

**Prospects of semi-cultivating
the edible weaver ant
*Oecophylla smaragdina***

Joost Van Itterbeeck

Thesis committee

Promotor

Prof. Dr A. van Huis
Personal chair at the Laboratory of Entomology
Wageningen University

Other members

Prof. Dr L. Poorter, Wageningen University
Dr A.A. Mabelis, Wageningen University
Dr C. Peeters, Université Pierre et Marie Curie, Paris, France
Dr P. Van Mele, Agro-Insight, Ghent, Belgium

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Production Ecology and Resource Conservation

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Thesis

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Joost Van Itterbeeck
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Abstract

An increased use of edible insects as human food and animal feed is a viable means to feed the growing human population and to tackle sustainability issues of the food production systems. The semi-cultivation of the edible weaver ant *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae) in Southeast Asia can assure a sustainably supply of the highly favoured queen brood; reduce the environmental costs and financial inputs associated with non-biological pest control methods; increase the agricultural productivity of plantations; and trigger a sustainable diversification of agricultural produce. This thesis explores the sustainable increase of the availability, predictability, and productivity of *O. smaragdina* colonies. Because the procurement of edible insects has been facilitated throughout history, a detailed account was made of the indigenous knowledge of *O. smaragdina* and the harvesting practices. This revealed that the queen, the vital organ of the colony, is not known to the collectors, and that the collectors refrain from removing large numbers of worker ants. The investigation of the location and external characteristics of the queen nest suggests that this nest is ignored by the collectors because it is small and therefore could only yield a very small amount of queen brood. This study provides an easy way to identify the queen nest and as such facilitates the introduction and long-term establishment of colonies in designated areas (e.g., plantation, home garden). This thesis also suggests that the location of the queen nest within the colony's territory is a structural adaptation that serves her protection, and shows the behavioural mechanisms (i.e., warning by worker ants, queen evacuation from her nest, and function of the retinue) that are involved to protect the queen from predators. A harvesting experiment was also conducted to investigate the resilience of a colony to harvesting its queen brood. This experiment showed that worker ant mortality can be very high and negatively affect subsequent brood production. Harvesting methodologies and techniques must thus be developed that avoid the loss of large numbers of worker ants. This thesis concludes that indigenous knowledge and modern science can benefit from working together to accomplish the semi-cultivation of *O. smaragdina*, but they require the support of governing bodies in the developed world and the developing world. Directions or future research are given. This includes the development of outdoor artificial weaver ant nests, analogous to artificial wasp and bee nests. Such a development can accelerate advances in our understanding of *O. smaragdina*, and, when well designed, can facilitate the queen brood harvest.

Keywords: entomophagy, edible insects, Formicidae, global food security, agricultural revolution, Lao PDR

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Chapter 1

General introduction

Insects as human food and animal feed: Evolution of a field of science

Modern science has its beginnings in the 19th century. At that time, eating insects (entomophagy) was considered primitive and disgusting in the developed world. Few scholars believed that modern humans did not have an innate aversion to eating insects but rather had *developed* an aversion to it. They questioned this aversion. One of them was Charles V. Riley. In the 1870s, Riley was appointed to combat the Rocky Mountain Locust which was a severe pest insect. The locust hindered agriculture and caused starvation among the settlers in the western USA. One of Riley's recommendations to farmers was to eat the insect that ate their crops. He drew on the dietary habits of the Native American Indians. They consumed a variety of insects including the Rocky Mountain Locust. Riley himself developed a taste for the locust but his enthusiasm was not shared by many (Lockwood 2004, Riley 1877). In 1885, in England, Vincent M. Holt published his booklet *Why not eat insects?* Holt's stance was similar to Riley's. He strongly questioned modern human food preferences. Holt suggested that poor peasants could avoid starvation by turning to their gardens and meadows for edible insects. Also Holt tried and liked edible insects (Holt 2007).

In 1951, Frederick S. Bodenheimer published a landmark book on human entomophagy: *Insects as human food: A chapter of the ecology of man* (Bodenheimer 1951). The book is of great scientific value because it clarifies the commonness and importance of insects as human food throughout the world and throughout human history. The book provided food for thought as is evidenced by one of its reviewers: "It may well be that in the future our rapidly growing human population may be forced to eat some of our highly successful agricultural pests rather than to destroy them by poison or otherwise. This possibility was early discovered by our ancestral stock who staved off famine by eating the invading hordes of locusts" (Brues 1952).

During the second half of the 20th century, the viewpoint on entomophagy slowly became more positive in the developed world. It also broadened in scope. Eating insects was not considered only as an option for the poor, the starving, and the developing world, but for all humans. A handful of scholars were the driving force behind the slowly changing viewpoint. These include Gene R. DeFoliart, Jacques Hardouin, Arnold van Huis, François Malaisse, Maurizio G. Paoletti, Julieta Ramos-Elorduy, and Victor Benno Meyer-Rochow. These scholars advocated the use of edible insects as a source of human food and animal feed. They questioned the rationale of the food culture of developed countries, and its adoption by developing countries. Attention was drawn to the favourable characteristics of insects, e.g., the nutritional values that are comparable to those of conventional meat, the higher efficiency in converting feed into biomass than conventional livestock, and the high reproductive rate. The late Gene R. DeFoliart stated: "Considering their efficiency in converting plant biomass to animal biomass, the failure to domesticate edible insects on any significant scale (except as a byproduct of silk and honey production) may have been a greater calamity in the global development of agriculture than we yet realize" (DeFoliart 1999).

In the beginning of the 21st century, positive attitudes towards entomophagy can be seen at various levels of society. An agricultural revolution is called upon to feed a growing human

population and tackle sustainability issues of the food production systems (Godfray et al. 2010). Entomophagy is increasingly seen as a valuable agricultural development that can benefit humankind. Edible insects are considered a viable and sustainable alternative to conventional meat which can make a major contribution to alleviating global food insecurity (van Huis et al. 2013). Developments in entomophagy have become widespread. Initiatives are taken worldwide by academic institutions (e.g., Wageningen University), the development sector (e.g., FAO), private companies (e.g., AgriProtein), and the public (e.g., girl-meetsbug.com). The scientific study of edible insects and their use as human food and animal feed is, however, in its infancy. To realize the potential benefits of edible insects to humankind implies that entomophagy must continue to grow as a field of science in the 21st century.

Semi-cultivating and farming edible insects: A key development

Edible insects are widely consumed by rural populations, they are sold in rural and urban fresh food markets, they are canned and bagged for urban consumers, and they are exported either fresh or processed (van Huis et al. 2013). The edible insect trade is highly lucrative and vast amounts of insects are collected. The far majority of the edible insects come from the wild. They are mainly a Non-Wood Forest Product (NWFP). The edible insect resource thereby depends on the size and condition of the insects' habitats, and on the sustainability of the harvesting methodologies. Habitat degradation and fragmentation pose a threat to the edible insect populations (van Huis et al. 2013). Concerns have been raised over the sustainability of the edible insect trade and a variety of species is already over-exploited (Ramos-Elorduy 2006). Ramos-Elorduy (2006) points to the adoption of unsustainable harvesting practices. Ecologically speaking, the sustainable collection of a species occurs when its reproduction and regeneration are not negatively affected in the long-run, and without deleterious effects on other species and the ecosystem (Sunderland et al. 2011). It should be noted that edible insects provide vital ecosystem services such as pollination (e.g., honeybees), waste removal (e.g., dung beetles), and pest control (e.g., weaver ants). The threats to the edible insect populations must be countered. The semi-cultivation and farming of edible insects are key developments herein (Durst et al. 2010, Hanboonsong et al. 2013, van Huis et al. 2013, Vantomme et al. 2012). These developments can reduce the pressure on wild edible insect populations and assure a continuous supply of the edible insect resource. We had made a distinction between these two concepts as follows (van Huis et al. 2013).

Farming implies that the insects are reared (i.e., tended) in captivity. Their living conditions and food are controlled. The insects are isolated from their natural populations. A consequence of farming may be that the farmed insects differ over time from their natural populations, e.g., a decrease in body size. This is said to be the case of cricket farming which is practiced in Laos, Thailand, and Vietnam (Yhoung-Aree and Viwatpanich 2005).

Semi-cultivation resembles cultivation which is a process that promotes the growth (or quality) of an organism through the use of labour and skill. Semi-cultivation involves manipulations of an edible insect's habitat, at a low or large scale, to improve the insects' availability, predictability, and/or productivity. The insects are generally not grown in captivity and are thus not isolated from their natural populations. Semi-cultivation rarely involves the tending of insects but it is a steppingstone towards a more controlled production, i.e., farming. This

activity has the potential to contribute to both edible insect habitat conservation and food security.

Developments in the semi-cultivation and farming of edible insects depend on a sufficient understanding of edible insect biology and ecology (van Huis et al. 2013).

Edible ants

Ants (Hymenoptera: Formicidae) are among the most diverse and abundant insects (Hölldobler and Wilson 1990). They perform provisioning, regulating, cultural, and supporting ecosystem services that benefit human society (Del Toro et al. 2012). Ants are used as human food, animal feed, and medicine throughout the world (provisioning services). They regulate ecosystem processes through, e.g., seed dispersal, pollination, animal community regulation, and biological control (regulating services). They are used in symbology, rituals, myths, literature, and film (cultural services). Ants support the former three services by, e.g., nutrient cycling, soil structure formation, and decomposition (supporting services). However, disservices are also identified such as stinging and the negative effects of invasive species on the environment (Del Toro et al. 2012).

The least amount of scientific study has been done on the cultural and provisioning services of ants (Del Toro et al. 2012). There are 48 edible ant species worldwide (Jongema 2012, Van Itterbeeck and Fisher 2013). An advantage to using ants and/or their brood as food is that large quantities can be collected in a short time. This is because ants live in colonies. However, concerns have been expressed of over-exploitation. The survival and productivity of individual colonies and the maintenance of ant populations may be under threat. This could affect the services that the ants provide. Ants that are potentially threatened include *Liometopum* spp. of which the brood ('escamoles') is collected in Mexico (Ramos-Elorduy 2006); the leaf-cutter ants *Atta* spp. and *Acromyrmex* spp. of which the winged females ('hormigas culonas') are consumed throughout Central and South America (Choo 2008); and the weaver ant *Oecophylla smaragdina* of which both brood and adult ants of all castes are used as human food and animal feed (Césard 2004, Hanboonsong et al. 2013, Sribandit et al. 2008).

The potential of the weaver ant *Oecophylla smaragdina*

The weaver ant genus *Oecophylla* consists of two extant species. *Oecophylla longinoda* occurs in tropical Africa, *O. smaragdina* occurs from India to the Pacific and from the south of China to the north of Australia (Figure 1.1). Weaver ants are arboreal, i.e., they live in the trees. This ant genus is well-known for the leaf nests it builds (Figure 1.2.a). These are constructed by pulling and rolling living leaves in place (Figure 1.2.b) and binding them together with larval silk. The ants hold a larva between the mandibles and use it as a 'weaving shuttle' (Crozier et al. 2009) (Figure 1.2.c). A *Oecophylla* spp. colony is polydomous, i.e., it consists of multiple nests. The nests of a single colony are frequently distributed in the crowns of several trees. The *Oecophylla* genus is a behaviourally dominant ant. The ants maintain close control of their colony's territory. This territory consists of the tree crowns where they nest (central territory) and the ground around the tree bases (secondary territory) (Rastogi 2007).

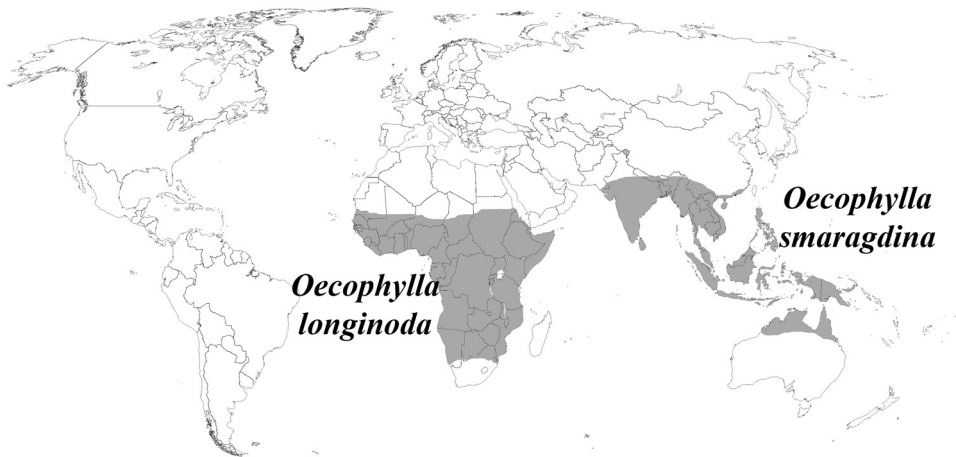


Figure 1.1. Geographic distribution of *Oecophylla longinoda* and *O. smaragdina* (based on Crozier et al. 2009).

