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Opening myrmecochory's black box: what happens inside the ant nest?

Abstract In the process of seed dispersal by ants (myrmecochory), foragers bring diaspores back to their nest, then eat the elaiosome and usually reject viable seeds outside the nest. Here, we investigate what happens inside the nest, a barely known stage of the myrmecochory process, for two seed species (*Viola odorata*, *Chelidonium majus*) dispersed either by the insectivorous ant *Myrmica rubra* or by the aphid-tending ant *Lasius niger*. Globally, elaiosome detachment decreased ants' interest towards seeds and increased their probability of rejecting them. However, we found marked differences in seed management by ants inside the nest. The dynamics of elaiosome detachment were ant- and plant-specific whereas the dynamic of seed rejection were mainly ant-specific. Seeds remained for a shorter period of time inside the nest of the carnivorous ant *Myrmica rubra* than in *Lasius niger* nest. Thus, elaiosome detachment and seed rejection were two competing dynamics whose relative efficiency leads to variable outcomes in terms of types of dispersed items and of nutrient benefit to the ants. This is why some seeds remained inside the nest even without an elaiosome, and conversely, some seeds were rejected with an elaiosome still attached. Fresh seeds may be deposited directly in contact with the larvae. However, the dynamics of larvae-seeds contacts were also highly variable among species. This study illustrates the complexity and variability of the ecological network of ant–seed interactions.

Keywords Seed dispersal · Ant nest · Elaiosome · *Myrmica rubra* · *Lasius niger* · *Chelidonium majus* · *Viola odorata*

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Introduction

Plants require the movement of pollen and seeds across space to fulfill their reproductive cycle, which is often carried out by animals (Herrera 2002). In pollination systems involving animal vectors, movements have distinctive and predictable targets (conspecific flowers) with incentives provided by the target (pollen, nectar) in order to control animal movements. On the other hand, seed dispersal systems have spatially unpredictable targets (germination sites) with no incentives, involving many disperser species that are seldom seed-specialized (Herrera 2002; Wheelwright and Orians 1982). Thus, the fate of seeds dispersed by animals is highly variable and depends strongly on vector traits such as diet, morphology, and behavior (Fenner and Thompson 2005; Howe and Westley 1988). In the case of seed dispersal by ants (myrmecochory), seeds are transported by many different species that—unlike harvester ants (Detrain and Tasse 2000)—are not interested in the seed itself but in a nutritious lipid-rich appendage called the elaiosome. Because ants are central place foragers—i.e., they collect food and bring it to the nest to be stored or shared with nestmates and larvae, three distinct stages can be recognized in the myrmecochory process. First, ants collect seeds in the surrounding areas and bring them back to the nest in a "centripetal" movement toward a central place (stage I). Second the elaiosomes are removed inside the nest and fed to the colony (stage II). Finally, once elaiosomes have been removed, seeds usually remain viable but are no longer of any interest to the ants. As waste items, seeds may be abandoned inside the nest or actively rejected outwards in a "centrifugal" movement from the nest to its surroundings (stage III).

A considerable amount of data in the literature deals with the first and third stages of the myrmecochory process. For example, a high removal speed reduces the impact of seed predation (Bond and Breytenbach 1985; Heithaus 1981) and depends on several ant traits such as their body size (Hughes and Westoby 1992), diet

(Hughes et al. 1994), foraging strategies (Peters et al. 2003), recruitment behavior (Zelikova and Breed 2008), learning abilities (Smith et al. 1989) and phenology (Boulay et al. 2007). Large dispersal distances reduce competition with the mother plant (Handel 1976, 1978) and also closely depends on ants' features such as their body size (Gomez and Espadaler 1998; Ness 2004; Pudlo et al. 1980), foraging behavior (Horvitz and Schemske 1986; Hughes and Westoby 1992; Kalisz et al. 1999), and community structure (Gomez and Espadaler 1998). After the nest stage, the quality of germination sites where seeds are secondarily relocated is determined by the propensity of ants to place seeds in nutrient-rich sites (e.g., refuse piles), i.e., features such as the territorial range of the ant colony or the dropping rates during seed transport (Beattie and Culver 1982; Gorb and Gorb 2003; Levey and Byrne 1993).

Although the nest is a place where ants make crucial decisions for seed fate, the ant-nest stage (II) remains a black box. The only study on the subject showed that for the same seed species, some ant species prefer to keep seeds inside the nest after removing the elaiosome, and others prefer to reject them (Gomez and Espadaler 1997). The present study investigates how seeds are managed by inner-nest workers and whether seed fates vary depending on the ant or the seed species involved. Therefore, we carried out a crossed experimental design in the laboratory involving two seed species (*Chelidonium majus* and *Viola odorata*) and two ant species, the aphid-tender *Lasius niger* and the more insectivorous *Myrmica rubra*. A previous study (Servigne and Detrain 2008) showed in the same conditions that, outside the nest, *Myrmica rubra* removes both *Chelidonium majus* and *Viola odorata* seeds in larger numbers and quicker than *Lasius niger*. The question remains as to whether these species-specific differences in seed retrieval are consistent with differences in seed management inside the nest. More specifically, this study aimed (1) to identify the dynamics of elaiosome detachment and seed rejection; (2) to determine the relative efficiency of these two phenomena in order to assess the expected benefits for the ants (in terms of elaiosome consumption) and for the plants (in terms of seed rejection); (3) to quantify the impact of elaiosome detachment on the ants' decision to reject seeds; and (4) to follow changes in seed location within the nest.

