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Fire ant myrmecophiles: feeding relationships of *Martinezia dutertrei** and *Euparia castanea* (Coleoptera: Scarabaeidae) with their host ants, *Solenopsis* spp. (Hymenoptera: Formicidae)**

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Summary

Feeding relationships of adult *Euparia castanea* Serville and *Martinezia dutertrei* Chalumeau with their ant hosts were studied in the laboratory using the radioactive tracer ^{32}P. *Euparia castanea* was tested with *Solenopsis geminata* (F.), *Martinezia dutertrei* Chalumeau was tested with *S. invicta* Buren, *S. richteri* Forel, and *S. geminata*. Unlabeled beetles were exposed to various radioisotope labeled conditions for 24 hr and then checked for acquired radioactivity. In whole colony tests, both species of beetles acquired radioactivity. *M. dutertrei* obtained food from live ants, but *E. castanea* did not. Both species of beetles ate ant larvae. *E. castanea* also obtained food from ant larvae by strigilation. Neither species of beetle fed on ant feces or other secretions on the substrate. Both species of beetles obtained food by strigilation from fresh and decomposed worker ant cadavers. *M. dutertrei* also ate both kinds of ant cadavers. Both species of beetles also ate dead house flies, indicative of scavenging or feeding on ant booty. *Martinezia dutertrei* showed no preference for any particular ant species. Ants did not obtain food by trophallaxis or glandular secretion from either species of beetle.

Introduction

Most information on the feeding habits of ectosymbionts is based on direct observation rather than experimentation. The validity of the information depends on

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* *Martinezia dutertrei* Chalumeau, 1983 (= *Myrmecaphodius excavaticollis* Auct., *nec* Blanchard 1843).

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the observer's ability, resourcefulness, and good fortune. Also this information may be biased by the observer's evaluation of the ectosymbiont's activities. Obtaining noteworthy observations usually requires an inordinate amount of time, especially for detecting infrequent behavior. For example, the senior author spent almost 200 hr observing *Martinezia dutertrei* Chalumeau before a beetle of this species was seen eating an ant larva.

Radioisotope tracers are useful for studying food relationships and trophallaxis in social insects (Wilson, 1971). However, only a few species of myrmecophiles have been studied using radioisotopes (Hölldobler and Wilson, 1990). The only Scarabaeidae known to prey on larvae of their host ants are *Cremastocheilus* spp. (Cazier and Mortenson, 1965; Alpert and Richter, 1975). Wojcik (unpublished) observed this behavior with *M. dutertrei*, both not with *Euparia castanea* Serville (50+ hr of observation time).

Many species of ectosymbionts have trichomes (tufts of long golden hairs associated with glands) on which their hosts apparently obtain glandular secretions (Wilson, 1971). *E. castanea* and *M. dutertrei* do not have any obvious trichomes. Even so, Travis (1941) observed *Solenopsis geminata* (F.) workers continually cleaning *E. castanea* at the junction of the prothorax and elytra (the usual location of trichomes). We observed this behavior often and saw similar behavior between *S. invicta* Buren or *S. richteri* Forel and *M. dutertrei*. Examination of both species of beetles failed to reveal trichomes or glandular areas at this junction (Wojcik unpublished).

These 2 species of scarabs occur in fire ant nests in the southeastern United States (Wojcik et al., 1977). *M. dutertrei* was evidently introduced into the United States from South America with one or both of its introduced hosts, *S. invicta* and *S. richteri* (Wojcik et al., 1977; Chalumeau, 1983). *E. castanea* is native to North and Central America in nests of the native fire ants, *S. geminata* and *S. xyloni* McCook (Chalumeau and Howden, 1984). As part of a continuing study of potential biological control agents of imported fire ants, this study was designed to elucidate the feeding relationships between these 2 species of beetles and their respective ant hosts.