Weaver ants are generalist predators (Figure 1.2.d). Their main prey is insects. These protein-rich sources of food are used to raise the brood. The adult ants do not require protein-rich food but energy-rich food. The sources of energy-rich food are the excretions of extrafloral nectaries and the honeydew that is produced by Hemiptera (trophobionts). Trophobionts are actively tended and protected from their natural enemies (Figure 1.2.e).

A single *O. smaragdina* colony can be very large and consist of thousands of worker ants (Crozier et al. 2009). The worker caste is made up of two subcastes. The large-sized major workers primarily forage and defend the colony. The small-sized minor workers primarily nurse the brood inside the nests. The colony produces worker ants year round whereas sexual forms are produced during certain times of year. These sexual forms have wings. Males are black and small. Queens are green and large. They are called virgin queens when they have not mated yet. This distinguishes them from the dealate, physogastric, gravid queens (Figure 1.3.a and 1.3.b) (Hölldobler and Wilson 1990). Both monogynous (single gravid queen) and polygynous (multiple gravid queens) colonies exist (Peng et al. 1998a, 1998b).

A large number of tree species is host to the weaver ants. Several of these trees have a high economic value, e.g., mango, citrus, coffee, tea, coconut, and cashew (Lim 2007). The ants are a viable alternative to chemical control of pest insects. The oldest record of biological control is the use of weaver ants in citrus trees in south China (Huang and Yang 1987). The presence of the weaver ants can increase tree crop yield and/or quality (Peng et al. 2004). The financial input associated with weaver ant pest control is also less than when using chemical control methods (Peng et al. 2013). The aggressiveness of the ants is however a nuisance to humans (Van Mele et al. 2009). Van Mele (2008) suggests that because of this nuisance little research has been done in using weaver ants in agroforestry practices.

Weaver ants are one of the most popular edible insects in Thailand and the Lao PDR (Sribandit et al. 2008, Yhoung-Aree and Viwatpanich 2005). Sribandit et al. (2008) show the importance of the *O. smaragdina* trade to local livelihoods. They estimate an average ant harvest of 219 (\pm 107.5) kg per year per ant collecting household. This yield constitutes an average



Figure 1.2.a. A weaver ant nest. (Picture: Joost Van Itterbeeck)

Figure 1.2.b. Weaver ants pulling together leaves. (Picture: Joost Van Itterbeeck)

Figure 1.2.c. A weaver ant binding together two leaves with larval silk. (Picture: Joost Van Itterbeeck)

Figure 1.2.d. Weaver ants pin down an ant of a conspecific colony. (Picture: Joost Van Itterbeeck)

Figure 1.2.e. Weaver ants tending trophobionts. (Picture: Joost Van Itterbeeck)

of 30% of the yearly household income among the ant collectors. The use of weaver ants as human food concerns predominantly the queen brood, i.e., large-sized larvae and pupae that are destined to become virgin queens. Worker brood and adult ants are also used. A variety of dishes is prepared with the brood and adult ants (Figure 1.4.a, 1.4.b, and 1.4.c). The brood

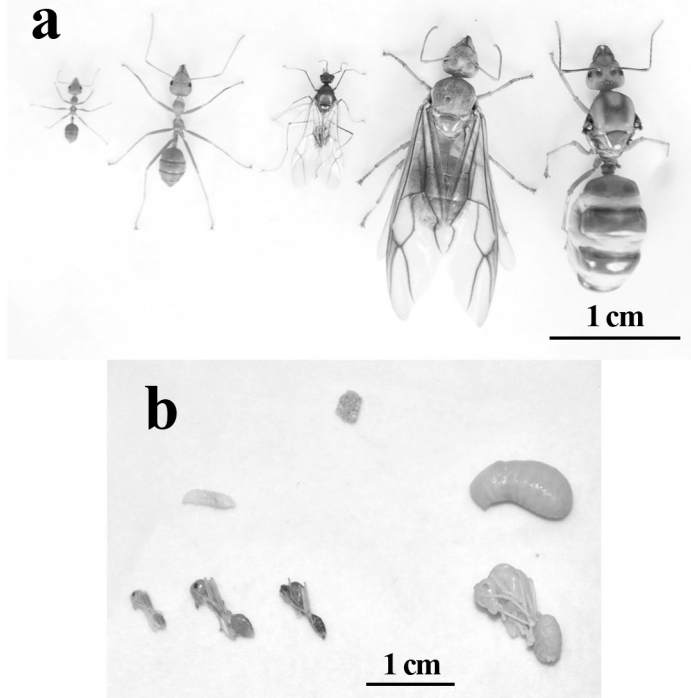


Figure 1.3.a. *Oecophylla smaragdina* castes; from left to right: minor worker, major worker, male, virgin queen, egg-laying queen. (Picture: Joost Van Itterbeeck)

Figure 1.3.b. *Oecophylla smaragdina* brood. Top: egg cluster. Middle row: small and large larva. Bottom row, from left to right: pupae of minor worker, major worker, male, and virgin queen. (Picture: Joost Van Itterbeeck)

and adults are also canned (Figure 1.4.d). In Indonesia, *O. smaragdina* is a high-priced feed for songbirds. For this purpose, the worker brood, i.e., small-sized larvae and pupae that develop into worker ants, is favoured. This brood is collected year round (Césard 2004).

Offenberg and Wiwatwitaya (2010) suggest that weaver ants could be kept in crop trees where they function both as a biological control agent and as a direct source of human food. Moreover, the ability to increase the predictability, availability, and productivity of *O. smaragdina* colonies may boost its use as a biological control agent (Decha Wiwatwitaya personal communication). When this development has been achieved, it is an innovation that should be classified as semi-cultivation (van Huis et al. 2013). Sribandit et al. (2008) indicate that there is a positive attitude towards such a development among rural peoples. The potential benefits are: a reduction in the environmental costs and financial inputs associated with non-biological pest control methods; an increase in the agricultural productivity of plantations both in terms of tree crops and queen brood; and a sustainable diversification of agricultural produce, e.g., by designing agroforestry practices with *O. smaragdina* host trees (Offenberg et al. 2013, Van Mele 2008). Besides its application in agroforestry practices (e.g., plantation, home garden), the weaver ant can potentially act as a keystone or umbrella species in nature conservation. Raina et al. (2010) have shown in Africa how commercially valuable insects (silk moth, honeybee) can provide nature conservation incentives. This possibility exists with



Figure 1.4.a A salad of fresh ant brood and herbs. (Picture: Joost Van Itterbeek)

Figure 1.4.b Fish soup with ant brood and adults. (Picture: Joost Van Itterbeek)

Figure 1.4.c Omelette with ant brood. (Picture: Luc Van Itterbeek)

Figure 1.4.d Canned queen brood. (Picture: Joost Van Itterbeek)

the weaver ant *O. smaragdina* in countries where it is a popular source of human food and/or animal feed.

Oecophylla spp. is considered a pinnacle of social evolution (Hölldobler and Wilson 1990). Both fundamental and applied research has been done on this ant genus. However, very little research has been done that relates to the semi-cultivation of *O. smaragdina*. Offenbergh and Wiwatwitaya (2010) investigated the effect of the ant brood harvest on the biological control potential of weaver ants in a Thai mango (*Mangifera indica*) plantation. They applied the traditional ant brood harvesting technique in Thailand and harvested queen brood twice during

the season when queen brood is available. They conducted this experiment with colonies that were offered additional food (sugar and cat food) and with colonies that were not offered additional food. The fresh mass yield from fed colonies was 258-377 g queen brood tree⁻¹ year⁻¹. From unfed colonies, the yield was 114-157 g queen brood tree⁻¹ year⁻¹. By offering a cheap source of additional food to colonies, the queen brood yield can thus be increased. The authors suggest that the queen brood harvest is a sustainable practice (whether or not additional food is offered) that is compatible with using the colonies as a biological control agent. This follows from observations on worker ant densities (it is the worker ants that capture pest insects). The harvest did not significantly affect worker ant densities that were estimated shortly after the harvest. Moreover, an increase in worker ant densities was found in the long-term (estimated up to 11 months after the first harvest). This could suggest that the queen brood harvest promotes worker production and potentially increases the effectiveness of pest control by weaver ant colonies. This research provides promising results but additional harvesting experiments need to be conducted. The use of the ant brood as human food probably has an ancient origin. Although there is no evidence in the literature that indigenous peoples manipulate the ant colonies and/or their habitat to facilitate the procurement of the queen brood, their knowledge may hold relevant clues to increase the predictability, availability, and productivity of queen brood by individual colonies. Ant management is also a relevant issue. The use and management of *O. smaragdina* colonies for pest control has been studied (Peng and Christian 2007, Peng et al. 2004, 2011, Van Mele and Cuc 2007). For example, issues that relate to the introduction of colonies to designated areas (e.g., plantation) have received attention. This includes identifying the location of the queen nest within the polydomous territory of mature colonies (Peng et al. 1998b), and increasing the growth rate of incipient colonies (Peng et al. 2013). It is suggested that incipient colonies could be raised to a suitable colony size in a controlled environment and then introduced into trees (Peng et al. 2013). Peng et al. (2013) show how new queens can be trapped shortly after the nuptial flight by using artificial nests. An incipient colony can contain a single queen but also multiple queens. It is beneficial if the growth rate of incipient colonies is increased because it takes up to three years under natural conditions for a colony to become mature and thus produce queen brood. Transplanting pupae of different colonies to the incipient colonies promotes early colony growth, especially when the incipient colonies have multiple queens (Offenberg et al. 2012b, Peng et al. 2013). An earlier study showed that larvae can be transplanted between different colonies (Krag et al. 2010). Offenberg et al. (2012b) and Peng et al. (2013) suggest that the fastest growth can be achieved with higher numbers of queens present in the incipient colonies, and with higher numbers of transplanted pupae. For example, four-queen colonies with 60-pupae transplantation produced 476% more brood than two-queen colonies without pupae transplantation (brood production was measured 12 days after the pupae transplantation) (Peng et al. 2013). This is in part explained by the higher production of new workers in incipient colonies with higher numbers of gravid queens as each queen contributes to the production of workers (Offenberg et al. 2012a). Food availability is also a relevant issue in the growth and maintenance of a colony. Lynegaard et al. (2014) propose to use insect traps to increase prey intake by ant colonies. The use of Malaise-like traps can increase prey intake by 5-14%. When a light source is used to attract more insects during the night, prey intake could be increased to 38-78%. More work is needed to improve the management and use of

O. smaragdina colonies.

Laos

Entomophagy is very common in Southeast Asia. A large variety of insects is consumed in the Lower Mekong Basin which comprises the countries of Cambodia, Laos, Myanmar, Thailand, and Vietnam. Edible insects are consumed in rural and urban areas, including metropolises such as Bangkok. International trade is common, especially in border regions (van Huis et al. 2013, Yhoung-Aree and Viwatpanich 2005). Southeast Asia has a lot of potential to develop the semi-cultivation and farming of edible insects on a wide scale. Some edible insects are already farmed, and research is ongoing to farm more different species (Han-boonsong et al. 2013). For my PhD project, I cooperated with the Faculty of Science of the National University of Laos (NUoL). The research was done in Xaythani District, Vientiane Municipality, Lao PDR. The Lao People's Democratic Republic (Lao PDR, Laos) is a communistic and landlocked country (UNPAN 2005). It is listed as a least developed country by the United Nations (UNCTAD 2012). Food production within Laos is dominated by rice cultivation and the collection of Non-Wood Forest Products (NWFPs). The rice-based eco-systems and NWFPs are of particular importance to livelihoods (Foppes 2008, Meusch et al. 2003, Nurhasan 2008). A large variety of insects is commonly consumed and marketed, and crickets are farmed (Nonaka, Boulidam et al. 2008, Nonaka, Sivilay et al. 2008, Yhoung-Aree and Viwatpanich 2005). A study by Meyer-Rochow et al. (2008) illustrates the high economic importance of edible insects. Agricultural development in Laos lags behind that of its neighbouring countries while forest and wooded land cover of the Lower Mekong Basin countries is highest in Laos (FAO 2010). There is scope for Laos to include edible insects in its development efforts. The weaver ant *O. smaragdina* could play a central role.