Methods

Ant species, collecting and rearing

The black garden ant *Lasius niger* (L.) (mean weight 1.9 mg; mean length approximately 4 mm; mandible gap 0.6 mm) is very common in European temperate regions and forms monogynous colonies that may contain up to 13,000 workers (Stradling 1970). This species feeds mainly on honeydew (Pontin 1958), but may occasionally eat aphids (Pontin 1958), dead or small alive insects (Pontin 1961), and elaiosomes (Oberrath and Bohning-Gaese 2002; Sernander 1906). The red ant *Myrmica rubra* L. (mean weight 2.3 mg; 4–6 mm length; mandible gap 0.8 mm) also lives in European temperate regions and forms polygynous colonies that may contain 6,000 workers (Elmes 1973). *Myrmica* workers are mainly insectivorous: they usually feed on dead or alive prey but may also consume sugars or elaiosomes (Bülow-Olsen 1984; Cammaerts 1977; Gorb and Gorb 2000; Le Roux et al. 2002).

We collected six colonies of *Lasius niger* from earth banks in Brussels (Belgium) and six colonies of *Myrmica rubra* in La Gorgue (France). In the laboratory, colonies were reared in plaster nests covered by a glass plate through which ants could be observed (Janet type, $10 \times 10 \times 0.4$ cm) and were placed in arenas ($50 \times 38 \times 10$ cm) whose walls were coated with Fluon to prevent the ants from escaping. Nests were regularly moistened and kept at $21 \pm 1°C$, $35 \pm 5\%$ relative humidity and a constant photoperiod of 12 h/day. All colonies contained 200–300 workers with brood covering around 10% of the nest area. *Lasius niger* colonies were queenless while *Myrmica rubra* ones contained 5–10 gynes. We supplied the colonies with water and sucrose solution (1 M) ad libitum, cockroaches twice a week (*Periplaneta americana*) and an artificial diet containing proteins, sugars, and vitamins (Bathkar and Whitcomb 1970).

Plant species and seed storage

Viola odorata L. is a perennial plant of temperate forests, shrubberies, and ruderal habitats (Lambinon et al. 1992). Its seeds are yellow–brown (seed weight 5.0 mg) with white-colored and soft cone-like elaiosomes (elaiosome width 1.74 mm). *Chelidonium majus* L. is a perennial plant that grows in ruderal habitats or groves (Lambinon et al. 1992). Its seeds are dark brown and small (seed weight 0.83 mg) with white and fleshy elaiosomes (elaiosome width 0.80 mm). Collected seeds were stored at $-18°C$ since cold-storage did not significantly alter seed attractiveness to the ants (Servigne and Detrain 2008).

Experimental procedure

Before the start of each experiment, we noted the presence of workers and larvae in each square of a 100 cm² grid covering the nest surface (10×10 cm). The grid was divided into three areas: the brood area (all squares containing at least one larvae or pupae), the workers' area (squares where at least one worker remained for at least 10 s and containing no brood) and empty areas (squares with no brood nor workers remaining for at least 10 s).

Four ant–plant pairs were compared: *Lasius/Chelidonium* (LC), *Lasius/Viola* (LV), *Myrmica/Chelidonium*

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(MC), and *Myrmica/Viola* (MV). Each colony was tested three times per seed species (i.e., 18 experiments for each ant–plant pair). For each experiment, we allowed the ants to take ten seeds to the nest. The experiment started as soon as those ten seeds were retrieved within the nest—i.e., at time $t = 0$. Then, every 30 min for 6 h, we observed the location of the seeds inside the nest—i.e., near workers, near brood or in empty nest areas, how many workers were in contact with them, and the presence or not of the elaiosome. We counted the number of ants in contact with the seeds—i.e., every worker that either antennated, manipulated, or moved a seed. A seed was considered as being in contact with the brood when it was laid by workers within a grid square containing at least one larva or pupa.

Data analyses

In order to compare how seeds are managed inside the nest, we quantified three decision-making processes in ants: (1) their probability of detaching an elaiosome (k_1); (2) their probability of rejecting a seed with its elaiosome still attached (k_{2e}); (3) their probability of rejecting a seed once its elaiosome was detached (k_{2d}).

These probabilities are constant values that could not be obtained by a direct observation of ant behavior but were calculated on the basis of experimental data as described below.

First, we assumed that the number of entire seeds inside the nest (E_{in}) decreases as a result of two concurrent processes: the removal of the elaiosome (k_1) and the rejection of entire seeds outside the nest (k_{2e}). Thus, the dynamics of entire seeds inside the nest since their retrieval ($t = 0$) can be described by Eq. (1):

$$E_{in} = e^{-(k_1+k_{2e})t} \tag{1}$$

The changes in the number of entire seeds within the nest over time were observed experimentally. After being log-transformed, these data were linearized (Fig. 3) and the slope of the regression line gave ($k_1 + k_{2e}$), the probability of entire seeds to "disappear" from the nest per unit time.

