

Mushroom harvesting ants in the tropical rain forest

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Abstract Ants belong to the most important groups of arthropods, inhabiting and commonly dominating most terrestrial habitats, especially tropical rainforests. Their highly collective behavior enables exploitation of various resources and is viewed as a key factor for their evolutionary success. Accordingly, a great variety of life strategies evolved in this group of arthropods, including seed harvesters, gardeners, and planters, fungus growers, nomadic hunters, life stock keepers, and slave makers. This study reports the discovery of a new lifestyle in ants. In a Southeast Asian rainforest habitat, *Euprenolepis procera* is specialized in harvesting a broad spectrum of naturally growing mushrooms, a nutritionally challenging and spatio-temporally unpredictable food source. While unfavorable to the vast majority of animals, *E. procera* has developed exceptional adaptations such as a shift to a fully nomadic lifestyle and special food processing capabilities, which allow it to rely entirely on mushrooms. As a consequence, *E. procera* is the most efficient and predominant consumer of epigeic mushrooms in the studied habitat and this has broad implications for the tropical rainforest ecosystem.

Keywords Dietary specialization · Formicidae · Fungi · Mycophagy · Spore dispersal

Introduction

Fungi play an important role in forest biomes (Dix and Webster 1995). Their mycelia decompose and recycle organic material during long-lasting vegetative phases of their life cycles. Being integral parts of nutrient cycles, fungi may also interact symbiotically with other organisms, such as in the widespread and ecologically important mycorrhiza–plant associations (Dell 2002; Newman and Reddell 1987; Wang and Qiu 2006). Being an integral part of many nutrient cycles, fungal mycelia also represent a stable and abundant food resource for several arthropod taxa, including mainly beetles, collembolans, leaf bugs, mites, and diplopods (Hammond and Lawrence 1989). Among mycelia-feeding arthropods we also find the important group of Attine ants in the New World that are specialized on the cultivation of symbiotic Lepiotaceae fungi in the stable environment inside their nests (Martin 1992; Mueller et al. 1998). Of equal importance are the Macrotermitinae termites who are known to feed exclusively on harvested plant material that is digested in their nests by symbiotic *Termitomyces* fungi (Aanen et al. 2002; Mueller and Gerardo 2002). Fungus farming has also been realized on a much smaller scale in groups such as the wood-dwelling ambrosia beetles (Farrell et al. 2001). Although vegetative fungus mycelia are ubiquitous in forest biomes, if they are not cultivated and controlled as by the insect groups listed above they will exist at densities so low that they are not suitable as a diet for most animals. In contrast to the mycelia, the reproductive fungal stages, or sporocarps, commonly referred to as mushrooms, consist of

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highly concentrated fungal hyphae. Even though more densely packed, this agglomeration does not render the sporocarp a better food source because the vast majority of this source is short-lived, patchily distributed, and grows spatiotemporally in a highly unpredictable manner. Thus, only a few specialized animals such as some rodents (North et al. 1997) and marsupials (Johnson 1994), various dipteran larvae, and some beetles (Jacobs and Renner 1998) are able to rely mainly or, less frequently, exclusively on sporocarps. Further characteristics of mushrooms contribute to their status of being an unfavorable or at least challenging food source, including the occurrence of potent toxins, a relatively low nutritional value, and chitinous cell walls which cannot be digested by most animals (Martin 1979). Consequently, it is not surprising that no reports exist of ants with a nutritional specialization of this type, although almost every other naturally available resource is utilized, at least to some extent, by them (Hölldobler and Wilson 1990). It was therefore surprising to observe ants of the genus *Euprenolepis* heavily exploiting fungal fruiting bodies and harvesting them regularly in the Malaysian rain forest. Up to date, virtually nothing is known about the biology of this ant genus. Consequently, we conducted a field study to assess the utilization of fungi by *Euprenolepis* ants and their overall impact on fungal sporocarps. Here we present the first results on the outstanding biology of mushroom-harvesting ants.