Materials and methods

Laboratory colonies of *S. geminata*, *S. invicta*, or *S. richteri*, each composed of 3000 to 5000 workers and larvae, were starved for 24 hr and then fed ^{32}P in beef baby food or laboratory ant diet (Banks et al., 1981). The uCi of ^{32}P in each diet, amounts fed to each colony, the species and numbers of specimens used in each experiment, and the numbers of replicates are listed in Tab. 1. *E. castanea* was tested with *S. geminata*, one of the 2 ant species that it is associated with in nature (Wojcik et al., 1977). *M. dutertrei* was tested with all 3 species of ants, as it is known to be associated with all 3 species (Wojcik et al., 1977).

The beetles were obtained by placing portions of field-collected ant mounds in 5 gal buckets and then separating the ants and beetles from the soil by water flotation (Jouvenaz et al., 1977). The beetles were isolated in moistened plaster-based vials

Table 1. Summary of 4 tests using fire ants fed ^{32}P labeled diet showing replicates and dosages used

Test	Species		No. beetles used	No. rep.	Diet (g)/ replicate	Specific activity (uCi/g diet)
	ant[a]	beetle[b]				
1	Sg	Ec	82	3	1	7.4
2	Si	Md	144	3	1	75.7
	Si	Md	39	1	1	70.2
3	Sg	Md	44	1	2	13.0
	Sr	Md	38	1	2	13.0
4	Sg	Ec	144	3	1	27.2

[a] Sg = *Solenopsis geminata*, Si = *S. invicta*, Sr = *S. richteri*.
[b] Ec = *Euparia castanea*, Md = *Martinezia dutertrei*.

without food for several weeks to prevent antagonistic behavior by new host ants (Vander Meer and Wojcik, 1982). Most beetles were collected from *S. geminata* mounds (Alachua Co. and Marion Co., Fl), but some *M. dutertrei* were collected from *S. invicta* mounds (Alachua Co., Fl) (collection records included in Wojcik et al., 1977). The numbers of each species of beetle used in each experiment were determined by availability at the time of the experiment and are given in Tab. 1.

Each test consisted of 8 experiments using a beetle species and the appropriate ant species (summarized in Tab. 2 and 3). In whole colony experiments, beetles were placed with the labeled colonies contained in Wilson cells (Banks et al., 1981). These labeled colonies, hereafter referred to as parent colonies, supplied the appropriate caste of ants for the remaining experiments. In all other experiments, the beetles were placed with the designated caste of ant or test condition in 150 ml plastic vials. The vials, with 3 equally spaced holes (5 mm in diameter) drilled in the bottom, had ca. 2 cm of a liquid 9 : 1 plaster of paris-cement mixture poured in the bottom of the vials. After hardening, a circular piece of Cellucotton® was placed in the vials on top of the plaster-cement. (Cotton was not suitable as the beetles became entangled in its strands, Wojcik unpublished). These materials were moistened prior to use and the vials were placed in a tray on a 5 cm layer of moist peat moss for moisture regulation. All experiments lasted 24 hr.

Thirty individuals of the required caste were removed from the parent colony and placed in the appropriate vials with the beetles for each replication in experiments 2, 5, 6 (worker ants), and 3 (larvae). After 10 ants and the beetles were removed for assay of radioactivity for experiment 2, the remaining 20 ants were left in the vial for an additional 24 hr before being removed. The beetles for experiment 4 were then placed in the same vial with the labeled ant feces or other secretions for 24 hr. For experiments 5 and 6, labeled ants were placed into the vials and then killed by freezing. For experiment 6, the vials containing the dead ants were held at room temperature for 3 days to allow decomposition. For each replication in experiment 7, insectary-reared house flies, *Musca domestica* L., were fed ad lib. with 10% honey-water containing ca. 20 uCi/ml of ^{32}P for 24 hr. The labeled flies were killed by freezing and 5 flies were placed in the appropriate vials with the beetles. For each replicate in experiment 8, the beetles were labeled by placing them in the labeled