In contrast, the number of entire seeds outside the nest (E_{out}) increases over time due to rejection by ants and can be described by Eq. (2):

$$E_{out} = \frac{k_{2e}}{k_1 + k_{2e}}\left(1 - e^{-(k_1+k_{2e})t}\right) \tag{2}$$

Based on our experimental values, a linear relationship was found between the number of entire seeds inside the nest and those outside the nest (*M. rubra/V. odorata*: $R^2 = 0.9788$; *L. niger/V. odorata*: $R^2 = 0.9089$; *M. rubra/C. majus*: not applicable; *L. niger/C. majus*: $R^2 = 0.9688$). The slope of the regression line gave the value of $\left(\frac{k_{2e}}{k_1+k_{2e}}\right)$ in Eq. (2). With the numerical values of the two coefficients ($k_1 + k_{2e}$) and $\left(\frac{k_{2e}}{k_1+k_{2e}}\right)$ we can deduce the constant values of k_1 and k_{2e}, which are, respectively, the probability of an ant detaching an elaiosome (k_1) and the probability of rejecting a seed with its elaiosome still attached (k_{2e}).

Finally, we assumed that the number of seeds without elaiosomes inside the nest (D) increases with the number of elaiosomes removed ($k_1 \cdot E$) and decreases with the rejection of seeds without elaiosomes ($k_{2d} \cdot D$). Thus, the dynamics of the number of seeds without elaiosomes follows Eq. (3):

$$\frac{dD}{dt} = k_1 E - k_{2d} D \tag{3}$$

By integrating Eq. (3), we obtained the number of seeds without elaiosomes inside the nest (4).

$$D = \frac{k_1(e^{-k_{2d}t} - e^{-(k_1+k_{2e})t})}{k_1 + k_{2e} - k_{2d}} \tag{4}$$

By using values of k_1 and k_{2e} as estimated above, we best-fitted the experimental data (dynamics of seeds without elaiosomes inside the nest, Fig. 1) with Eq. (4). From this fitting, we obtained a good approximation of the probability (k_{2d}) for an ant to reject seeds without their elaiosomes.

All these estimated probabilities can be used to highlight differences between ant species in terms of seed management. In particular, the ratio (r) between the probability of detaching an elaiosome (k_1) and the probability of rejecting a seed with its elaiosome still attached (k_{2e}) gives an assessment of expected nutritive benefits for the ants. If $r > 1$, ants are likely to consume the elaiosome before rejecting the seeds. If ants reject the seeds before detaching the elaiosome ($r < 1$), then the nutritional benefits obtained from the seeds are expected to be poor.

In addition, the inverse values of these probabilities give, respectively, the mean time taken for a seed to have its elaiosome detached (T_1) and for a seed to be rejected either entire (T_{2e}) or without its elaiosome (T_{2d}).

Statistical analyses

The slopes of the regression lines (calculated on log-transformed data) were compared with a test of comparison of several slopes followed by a post-hoc test (q), using Microsoft Excel Software version 2004. Other statistical tests were performed using GraphPad InStat version 3.05. Considering that most data were not normally distributed, we used non-parametric statistics. The mean number of items removed was compared between ant and plant pairs with Mann–Whitney unpaired tests ($n = 18$ experiments). For all the experiments, the percentages of the nest area occupied by workers/brood were compared to those occupied by seeds by using Wilcoxon signed-rank tests.

Fig. 1 Dynamics of the mean number ($\pm$ SE, $n = 18$) of seeds with their elaiosome (*full*) and without elaiosome (*hatched*) within the nest for the *Lasius/Viola* (**a**), *Myrmica/Viola* (**b**), *Lasius/Chelidonium* (**c**), and *Myrmica/Chelidonium* (**d**) ant–plant pair
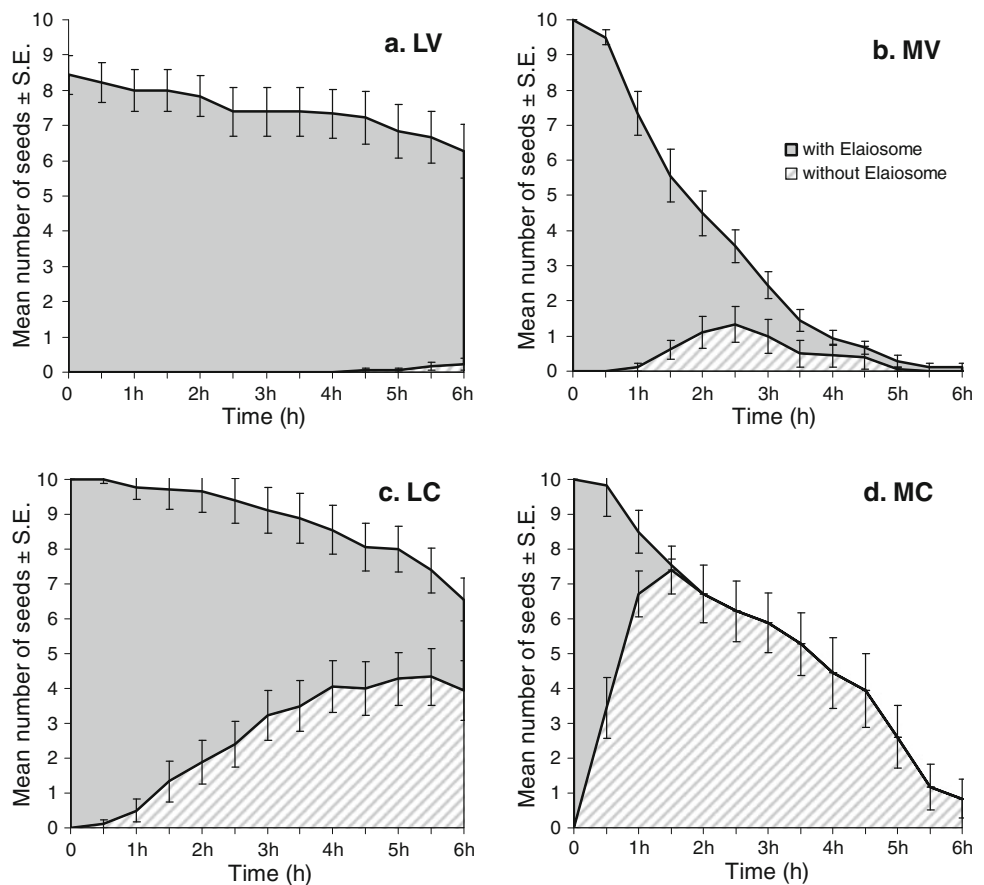
Results