Materials and methods

Field observations were carried out during 3 weeks each in March and September 2006, in March 2007, and 2008 at the Field Studies Centre of the University Malaya in Ulu Gombak, Malaysia (03° 19.4796' N, 101° 45.1630' E, altitude 228.8 m). Nests were located at night in the field by back-tracking foraging columns, in particular during baiting sessions (see below). All nest sites were marked and monitored regularly 2–5 times every night from discovery until the colonies moved away. As nests were located on different days throughout the observation period and emigrations occurred frequently, total observation times were different for each colony. Foraging activity was recorded by counting in- and outbound ants as well as retrieved food particles during 1 min intervals. Categories were built for ordinal statistical evaluation (0=no visible ant, 1=1–5 ants, 2=6–15 ants, 3>15 ants). In addition, the activity was checked randomly during daylight. To examine dietary preferences, food particles were collected from the ants when available and determined under a stereo microscope ($N=266$). If possible, the food source was located and the distance from the nest measured. In addition, we offered experimentally honey or freshly killed

insects both in the field ($N=10$ each) and in the laboratory ($N>70$ each). To assess nest density and resource utilization we randomly placed 92 baits of edible mushroom (*Pleurotus* sp. pieces of ca. 4×6 cm \approx 20 g) on the rainforest floor between 9:00 and 10:00 P.M. at distances of 10 m from each other in six different baiting sessions. The baits were checked every 60 min over 4 h and the abundances of all feeding animals were recorded as well as the percentage of consumed bait. Nest sites were located by back-tracking foraging columns, and the distances of nests to the baits were measured. Nest density (ND) was calculated as $ND = (n_b \times \pi d_n^2) / n_n$; with n_b =total number of baits, d_n =median nest distance from baits as measured in the experiments, and n_n =number of detected nests. The presence of the baits and the consumed biomass was checked finally after 24 h.

The efficiency of mushroom exploitation was studied in a field recruitment experiment. A large *Pleurotus* mushroom (diameter 10 cm, weight \sim 40 g) was placed 3 m from a nest entrance and the number of retrieving ants was monitored until the mushroom was fully depleted (11 counts during 3 h).

Eight *Euprenolepis procera* nests were collected successfully, maintained at the field station first and later in the laboratory at the Ludwig-Maximilians Universität in Germany. They were kept in a climate chamber with 12/12-h light/dark cycle at 26°C and 80% relative humidity. Laboratory nests were constructed of gypsum covered by glass panes and darkened with carton covers. Colonies were either fed exclusively with edible mushrooms (*Agaricus* spp. and *Pleurotus* spp.) over periods of up to 13 weeks or with honey and frozen crickets as additional diet during other times. Treatment of fungal material inside the nests was observed visually through the glass covers. Fungal pulp of different stages was also removed from the nests, studied under a microscope (Carl Zeiss AG, Axioplan) or used for further experiments. Nonparametric statistics were used for data evaluation and description since normal distribution was not given.

Results

Specialization on mushrooms Our baiting experiments revealed a surprisingly high abundance of one species, *E. procera* (Emery 1900), in the field. Within 4 h, 44% of the baits ($N=92$) were located by these ants and most of them were heavily exploited (Fig. 1). A median nest distance to exploited baits of 4.5 m (range, 1–25 m; $N=40$) indicated a high population density. We calculated a nest density of one nest per 146 m², which is probably still an underestimation because some colonies might have remained undetected. The fact that 86% of the undiscovered baits ($N=36$) were

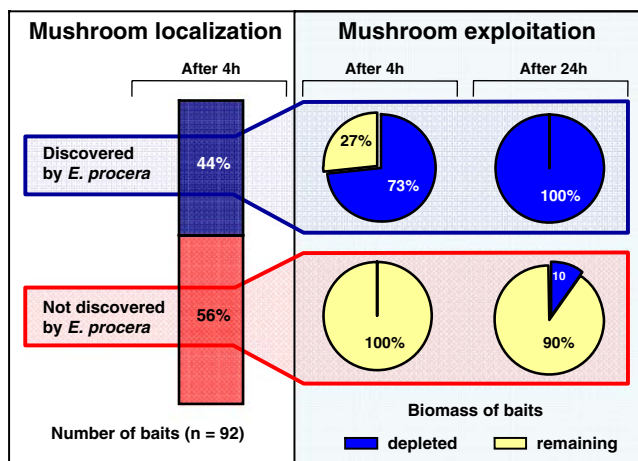


Fig. 1 Localization (*left*) and exploitation (*right*) of mushroom baits by *E. procera*. *Left* Proportion of baits discovered; *Right* depleted biomass after 4 h and 24 h. While baits occupied by *E. procera* were typically dominated by these ants, consumption of other baits includes all animals

still in place after 24 h and showed only 10% loss in biomass demonstrates a low impact of potentially competing mycophagous animals. In contrast, mushroom exploitation by *E. procera* was very efficient, with 73% depletion of all discovered baits ($N=40$) within the first 4 h.