Table 2. Summary of the 8 experiments conducted to define the feeding relationships between *E. castanea* and its ant host

Experiment	*S. geminata*	
Labeled ants		
1 whole colonies	27/28[a]	96%[b]
2 worker ants	1/30	3%
3 larvae	12/30	40%
4 ant feces or other secretions	0/30	0%
5 fresh dead ants	9/30	30%
6 decomposing ants	20/30	67%
7 labeled house flies	13/18	72%
8 labeled beetles	5/210[c]	2%
Total beetles	226	

[a] Number of beetles acquiring radioactivity/no. beetles tested.
[b] % of beetles acquiring radioactivity.
[c] Numbers and % for ants tested with 30 beetles.

Table 3. Summary of 8 experiments conducted to define the feeding relationships between *M. dutertrei* and its ant hosts

Experiment	*S. invicta*		*S. richteri*		*S. geminata*	
Labeled ants						
1 whole colonies	18/18[a]	100%[b]	6/6	100%	5/5	100%
2 workers ants	15/35	43%	0/7	0	0/6	0
3 larvae	29/37	78%	6/6	100%	6/7	86%
4 ant feces or other secretions	3/21	14%	0/4	0	0/4	0
5 fresh dead ants	14/21	67%	6/6	100%	4/6	67%
6 decomposing ants	21/21	100%	4/5	80%	5/5	100%
7 labeled house flies	16/22	73%	–	–	–	–
8 labeled beetles	0/90[c]	0	–	–	1/30	3%
Total beetles	192		34		39	

[a] Number of beetles acquiring radioactivity/no. beetles tested.
[b] % of beetles acquiring radioactivity.
[c] Numbers and % for ants tested with 30 beetles.

parent colonies for 3 days. The beetles were then removed and placed in vials containing 10 unlabeled ants. After 24 hr, these 10 ants were removed for assay and 10 additional unlabeled ants were placed in the vial. This was repeated until a total of 30 unlabeled ants were exposed to the labeled beetles. After the last 10 ants and beetles were removed in experiment 4, 10 unlabeled ants were added to the vials. The Cellucotton®, from the vials in experiments 4 and 8, was removed from the vials and air dried for 3 days. It was then quartered and each quarter was assayed separately.

All assays of radioactivity were made in a Packard Tri-Carb® Liquid Scintillation Spectrometer using low K vials. The scintillation cocktail was made in 3-liter quantities by mixing 2250 ml scintillation grade toluene, 750 ml absolute ethanol, 15 g PPO (2,5-diphenyloxazole), and 900 ml Cab-O-Sil® thixotropic gel powder. The counting efficiency was ca. 80 %. Samples were counted for 10 min when possible and reported as counts/min. Background counts were made and any counts below 81 counts/min were considered in the background range.

Preliminary experiments (Wojcik, 1975) indicated that beetles could acquire radioactivity through simple contact with the ^{32}P labeled ant larvae. Ant larvae have anal and mouth secretions which can be transferred to their own or other larval bodies. In these experiments, the anal secretions of the labeled larvae were highly radioactive (Wojcik, 1975). A washing procedure to remove external radioactive contamination was used on the beetles (Wojcik, 1975).

Results and discussion

The results of the 8 experiments using *E. castanea* and *S. geminata* are summarized in Tab. 2. The results of the 8 experiments using *M. dutertrei* and *S. invicta*, *S. richteri*, or *S. geminata* are summarized in Tab. 3.

Experiment 1 for both beetle species was used as a positive check and all beetles became labeled with radioactivity in all replicates. Washing the beetles resulted in a 0 to 100 % reduction in radioactivity for *E. castanea* and a 14 to 49 % reduction in radioactivity for *M. dutertrei*, indicating that only part of the radioactivity was due to external contamination. The radioactivity of only 1 *E. castanea* was reduced into the background range. In experiment 1 and subsequent experiments, no preference was shown by *M. dutertrei* for any single ant species. No differencies in acquiring radioactivity were demonstrated by either sex of either beetle species in any of the experiments.