Dynamics of elaiosome detachment and seed rejection

Once seeds were retrieved in the nest, they underwent the detachment of their elaiosome and/or their rejection outside the nest by ant workers. However, the dynamics and intensity of these two phenomena were highly variable, depending on the ant and plant species (Fig. 1).

The detachment of elaiosomes by *Myrmica rubra* (Fig. 1b, d) occurred significantly earlier than by *Lasius niger* (Fig. 1a, c) (for *Viola odorata* $p = 0.0147$; for *Chelidonium majus* $p < 0.0001$; Mann–Whitney test). Likewise, a higher number of elaiosomes was removed by *Myrmica* than by *Lasius* workers (for *V. odorata* $p < 0.0001$; for *C. majus*, test not applicable; Mann–Whitney test).

Chelidonium majus elaiosomes (Fig. 1c, d) were detached significantly earlier than those of *V. odorata* (Fig. 1a, b) by both ant species (for *M. rubra* $p < 0.0001$; for *L. niger* $p = 0.0336$; Mann–Whitney test) and in higher quantities (for *M. rubra*, test not applicable; for *L. niger* $p < 0.0001$; Mann–Whitney test). When combined, ant and seed effects gave strong differences in the global outcomes. For example *M. rubra* ants succeeded in removing all *C. majus* elaiosomes within only 2 h, whereas

it took about 6 h for *L. niger* ants to remove only 3.3% of the total number of *V. odorata* elaiosomes.

Regarding the dynamics of seed rejection, they were mostly ant-specific, and were unexpectedly less dependent on plant species (Figs. 1, 2). *L. niger* ants rejected both seed species (all items pooled) at a constant probability per minute of 2×10^{-4} for *C. majus* and of 10^{-4} for *V. odorata*. In contrast, *M. rubra* ants rejected seeds at a probability of 9×10^{-4} for *C. majus* and 21×10^{-4} for *V. odorata*. Hence, seeds treated by *M. rubra* were almost all rejected within 6 h with a mean length of stay in the nest of 132 min (*V. odorata*) and 22 min (*C. majus*). The speed of rejection was nearly ten times faster in the carnivorous *Myrmica* species than in the aphid tending *Lasius* species (test of comparison of slopes: for *C. majus*: $F = 21.88$, $p < 0.001$; for *V. odorata*: $F = 27.39$, $p < 0.001$). The rejection of entire seeds followed a similar pattern (Fig. 3), increasing the inter-pair differences.

Elaiosome consumption versus seed rejection: an assessment of myrmecochory efficiency

The number of entire seeds inside the nest (E_{in}) decreased as a result of two concurrent phenomena: elaiosome removal (k_1) and the rejection of entire seeds
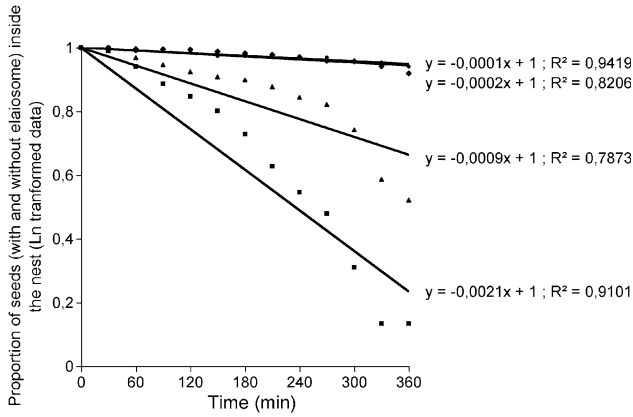
Fig. 2 Dynamics of the percentage of items still present inside the nest (entire seeds + seeds without elaiosome) for *Lasius/Viola* (*circles*), *Lasius/Chelidonium* (*diamonds*), *Myrmica/Viola* (*squares*), and *Myrmica/Chelidonium* (*triangles*). Exponential fittings are based on the dynamics of the pooled number of items ($n = 18$ experiments) calculated on log-transformed data
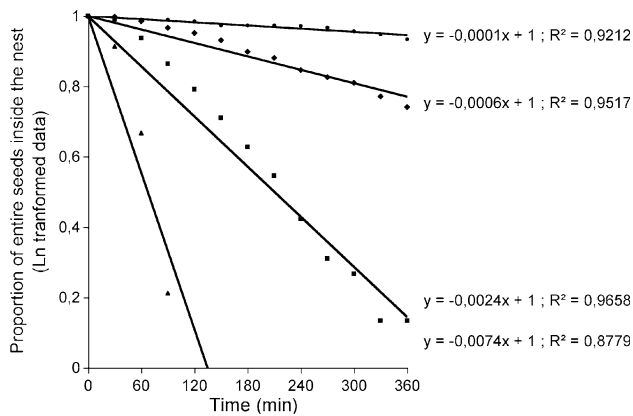
Fig. 3 Dynamics of the percentage of entire seeds (*E*) still present inside the nest for *Lasius/Viola* (*circles*), *Lasius/Chelidonium* (*diamonds*), *Myrmica/Viola* (*squares*), and *Myrmica/Chelidonium* (*triangles*). Exponential fittings are based on the dynamics of the pooled number of items in the nest ($n = 18$ experiments) calculated on log-transformed data

