Aphaenogaster senilis Mayr (Hymenoptera, Formicidae): a Possible Parasite in the Myrmecochory of Euphorbia characias (Euphorbiaceae)

by

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ABSTRACT

The response of Aphaenogaster senilis workers to the seeds of the myrmecochorous Euphorbia characias shows a remarkable degree of variation. Two factors influencing this response are: 1) worker age: young workers, still performing nest cleaning activities, may transport the seed and discard it intact far from the nest; older workers may ignore the seed or take it to the nest; 2) seed location in relation to the nest entrance and the external midden: seeds fallen between the nest entrance and midden may be carried into the nest or taken outside the midden; seeds found outside of the refuse pile are ignored or transported to the nest. Seeds transported to the nest are deprived of the elaiosome and discarded in the refuse heap or farther away within a short time. Those remaining amid the debris may germinate but do not establish. Since seeds that are discarded far from the midden are attractive nearly only to granivorous ants. Aphaenogaster senilis may act as a parasite of the diffuse mutualism between ants and a myrmecochorous plant.

Keywords: ant, Aphaenogaster, Euphorbia, insect-plant interaction, interference, myrmecochory, seed dispersal, worker age.

INTRODUCTION

Myrmecochory, the dispersal of seeds by ants, is not a coevolved system: it does not appear as a specific relation of a single ant species and a single plant species. The reverse is the rule: several ant species are usually involved in the dispersal of a plant, and one ant species may disperse seeds of several species (Sernander 1906). The same rule applies locally: in a given area, several ant species establish a myrmecochorous relationship with seeds of several plants (Beattie 1985). It is likely that ant species differ in the benefits they provide

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plants.

Previous observations have shown a variable reaction of *Aphaenogaster senilis* Mayr to the seeds of *Euphorbia characias*: upon finding a seed, workers ignore it half the time and take it to the nest on the other half (Espadaler & Gómez 1997). This variable response of individual ants to elaisome-bearing seeds is well known (Beattie 1985), though the reason(s) for such varibility have not been investigated. In addition, preliminary observations reveal that some workers of *Aphaenogaster senilis* show an active refusal of elaisome-bearing seeds by transporting them far from the nest upon encountering them (Espadaler & Gómez 1996). Those workers discard the seeds at a short distance—up to 1 m—from the nest, leaving them directly available to other ants. Here we show that the unusual response—discarding of intact seeds—in *A. senilis* is related to the age of individual ants. We also studied the destiny of the diaspores when taken to the nest and provide evidence that shows that *A. senilis* (*Aphaenogaster* hereafter) may act as a detrimental agent in the myrmecochorous relationship of *E. characias* (*Euphorbia* hereafter) with other ants.

**MATERIALS AND METHODS**

Observations were made in a field abandoned in 1981 and occupied by an herbaceous community (*Inula-Oryzopsis* *miliaceae*, Boló, 1962) at Can Llavlópol in the Collserola Park, near Barcelona (41°25' N, 2°6' E, elevation 315 m). Climate is Mediterranean, with 620 mm of annual rainfall. The old field is bordered by a mixed wood of *Pinus pinea* L., *Pinus halepensis* Mill and *Quercus ilex* L. The study site has a high density (4.4 nests/100 m²) of *Aphaenogaster* nests (Gómez & Espadaler 1997). Other ant species gather seeds of *Euphorbia* to their nest and discard them in chambers or galleries (*Pheidole pallidula*, *Tapinoma nigerrimum*) or eat them (*Messor barbarus*, *M. bouvier*). Following ballistic dispersal, seeds are arranged singly on the ground and there is no seed bank (Espadaler & Gómez 1996). All mature shoots of *Euphorbia* were cut before the dehiscence period to ascertain that no seeds could be retrieved by the ants before field observations and interfere with our results. Seeds used were obtained by allowing mature shoots to desiccate in the laboratory.

**Worker age**

The age was estimated by the ovary condition, a widely used marker. ovaries in workers typically pass through a series of different developmental stages, with callow workers having fully developed ovaries and laying eggs; as they age, and when all possible ovules have been laid,
worker's ovaries become non functional, showing one or more yellow bodies, the remains of trophic chambers that provided food for the maturing oocytes (Hölldobler & Wilson, 1990). It is known that in orphaned colonies of *Aphaenogaster* some workers develop the ovary to an egg laying condition (Ledoux 1975) and become physogastric (gaster length 2.1 mm, Ledoux & Dargagnon 1973; non-laying worker gaster length 1.5 mm). This could obscure the correlation age-ovary development. We checked visually that workers to be dissected were not physogastric. Workers performing three different activities were sampled: a) foraging workers, usually with items in their mandibles, returning in a straight path to their nest, b) workers laden with debris, exiting from the nest and c) workers seen taking a seed of *Euphorbia* and transporting it far from the nest. Dissections were done in Ringer solution, and ovary development was scored according to the following convention: 1. Ovaries well developed, with 6-7 ovules with their trophic chambers; no yellow bodies present. 2. Ovaries shorter, with 2-5 ovules; one or two yellow bodies. 3. Ovaries regressed, reduced to two short and transparent tubes, rarely with a single ovule; with two yellow bodies. From laboratory nests, darkened nymphs and workers recently eclosed -still not deep black in colour- were dissected to fix the initial level of ovary development.