Objectives of this thesis

The research of the combined use of the weaver ant *O. smaragdina* as a biological control agent and as a direct source of human food, as well as the research in increasing the predictability, availability, and productivity of *O. smaragdina*, is in its infancy. This thesis aims to identify the possibilities and constraints to *O. smaragdina* semi-cultivation from a biological, practical, and technical viewpoint, and further facilitate the management and use of *O. smaragdina* colonies. I do this by focussing on three main themes.

First, because **indigenous knowledge** is an adaptive body of knowledge, practice, and belief that people use to maintain or improve their livelihoods, and therefore can be an important complement to modern science and the development process (Berkes 2008), I aim to:

- review the semi-cultivation of edible insects in a historical perspective; and
- investigate the indigenous knowledge of the weaver ant and the harvesting practices applied by collectors from the Vientiane Plain, Lao PDR.

Second, because the **gravid queen** is vital to the colony, is required for the production of queen brood, and is required for the long-term establishment of the colony (Hölldobler and Wilson 1990, Franks and Sendova-Franks 1999), I aim to:

- identify the location of the queen nest within the colony's territory; and
- investigate the vulnerability of the gravid queen to the harvesting practices.

Third, because the **resilience** of the colony to its use as a source of human food is an important determinant of the colony's potential as a semi-cultivated (and farmed) product, I aim to experimentally investigate the effect of the queen brood harvest to the weaver ant colony.

Thesis outline

Chapter 2 reviews the semi-cultivation of edible insects in a historical perspective. The chapter focusses on three cases: (1) eggs of aquatic Hemiptera in Mexico which are semi-cultivated by water management and by providing egg laying sites; (2) palm weevil larvae in the Amazon Basin, tropical Africa, and New Guinea of which the collection is facilitated by manipulating host tree distribution and abundance and which are semi-cultivated by deliberately cutting palm trees at a chosen time at a chosen location; and (3) arboreal, foliage consuming caterpillars in sub-Saharan Africa for which the collection is facilitated by manipulating host tree distribution and abundance, shifting cultivation, fire regimes, host tree preservation, and manually introducing caterpillars to a designated area. This chapter highlights the importance of indigenous knowledge to developments with edible insects.

Chapter 3 describes the indigenous knowledge of *O. smaragdina* that is held by expert collectors from the Vientiane Plain, Lao PDR, and provides details of the experts' collection practices. The chapter reflects on sustainability and conservation issues, and on semi-cultivating constraints and possibilities embedded in indigenous knowledge and collection practices.

In Chapter 4, the location and external characteristics of the queen nest in a mature *O. smaragdina* colony are studied. The chapter describes an accurate way to identify the queen nest. This facilitates the translocation of mature colonies to designated areas for the biological control of pest insects and/or their use as human food and animal feed. The chapter also reflects on queen defence mechanisms.

Chapter 5 investigates how *O. smaragdina* workers safeguard the gravid queen against a predator. This chapter shows the function of specific behavioural mechanisms in the defence of the gravid queen.

In Chapter 6, the resilience of a *O. smaragdina* colony to its use as human food is investigated. This is done by conducting a harvesting experiment where an intensive harvesting pressure is applied. This chapter contributes to the issue of sustainable use of an individual colony and reflects on semi-cultivation constraints and possibilities from a biological and technical point of view.

Finally, Chapter 7 provides a general discussion and conclusion of the thesis and highlights priorities of future research in *O. smaragdina* semi-cultivation. The chapter calls for an investment in fundamental research of *O. smaragdina*, it proposes an *O. smaragdina* development analogous to wasp- and beekeeping, and it advocates that research in the evolution and

dynamics of the perceptions of insects as human food are vital to realize the potential benefits of entomophagy to humankind.

Chapter 2

Environmental manipulation for edible insect procurement: A historical perspective

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Abstract

Throughout history humans have manipulated their natural environment for an increased predictability and availability of plant and animal resources. Research on prehistoric diets increasingly includes small game, but edible insects receive minimal attention. Using the anthropological and archaeological literature we show and hypothesize about the existence of such environmental manipulations related to the procurement of edible insects. As examples we use eggs of aquatic Hemiptera in Mexico which are semi-cultivated by water management and by providing egg laying sites; palm weevil larvae in the Amazon Basin, tropical Africa, and New Guinea of which the collection is facilitated by manipulating host tree distribution and abundance and which are semi-cultivated by deliberately cutting palm trees at a chosen time at a chosen location; and arboreal, foliage consuming caterpillars in sub-Saharan Africa for which the collection is facilitated by manipulating host tree distribution and abundance, shifting cultivation, fire regimes, host tree preservation, and manually introducing caterpillars to a designated area. These manipulations improve insect exploitation by increasing their predictability and availability, and most likely have an ancient origin.

Keywords: edible insect, entomophagy, facilitation, environmental manipulation, semi-cultivation, aquatic insect egg, *ahuahuite*, palm weevil, palm larvae, caterpillar

Introduction

Much research on prehistoric diets focused on the procurement of meat, i.e. hunting of large animals. This has been considered to be an important socio-cultural aspect in antiquity. McGrew (2001) warns for a skewed picture of early diets. Regarding animal protein, archaeological evidence throughout the world points to a broad diet that included fish, birds, lizards, rodents, rabbits, turtles, crabs, molluscs, and shellfish (Barker 2009). Coprolite analyses provide direct evidence also of the consumption of insects e.g. termites and predaceous diving beetles by early American Indians (up to 9,500 B.P.) and ants, dung beetle larvae, and caterpillars in Mexico (> 5,400 B.P.) (Elias 2010 and references therein, see also Sutton 1995). For an understanding of prehistoric life, archaeologists increasingly consult anthropological studies of traditional forager societies today, such as the !Kun-San of the Kalahari and Australian Aborigines, which are believed to resemble such life most closely (Barker 2009, Sutton 1990a). It is increasingly acknowledged that studies on the ecological anthropology of these peoples have usually wrongfully ignored the use of insects as food. The !Kun-San consume termites, grasshoppers, caterpillars, and ants (Nonaka 1996). Australian Aborigines consume e.g. termites, grasshoppers, moths, caterpillars, beetle larvae, wasp larvae, and ant brood (Meyer-Rochow and Changkija 1997, Reim 1962, Yen 2005). Their practice of entomophagy has decreased though over the past 200 years through western influence (Yen 2010).

Some other studies incorporating insect use were conducted in the Amazon Basin (Dufour 1987, Milton 1984, Ruddle 1973). The indigenous groups under investigation practice forms of cultivation and horticulture in combination with hunting and gathering. Such combinations in subsistence have been practiced throughout history (Barker 2009). These records indicate intensive use of insects as food source. Based on such archaeological and ethnographic records, Sutton (1995) stresses that "... we must *expect* [original italics] the use of insects in antiquity and so we must subject insect remains to the same examination and analysis that we do for other small animals".

From the perspective of hunting *individual* animals in relation to their body size, insects can generally be considered low ranked food sources as the return rates (energy gained minus energy costs from searching, handling, and processing) of large animals is higher (Madsen and Schmitt 1998). Madsen and Schmitt (1998) reason that an increased abundance in such lower ranked resources (e.g. in patches, clumps, and swarms where the collected/hunted unit is no longer an individual) and/or the introduction of mass collecting technology increases their return rates to an equally high or higher level than that of large animals collected individually. This theory is based on evidence from Lakeside Cave (Great Salt Lake, Utah, USA) where excavations and analyses of faecal remains covering the past 5,000 years indicate a switch from mammal to locust consumption when abundant (Madsen and Schmitt 1998). Following outbreaks, vast numbers of locusts (e.g. *Melanoplus sanguinipes*) drowned in the lake and washed up on the shores in windrows numerating to tens-of-thousands, making collection easy. The lake served as a natural collecting mechanism enabling mass collection. The return rates of locusts increased, at least in the direct vicinity of the lake, even with unchanged abundance in large animals. This caused a shift in focus from large, high ranked animals to small, normally low ranked animals (Madsen and Schmitt 1998). Such locust outbreaks were

no annual phenomena, but occurred twice every decade since the 1850s. They have been relatively common yet unpredictable (Madsen and Kirkman 1988).

In an anthropological study of indigenous populations Steward (1938 in Dyson-Hudson and Alden Smith 1978) indicates though that in this area small game, i.e. rodents, lizards, and insects, probably already contributed more to the diet than large animals due to the latter's scarcity and lack of occurrence in herds. Families exchanged useful information on locations of those resources including locust concentrations.

Seemingly in line with Madsen and Schmitt's (1998) findings and theory, the Bogong moth (*Agrotis infusa*) was the most reliable summer food source for some Australian Aborigine tribes and preferred over other food sources available at the same time. As Bogong moths naturally congregate by the masses in crevices in rocks and in recesses between rocks in the Australian Alps, large quantities could easily be collected (Flood 1980). The traditional Aborigine diet was furthermore overall low in fat (O'Dea et al. 1991, Reim 1962, Yen 2005) and fat-rich grubs and moths such as the Bogong moth may have been important nutritionally (Flood 1980). These moths also played a role in socio-cultural aspects: ceremonial life, marriage, and trade. Their collectors are now referred to as moth hunters (Flood 1980).

McGrew (2001) points to such importance of energetic efficiency and suggests that a large-brained forager could be able to deal with this issue by means of intelligent strategies making the gathering/collecting of animal protein as productive as the strategies of hunting. Such strategies may have very well included, as it was for various other resources (Barker 2009), besides the construction of tools, the manipulation of the environment related to insect procurement (McGrew 2001, Sutton 1990b).

This invites us to review findings of facilitating edible insect procurement beyond the construction of tools and reinterpret environmental manipulation reported in the archaeological and anthropological literature. As examples we use the following semi-cultivated edible insects: eggs of aquatic Hemiptera in Mexico, palm weevil larvae in the Amazon Basin, tropical Africa, and New Guinea, and arboreal, foliage consuming caterpillars in sub-Saharan Africa. The prime source of literature was the entomophagy bibliography of the authors containing more than 1500 publications (the majority peer reviewed) of which more than 600 publications deal specifically with edible insects and the human practice of entomophagy – eating insects. The aforementioned examples were chosen based on the amount of information available. Additional information on these examples was collected via internet search engines. This review excludes edible insects that are primarily semi-cultivated or domesticated for their products such as bees, wasps, and silkworms.

Water management for eggs of aquatic Hemiptera in Mexico

When comparing food procurement of ancient civilizations it is remarkable that only pre-Hispanic Mesoamerica achieved organizational complexity and high population density without a domesticated herbivore. Non-agricultural high-protein resources are expected to have been used intensively (Parsons 2010). These are particularly aquatic fauna and flora of which the eggs of aquatic true bugs (Hemiptera: Neopomorpha) (*ahuaquhtle*) are regarded of specific importance (Parsons 2010). A delicacy among the Aztecs, the eggs were called 'Mexican caviar' by the Spanish conquistadores (Bachstetz and Aragon 1945).

The ponds and marshes of the valleys and basins of the Mexican Mesa Central were of such importance to subsistence, energetically, nutritionally, and economically, that their contribution holds comparison with agriculture. Fish and birds were caught, algae collected, and insects netted. These activities were conducted by large numbers of people (Parsons 2010). The *ahuauhtle* (the adults are called *axayacatl*), measuring about 0.5 to 1.0 mm, are the eggs of particularly *Krizousacorixa* spp., *Corisella* spp., and *Corixa* spp. (Hemiptera: Nepomorpha: Corixidae) and *Notonecta* spp. (Hemiptera: Nepomorpha: Notonectidae) (Bachstez and Aragon 1945, Bergier 1941, Parsons 2010). They are deposited on aquatic vegetation throughout the year (Parsons 2010); according to Bachstez and Aragon (1945) from June to October in Lake Texcoco (located in that same area). Insects and their products (and algae) were collected in higher amounts than other aquatic resources while their nutritional value exceeds that of those other food resources. Parsons (2010) roughly estimates an insect harvest of 10 kg and insect egg harvest of 5 kg every two weeks per ha reaching an annual insect/insect-egg harvest of 3,900 metric tonnes for an assumed available lake surface area of 10,000 ha. This is an impressive figure in regard of the nutritional value: insects are generally good sources of protein, minerals, vitamins, fibre, fatty acids, and essential amino acids (Bukkens 2005) and *ahuauhtle* (eggs) contains 0.20% Ca, 0.33% Fe, 5.7% fat, 77% protein, and 0.73% phosphor (Bachstez and Aragon 1945). Bergier (1941) claims from examining samples from the 1850s that only the egg shells would have been collected. That would be rather unlikely although it is certainly possible that part of the eggs had already hatched.