Various naturally occurring fungi were accepted as diet (Fig. 2a–c). Feeding was observed on 30 different mushroom species in the field, while at least 50 species were ignored by the ants. Many mushrooms occurred regularly in association with the root system of rainforest trees, indicating that mycorrhizas are included in the diet. Additional feeding experiments ($N>80$) with honey or freshly killed insects were successful, demonstrating that a typical ant diet is also accepted by *E. procera*. However, the proportion of naturally retrieved food other than fungi was negligible (99.6% fungal sporocarp, $N=266$ food items). Only two occasions were observed in the field in which colonies foraged on dead animals (a grasshopper and a snail). This provides strong evidence that the natural diet of *E. procera* is based almost exclusively on mushrooms, which is further supported by the fact that laboratory colonies provided exclusively with mushrooms and water ad libitum thrived well over more than 3 months including egg laying and brood development.

Lifestyle adaptations All of 40 located *E. procera* colonies nested opportunistically inside preformed cavities, without obvious nest construction. Furthermore, these ants migrated with high frequency. Eight out of 12 field colonies, which were monitored regularly without disturbance, disappeared within a few days (median, 4 days; range, 1–9 days). Three nest relocations were encountered in the field with emigration columns reaching over several meters and

sporadic brood caches on the leaf litter surface. Captured colonies varied considerably in size, comprising approximately 500–5,000 workers and up to three queens. Much larger colonies were observed in the field with estimated colony sizes (based on the duration of their emigrations) of up to 20,000 workers or more.

All activities of *E. procera* were strictly nocturnal and laboratory colonies also remained inactive under light conditions in the climate chamber. Natural foraging distances reached over 12 m in median (range, 4–30 m; $N=22$). Monitored colonies showed low or no activity on some days in contrast to massive mushroom foraging on other days. The foraging activities were significantly higher when colonies retrieved fungi compared to all other food types (exact Mann–Whitney U -test, $U=422.0$, 2-tailed $P<0.001$, retrieving fungi ($N=35$) vs. not retrieving fungi ($N=13$)). Furthermore, lower activity was observed 0–3 days before emigration events compared to 4–9 days before emigrations (exact Mann–Whitney U -test, $U=876.5$, 2-tailed $P=0.031$, $N_{(0-3)}=49$, $N_{(4-9)}=46$), indicating that colony movement was caused by a lack of resources in the vicinity.

E. procera exploited experimentally offered mushrooms both in the field and in the laboratory with high efficiency. In the recruitment experiment, the entire mushroom of 10 cm diameter (40 g) was depleted almost completely within 3 h (Fig. 2d–f). During this experiment, a median of 35 sporocarp pieces were retrieved per minute ($N=11$ counts). Consequently, the naturally observed retrieval of 17.5 pieces per minute, (range, 1–61, $N=16$, observed over nine different days for the same colony), equals a consumption of 67 g sporocarp (or two large mushrooms) per night (10 h).

The fate of retrieved fungal material inside the nests was observed in laboratory colonies. After transporting sporocarp pieces into the nests, the workers arranged the mushroom particles into piles of 1–4 cm diameter (Fig. 2g). These piles of fungal material subsequently changed its consistence into a soft pulp and its color from whitish to brown or almost black (Fig. 2h,i). Worker ants continuously processed fungal piles by intense mandible chewing and also apparently fed from the material as their gasters expanded notably (Fig. 2g,i). While larvae never came in direct contact with fungi, workers were observed feeding larvae after leaving the fungal piles ($N=2$). When retrieved from the nests, the fungal pulp had a characteristic sweetish-sour smell which was suggestive of fermentation. Food processing inside the nests could last up to 1 week, depending much on the initial amount and the nutritional condition of the colony. Symptoms of spoilage in processed material were never observed. In contrast, fungal material which did not come into contact with the ants but was kept under identical conditions in the climate chamber turned

