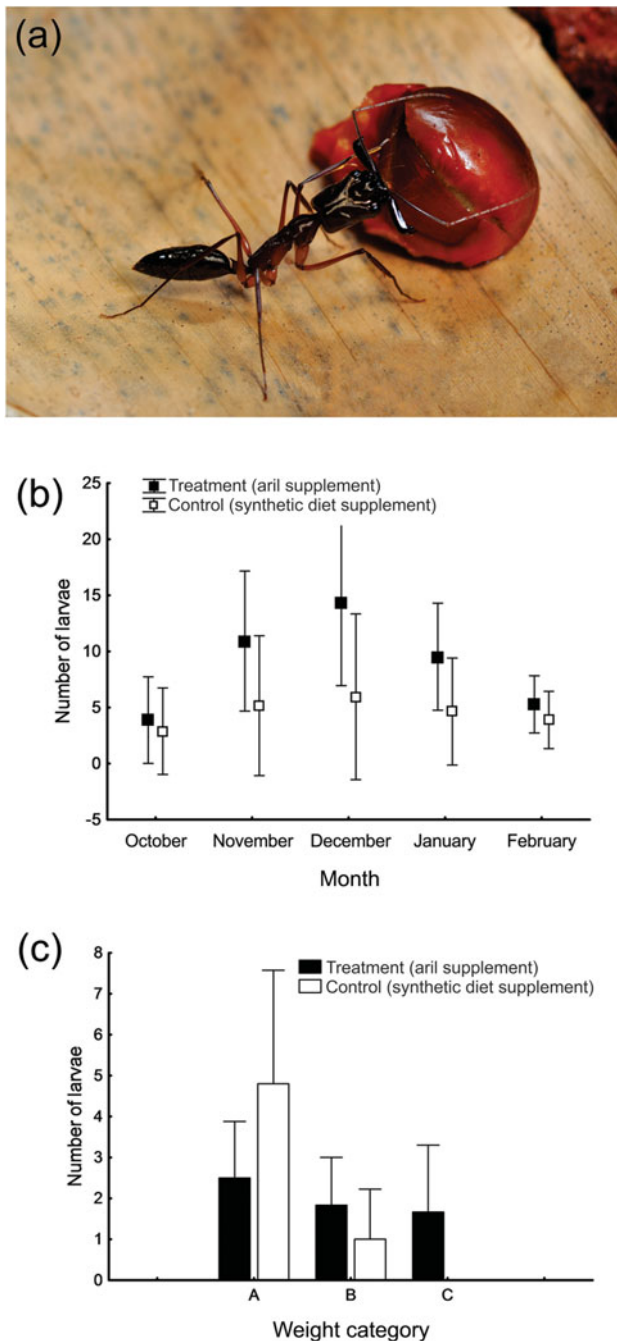


Figure 1. Interaction between *Odontomachus chelifer* and arillate seeds of *Cabralea canjerana*. Worker inspecting a fallen seed on the leaf litter; the red lipid-rich aril eventually will be removed and fed to larvae in the nest (photograph by H. Soares Jr.). (a). Mean number of larvae through time (October 2009–February 2010) in ant colonies treated with a food supplementation of 1 g of seed arils, and in control colonies supplemented with 1 g of synthetic ant diet; aril supplementation did not affect total number of larvae produced ($F_{(1)} = 2.61$, $P = 0.137$); diet treatment \times time was not significant ($F_{(4,1)} = 1.90$, $P = 0.130$) ($N = 6$ colonies per group; whiskers represent 95% confidence intervals) (b). Mean number of larvae per weight category in experimental colonies after 5 mo of diet supplementation; larval size categories range 7.52 mg: A = 0.06–7.58 mg; B = 7.59–15.1 mg; C = 15.1–22.6 mg ($F_{(2,1)} = 4.85$, $P = 0.016$; whiskers represent 95% confidence intervals) (c).

away from this predation-prone zone and in the nest feed the aril to larvae. Aril removal by *O. chelifer* greatly facilitates seed germination in *C. canjerana* (Pizo & Oliveira 1998). Although *O. chelifer* is predominantly carnivorous (Raimundo *et al.* 2009), foragers collect fleshy diaspores of several plant species on the ground (Passos & Oliveira 2003). Nonetheless, the potential benefits of feeding on lipid-rich arils are uncertain for *O. chelifer*.

Gammans *et al.* (2005) demonstrated that consumption of lipid-rich elaiosomes of true myrmecochores (*Ulex* spp., Fabaceae) substantially increased larval weight in colonies of *Myrmica ruginodis* (Myrmicinae). The degree to which a similar benefit could be conferred to larvae in unspecialized ant-seed interactions is unknown. Here, we hypothesize that consumption of lipid-rich arils of *C. canjerana* by primarily carnivorous *O. chelifer* will improve larval development, as shown for specialized granivorous ants.

The effect of seed arils on larval development was investigated using captive colonies of *O. chelifer* reared under controlled diet protocols. In July 2009, 12 ant colonies were collected in the rain forest on sandy plains at the Parque Estadual da Ilha do Cardoso (hereafter PEIC), a 22 500-ha island located off the coast of south-east Brazil (25°03'S, 47°53'W; Passos & Oliveira 2003). Colonies contained one queen each, 27–200 workers, and plenty of brood (eggs and larvae). The ants were cultured in the laboratory at 25 °C, and housed in artificial nests consisting of four test tubes (2.2 cm diameter \times 15 cm length) containing water trapped behind a cotton plug. Nest tubes were placed in a box (30 \times 20 \times 8 cm) where the ants foraged for food.