outside the nest (k_{2e}). Since these two processes compete within the nest, their relative speed should determine the outcome in terms of elaiosome consumption by ants as well as in terms of content of dispersed items. Such variability in the outcomes is shown by the ratio *r*—i.e., the probability of detaching the elaiosome (k_1) divided by the probability of rejecting a seed with its elaiosome still attached (k_{2e}) (Table 1).

For *Lasius niger*, the probability of rejecting entire seeds outside the nest (k_{2e}) was similar for both seed species (Table 1), but the probability of detaching the elaiosomes (k_1) was very low for *V. odorata*. As a result, the ratio *r* was very low (0.02) for *V. odorata* seeds, which was confirmed by the fact that very few elaiosomes were detached within 6 h (Fig. 1a) and that all rejected items were entire seeds (Fig. 4a).

For *C. majus* seeds, the *r* value reached 4.48, which means that *L. niger* ants showed a slight preference for

detaching the elaiosome rather than rejecting the entire seed. Then, the quantities of each type of item (seeds with or without elaiosomes) that were rejected outwards were similar at the end of the experiment (*L. niger/C. majus*, $p = 0.6571$; Mann–Whitney test; Fig. 4c). This was also the case for the pair *M. rubra/V. odorata*: a *r* value slightly higher than 1 ($r = 1.18$) resulted in a similar number of *V. odorata* seeds rejected with or without their elaiosome (Fig. 4b) (*M. rubra/V. odorata*, $p = 0.2722$; Mann–Whitney test). Finally, the pair *M. rubra/C. majus* was very efficient at detaching elaiosomes ($k_1 = 45.3$ E-3): *Myrmica* ants, never rejected entire seeds ($k_{2e} = 0$) but only items deprived of their elaiosome (Fig. 4d).

Influence of elaiosome detachment on seed rejection

A variable number of inner-workers (up to four ants) could be seen manipulating and chewing the same seed, but on average, there was a significantly lower number of workers in contact with a seed without elaiosomes than with an entire seed of the same species (Table 2) (*M. rubra/V. odorata*: $p < 0.0001$; *M. rubra/C. majus*: $p < 0.0001$; *L. niger/V. odorata*: N.A.; *L. niger/C. majus*: $p < 0.0001$; Mann–Whitney test). This indicates that ants were less interested in seed items as soon as the elaiosome was removed. This was also shown by their higher probability of rejecting a seed without its elaiosome (k_{2d}) than an entire seed (k_{2e}) (Table 1). This demonstrates that the loss of the elaiosome enhances seed rejection by inner-nest workers.

Spatial dynamics of seeds in the nest

The proportions of seeds left near workers, near brood, or in empty nest areas changed over time and depended on each ant–seed pair (see Fig. 5). Soon after the seeds were brought inside the nest, the percentages of seeds left alone in empty nest areas (0% for both plant species with *M. rubra*; 31.2% for *V. odorata* and 17.5% for *C. majus* with *L. niger*) was significantly lower than expected from a random distribution ($52.2 \pm 8.1\%$, $n = 36$ for *L. niger*, and $42.2 \pm 7.8\%$, $n = 36$ for *M. rubra*, which represents the percentages of nest area unoccupied by workers) (Wilcoxon signed-rank test, two-tailed $p < 0.0001$ for *L. niger/V. odorata*; $p < 0.0001$ for *L. niger/C. majus*; $p < 0.0001$ for *M. rubra/V. odorata*; $p < 0.0001$ for *M. rubra/C. majus*). Hence, the majority of retrieved seeds were actively directed to locations in which they could be treated and/or eaten by either nestmates or larvae.

However, we found an ant-specific trend in the way seeds were brought near the brood. Once seeds were retrieved inside the nest ($t = 0$), *L. niger* workers left some seed items in empty nest areas (*V. odorata*: 2.9 ± 2.7 seeds, $n = 18$; *C. majus*: 1.7 ± 0.5 seeds, $n = 18$) and gradually brought them closer to the

Table 1 Probabilities (seeds/min) of elaiosomes removal (k_1), of rejection of intact seeds (k_{2e}), of rejection of seeds without elaiosome (k_{2d})

	k_1	k_{2e}	r	k_{2d}	T_1	T_{2e}	T_{2d}
Lasius/Viola	8.3 E-6	4.92 E-4	0.02	No data	12 E4	2,034	No data
Lasius/Chelidonium	2.7 E-3	6.03 E-4	4.48	1.4 E-3	371	1,660	706
Myrmica/Viola	7.6 E-3	6.42 E-3	1.18	33 E-3	132	156	30
Myrmica/Chelidonium	45.3 E-3	No data	–	5.1 E-3	22	No data	196