The intensive exploitation of *ahuauhtle* is facilitated, referred to as semi-cultivation (Ramos-Elorduy 1997, Parsons 2010). The techniques employed reflect indigenous knowledge of the oviposition behaviour of aquatic Hemiptera. Locals made bundles of twigs of grasses or reeds, e.g. *Carex* (Guérin-Méneville 1858), bound them together with a rope, and placed many of these bundles on the bottom of the lakes at certain distances from each other. To keep them in place, a stone was tied to the rope or the bundles were simply pushed in the lake bottom (Guérin-Méneville 1858, Parsons 2010, Ramos-Elorduy 1993). More recently, long U-shaped grass/reed bundles were placed at one meter intervals. The female Hemiptera lay their eggs on these bundles which can be harvested shortly after. This facilitation works best in still and shallow water which requires skills in engineering and management practices of the lakes, skills that may well have been present in ancient times (Murrell 2006, Parsons 2010). A map of Lake Texcoco from 1550 suggests a division of activities to two sides of the lake divided by, possibly, a reed barrier. The nature of the activities depicted suggests that the shallow side was used for insect farming, as it was more suitable for netting insects and exploiting eggs of aquatic Hemiptera (Parsons 2010).

Host plant manipulation for palm weevil larvae in the Amazon Basin, tropical Africa, and New Guinea

The larvae of several species of palm weevils (Coleoptera: Curculionidae) are edible including the Asian *Rhynchophorus ferrugineus* (Mercer 1997) and *R. bilineatus* (Meyer-Rochow 1973), the African *R. phoenicis* (Banjo et al. 2006, Malaise 1997, Tchibozo et al. 2005), and the American *R. palmarum*, *Rhinostomus barbirostris*, *Dynamis borassi* and *Metamasius* sp.

(Cerde et al. 2001). While the soft-bodied larvae are favoured, Townsend (1973) reports that adults may also be eaten. Facilitation of palm weevil larvae procurement, also referred to as semi-cultivation, is practiced by various indigenous groups and well reported in Asia (Mercer 1997, Tommaseo-Ponzetta and Paoletti 1997, Townsend 1973), Africa (Dounias 2003), and South America (Cerde et al. 2001, Choo 2008, Choo et al. 2009, Dufour 1987, Onore 2005, Ruddle 1973). The detailed work of Choo et al. (2009) is highlighted here who report that based on their Traditional Ecological Knowledge of palm weevils (*Rhynchophorus palmarum* and *Rhinostomus barbirostris*) the Jotĩ can exercise controlled supply by deliberately felling palm trees for harvesting the larvae.

Aforementioned weevils differ in their ovipositing biology. Whereas *R. palmarum* adults are attracted to exposed inner palm tissue of felled or naturally fallen palms where they feed, mate, and oviposit, *Rh. barbirostris* females oviposit on the intact surface of the trunk and are thus able to use the entire trunk length for oviposition. When harvesting 1 – 3 months later, larvae of *Rh. barbirostris* are then more abundant than those of *R. palmarum*. With the flavour and fat content of these weevil larvae differing, the Jotĩ, depending on personal preferences, manipulate species abundance in favour of *R. palmarum* which arrives at the trunks earlier than *Rh. barbirostris*. This requires an increased availability of softer inner tissue which is provided by making deep cuts in the trunk (Choo et al. 2009). Paraguayan Indians cut the trunk in smaller pieces for the same goal (DeFoliart 1993) while the Korowai of Western Papua make holes in the trunk to ease weevil access (Abd-Aziz 2002). Palm weevil mating behaviour is gregarious which easily results in 100 larvae and more in a single trunk. As adult weevils only oviposit in unworked portions of trees that are cut to harvest starch (Townsend 2003), deliberately felling trees for harvesting of larvae would thus increase their numbers.

A variety of palm trees is used but in the Amazon Basin the *moriche* palm (*Mauritzia flexuosa*) renders highest larval density (Cerde et al. 2001). In Papua New Guinea sago palm species (*Metroxylon* spp.) that render lower amounts of starch are rather felled for palm weevil larvae (*Rhynchophorus ferrugineus* and *R. bilineatus* [Ellen 2006]) semi-cultivation presumably for the matter of energetic efficiency (Townsend 1973).

The products provided by palm trees, e.g. fruit, starch, building material, and palm weevil larvae, are of general importance to many indigenous groups, and were of importance in pre-history (Abd-Aziz 2002, Barker 2009, McKillop 1996). Manipulation of the forest, and palm tree distribution and abundance specifically, has occurred throughout history (Barker 2009). The Nukak are a South American hunting and gathering group that seem to manage or manipulate various plant species including palm trees. Politis (1996) suggests that this observation of manipulation is due to the Nukak being a mobile group that frequent previous camp sites. High concentrations of seeds are left each time thus favouring those species. In a similar way Hurtado et al. (1985) found palm trees to occur in a somewhat uniform distribution in the forest inhabited by Aché Indians. Explanations of such manipulation are primarily in light of starch which may be a staple. Though the larvae are an acclaimed delicacy for which even large feasts are held and in preparation of it numerous trees are deliberately felled, they are considered a by-product by Abd-Aziz (2002). Dufour (1987) refers to the larvae as a by-product when the Tatuyo Indians fell trees for harvesting the fruits, and refers to them as “cultivated” only when trees are felled specifically for palm weevil larvae facilitation. In Africa, *R. phoenicis* larvae are harvested from trunks that have been cut down to extract sap which

is used for making palm wine. Adult weevils are attracted to the rotting trunk and invade it (Dounias 2003, Fasoranti and Ajiboye 1993). Since after harvesting the fruits or extracting the sap the trunks are left and visited at a later date to harvest the larvae, it is possible that a dual production of both fruits and larvae is intended (Townsend 1973). Bodenheimer (1951) therefore refers to the larvae as “a second crop”. Johnson (1983) made an assessment of various palm species in terms of the number of products they provide (multipurpose palms) but did not include palm weevil larvae. More careful consideration is required when classifying weevil larvae as a by-product. We thus argue that the role of these larvae in palm tree distribution and abundance manipulation may be underestimated.

Archaeological evidence suggests that the strategies and technologies observed today could very well be similar to those practiced several thousands of years ago, making rainforest exploitation more efficient and effective (Barker 2009). As described, the Jotí fell palm trees deliberately, and they do so at a chosen location at a chosen time thereby controlling the place and time for harvesting the larvae (Choo et al. 2009). The Yapú employ this as a strategy in long hunting and fishing trips that lead them away from their village (Dufour 1987). By felling palm trees beforehand the weevil larvae are a more predictable resource than by natural collection as search time is decreased (Choo et al. 2009). Moreover, during periods of game and fish scarcity, the larvae are expected to become a more important source of protein and fat (Bukkens 2005, Collins and Novotny 1991, Milton 1984). In both cases, the availability of concentrated patches and/or a uniform distribution of palm trees, as seems to occur among the Nukak (Politis 1996) and Aché Indians (Hurtado et al. 1985) respectively, would then ‘facilitate the facilitation’ of the palm weevil larvae harvest. Indications exist in the archaeological evidence of the management and protection of at least sago palms while interventions such as forest clearance, planting, and plant-tending were common throughout history (Barker 2009).

Host plant manipulation for arboreal, foliage consuming caterpillars in sub-Saharan Africa

The far majority of edible caterpillars (Lepidoptera) reported in sub-Saharan Africa are leaf feeders (Malaisse and Parent 1980, Silow 1976). Latham (2003) reports from the Bas Congo the introduction of caterpillars *Imbrasia obscura*, *Imbrasia ertli*, and *Cirina forda* in selected areas. People collect or buy young caterpillars and place them in trees near their home and on their land. Similarly, Malaisse (1997) mentions the translocation of a branch with many caterpillars to the same tree species closer to one’s village. When the caterpillars reach maturity (final larval stage and largest size) they are collected to eat (Latham 2003). In this system people can keep an eye on their caterpillars thus improve the timing of collection: only those caterpillars large enough will be taken. Furthermore, some of the caterpillars are allowed to pupate below the trees (Latham 2003). Provided that during this stage the area is protected from fire, the resulting moths of certain species will lay eggs on the tree below which they pupated or on nearby trees (Latham 2003). A farmer followed this system of facilitation by introducing *Cirina forda* caterpillars to an area of savannah abundant in the tree species *Crossoteryx febrifuga* and could harvest caterpillars on a regular basis since this introduction

(Latham 2003).

Silow (1976) mentions a traditional tendency to domesticate caterpillars but does not provide details. Though, he does explain that those caterpillars that feed on more than one host tree differ in flavour and thus may be translocated by epicures from one host tree to another to improve the caterpillars' flavour. It would indeed be most convenient to translocate them closer to home.

Four forms of traditional facilitation through habitat management, all of which increase caterpillar yields, whether or not employed in combination, are reported. First, host tree planting in predetermined areas attracts moths (Latham 1999) which oviposit eggs on the foliage. Second, shifting cultivation stimulates re-growth of caterpillar host trees. Re-growth of miombo woodland (*Brachystegia-Julbernardia*) on land previously under cultivation in Zambia is shown to be dominated by *Julbernardia paniculata* (the main host tree of e.g. *Gonimbrasia (Imbrasia) zambesina* and *Gynanisa maja* caterpillars) in comparison to old-growth woodland such as forest reserves (Chidumayo and Mbata 2002). Third, applying correct fire regimes protects host trees, and avoids destruction of moth eggs on foliage and the pupae underground (Gade 1985, Leleup and Daems 1969, Mbata et al. 2002). Fourth, not cutting down host trees preserves caterpillar 'breeding sites' (Takeda 1990).

In addition, traditional regulations may involve the monitoring of caterpillar development and abundance. Mbata et al. (2002) provide a detailed account of such activities on caterpillars (e.g. *Gynanisa maja* and *Gonimbrasia zambesina*) in the Kopa area in Zambia which include the identification of high density moth egg sites, the appearance of first instar larvae, and the appearance of final instar larvae (the only instar allowed to be collected). The monitoring is well organised and continuous throughout the season whereby all levels of society are involved, rituals are performed, and ceremonies held. When necessary, the observations result in temporal restrictions in harvesting (Mbata et al. 2002).

Caterpillars are a widely consumed resource, particularly in Africa, with over 40 edible species reported for Congo DR alone (Latham 2003, Munyuli Bin Mushambanyi 2000). They are a popular food, harvested in large quantities by numerous people, and in lean times are of specific importance. The facilitation techniques employed are straightforward and stem from indigenous knowledge of caterpillar biology (e.g. Mbata et al. 2002). These types of strategies – plant manipulation, use of fire – have been employed in antiquity throughout the world (Barker 2009). It seems highly unlikely that forms of landscape management in support of caterpillar exploitation is a recent development.

Discussion

Revaluating the importance of insects as food in history

Combining archaeological evidence from coprolites and excavations with ethnographic studies of indigenous peoples suggests human entomophagy – eating insects – to be an ancient habit (McGrew 2001, Sutton 1995). A large variety of plants and animals were manipulated in antiquity (Barker 2009), but edible insects have so far received little attention concerning this matter. Barker (Barker 2009) points in his extended review to zeitgeist, methodologies applied, and techniques available: "... the questions being asked [by an archaeologist] affect the kind of methodologies used in the field, prioritizing the recovery of certain classes of in-

formation over others. New theories will pose new questions about past societies, and these questions will result not just in reinterpretations of existing data but also in new kinds of data being collected in the field or the laboratory. New data, whether searched for explicitly or thrown up by unexpected discoveries, feed back into theoretical frameworks.”

The general Western cultural and historical bias towards male activities and hunting of large game is gradually dissolving, the data providing a fuller picture of the various modes of subsistence. Particularly Sutton (1990a, 1990b, 1995) has previously made a case for (edible) insects, arguing the bias against this one category of ‘small game’. The retrieval, identification, and interpretation of insect remains is problematic, Sutton (1995) clarifies practical archaeological issues. The reinterpretations made here are problematic, particularly for the cases of palm weevil larvae and caterpillars, as it is difficult, if not impossible, to provide direct evidence of the manipulations to be also insect related. Thus the majority of hypotheses concerning such landscape manipulations must come forth from reinterpretations fed by anthropological findings of insect use, and if available, supported by ancient scripture (as in the case of *ahuauhtle*) and ancient depictions (such as Egyptian hieroglyphs of honey extraction). The cases described here can contribute to new archaeological theories on insect use and its role, leading to reinterpretations of existing evidence (e.g., fire, tree distribution, tools), the collection of new data, and a feedback into theoretical frameworks.

The path to domestication

It was not a sudden event that transformed foragers into farmers, nor was it a discovery or invention, but a long process, a transition, spanning several thousands of years. Landscape manipulations of the kind described above are steppingstones in the development of domestication/agriculture and have their origin most likely in a need to increase resource predictability and availability (Barker 2009).