The results of experiment 2 for *E. castanea* (Tab. 2) demonstrated that this species did not obtain any food from live worker ants. The 1 beetle that became radioactive was in a replicate where 1 of the ants died during the test period. The radioactive level of this beetle was in the range of those that fed on fresh dead ants (Experiment 5). The results from experiment 2, using *M. dutertrei* and live ants (Tab. 3), indicated that these beetles obtained food from live worker ants. In 1 replicate, none of the ants died and 4 of the 7 beetles acquired sufficient radioactivity to indicate uptake from the ants. Whether this was by trophallaxis or strigilation (feeding on or licking surface secretion of other animals, Wilson, 1971) could not be determined, and neither behavior was observed.

The results from experiment 3 demonstrated that the beetles obtained food from ant larvae in 2 ways. One way was by strigilation. One specimen of *E. castanea* (Tab. 2) acquired radioactivity even though all 30 ant larvae were still present in the vial at the end of the experiment. It could not be determined if the beetles actually solicited larval secretions as do worker ants (O'Neal and Markin, 1973). *Solenopsis* spp. larvae have mouth and anal secretions and cuticular deposits which are available for the beetles to feed on. According to O'Neal and Markin (1973) the late

larval instars produce 2 types of anal secretions. The first, a clear sticky viscous secretion, is always discarded, and the second, a milky liquid secretion, is always consumed by the ants. This milky secretion was often extruded by the larvae in response to handling with forceps and was highly radioactive (Wojcik, 1975). It is possible that beetles could stimulate the larvae to produce this secretion by walking through a brood pile. In a brood pile, this secretion can easily be transferred from the surface of 1 larva to another (Wojcik unpublished) and become available to the beetles.

Strigilatory behavior is known to occur in a variety of ectosymbionts (Kistner, 1982), but has not been previously reported from scarab beetles.

A second method by which the beetles may obtain food from ant larvae is predation. This occurred in 1 replication with *E. castanea* (Tab. 2) where one larva was missing and 5 others appeared crushed with the integuments ruptured. Four of the beetles, from this replicate, had high levels of radioactivity after washing (8323, 11860, 12725, and 16590 count/min) which could only have been acquired by actually feeding on the larvae. *M. dutertrei* also ate ant larvae (Tab. 3). In 6 of 7 replications, 3 to 25 larvae were consumed. One beetle reached the highest level of radioactivity (183720 count/min) of all the beetles tested of both species. Predation on host ant larvae was previously reported for scarabs only in the genus *Cremastocheilus* (Cazier and Mortenson, 1965; Alpert and Richter, 1975). Some beetles of both species acquired substantial amounts of radioactivity apparently from external contamination. The external radioactivity could have been acquired while feeding, brushing against, or crawling over larvae since 1 beetle of each species had all their radioactivity removed by washing, indicating that they had not fed on any larvae during the 24 hr test period.

The results of experiment 4 (Tab. 2 and 3) indicated that neither species of beetle fed on ant feces or other secretions deposited on the substrate. All of the quarters of Cellucotton® had substantial radioactivity present (125 to 22366 counts/min) demonstrating the presence of ant feces or other secretions. However, all of the *E. castanea* used in this experiment were within the background range. No radioactivity was recorded from 6 beetles exposed in a vial in which 1 ant died and probably voided its gut contents onto the Cellucotton® (as indicated by the level of radioactivity on a Cellucotton® quarter). Three of the *M. dutertrei* used in 1 replicate of this experiment were above background. These 3 beetles were not washed and probably were externally contaminated.