**Seed location**

Nests of *Aphaenogaster* have a highly visible single entrance, and a more or less developed semicircular refuse heap near the entrance. Limits are not sharp but there is a clean zone (up to 5 cm) between the midden and the entrance. Since age is known to influence ant behaviour, and it is expected that younger workers (Innendienstiere) do not engage in foraging activities outside the nest limits, including midden, we tested whether seed location has any influence on the response of workers upon finding the seed. Seeds were deposited on top of soil, each seed 1 cm apart from its neighbour: a) between the midden and the entrance (n=96) and b) 3-5 cm out from the midden (n=73). Three nests were used for these observations, and the number of seeds during the tests were fewer than ten at any given moment for each situation. Data for the three nest were not found to be statistically different and are considered together. Given the precise location of the seeds, it was possible to follow how many were taken into the nest or discarded outside the nest.

**Short term seed fate**

A seed of a myrmecochore that has been taken to a nest may follow different routes: it can be eaten -if the ant is granivorous-, deprived of
its elaiosome and left in inside chambers or galleries or deposited outside the nest, in the midden, or at some distance from it. To know what happens to *Euphorbia* seeds we followed the method of Hughes & Westoby (1992): a circular plastic collar of 35 cm diameter and 15 cm height was inserted in the soil, centred on a nest that had previously received 50 seeds. The interior side of the cylinder was coated with Rata-Stop (a sticky substance with the same effect as Tanglefoot) to prevent any ant eventually transporting a seed outside the nest area and to prevent ants from other colonies removing any discarded seeds. Nests were offered 50 seeds early in the morning and were observed until all seeds had been taken into the nest. The collared nests were inspected 12 h (Can Llavallol) or 24 h (Sant Cugat) after. We tested three nests at the study site and six nests at a nearby locality (Sant Cugat del Vallès, 4.5 km). Two non-collared nests were offered 50 seeds and excavated three days later to ascertain the presence of seeds in deep galleries. In addition, refuse material from eight middens of other nests was collected and inspected in search of seed remains.

**Seedling emergence and establishment**

*Euphorbia* seeds discarded by *Aphaenogaster* after elaiosome removal are deposited in the midden or outside, at some distance, and are, thus, available to other seed gatherers (see Results); in our site we have shown that seeds without elaiosome are taken nearly exclusively by the granivorous *Messor barbarus* (Espadaler & Gómez 1997) because other ant species do not respond at all (*Tapinoma nigerrimum*) or in a very low proportion (*Aphaenogaster, Pheidole pallidula*) to seeds without an elaiosome. Since any *Euphorbia* seed is retrieved in less than an hour (Espadaler & Gómez 1996), we assume that no seeds without elaiosome escape ants if deposited on the soil surface. From the seeds taken by *Aphaenogaster* only those that become buried in the midden of *Aphaenogaster* have a possibility to germinate. To ascertain whether seeds that become buried in the midden can germinate and establish, we planted seeds on middens and followed survival by bi-weekly counts after germination occurred. Forty seeds with the elaiosome artificially removed were inserted with forceps spaced 1 cm in a midden; depth was variable—from 1 to 3 cm— in direct proportion to the thickness of the refuse pile. Ten middens were subjected to this treatment. In some cases the removal of elaiosome affects germination (Lobstein & Rockwood 1993; Lisci *et al.* 1996). We have previously shown that seeds with elaiosome removed by ants have visible scratches on the seed cuticula and reach a 82.7% germination but intact seeds with elaiosome have a significantly lower 63.9% germination (Gómez & Espadaler 1998).
Seedling emergence was monitored in October, after the first autumn rains. Total precipitation was 273.5 mm (September 1994) and 156.7 mm (October 1994).