The development of domestication/agriculture is considered a revolution with regard to the effect it had on human societies (Barker 2009) though it has its negative effects as well e.g. through rapid population growth (Diamond 2002). DeFoliart (1999) states: “Considering their efficiency in converting plant biomass to animal biomass, the failure to domesticate edible insects on any significant scale (except as a by-product of silk and honey production [and carmine production for e.g. cloth and food colouring]) may have been a greater calamity in the global development of agriculture than we yet realize.” Research is and has been carried out to semi-cultivate and domesticate edible insects and to optimize existing techniques, e.g.: bamboo worm (*Omphisa fuscidentalis*) (Kayikananta 2000), Mopane worm (*Imbrasia belina*) (Gardiner 2006, Ghaly 2009), termite (Isoptera) (Ayieko et al. 2011, Farina 1991), palm weevil larvae (e.g. *Rhynchophorus palmarum* and *R. ferrugineus*) (Cerde et al. 2001, Yupa Hanboonsong personal communication), cricket (*Acheta domesticus*) (Yhoun-Aree and Viwatpanich 2005, Yupa Hanboonsong personal communication), and weaver ant (*Oecophylla smaragdina*) (Offenberg and Wiwatwitaya 2009, Krag et al. 2010). In fact, weaver ant nests (Hymenoptera: Formicidae: *Oecophylla smaragdina*), made by worker ants by ‘weaving’ together living leaves with larval silk (Hölldobler and Wilson 1990), are traditionally taken from the forest and placed in trees in gardens and plantations in Thailand as their brood is a valued source of food (Yhoun-Aree and Viwatpanich 2005, Yupa Hanboonsong personal communication). As weaver ants nest in a large variety of trees (Lim 2007) environmental

manipulations in the sense described above are however less likely to have occurred in history.

Some scholars advocate an increased use of small game (mini-livestock) (DeFoliart 1995, Hardouin 1995, Paoletti and Dreon 2005, Ramos-Elorduy 2005). Breeding edible insects has for example environmental advantages over breeding conventional livestock such as their efficiency in converting feed into biomass (Nakagaki and DeFoliart 1991) and a lower greenhouse gas production (Oonincx et al. 2010). Research is showing positive results yet issues remain to be tackled to assure successful edible insect production e.g. susceptibility to viral and bacterial diseases (Gardiner 2006, Sikorowski and Lawrence 1994). Perhaps in the beginning of next century a scholar will review these developments and adapt Graeme Barker's (2009) title: *The Agricultural Revolution in the 21st Century – Why did livestock farmers become mini-livestock farmers?*

Conclusions

Three examples were presented here that show and hypothesize about manipulations of the environment related to edible insect procurement. The exploitation of eggs of aquatic Hemiptera in Mexico, palm weevil larvae in the Amazon Basin, tropical Africa, and New Guinea, and arboreal, foliage feeding caterpillars in sub-Saharan Africa is facilitated, enhancing the predictability and availability of these edible insects, up to a form of semi-cultivation. These manipulations allow for large numbers of small animals to be collected efficiently, thereby increasing return rates. The facilitation of harvesting the edible insects dealt with in this paper can be regarded as an intelligent strategy for the procurement of, particularly, animal protein and fat. However limited the evidence presented here may be, the likeliness of insect related manipulations of the environment both in the present and the past is not that farfetched, even when it concerns but few species and insects not necessarily being the only or main reason for the manipulations.

Chapter 3

Indigenous knowledge of the edible weaver ant *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae) from the Vientiane Plain, Lao PDR

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Abstract

Of major importance in realizing the potential of edible insects as a core element in improving food security, sustainable food production, and biodiversity conservation, are developments in sustainable exploitation of wild edible insect populations and in (semi-)cultivating and farming edible insects. Such developments can draw on both western science and indigenous knowledge. *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae), of which particularly the queen brood is commonly consumed in Thailand and the Lao PDR, is believed to have the potential to act as flagship/umbrella species in forest conservation and management, to be incorporated simultaneously as biological control agent and direct source of human food in agroforestry practices, and to be (semi-)cultivated. We provide a detailed account of indigenous knowledge of *O. smaragdina* and ant brood collection practices from the Vientiane Plain, Lao PDR, through focus group discussions and participant observations, and then reflect on sustainability and conservation issues, and on (semi-)cultivating constraints and possibilities embedded in indigenous knowledge and ant brood collection practices.

Keywords: *Oecophylla smaragdina*, entomophagy, edible insects, indigenous knowledge, Lao PDR, semi-cultivation, agroforestry

Introduction

Edible insects are a possible core element in improving food security, sustainable food production, and biodiversity conservation, in particular in developing countries accustomed to entomophagy. Of major importance in realizing these potentials are developments in sustainable exploitation of wild edible insect populations and in (semi-)cultivating and farming edible insects (defined in van Huis et al. 2013), which can draw on both indigenous knowledge and western science (Durst and Shono 2010, van Huis et al. 2013). As such, Ramos-Elorduy (2006) points to non-traditional collection practices threatening 14 edible insect species in Hidalgo, Mexico, and the need for preservation measures; Ayieko et al. (2011) combine indigenous technological knowledge and modern technology in Kenya in developing a termite (*Macrotermes subhyllanus*) mass collection device; and Choo et al. (2009) show how Amerindians use traditional ecological knowledge in the cultivation of palm weevils (*Rhynchophorus palmarum*, *Rhinostomus barbirostris*).

The Asian weaver ant *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae) (Figure 1.3.a) is one of the most favoured edible insects in the Lao PDR (H. Barennes 2010 personal communication) and Thailand (Sribandit et al. 2008). The *O. smaragdina* use as human food concerns predominantly the brood (i.e., eggs, larvae, pupae), particularly the seasonally available large larvae and pupae – the queen brood – that develop into winged reproductive females (i.e., virgin queens) (Figure 1.3.b). Sribandit et al. (2008) showed the economic importance of *O. smaragdina* queen brood trade to rural livelihoods in Northeastern Thailand. There is every reason to assume a comparable economic importance in the neighbouring, and culturally similar, Vientiane Plain, Lao PDR, where rural people are highly dependent on non-agricultural resources, including edible insects (Meyer-Rochow et al. 2008).

Oecophylla smaragdina is an arboreal ant that builds nests by binding living leaves together. Its colonies are polydomous, i.e., consisting of multiple nests. As an aggressive and generalist predator it can control a number of pest insects of e.g. mango, cashew, and citrus (Crozier et al. 2009). Offenberg and Wiwatwitaya (2010) suggest that the traditional Thai collection practice of *O. smaragdina* does not conflict with the biological control capability of this ant species in orchards. Considering the large variety of commercially valuable *O. smaragdina* host plants, its potential in agroforestry practices is apparent (Lim 2007, Van Mele 2008). Currently though, *O. smaragdina*, of ecological importance as an abundant and territorially dominant ant species in Southeast Asia, northern Australia, and the Pacific (Crozier et al. 2009), is primarily collected in forests.

The abundance, biological control capability, and relative sessile character of *O. smaragdina* colonies (Rastogi 2007) may be particularly beneficial to further developments in the exploitation of *O. smaragdina*. This species may have the potential to act as a flagship and/or umbrella species in forest conservation and management, it may be used both as biological control agent and human food source in agroforestry practices, and it might be (semi-)cultivated to increase queen brood predictability and availability (van Huis et al. 2013, Offenberg and Wiwatwitaya 2010). Issues pertaining to sustainable use and biodiversity conservation, and biological mechanisms underlying the predictability and availability of the *O. smaragdina* queen brood resource are likely embedded in indigenous knowledge which can provide the basis for such further developments (Berkes 2008, Durst and Shono 2010, van Huis et al.

2013). The aim of this paper is therefore to provide a detailed account of indigenous knowledge of the weaver ant *O. smaragdina* from the Vientiane Plain, Lao PDR, and to reflect on sustainability and conservation issues, and on (semi-)cultivating constraints and possibilities from a biological and practical perspective.

Methods

The research was conducted in the southern half of Xaythani District, Vientiane Municipality, Lao PDR. Three rural villages, Ban Dongbang, Ban Dongmakkhai, and Ban Sanghouabor (Figure 3.1), were randomly sampled. Land use and demographic data are summarized in Table 3.1.

Our informants were expert ant brood collectors, who are well-known for their expertise in their village. Each head of village, with aid of his assistants, invited the experts to participate in the research, and most responded positively. All the expert collectors stated they are farmers growing rice in the rainy season (May – October). Some farmers irrigate their fields in the dry season (November – April) allowing for a second, yet shorter, period of rice cultivation. All collect a large variety of Non-Wood Forest Products throughout the year, including fish, edible insects, mushrooms, bamboo shoots, and fruits. Most informants supplement this with keeping a vegetable garden. Informants' ages ranged between 25 and 70, most were female. Information was compiled through focus group discussions and participant observation. Focus group discussions were held in December 2010 using a semi-structured questionnaire. To cover all topics of the questionnaire sufficiently, two group discussions were conducted in each village. At the start of each session, informed consent was received collectively on recording the discussion (digital voice recorder). Attendance ranged between 7 and 21 people. The questionnaire focussed on biological and ecological aspects of *O. smaragdina* including reproduction, castes, developmental stages, behaviour, nest characteristics, and host tree characteristics, and on harvesting methodologies and their effect on the ants. Using an A1 size print of the village and its direct surroundings, different areas were discussed in terms of *O. smaragdina* abundance and yields.

Participant observations were conducted from January to April 2011. At least four expert collectors were followed in each of the three villages independently of each other and on at least two separate ant brood collecting trips. All but two expert collectors attended the focus group discussions. Collecting trips typically lasted from morning (8 AM) until noon (12 AM), but sometimes continued into afternoon (between 2 – 4 PM). Observations were made on harvesting tools and techniques, localities, external characteristics of nests and their contents, and informal discussions were held during the trips using the aforementioned questionnaire. The focus group discussions were thus verified with individuals during these collecting trips, and additional and more detailed information could be acquired. When an opposing view was encountered, it was verified with other experts.

Lao words are written in standard international phonetic alphabet but excluding signs for intonation and taken from the Lao-English/English-Lao Dictionary (Mingbuapha and Poomsan Becker 2003). Spelling marked * is written by the first author. The most frequently used vernacular names are given.

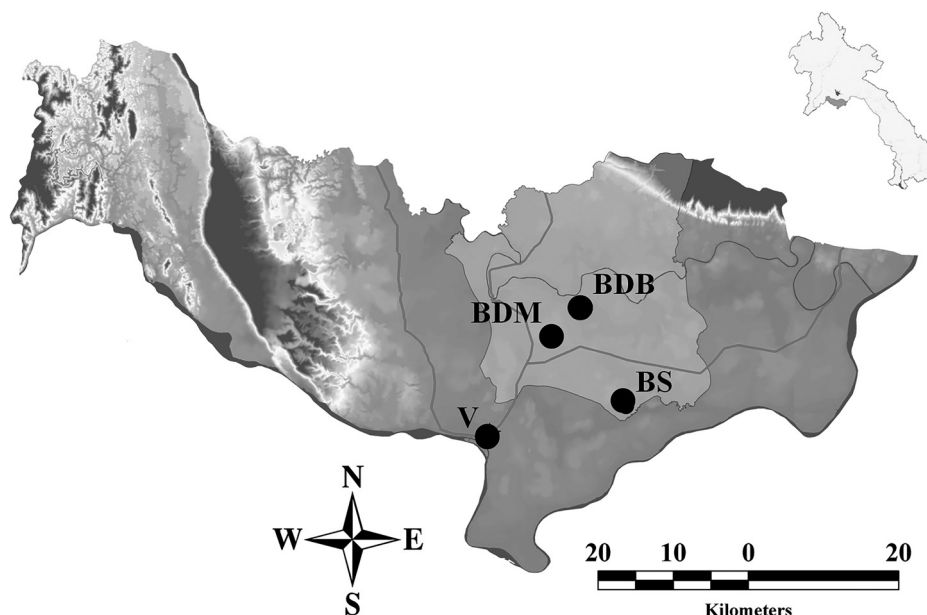


Figure 3.1. The location of the three research sites, Ban Dongbang (BDB), Ban Dongmakkhai (BDM), and Ban Sanghouabor (BS), in Xaythani District (shaded area), Vientiane Municipality, Lao PDR. V = Vientiane Capital. (Source: Soulixay Inthasone, Faculty of Environmental Sciences, National University of Laos).

Table 3.1. Land use and demographic data of the three research sites.