The ratio of these probabilities gives r ($r = k_1/k_{2e}$)
Mean time (min) before elaiosome detachment (T_1), entire seeds retention in the nest (T_{2e}), and retention of seeds without elaiosome (T_{2d})

Fig. 4 Cumulative number (mean ± SD, $n = 18$) of entire seeds (*black*) or seeds without elaiosome (*grey*) that are rejected outside the ant nest for the *Lasius/Viola* (**a**), *Myrmica/Viola* (**b**), *Lasius/Chelidonium* (**c**), and *Myrmica/Chelidonium* (**d**) ant–plant pair
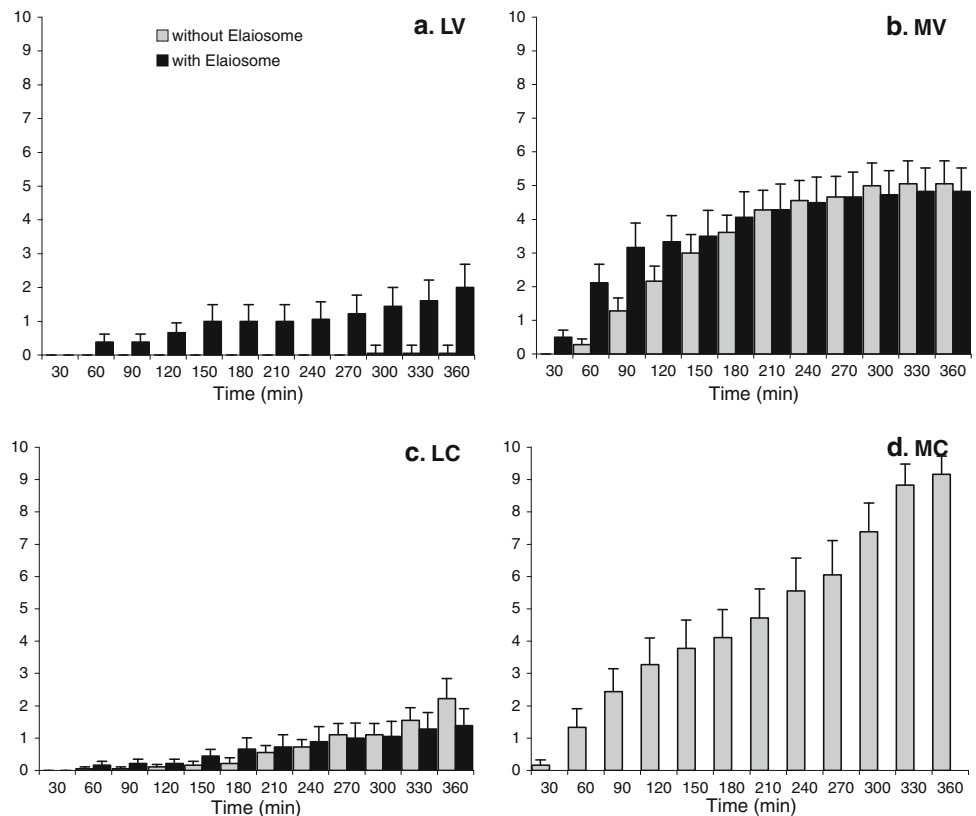
Table 2 Mean number of ants (± SD) in contact with entire seeds (E) and seeds without elaiosome (D) (all observations pooled per ant-seed pair); Mann–Whitney test

|	E	n	D	N	*p*-value
Lasius/Viola	1.03 ± 0.92	1473	1 ± 0.82	4	Too little data
Lasius/Chelidonium	0.61 ± 0.62	1227	0.31 ± 0.48	498	< 0.0001***
Myrmica/Viola	1.89 ± 1.17	736	0.33 ± 0.53	99	< 0.0001***
Myrmica/Chelidonium	1.51 ± 0.73	326	0.55 ± 0.61	970	< 0.0001***

brood. As a result, the number of seeds in contact with the larvae increased progressively from 0.3 to 2 for *V. odorata* and from 1.4 to 6.3 for *C. majus* ($n = 18$) and reached a maximum after 3 h (Fig. 5a, c). In contrast, *M. rubra* workers immediately treated all the retrieved seeds: the maximum number of seeds placed in contact with larvae [0.7 ± 0.3 ($n = 18$) *V. odorata* seeds and 3.6 ± 3.3 ($n = 18$) *C. majus* seeds] was reached in a few seconds for *V. odorata* and after 30 min for *C. majus*

seeds. Over time, the number of seeds near larvae decreased while their number concurrently increased at empty locations due to the loss of interest of workers in seeds from which elaiosome had been removed.