RESULTS

Worker age

The nymphs and undarkened workers have their ovaries in stage 1, with ovules, trophic chambers and no yellow bodies. We assume, thus, the age-ovary development relationship. The three groups of workers show different degrees of ovary development (Table 1: G test = 39.4; 4 d.f., p< 0.01). Foraging workers (group A), as expected, have ovaries usually in an advanced stage, indicating an older age. However, both workers with debris (group B) and workers that took intact seeds out of the nest (group C) have ovaries in an earlier state of development, suggestive of a younger age; these two last groups did not differ in their ovary development (G test = 4.3; 2 d.f. p = 0.11).

Seed location

The precise location of seeds in relation to the nest entrance has a strong influence on the final site where seeds are taken (Table 2). All 73 seeds offered outside of the midden were transported inside the nest. In contrast, twelve of 96 seeds deposited between the midden and the nest entrance were taken and left untouched at some distance outside the refuse pile, presumably by non-foraging, young workers; the other 84 seeds were transported to the nest (G test = 14.2; p<0.01). It is likely that worker age is the actual factor underlying this different destiny of

Table 1. Number of workers (percentage) with varying degree of ovary development in *Aphaenogaster sanilis* workers performing three distinct activities. A: foraging, far from a nest. B: exiting the nest with debris and depositing it in the midden. C: transporting a seed of *Euphorbia characias* with its elaisome out of the nest, to the midden or farther away. Scale of ovary development: 1. Ovaries well developed, with 6-7 ovules with trophic chambers; no yellow bodies present. 2. Ovaries shorter, with 2-5 ovules; one or two yellow bodies. 3. Ovaries reduced to two short and transparent tubes, rarely with a single ovule; with two yellow bodies. Ovary development is different in the three groups (G test = 39.4; 4 d.f., p<0.01) but groups B and C are not distinct (G test = 4.3; 2 d.f., p=0.11).

<table>
<thead>
<tr>
<th>Worker group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
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<tr>
<td>A</td>
<td>0</td>
<td>15</td>
<td>36</td>
<td>51</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>29</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>26</td>
<td>9</td>
<td>41</td>
</tr>
</tbody>
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Table 2. Fate of seeds of *Euphorbia* deposited between the midden and the nest entrance (Midden) or deposited 3-5 cm outside of the midden (Outside) of *Aphaenogaster*. Seeds were retrieved by workers to the nest (To nest) or transported outside the nest and deposited up to 1 m away (Far from nest). The initial location strongly influences the short term destiny of a seed (G test = 14.2; p<0.01).

<table>
<thead>
<tr>
<th></th>
<th>To nest</th>
<th>Far from nest</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midden</td>
<td>84</td>
<td>12</td>
<td>96</td>
</tr>
<tr>
<td>Outside</td>
<td>73</td>
<td>0</td>
<td>73</td>
</tr>
</tbody>
</table>

seeds depending on its location.

**Short term seed fate**

The seeds that have been transported to a nest are manipulated inside the galleries, deprived of the elaiosome and discarded out of the nest, in the midden or farther away. The process is rapid, as a mean ± s.d. of 41.3 ± 11.6 seeds (range 20-50) had been taken out of the nest in 24 h. The minimum number of 20 discarded seeds corresponds to a nest that begun to split during the observation period.

No seeds were found during the excavation of the two nests that had received 50 seeds three days before. The analysis of the eight middens showed the presence of some seeds (1 ± 0.5, mean ± s.e), deprived of the elaiosome, but otherwise intact. Depth of seeds was shallow (1-2 cm) since the middens are rather superficial.

**Emergence and establishment**

Seeds germinated after the first autumn rains. From 400 seeds, a total of ten seedlings (2.5 %) emerged from five of the ten nests (1, 1, 2, 3 and 3 seedlings respectively). Survivorship of these seedlings was: 10 (06.10.1994), 7 (21.10.1994), 4 (09.11.1994), 2 (23.11.1994), 2 (07.12.1994). None remained on 17.02.1995.