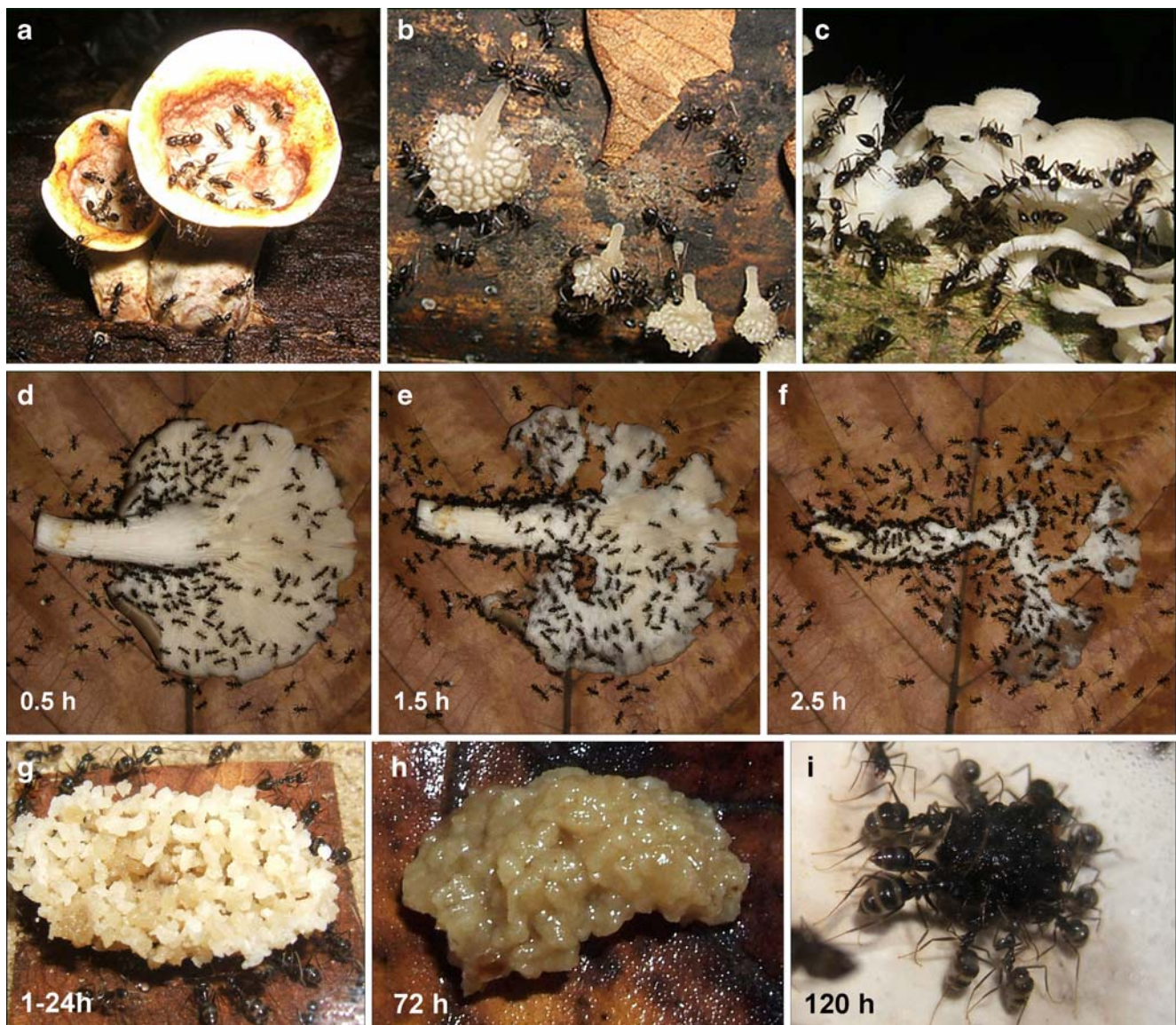


Fig. 2 Mushroom utilization by *E. procera*. **a–c** Diversity of exploited mushrooms; only three of at least eight mushroom species which were retrieved in the field are shown. **d–f** Efficiency of exploitation: recruitment of an *E. procera* field colony on a *Pleurotus* sp. (10 cm in diameter, 1 m distance from nest). **g–i** Food processing

inside the nests. **g** Fresh sporocarp pieces are attached to piles inside the ant nest. **h** These piles subsequently turn into a pulpy mass (photo after 72 h). **i** The pulpy mass loses volume and turns to almost black (photo after 120 h). Workers are attached with their mandibles most times

bad within 24 h, emitted a strong displeasing smell, and was completely overgrown by bacteria.