There was also a plant-specific effect on seed location within the nest. For both ant species, more *C. majus* than *V. odorata* seeds were placed in contact with the larvae. Indeed, when nearly all seeds still had their attractive elaiosome (30 min after seed retrieval), *C. majus* seeds

Fig. 5 Mean number (±SE, *n* = 18) of seeds inside the nest in contact with larvae (*triangle*), workers (*square*), or in empty places (*circle*) for the *Lasius/Viola* (**a**), *Myrmica/Viola* (**b**), *Lasius/Chelidonium* (**c**), and *Myrmica/Chelidonium* (**d**) ant–plant pair
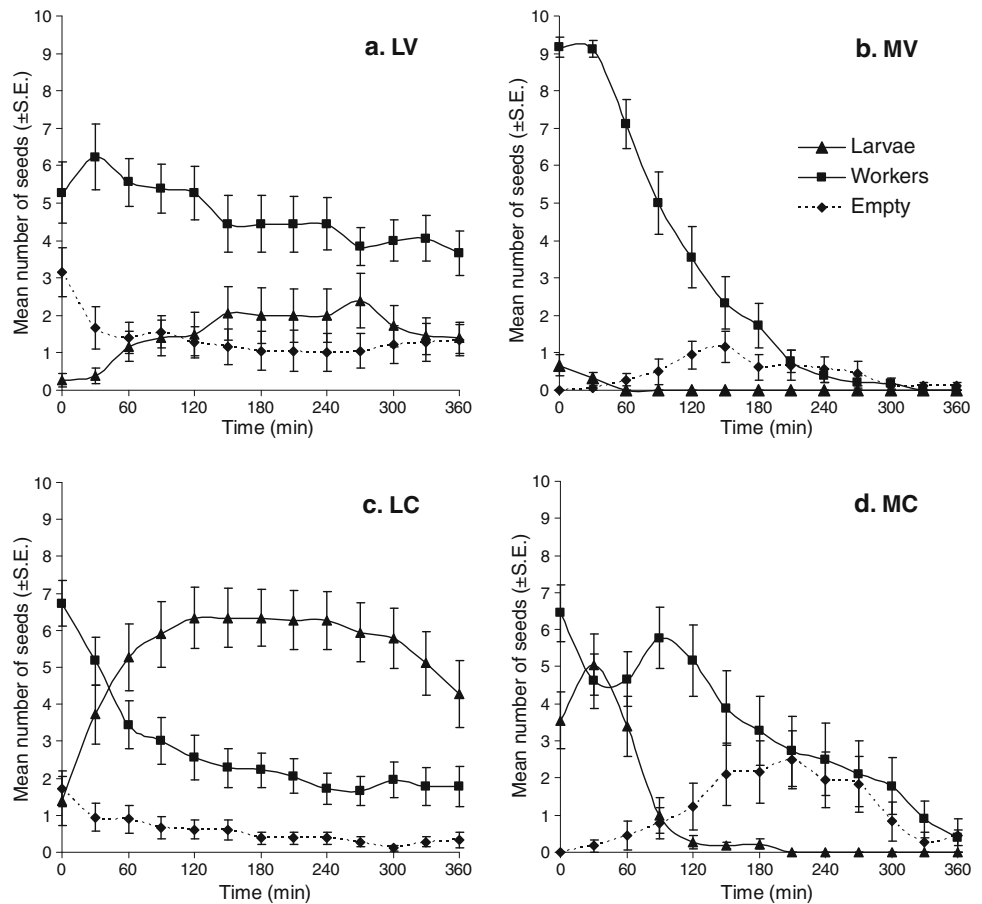
were deposited close to larvae at significantly higher proportions (*L. niger*: 37.2% of seeds; *M. rubra*: 51.4% of seeds) than expected from a random distribution (*L. niger*: 12%; *M. rubra*: 9% —i.e., % of nest area occupied by brood) (Wilcoxon signed-rank test, two-tailed *p* = 0.0003 for *M. rubra* and *p* = 0.0304 for *L. niger*). Thus, workers perceived the brood area as a preferred site for depositing *C. majus* seeds. Conversely, for *V. odorata*, the percentage of seeds brought close to larvae (*L. niger*: 4%; *M. rubra*: 3.5%) was significantly lower than expected from a random distribution (*L. niger*: 12%; *M. rubra*: 9%) (Wilcoxon signed-rank test, two-tailed *p* = 0.0003 for *M. rubra* and *p* = 0.0007 for *L. niger*). *V. odorata* seeds were scattered all over the nest but mainly near the nest entrance, far from the larvae.

The combined effects of ant- and plant-specificities resulted in extremely different patterns of seed processing within the nest. On one hand, seeds can be immediately treated by workers and left in contact with larvae for a short period of time (less than 1 h) in small numbers (0.08 ± 0.19 seeds during the 6 h experiment; *n* = 13) as for the *M. rubra/V. odorata* pair. Such a short lasting contact with larvae did not prevent high rates of elaiosomes' detachment after which seeds were left in empty areas before being quickly rejected. On the other hand, seeds can be progressively placed over the brood pile where they were accumulated before being slowly rejected outside

the nest as for the *L. niger/C. majus* pair. Around half of the seeds (5.3 ± 1.4 seeds) remained in contact with larvae during the whole experiment. Even though different seed processing was involved, at the end of the experiment, the number of elaiosomes being then detached for *L. niger/C. majus* was not significantly different from that for *M. rubra/V. odorata* (Mann–Whitney test, *p* = 0.1991).