DISCUSSION

Aging influences the behaviour of ants and the spatial distribution of workers (Billen 1984; Minkenberg & Petit 1985, Hölldobler & Carlin 1989). Since most foraging workers are old individuals in most ant species, actually seeds might be gathered by them and young workers have little chance of encountering the seeds outside the nest. However, for seeds transported already into a nest or in the small zone between nest entrance and the midden, the difference of response may be effected by age. We have shown that young workers of *Aphaenogaster*, still performing nest cleaning activities, respond to seeds of *Euphorbia*
by treating them not as food but as refuse material, perhaps functionally a corpse carrying behaviour. An active refusal of seeds has been also observed in A. senilis when encountering seeds of Euphorbia nicaeensis in Girona (NE Spain) or seeds of Euphorbia biumbellata in Turó d’en Cors, near the city of Barcelona (unpub. observ.). This unexpected response is in agreement with similar observations by Berg (1975; ant species not specified) in which diaspores of several plant species were occasionally seen to be taken out of the nest and deposited on a refuse heap. O’Dowd & Hay (1980) mention nonforagers of Pogonomyrmex californicus moving the diaspores of Datura encountered on the midden further from the nest entrance and discarding them without removing the elaiosome. Similarly, Oostermeijer (1989) found Lasius niger workers to either ignore, take to the nest or to the garbage heap the seeds of Luzula, Polygala and Viola. Smith (1989) describes how some seeds of Viola were dropped at the edge of the nest mound by Iridomyrmex purpureus and then they were picked up again and carried further away. In this last case, however, the seeds had only partial, shrunk elaiosomes still attached to them. It would be interesting to check the age of ants performing the reverse transport to ascertain if the same mechanism applies in those cases. The overall effect of those activities is to put the seeds again at the disposal of any predator or secondary dispersal agent. Thus, they generate a mere reubication and delay in the final destiny of a seed.

A rather different situation is produced when seeds are deprived of their elaiosomes; as shown here, Aphaenogaster senilis discards seeds in the midden or far from the nest. This refusal of seeds without elaiosome has also been described in laboratory conditions by Handel (1976; Aphaenogaster rudis), Horvitz & Beattie (1980; Pachycondyla harpax), Horvitz (1981; Pachycondyla spp., Odontomachus laticeps, Solenopsis geminata) or in field observations by Kjellsson (1985; Myrmica ruginodis) and Hughes & Westoby (1992 (Aphaenogaster longiceps, Rhytidoponera “metallica”), Pogonomyrmex californicus ejected a majority of Datura seeds previously brought into the nest within 24 h (O’Dowd & Hay 1980). In this situation, a seed is directly exposed to predation since it lacks the attractor for a dispersing ant. In effect, granivorous ants at our site (Messor barbarus) do not respond differently to seeds with or without the elaiosome -they take similarly both types of seeds- but a seed of Euphorbia without its elaiosome is much less attractive to other ants: it has a seven-fold decrease in probability of being taken to the nest if found by a non-granivorous ant (Espadaler & Gómez 1997).

As we have shown, seeds buried in the midden of Aphaenogaster
*serilis* eventually germinate in a small proportion but do not establish. This route seems to be a dead end for seeds. In addition, a small level of seed robbing by *Aphaenogaster* of the seeds taken by *Pheidole* or *Tapinoma* adds to the negative effect of this ant on *Euphorbia* (Espadaler et al. 1996). *Pheidole pallidula*, a partially granivorous ant, does not consume *Euphorbia* seeds (Gómez & Espadaler 1995). In short, *Aphaenogaster*, by depriving *Euphorbia* seeds of the elaiosome and putting them at disposal of predators is short-circuiting the myrmecochorous relationship with *Tapinoma* or *Pheidole*. This ant may be acting as a parasite in the sense suggested by Davidson & Morton (1981) for the effect of a species from the *Iridomyrmex purpureus* complex on the dispersal of some chenopods or by O’Dowd & Gill (1986) for some small *Monomorium* (also misidentified as *Mayriella*; Andersen, pers. com.) that eat the elaiosome of some *Acacia* in situ. Bond and Slingsby (1984; *Linepithema humile* on Proteaceae) and Mossop (1989; *Meranoplus, Crematogaster, Iridomyrmex* on *Acacia* and *Bossiaea*) offer similar examples of this interference. A more extreme example of an ant-plant relation in which *Pseudomyrmex nigropilosa* -a non-protecting ant- acts as a parasite of the ant-acacia mutualism is described by Janzen (1975).

The wide natural variation at temporal and spatial scales of ant responses to myrmecochorous seeds (Beattie 1985 and refs. therein) and the examples of interference leading to seeds without elaiosome that are subsequently exposed to predation (present work, Ohara & Higashi 1987; Ohkawara et al. 1996) may be construed as an impediment to the establishment of coevolved species. There should be strong selection to avoid those ant species and to favour any of the mutualists but it is difficult to imagine how a plant could select for “good” ants and escape “bad” ones. Timing of seed release may promote removal by beneficial species (Turnbull & Culver 1983) but this cannot be effective in our system since seeds of *Euphorbia* are scattered all along the day (Espadaler & Gómez 1996). A diffuse and facultative mutualism, such as the one proposed for the relationship between fruits and their dispersers (Herrera, 1985), in which selection favors the evolution of broad assemblages of ant species, that exploit occurring opportunities and plants that are served haphazardously by ants, explains much better the relationship of ants and myrmecochorous plants.

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