	Number of households ^a	Population ^a		Forest ^b (km ²)	Paddy field ^b (km ²)	Water body ^b (km ²)	Residential area ^b (km ²)	Vacant / arable land ^b (km ²)	Rice production (tonne) ^c	
		Male	Female						Rainy season / head	Dry season / head
Ban Dongbang	163	560	433	1.38	3.13	0.05	0.37	0.00	0.47	0.05
Ban Dongmakkhai	314	861	823	6.51	5.91	0.19	0.64	0.76	0.89	0.03
Ban Sanghouabor	157	537	415	4.81	5.10	0.40	0.19	0.29	0.40	0.02

^a National Statistics Center (2009)

^b Calculated by Mr Soulixay Inthasone, Faculty of Environmental Sciences, National University of Laos, using ArcMap

^c Office of Agriculture of Xaythani District (2007-2008)

Results

Tools and techniques

To collect weaver ant larvae and pupae, a bamboo pole of about 4 – 6 m long is used with a sharpened ending, and behind this tip a strong bag or a bamboo basket is attached with a string. The tip is pierced through a nest and, by shaking the bamboo pole and/or hitting it



Figure 3.2. Collecting the ant brood. (Pictures: Joost Van Itterbeeck)

with flat hand, the nest contents (all castes and developmental stages) falls into the bag/basket which hangs beneath the nest (Figure 3.2). The collection of queen brood thus implies the collection of worker ants and small-sized brood which develops into minor workers, major workers, and males; these are also consumed and marketed. Narrow trees and branches may be cut down to facilitate collection.

Two methods were observed to separate ant brood from adult ants. The dry method (applied during a collecting trip) involves emptying the bag/basket on a plate, removing debris, shaking the plate with upward movements thereby separating light-weighting ants from brood, and blowing the ants off the plate (Figure 3.3.a-d). The wet method (applied at home) involves emptying the bag/basket in a bowl of water, removing debris, swirling a piece of cloth through the water, and removing attached ants (Figure 3.3.e). Queen brood may be separated further from small-sized brood using a colander (Figure 3.3.f). All the brood is consumed and marketed, and a few adult workers and virgin queens may be used as condiment. Adult males are rarely consumed for they are said to taste foul. Worker ants may also be used to cure dizziness by crushing them between fingers and inhaling the released smell. One kg of queen brood is for sale in the markets for up to 12USD.

A variety of measures is used, whether or not in combination, to reduce nuisance from the aggressive ants. First, collectors wear long trousers and long-sleeved shirts to cover bare skin. Second, wearing rubber boots avoids ants to crawl up one's leg as is said that ants have difficulties to adhere to them, especially when heated by the sun or when covered with fine sand or tapioca starch. Third, whilst walking, the bamboo pole is hit regularly with e.g. flat hand to make ants that crawl out of the bag/basket and onto the pole fall to the ground.

Fourth, part of the pole is covered with fine sand or tapioca starch to avoid ants crawling along it. Fifth, tapioca starch is sprinkled on the ants inside the bag/basket and during the dry method separation which is said to decrease their aggressiveness (in addition to the said difficulty of the ants to carry away brood between mandibles). Van Mele et al. (2009) have recently reviewed nuisance reducing measures. Tools and techniques are similar in Northeast Thailand (Sribandit et al. 2008).



Figure 3.3.a. A basket is emptied of brood on a plate. (Picture: Joost Van Itterbeeck)

Figure 3.3.b. Brood is separated from light-weighting ants by shaking the plate with upward movements. (Picture: Joost Van Itterbeeck)

Figure 3.3.c. Ants are blown off the plate. (Picture: Joost Van Itterbeeck)

Figure 3.3.d. The brood is carried along. (Picture: Joost Van Itterbeeck)



Figure 3.3.e. A piece of cloth is swirled through water to separate brood from ants. (Picture: Joost Van Itterbeeck)
Figure 3.3.f. Queen brood is separated from small-sized brood by using a colander. (Picture: Joost Van Itterbeeck)

Perception on caste and life cycle

The prime vernacular name of the Asian weaver ant, *mot som* (*mot* = ant, *som* = sour), comes forth from its use as food (worker ants taste sour). The secondary name, *mot deeng* (*deeng* = red), is derived from appearance (worker ants are coloured red).

The expert collectors indicate that major workers guard the nests to protect its contents be-

cause many run out of a nest when disturbed and bite fiercely while releasing a liquid, “pee” (this is formic acid). They construct nests by pulling leaves together e.g. through forming a living chain, thereafter held in place by a white substance (“pee” as above but in this case larval silk). Major workers collect food as the collectors see them carry insects, and various insect remains are found among the collected brood.

Because of these behaviours, our respondents believe that major workers are mothers that lay eggs and care for their brood. They regard each ant an individual that cooperates with any other *mot som* – “all are family” – and compare ants to higher organisms, e.g. humans and their protective behaviour towards their young. Major workers are hence specified as *mee mot som* or *mee mot deeng* (*mee* = mother, and refers to ‘female’). They may also be called *mee phan** (*phan** refers to sexual intercourse and fertilization) as several collectors indicate that only after an act of sexual intercourse ants can lay eggs. Sexual intercourse is assumed to occur inside a nest; undisturbed behaviour inside the nests cannot be witnessed by the respondents and they acknowledge it remains open to speculation.

Consequently, identification of a father shows less consensus. Some respondents do not know of the existence of a father. Others claim that major workers with a larger gaster (the hind body part) are mothers, and those with smaller gaster are fathers. Minor workers (no specific vernacular name), which are rarely seen outside a nest, are believed to be young ants due to their smaller body size than major workers.

The informants refer to the brood as ant ‘eggs’, *kai mot som* (*kai* = egg). True eggs are specified as *kai nɔɔi nɔɔi* (*nɔɔi nɔɔi* = very small). When collecting ant brood, these are seen sticking on the leaves in the nest interior and in chambers made from “pee” (larval silk). Eggs are indicated to become larvae, *kai fa** (*fa** refers to small size). Some of them become large larvae, *kai khok** or *kai teung** (*khok** and *teung** refer to large size) (Figure 1.3.b, and see seasonality below). The informants explain the difference in size of the larvae by the amount of food and water received.

Larvae then develop into pupae, specified as *naang*, meaning young lady, one which is not married yet (as used for human girls). Small pupae are called *kai naang* or *naang fa**, large, queen destined pupae are called *mee naang*. Small pupae change their white colour into either red or black. The former develop into major workers (*mee mot som*, *mee mot deeng*) and minor workers, the latter into winged black ants, *mee dam* (*dam* = black) (these are males). Male pupal wing formation is also recognized. Large larvae (*kai khok**, *kai teung**) and large pupae (*mee naang*) become virgin queens, *mee peng** (*peng** refers to large size). These pupae change their white colour first into yellow.

An explanation for the existence of either of the two winged forms (*mee dam* and *mee peng**) is lacking. Our informants merely state that “it is nature” to produce them. Winged forms are known to leave their nest of origin by flight. Prior to their flight, the virgin queens are indicated to change their yellow colour to green and strengthen their wings. No collector could provide neither a reason for them leaving their nest nor indicate where they fly to. They assume that these will become food for other animals and thus do not regard them to have long life. Very few collectors know of a big-bottomed ant, lacking wings but able to crawl (this is the egg-laying queen). She has been called *hua-naa nyai* (*hua-naa* = boss, *nyai* = big) by one respondent on our only occasion encountering her during participant observation. A similar description is given to a caterpillar (*Liphyra brassolis brassolis* Westwood Lepidoptera:

Lycaenidae) occasionally found live in large nests though it is not recognized as such. They say it looks like a pillow and is hence called *มอญ mot deeng* (มอญ = pillow). Pictures of both egg-laying queen and *L. brassolis brassolis* butterflies were shown to Ban Sanghouabor informants but none recognized one or the other. Since rarely encountered, major workers are believed to produce neither *hua-naa nyai* nor *มอญ mot deeng* often. Their existence and function remained unexplained, and they are, rather jokingly, referred to as “big boss”, drawing comparison to human social organization. When found, they may be consumed. Total development time of the castes could not be clearly indicated by our respondents.

When and where to collect?

Seasonality and the role of water – Queen brood availability determines the ant brood collecting period which peaks in March. Worker brood production is known to occur throughout the year. However, the collectors observe an increase in major worker numbers in January and February, prior to the production of virgin queens in February, March, and April, and males in May. Overlap occurs. They explain the increase in workers by the need of a lot of mothers to produce many large ‘eggs’ (queen brood).

Local differences in availability are indicated: in the vicinity of water (e.g., a pond) queen brood production is sooner than elsewhere as queen brood is believed to drink a lot of water to become large. These areas are frequented from February onwards. Further, in February/March periods of rain are expected which is said to accelerate larval growth.

The onset of frequent collecting trips is determined, in order of importance, by (1) the seasonal cycle (attention is given to rainfall) and (2) preliminary yields of few nests. Decisions are made individually and with companions, information may be shared. The end of the season is determined by visual judgment of queen brood yield and is independent of the type of forest and its distance from a water source.

Ant ‘eggs’ is an open-access resource which can be collected outside one’s own village border. Non-fixed rotation schemes are applied.

Forest and tree characteristics – The majority of collection occurs along forest edges and paths mainly due to the ease of manoeuvring with a long bamboo stick. Many nests can be found away from these zones but are generally only harvested from or sought for when (1) a large nest is seen from the edge/path, (2) promising trees are seen from the edge/path (e.g., the evergreen *Syzygium cumini* Skeels Myrtaceae (*ton wa**)), (3) the collector is not satisfied with the number of nests found along the edge/path, and (4) trees are scattered. Trees in dry dipterocarp forest (*kho**) in the research sites are particularly scattered, edges and paths may be disregarded altogether. Dry evergreen forest (*dong**) is said to be less suitable for nesting because it is too dense. Collectors do ‘island hopping’: nests are found in trees amid paddy fields, usually growing on termite hills.

The collectors state that leaf characteristics are of influence on ant nest site selection. They relate leaf thickness and age to moisture contents of the leaf. The leaves are believed to provide water for the ants. The moisture is also said to cool down the interior climate of the nest. The respondents believe that in nests of old or very thin leaves the temperature becomes too high. Very thick leaves are said to be unsuitable for nest building as the ants cannot bend these.

Forest sections abundant in *Crematogaster (Physocrema) aurita*, called *mot dam*, are indi-

cated to be scarce in *O. smaragdina* as these ants dominate weaver ants in direct fights. Such areas are said to be less frequented. *Crematogaster (Paracrema)* sp., called *mot hai**, is said to be able to co-inhabit a weaver ant occupied tree but only when each species uses different branches for food procurement. Such co-inhabitation is said to occur rarely.

Which nests to collect from?

Collectors use three prime criteria on the external appearance of nests to indicate relative high queen brood yield: (1) nest size, (2) leaf colour, and (3) external visibility of larval silk. Relative refers to the ratio of large-sized brood (i.e., late queen larvae instars and queen pupae) to small-sized brood (i.e., worker and male brood, and early queen larvae instars), and the positive relation between nest size and nest contents.

Although all nests are said to contain queen brood, it is acknowledged some do not (“built only to live in”). Large nests (estimated > 30 cm diameter) are aimed for. However, it is known that medium- and small-sized nests are more abundant. While medium nests (estimated 10 – 20 cm diameter) are also targeted, small nests (<10 cm diameter) are generally ignored due to a very small yield. Relative queen brood yield is expected to be high from any nest with partly green and partly dried leafs and plenty of larval silk visible on the nest exterior.

Collectors mention two additional criteria: (1) bending of the supporting branch indicates a heavy nest thus with high relative queen brood contents and (2) nests easily shaken by wind are light thus contain little small-sized brood only or no brood at all.

Apart from the small nests, few along the route taken may escape (severe) disturbance: (1) recently disturbed nests as they contain only small-sized brood (but only very recent disturbance is recognized easily), (2) nests that are too high up in the tree (but sometimes two bamboo poles are attached to each other to reach them), (3) unnoticed nests (e.g. ‘hidden’ behind branches and leafs), (4) nests that are not emptied when probing yields only small-sized brood (nests are then allowed to be repaired such that the remaining brood can develop further), (5) nests that are ignored when others in the same tree lack queen brood, and (6) nests close to a large nest containing queen brood (not expected to contain such brood). Correct predictions of nest contents are difficult to make, therefore the collectors usually refrain from doing so.

Nests in home gardens

When separating brood from ants at home (wet method), ants are left at the base of a tree in the garden. These are said to build nests in the tree and produce brood. When the tree is already occupied by weaver ants, the ants are believed to join forces. Local collectors know that ants also naturally inhabit trees close to human settlements. Some collectors do not collect ant ‘eggs’ from these nests, others do so once or twice between February and April.