The results of experiments 5 and 6 (Tab. 2) using fresh killed and decomposing ants, respectively, demonstrated that *E. castanea* obtained food by strigilation from ant cadavers. None of the dead ants were broken apart and 3 of the beetles were observed feeding on the mouthparts or thorax of the ants. All radioactivity from several beetles was removed by washing in each experiment, indicating that the beetles had contacted the radioactive ant cadavers. The results of experiment 6 also demonstrated that *E. castanea* may act as scavengers, since they fed by strigilation on the decomposing cadavers. Considerable decomposition had occurred in ants dead for 3 days at room temperatures (Blum, 1970) and fungal conidia were often visible on the cadavers. The results of experiments 5 and 6 indicated that *M. dutertrei* (Tab. 3) acted as a scavenger, since they fed on dead ants. All of the replicates

contained some ants which were broken apart or entirely eaten, with the exception of a single replicate. In that replicate, all of the ants were present and undamaged, and 4 of the beetles acquired radioactivity, indicating feeding by strigilation.

The results of experiment 7 (Tab. 2 and 3), using dead labeled house flies, support the conclusion that both species of beetles act as scavengers or feed on booty brought in the nests by the ants. Some myrmecophilous Thysanura and Histeridae are known to feed on host booty (Wilson, 1971). Several beetles of both species were observed with their mouthparts on various parts of the flies. One *E. castanea* was feeding on a fly, which remained suspended from the beetle's mouthparts when the beetle was lifted out of the vial. Two *M. dutertrei* were found with their mouthparts on flies' bodies, and 2 flies were found broken apart. Both species of beetles also could have acquired radioactivity by strigilation on the flies. Five flies were washed with a 0 to 15% reduction in radioactivity, indicating considerable surface radioactivity on the flies.

The results of experiment 8 (Tab. 2 and 3) using labeled beetles of both species and unlabeled ants indicated that no food or glandular secretion was transferred from either beetle species to their ant hosts. Other experiments, particularly experiment 1, showed that the beetles can acquire external radioactive contamination from contact with ants or brood. The reverse could also be true. This external contamination probably accounted for the 5 ants with very low radioactivity that were recorded in the experiments with *E. castanea* and the 1 ant with very low radioactivity with *M. dutertrei.* The ants were often observed intensively grooming the beetles, particularly at the junction of the prothorax and the elytra (the usual location of trichomes, Wilson, 1971). If the ants were actually feeding on some sort of glandular secretion (as demonstrated for a Staphylinid by Hölldobler, 1970) many more ants should have become labeled, and labeled with much higher levels of radioactivity. It can also be concluded that direct food exchange by trophallaxis from the beetles to the ants did not occur, as has been recorded for a myrmecophilous brentid beetle (Le Masne and Torossian, 1965).

Conclusions

The results of all the experiments indicate that both species of beetles are predaceous myrmecophiles which can also obtain food by strigilation and scavenging.

Both beetle species exposed in radioactive whole colonies acquired radioactivity.

E. castanea did not obtain food from live ants. *M. dutertrei* did obtain food from live ants but neither strigilation nor trophallaxis was observed.

E. castanea obtained food from ant larvae by strigilation and eating host larvae. *M. dutertrei* ate host ant larvae.

Neither species of beetle fed on host feces or other secretion on the substrate.

E. castanea obtained food by strigilation from fresh and decomposed ant cadavers. *M. dutertrei* fed primarily on the fresh and decomposed ant cadavers, and secondarily by strigilation. Both species fed on house fly cadavers, indicating scavenging by the beetles. This also indicates that both species of beetles may feed on booty brought into the ant mounds by the hosts.

There was no transfer of food by trophallaxis or glandular secretion from either species of beetle to their ant hosts.

The use of radioisotopes to study relationships between ectosymbionts and their hosts has great potential. Carefully designed and controlled experiments could aid in defining ectosymbiotic relationships and quickly prove or disprove previously reported relationships.

The biological control potential of these 2 species of beetle is limited. Although they consume ant larvae, their effect is probably minimal (Jouvenaz, 1983), as the percentage of colonies that they inhabit is low (Wojcik unpublished), and their numbers are small in comparison to the numbers of ants in a colony (Markin et al., 1973).

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