Discussion

Seed rejection

Previous studies have shown variations in ant–seed interactions depending on partner species (Servigne and Detrain 2008). In this study, we also found marked differences in seed management by ants inside the nest. In particular, the dynamics of seed rejection seems to be highly ant-dependent, with *Myrmica rubra* rejecting seeds faster than *Lasius niger*. This fast seed rejection could be related to a more insectivorous diet of *M. rubra* compared to the aphid-tending ant *L. niger*. One may assume that carnivorous ant species have developed "hygienic" behaviors by which they quickly reject preys and waste items: this "prophylactic" behavior may have facilitated the seed-rejection phase of the myrmecochory process (Hölldobler 1982).

The seed-rejection dynamics could also influence the level of seed scarification, which is assumed to increase with the stay duration of seeds within the nest. However, the impact of scarification on seed fitness remains unclear, given that scarification may lead to worse or better germination rates depending on the ant/plant pair involved (e.g., Culver and Beattie 1980; Zettler et al. 2001).

Seed rejection was not as closely coupled to elaiosome detachment as initially expected from literature data. Indeed, some seeds remained in the nest even though they had no elaiosome left (see *M. rubra/C. majus*), and conversely, some seeds were rejected even with an elaiosome still attached (see *L. niger/V. odorata*). Our results confirm that not all elaiosomes were eaten by ants during the nest stage (Byrne and Levey 1993; Servigne and Detrain 2008; Weiss 1908). The consequences of such variability in the dynamics of elaiosome detachment and seed rejection may be important in terms of fitness for each partner. On the ant side, the benefits associated with myrmecochory can be measured by the efficiency of elaiosome detachment (high *r* values) and thus nutritional income to larvae (Fischer et al. 2005; Fokuhl et al. 2007; Gammans et al. 2005). On the seed side, elaiosome detachment can increase or decrease germination rates (e.g., Culver and Beattie 1980; Imbert 2006). In addition, when rejected seeds still bear their elaiosome (low *r* values), they might retain their attractiveness to secondary seed dispersers.

Elaiosome detachment

Elaiosome detachment was highly dependent on both ant and plant species. The *M. rubra/C. majus* pair appeared to be the most efficient in the myrmecochory process. The highly efficient treatment of *C. majus* seeds by *M. rubra* ants within the nest is coherent with the fast retrieval of those seeds (compared to those of *Viola odorata*) as well as with the high proportion of discarded elaiosomes after the nest stage (Servigne and Detrain 2008). As suggested by Gomez and colleagues (Gomez et al. 2005), the combination of a small seed and a large ant may explain such efficiency. Here, the small size of *C. majus* elaiosomes (0.8 mm wide) compared to those of *V. odorata* (1.65 mm wide) as well as the large opening width of mandibles could facilitate the detachment of seed elaiosome by *M. rubra* ants compared to *L. niger*.

However, the ant/seed size ratio is probably not the only factor explaining such variability among species. Due to the convergence of elaiosome and prey chemical compounds (Carroll and Janzen 1973; Hughes et al. 1994), ant species with a carnivorous diet may show a higher motivation and efficiency for detaching seed elaiosomes. Concerning plant seeds, *V. odorata* has a higher concentration of lipids in the external cells of the elaiosome whereas lipids are present in all cells of *C. majus* elaiosomes (Bresinsky 1963). Ants may thus be interested mainly in the external zone of *V. odorata*

elaiosomes explaining why these nutritional bodies were only partially consumed and poorly detached.

The larvae

Regarding seed management within the nest, diaspores were deposited directly in contact with the larvae. A similar phenomenon was observed in the ant *Aphaenogaster rudis*, in which inner-nest workers brought directly larvae onto *Carex pedunculata* seeds (Handel 1976). This suggests that ant larvae could feed directly and efficiently on *Chelidonium* elaiosomes. Since larvae are the final sinks of the elaiosomes (Fischer et al. 2005; Fokuhl et al. 2007), they are key individuals in the regulation of elaiosome detachment by foragers, and thus in the whole myrmecochory process.

The dynamics of larvae–seeds contacts were highly variable among species. *C. majus* seeds were preferentially placed in contact with larvae and their elaiosomes were detached at higher rates than those of *Viola odorata*. The cause of the differences in seed–larvae contacts may be chemical. The "brood mimicry hypothesis" (Fischer et al. 2008; Sernander 1906) assumes that ants carry seeds to brood chambers because they mimic larval odors. Indeed, seed removal can be elicited by the compound triolein (Boulay et al. 2006; Brew et al. 1989), which is known to be a major brood-tending pheromone in *Solenopsis invicta* (Bigley and Vinson 1975). This hypothesis should be further investigated by comparing chemical compounds in *Viola* and *Chelidonium* seeds as well as those of *Myrmica* and *Lasius* larvae, and by testing them in behavioral assays.

Perspectives

The nest stage of the myrmecochory process is far from being clear and opens new research perspectives in ant physiology and behavior, as well as in the ecology of seed dispersal. Future investigations on ant behavior could focus on how the dynamics of seed rejection and dispersal by ants are related to the ways ant colonies manage waste items. For instance, to what extent do seeds without elaiosomes have similar rejection dynamics to other items such as nest-building materials, inedible parts of prey, or nestmate corpses (Gordon 1984; Robinson et al. 2008)? Future studies should expect high inter- and intra-pair variability in outcomes, with each case lying within a continuum that ranges from "parasitism" to "mutualism" (Thompson 1982). The challenge of future myrmecochory studies will be to analyze the mechanisms of such variability and to link them with the geographic mosaic of ant–seed interactions (Thompson 1994).

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