Discussion

Ecological implications

The collection practices applied by our informants seem to assure colony survival for two reasons. First, the queen, who is the principal egg-layer (Hölldobler and Wilson 1990), is

very rarely, yet unintentionally, removed and she is unknown to most collectors (colonies that produce sexual forms have a single queen [Chapter 4]). This is because her nest is small and located near the top of a tree (Chapter 4). Small nests are generally ignored – a positive relation exists between nest volume and contents (Gupta 1968) – and may remain undetected when near the top of a tree. Removal of the queen would result in the death of the colony since *Oecophylla* spp. workers can only produce males (Hölldobler and Wilson 1983) and queen turnover – the acceptance of a new queen (Heinze and Keller 2000) – does not seem to occur in *O. smaragdina* (Chapter 5).

Second, the participant observations made on collection practices suggest that a large fraction of a colony remains undisturbed, although most nests are easily detected and the average tree height allows most nests to be reached. This is intentional as small nests yield small amounts of queen brood (or none at all) and collectors refrain from removing large amounts of worker ants, ‘mothers’. Therefore, it is likely that the fraction of workers and new worker brood removed by collection does not impede foraging nor defence, and thus does not impede the maintenance of an individual colony and its role in forest ecology.

The maintenance and survival of a population of *O. smaragdina* colonies on the other hand greatly relies on the reproductive castes – not required for the survival of parent colonies – as an ant colony could be viewed as a single reproductive organism, a ‘superorganism’, which produces daughter colonies (Hölldobler and Wilson 2009). In ants sexual intercourse occurs between males and virgin queens which conduct a mating flight (nuptial flight) after which the males quickly die and the now fertilized females attempt to establish new colonies (thus to become egg-laying queens). Colony founding is the most vulnerable phase in an ant colony’s life; it often fails due to, for example, predation (Hölldobler and Wilson 1990). Thus, a colony should maximize its reproductive success by producing an optimal number of the reproductive castes.

Our informants yet point to a decrease in queen brood availability (compared to five and ten years before the time of investigation) and explain it by an increase in number of collectors and a decrease in forest area. An increase in number of collectors likely causes more frequent brood removal from a single colony, which may obstruct the development of queen brood into mature brood (final instar larvae and pupae). However, the effect of queen brood removal on subsequent brood production remains largely unknown (see Chapter 6). A decrease in forest area indicates loss of suitable nesting sites and thus a decrease in queen brood availability via a decrease in colony abundance. This, in turn, likely causes more frequent brood removal from a single colony. The collectors nevertheless do not deem a conservation strategy for this resource necessary. A collector stated: “the ants always have been and always will be present [as merely relatively few worker ants (‘mothers’ according to the collectors) are removed during collection]”. *Oecophylla smaragdina* then currently does not act as a flagship/umbrella species in indigenous practices and its potential may not be strong in the Vientiane Plain, Lao PDR.

Oecophylla smaragdina (semi-)cultivation and agroforestry

The development of *O. smaragdina* (semi-)cultivation can benefit from an input of indigenous knowledge which is relatively detailed due to the intensive use of *O. smaragdina* as human food. The collectors’ knowledge of *O. smaragdina* seasonality is consistent with

scientific findings in Wongwiggarn and Leksawasdi (1988). Yet, making correct predictions of nest contents remains challenging. The factors inducing the onset and conclusion of *O. smaragdina* queen brood production need to be discovered and understood. This understanding can in turn form the basis in developing means to predict and manipulate queen brood production of an individual colony. As such, the collectors' belief in the role of water bodies and rain (water promoting larval growth) deserves examination. Further, the collectors' knowledge of *O. smaragdina* favoured host tree species and their nesting preference near open spaces, as confirmed by Hölldobler (1983), can form the basis in developing agroforestry practices of *O. smaragdina* queen brood and tree crops in accordance with local needs. A number of additional constraints and possibilities to *O. smaragdina* (semi-)cultivation are identified from the collection practices and the indigenous knowledge embedded therein. First, *O. smaragdina* is an open-access resource. Ownership does not occur and, since not even in home gardens, (semi-)cultivating *O. smaragdina* would require a conceptual change: from open-access natural resource to privately owned (semi-)cultivated product. Second, the aggressiveness of *O. smaragdina* to humans is a possible reason why the potential of *O. smaragdina* in agroforestry is under-researched (Van Mele 2008). Some informants do not wish *O. smaragdina* to inhabit the trees in their home garden. However, measures to reduce nuisance are applied by collectors, such as the use of starch powder on the collecting pole preventing ants crawling along it and attacking the collector (see also Sribandit et al. 2008, Van Mele et al. 2009). Such measures can be improved as they have been in beekeeping. Third, the land area owned by a family may not be sufficiently large to make *O. smaragdina* (semi-)cultivation feasible. Agroforestry practices in which *O. smaragdina* (semi-)cultivation is embedded are economically likely most beneficial. Last, due to the respondents' belief that each major worker ant is an individual able to reproduce and cooperate with any other major worker, an input from modern science on ant biology and ecology including queen biology and the concept of individual colonies is required either through formal education systems or training and workshop programs.

Conclusions

The indigenous knowledge of *O. smaragdina* from the Vientiane Plain, Lao PDR, is relatively detailed due to its intensive use as human food. Ant brood collection practices seem to assure colony survival as the gravid queen is rarely removed and unknown to most ant brood collectors, and the fraction of workers and worker brood removed does not likely impede the maintenance and ecological role of a colony. Yet, with decreasing forest area the population of *O. smaragdina* colonies seems in decline. Whether the ant brood collection itself negatively affects subsequent queen brood production and the number of newly founded colonies remains uncertain. The indigenous knowledge and practices provide scope for further developments in *O. smaragdina* exploitation, notably in the (semi-)cultivation of *O. smaragdina* incorporated in agroforestry practices whereby the predictability and availability of queen brood is increased.

Chapter 4

Location and external characteristics of the *Oecophylla smaragdina* queen nest

Submitted in slightly modified form as Van Itterbeeck J, Sivongxay N, Praxaysombath B, van Huis A (2014) Location and external characteristics of the *Oecophylla smaragdina* queen nest.

Abstract

Oecophylla smaragdina is a cheap and ecologically sound alternative to chemical control of pests in various tree crops while its brood developing into virgin queens is also an important source of human food and income in Thailand and the Lao PDR. Relocating established colonies from natural areas is one proposed method to introduce *O. smaragdina* at designated sites for either or both purposes. Long-term inhabitation and production of queen brood require the inclusion of the gravid queen. We investigated the location of the queen nest and its external characteristics in the Lao PDR to facilitate the relocation. Mature colonies were monogynous. The tree housing the queen could be most easily identified by taking into account both tree height and number of nests in the tree: the queen tree housed more nests than any non-queen tree that was taller than or equally tall as the queen tree in a given colony, and the queen tree was taller than any non-queen tree that housed more nests than the queen tree in a given colony. The queen nest was located near the top of the queen tree, and was very small. It could be distinguished from non-queen nests of similar size by the higher number of major worker ants present on its exterior. Eliciting queen evacuation, which was rapid when disturbing the queen nest, is recommended to verify the presence of the queen and facilitates her capture.

Keywords: weaver ants, edible insects, entomophagy, biological control.

Introduction

The Asian weaver ant *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae) is an arboreal and territorially dominant ant species (Crozier et al. 2009). As a generalist predator, it is a cheap and ecologically sound alternative to chemical control of pests in various tree crops including mango, cashew, and citrus (Van Mele 2008). *Oecophylla smaragdina*, but notably the queen brood (large-sized larvae and pupae developing into virgin queens), is also an important source of human food and income in Thailand and the Lao PDR (Nonaka, Boulidam et al. 2008; Sribandit et al. 2008). Offenberg and Wiwatwitaya (2010) suggest that collecting the *O. smaragdina* queen brood does not negatively affect the biological control capability of this ant species. They propose a sustainable multi-production system of tree crops and *O. smaragdina* queen brood. Considering the economic benefit of the queen brood trade (Sribandit et al. 2008), the prime interest of Thai and Lao farmers in such system may be the production of queen brood (Decha Wiwatwitaya, personal communication).

Whether aiming for a multi-production system or solely the biological control capability of *O. smaragdina*, the ant species may need to be manually introduced. Long-term inhabitation by large, mature colonies is most beneficial. This requires the inclusion of the gravid queen. Weaver ants and their gravid queen can be introduced in two ways. First, newly mated queens can be trapped and relocated (Peng et al. 2013). The maturation time of an incipient colony is long however (Vanderplank 1960), and research is ongoing to speed up early colony growth (Peng et al. 2013). Second, mature colonies can be relocated (Peng et al. 1998a, Van Mele and Cuc 2007). These colonies consist of numerous nests and inhabit several trees (Crozier et al. 2009, Peng et al. 1998a). A thorough understanding of the location of the queen nest and its external characteristics is required (Peng et al. 1998a).

Peng et al. (1998a) provide details of the location of the gravid queen(s) in mature Australian *O. smaragdina* colonies. The authors found both monogynous and polygynous colonies, but a colony always has a single queen nest. They found that the tree with the queen nest, the queen tree, houses more nests than other trees occupied by the colony, and has more ant trails connecting it to other trees occupied by the colony. Tree height, species, or position in the colony could not be used to identify the queen tree. Peng et al. (1998a) also indicate that the queen nest has a higher number of workers patrolling its entrance than other nests, it is located near the tree top, and it is of medium size.

Queen brood is commonly collected from medium-sized nests in the Lao PDR and most nests of a colony are within reach of the ant brood collectors. Yet, the gravid queen is unknown to them (Van Itterbeeck et al. 2014). Is the location and size of the queen nest in the Lao PDR different from Australia? Van Itterbeeck et al. (2014) indicate, for example, that nests located near the top of a tall tree and nests of small size are ignored by collectors. However, there may also be a behavioural mechanism that prevents the loss of the queen from a conspicuous nest, targeted by ant brood collectors. The *O. smaragdina* queen is tightly covered by worker ants, a retinue, all of which have strong adhesive abilities (Dirks and Federle 2011, Peng et al. 1998b). It is possible that this ball-shape of ants does not easily fall down during the collection of ant brood.

In this paper, we investigate the *O. smaragdina* queen nest location and its external characteristics in mature colonies in the Lao PDR. Based on the aforementioned findings of Peng

et al. (1998a) and Van Itterbeeck et al. (2014), we hypothesize that (1) the queen tree is the tallest of the trees occupied by a colony, (2) the queen tree has more nests than other trees occupied by the colony, (3) the queen nest is located near the top and in the centre of the queen tree crown, (4) the queen nest is of small size, and (5) the queen nest has a higher number of workers patrolling its entrance than other nests.

Methods

Fieldwork

The fieldwork was conducted in Sanghouabor village, Xaythani District, Vientiane Municipality, Lao PDR, in February and March 2012. Nine mature colonies ($n = 9$; colonies A – I) were sampled along edges of dry dipterocarp forest and on termite hills amid paddy fields. We chose these locations to assure visibility of and accessibility to nests: (1) colonies occupying trees ≤ 8 m can easily be sampled and (2) trees are more scattered than in e.g. dry evergreen forest. Roux et al. (2010) ensured the capture of gravid *O. longinoda* queens in Cameroon by collecting all nests of colonies each inhabiting an isolated tree. Though occasionally encountered, we did not sample such colonies.

All the trees occupied by a colony, i.e., trees housing nests that are occupied by ants, were identified by following ant trails or by an aggression test in which worker ants from one tree were transferred to another tree. We concluded that two trees were occupied by different colonies when at least four major workers were overrun within a few minutes and bitten by an ant on each of their legs, antennae, and mandibles (see Figure 1.2.d). Fully abandoned nests were ignored. Each colony was mapped and the following was recorded: (1) the number of trees, (2) tree height, (3) the number of nests in a tree, (4) nest size (small – medium – large), (5) nest position in the vertical plane (low – middle – high), (6) nest position in the horizontal plane, considered from tree crown edge to trunk (edge – middle – trunk), and (7) worker ant presence on the nest exterior (low (0-9 ants) – medium (10-19 ants) – high (> 20 ants)). A colony was mapped between 8 and 10 AM. *Oecophylla smaragdina* inhabits trees but also bushes and lianas; all are here termed tree. Tree species were identified at the Department of Biology, Faculty of Science, National University of Laos. Each nest of a colony was inspected for the presence and number of gravid queens by cutting the nest from its supporting branch (with pruning shears). All nests of a single colony were inspected on the same day.