Discussion

Our study demonstrates that *E. procera* is able to live exclusively on mushrooms over considerable time spans and that a generalistic harvest of fungal fruiting bodies is the realized niche in its natural environment. This lifestyle distinguishes clearly from those of fungus-farming insects, which have developed highly specialized and obligate mutualisms with their cultivated fungi (Farrell et al. 2001;

Mueller et al. 2005; Schultz and Brady 2008). The mushroom harvesting *E. procera*, in contrast, selects its diet from a variety of naturally growing fungi that have evolved independently.

Such an exceptional dietary specialization can be understood as a strategy of avoiding competition, a condition that is believed to be ubiquitous among ants (Davidson 1998; Hölldobler and Wilson 1990). Competition might be especially severe in a densely populated rainforest ecosystem like our study site. Competition for mushrooms, however, is apparently low since in our baiting experiments *E. procera* was by far the most dominant consumer and other animals had negligible impact. The nest density of *E. procera* was

very high at the study site, with about half of the baits in close vicinity of a mushroom harvester ant colony. Furthermore, a single colony of *E. procera* had the capacity to harvest several mushrooms during one night. These ants must therefore have a significant impact on fungal fruiting bodies (see below). The foraging activity of *E. procera* depended on the availability of mushrooms and reduced foraging success over several consecutive days raised a colony's tendency to migrate. Consequently, the irregular foraging pattern, the opportunistic nesting habits, and the observed migratory lifestyle appear to be adaptations to the challenging food source of fungi. Such remarkable shifts in life habits in adaptation to unpredictable food resources are unique within the subfamily Formicinae and occurred only three times in ants; first in the dolichoderine herdsmen that transport their pseudococcid trophobionts frequently to freshly growing plant parts (Maschwitz and Hänel 1985), second in the mass raiding army ants which shift their nests frequently into new foraging areas (Gotwald 1995; Maschwitz et al. 1989), and third in the nomadic mushroom harvesters as described here for the first time.

Besides the remarkable biology of *E. procera*, the mode of gaining nutritional value from low quality fungal material is of future interest. Once retrieved into the nest, fungal material is "conserved" by the ants for many days through special processing which might help to overcome periods of mushroom shortages. The exact mechanism of the food processing is still under investigation. Taken together, our findings document an exceptionally high degree of specialization on fungi in *E. procera*, including efficient localization and exploitation of this challenging food resource. The benefits of collective exploitation in combination with dietary shifts and expansions were even regarded as key factors in the evolutionary success story of ants (Ward 2006). In this respect, the discovery of a novel lifestyle demonstrates once more the high potential of sophisticated social systems to develop success strategies for utilizing all kinds of ecological niches on earth. In addition, identifying mushroom harvesting ants as dominant consumers of fungal fruiting bodies has important implications for an understanding of basic ecosystem processes. An effective harvest of fungal fruiting bodies must impact spore development and distribution and thus should have significant influences on the fungus community, analogous to seed harvesting ants that are shown to strongly affect plant community structure (MacMahon et al. 2000; Rissing 1986). It is also known that other mycophagous animals act as important fungal spore dispersers (Reddel et al. 1997) and consequently, such effects can be expected from *E. procera* in the studied ecosystem. Most importantly, the majority of vascular plants, including the Dipterocarpaceae, which characterize the studied Southeast Asian rainforest ecosystem, have mutualistic relationships

with mycorrhizal fungi and have a high nutritional dependence on these symbionts (Dell 2002; Newman and Reddell 1987). In this sense, plant diversity and productivity appear to be directly connected to the occurrence of fungal symbionts (van der Heijden et al. 1998). In conclusion, ants might play another key role in ecological interactions which has not been recognized before. Further studies are necessary and are planned to clarify the exact role of mushroom harvesting ants in Southeast Asian ecosystems.

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