Since colony size (i.e. number of workers) can significantly affect several components of colony social structure (Morales & Heithaus 1998), in August 2009 we standardized the size of the captive *O. chelifer* colonies at 27 workers and one queen (all immatures removed). The colonies were randomly assigned to either treatment or control groups ($N = 6$ for each), both of which were fed every 2 d with *Tenebrio* beetle larvae and synthetic ant diet (Hölldobler & Wilson 1990), totalling 1 g of food. Colonies in the treatment group had their diet supplemented with 1 g of aril from *C. canjerana* seeds, whereas colonies in the control group were supplemented with 1 g of synthetic diet. Experimental colonies were monitored regularly for 5 mo, from October 2009 to February 2010, which corresponds with the fruiting period of *Cabralea canjerana* (Pizo 1997). The number of eggs and larvae was counted weekly for every colony in each experimental group. After 5 mo the larvae were kept frozen in separate vials and were then weighed on a precision Cahn C-30 Microbalance. The number of eggs produced in treatment and control colonies was compared for effects of seed aril consumption using one-way ANOVA. The change in number of larvae through time in experimental colonies was compared

using repeated-measures ANOVA. Larval weight through developmental categories was analysed using a factorial ANOVA. Due to the short duration of the experiment, production of pupae and workers by ant colonies was too small for consideration in the analyses.

Egg production did not differ between experimental colonies ($F_{(1)} = 0.17$, $P = 0.69$). Production of larvae through time was similar in both experimental groups, increasing in November and December (Figure 1b). After 5 mo of diet treatment, however, larval weight in *O. chelififer* colonies supplemented with seed arils was 3.5 times higher than in those supplemented with synthetic diet (mean \pm SD; 7.49 ± 4.65 vs 2.15 ± 1.82) ($F_{(1)} = 5.77$, $P = 0.04$). In addition, colonies feeding on seed arils presented increased numbers of large larvae through time than those receiving control diet (Figure 1c).

Results showed that food supplementation with lipid-rich arils of bird-dispersed *Cabrlea canjerana* positively affected larval development in *Odontomachus chelififer*. Using sticky traps to evaluate overall availability of arthropod prey at our study site (PEIC), Cogni & Oliveira (2004) detected a decreased quantity of arthropods during the dry/cold period (July), which makes the consumption of lipid-rich arils especially valuable for *O. chelififer* colonies by the end of the wet/warm period (February) when there are still plenty of *C. canjerana* seeds on the forest floor (Bottcher 2010). Moreover, predation experiments carried out in Atlantic forest sites indicate that despite being primarily carnivorous, *O. chelififer* increases consumption of nutritious fleshy diaspores in areas with a low supply of preferred litter arthropod prey, as reported for the sandy forest at PEIC (Pizo *et al.* 2005, see also Clark & King 2012). The importance of lipids as mediators of ant-diaspore interactions has already been highlighted for a variety of ants, which respond with a stereotyped carrying behaviour upon contact with a small suite of oleyl-containing compounds present in seed elaiosomes of myrmecochores (Beattie 1985). The aril of *C. canjerana* seeds has a marked predominance of oleic and linoleic fatty acids (Pizo & Oliveira 2001), making them chemically analogous to insect prey (Hughes *et al.* 1994) and thus especially attractive to carnivorous ponerines such as *Odontomachus* and *Pachycondyla* species (Passos & Oliveira 2002, Pizo & Oliveira 1998).

Essential fatty acids are nutritional requirements for ants and are important for growth and reproduction (Bono & Heithaus 2002). Improved larval development in *O. chelififer* colonies fed with seed arils suggests that this food supplement increased the quality of the diet compared with colonies fed with a controlled food intake. The net benefit of lipid-rich arils and elaiosomes to ants likely derives from the easy way with which foragers can carry and store them in the nest, associated with their importance as nutrient-rich larval supplements (Fokuhl *et al.* 2012, Gammans *et al.* 2005). A qualitative dietary

enrichment via *C. canjerana* arils is supported by the fact that treatment and control colonies of *O. chelififer* received an equal weight (1 g) of supplemented food. In fact, not all the food was eaten each time the ants were fed, suggesting that the colonies in either experimental group had more than needed.

Despite the dominance of ants in many apparent seed dispersal mutualisms with plants (Beattie 1985, Rico-Gray & Oliveira 2007), demonstration of plant-derived benefits to ants is still rare and studies focus mostly on mutual effects from specialized elaiosome-bearing myrmecochores on temperate omnivorous ants (Bono & Heithaus 2002, Fokuhl *et al.* 2012, Gammans *et al.* 2005, Morales & Heithaus 1998). Although *C. canjerana* is primarily dispersed by birds (Pizo 1997), secondary seed dispersal followed by aril removal by *O. chelififer* can benefit seeds through displacement from a predation-prone zone below parent trees, and enhanced germination (Pizo & Oliveira 1998). Through improved larval development, our study unequivocally demonstrated a reciprocal benefit to *O. chelififer* due to consumption of lipid-rich seed arils of *C. canjerana*, thus confirming the mutualistic nature of this interaction. This result is meaningful in view of the predominantly carnivorous diet of ground-dwelling *O. chelififer* (Raimundo *et al.* 2009), and the generalized nature of the interactions between ants and fallen fleshy diaspores in tropical forests (reviewed by Rico-Gray & Oliveira 2007). Measuring the fitness of ant colonies requires an assessment of the production of reproductive castes (Hölldobler & Wilson 1990), and whether or not benefits to larval development translate into significant colony gains to *O. chelififer* is an open question awaiting further investigation.

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