Laboratory

Additional data was collected from colonies that were housed indoors. Fragments of three colonies ($n = 3$; colonies F, H, and I) were relocated and kept indoors in artificial nests (glass test tubes, 15 x 2.2 cm) subsequent to investigation in the field. Water and an artificial diet were offered. The diet was based on Bhatkar and Whitcomb (1970) and consisted of agar, water, chicken egg, amino acids, honey, vitamins, and minerals. Several days after colony establishment, the queens lost their physogastricity and their body colour darkened. This is probably due to a delayed heat effect during transport in sealed plastic boxes that yet contained a lot of nest material and moistened paper towelling.

Records were made of (1) the number of ants positioned on the nest exterior in an undisturbed state (actual counts) and (2) the nest opening size (using the formula to calculate the

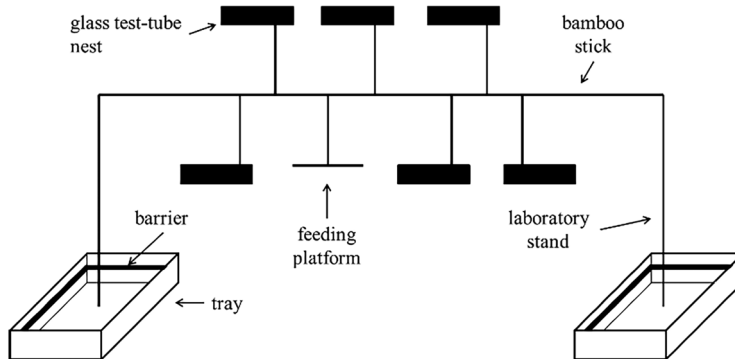


Figure 4.1. Laboratory colony set-up.

area of an ellipse $E = \pi \cdot a/2 \cdot b/2$ where E is the nest entrance size, a is the longest diameter, and b the shortest diameter). These records were made between 10 and 12 AM on four consecutive days and at least three months after establishment of the colony fragments.

I developed an original and practical laboratory set-up which proved suitable for long-term housing of colony fragments (Figure 4.1). Laboratory stands and bamboo sticks were used to suspend the nests and a feeding platform above an area inaccessible to the ants. The laboratory stands were placed in aluminium trays. Escape was prevented by a rubber strip or duct tape (2 cm wide) on the upper and inner edges of trays and coated in tapioca starch (rice powder works less well). Waste was discarded by the ants mostly by dropping it from the nest and feeding platform. The waste was easily removed without killing worker ants. I collected waste from three colonies ($n = 3$) after three weeks from (1) the area below the nests and the feeding platform, and (2) inside the trays. The average amount of waste discarded from the nests and feeding platform was 43.70 ± 38.17 grams, and the average amount of waste discarded in the trays was 0.67 ± 0.40 grams. The weight of the waste (grams) discarded from the nests and feeding platform (median = 33.60) was just not significantly higher than the amount discarded in the trays (Wilcoxon signed ranks test, median = 0.60, $z = -1.604$, $p = 0.125$, Exact significance, one-tailed). Significance values are affected by the small sample size and the large variation in the amount of discarded waste due to large size differences in colony fragments. Further improvements can be made to the set-up. For example, refraining the ants access to the trays would imply that waste is only discarded below the nests and feeding platform. This would further facilitate the removal of waste by experimenters.

Statistical analyses

Statistical analyses of the data collected in the field are performed in two stages: tree level (i.e., identifying the queen tree) and nest level (i.e., identifying the queen nest in the queen tree, thus excluding nests in non-queen trees).

The paired-sample Wilcoxon signed ranks test is used to analyse tree height and number of nests in a tree. Each variable is tested independently. The height of the queen tree is compared to the height of the tallest non-queen tree in a given colony. The number of nests in the queen tree is compared to the number of nests in the non-queen tree with most nests in a given col-

ony. Descriptive statistics are also given.

Descriptive statistics are provided of queen nest size and queen nest location in the vertical and horizontal plane (frequencies are given). The number of ants positioned on the nest exterior is analysed with the paired-sample Wilcoxon signed ranks test. The number of ants on the queen nest exterior of a given colony is compared to the highest recorded number of ants on the exterior of a non-queen nest in the queen tree of the same colony, but excluding non-queen nests larger than the queen nest and non-queen nests in the direct vicinity of the queen nest (i.e., on the same supporting branch and only few centimetres away).

The number of ants positioned on the nest exterior and the nest opening size in colony fragments kept indoors are analysed with the paired-sample Wilcoxon signed ranks test. The queen nest value of a given colony is compared to the highest recorded non-queen nest value in the same colony.

Statistical tests are performed with the software package SPSS 20. All the given significance values are one-tailed (exact test).

Results

Identifying the queen tree

The queen tree (median = 6.50) was not significantly taller than the tallest non-queen tree occupied by a colony (median = 6.00, $z = -0.178$, $p = 0.445$). Moreover, this tree was the tallest tree occupied by a colony in only four of the nine sampled colonies (44%; Table 4.1). Table 1 shows that the difference in tree height between the queen tree and non-queen trees was large (e.g., minimum difference of 2.5 m in colony A), yet also small (e.g., minimum difference of 0.2 m in colony F).

The number of nests in the queen tree (median = 13.00) was not significantly higher than that of the non-queen tree with most nests in a given colony (median = 13.00, $z = -0.059$, $p = 0.492$). The queen tree housed more nests than any other occupied tree also in only four of the nine sampled colonies (44%; Table 4.1). Table 1 shows that the difference in nest number between the queen tree and non-queen trees was large (e.g., minimum difference of 17 nests in colony A), yet also small (e.g., minimum difference of one nest in colony B).

The queen tree could however be identified by considering both tree height and number of nests in a tree: the queen tree housed more nests than any non-queen tree that was taller than or equally tall as the queen tree in a given colony, and the queen tree was taller than any non-queen tree that housed more nests than the queen tree in a given colony (Figure 4.2). Colony H was an exception (Table 4.1; Figure 4.2 – see for example the data point indicated by the arrow).

All the colonies occupied both evergreen and deciduous tree species. The evergreen *Syzygium cumini* was included most in the colonies' territories. The queen trees were predominantly evergreen species, viz., *S. cumini* (five colonies) and *Morinda coreia* (three colonies). One colony housed the queen in the deciduous *Butea monosperma* (Table 4.2). Trees neighbouring the queen tree were always incorporated in the colony's territory. In some colonies, the queen tree bordered an open area, e.g., a paddy field.

Table 4.1. Number of trees, number of nests, tree height, and number of nests per tree of nine *O. smaragdina* colonies. NQ = non-queen tree; Q = queen tree.

	Total # of trees ^a	Total # of nests	Tree Height (m)			Q ^b	Number of Nests / Tree			Q ^c
			NQ				NQ			
			Mean ± SD	Min	Max		Mean ± SD	Min	Max	
Colony A	8	47	1.24 ± 1.34	0.35	4.00	6.50	2.9 ± 3.3	1	10	27
Colony B	11	54	5.30 ± 1.92	2.50	8.50	7.00	4.1 ± 4.0	1	14	13
Colony C	13	85	3.58 ± 2.14	0.70	7.00	6.50	5.6 ± 4.3	1	15	18
Colony D	21	98	3.48 ± 1.10	1.80	6.00	5.70	3.7 ± 2.9	1	13	24
Colony E	8	20	2.41 ± 1.19	0.90	4.25	4.80	2.0 ± 0.8	1	3	6
Colony F	20	116	4.21 ± 1.61	1.20	7.00	7.20	5.6 ± 4.3	1	18	9
Colony G	10	97	3.71 ± 1.93	1.50	6.00	8.00	9.1 ± 9.5	2	28	15
Colony H	5	25	5.69 ± 2.01	3.75	8.50	5.00	5.8 ± 3.5	4	11	2
Colony I	9	30	2.04 ± 1.03	0.30	4.00	3.50	3.0 ± 2.7	1	9	6

^a Includes mature trees, young trees, bushes, sprouts, and lianas.

^b Bold values highlight that the queen tree is the tallest tree.

^c Bold values highlight that the queen tree houses most nests.

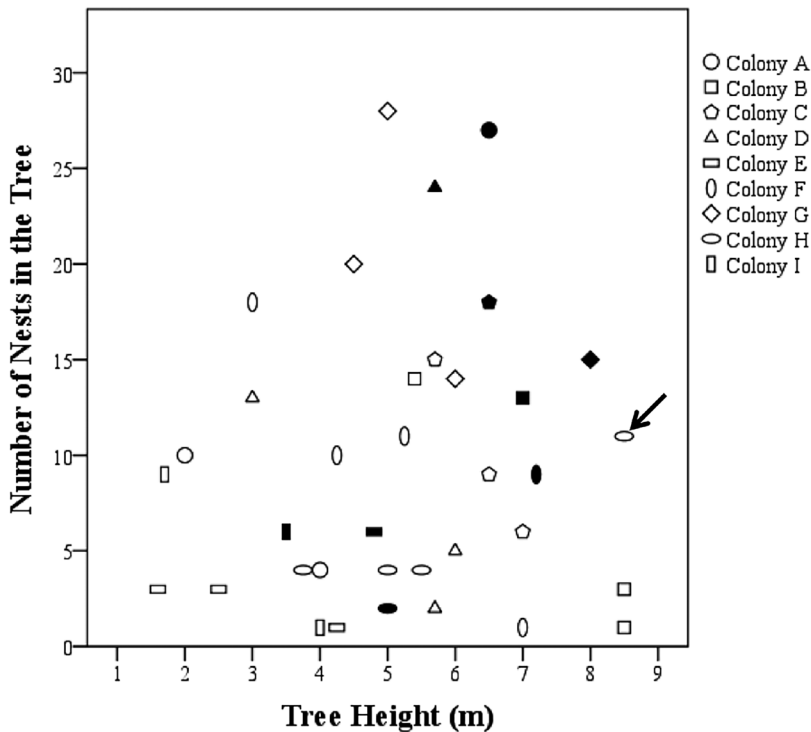


Figure 4.2. Scatterplot of tree height (m) and number of nests in the tree of queen trees (black-filled patterns) and non-queen trees (non-filled patterns) of nine *O. smaragdina* colonies. Due to overlapping data points, the graph displays a selection of non-queen trees of a given colony to increase clarity: (1) non-queen trees equally tall as and taller than the queen tree (when not available, the tallest non-queen tree(s) is selected) and (2) non-queen trees with number of nests equal to and higher than the queen tree (when not available, the non-queen tree(s) with the highest number of nests is selected). Both non-queen tree selection criteria are applied to the data set independently.

Table 4.2. Tree species and their occurrence in nine *O. smaragdina* colonies.

Tree species	Colony ^a									Total	Percentage
	A	B	C	D	E	F	G	H	I		
<i>Aganosma marginata</i>					1					1	1
<i>Butea monosperma</i>			1							1	1
<i>Cananga latifolia</i>				1						1	1
<i>Careya arborea</i>									1	1	1
<i>Croton</i> sp.	2		1							3	3
<i>Ficus religiosa</i>								1		1	1
<i>Glochidion eriocarpum</i>			1					1		2	2
<i>Gluta</i> sp.									1	1	1
<i>Ichnocarpus frutescens</i>	1		1							2	2
<i>Ixora cibdela</i>						2	2			4	4
<i>Lagerstroemia floribunda</i>				1						1	1
<i>Meyna</i> sp.									1	1	1
<i>Mitragyna diversifolia</i>							1			1	1
<i>Morinda coreia</i>	1	1		1			1			4	4
<i>Salacia chinensis</i>	1	1								2	2
<i>Shorea siamensis</i>	1			1	1		1		3	7	7
<i>Syzygium cumini</i>	2	7	7	16	6	13	2	3	2	58	55
<i>Tamilnadia uliginosa</i>									1	1	1
<i>Terminalia</i> sp.			1				1			2	2
<i>Vitex</i> sp.		1				1	1			3	3
n.a.		1	1	1		4	1			8	8

^a Bold indicates queen tree species.

Identifying the queen nest

All investigated colonies (100%) had a single physogastric, dealate, gravid queen. All investigated queen nests were small (100%) and built out of a few leaves, or even a single rolled-up leaf. The queen nest was located near the top of the queen tree (100%) and generally near the trunk (67%). However, many nests in the queen tree complied with these characteristics. The queen nest could be distinguished from small non-queen nests by the higher number of major workers positioned on the nest exterior in an undisturbed state ($z = -2.070$, $p = 0.031$) (minor workers are rarely observed here). Non-queen nests located in the queen nest's direct vicinity (i.e., on the same supporting branch and only few centimetres away) were possible exceptions. Colonies A, B, and C were excluded from the analysis because the queen nest was only detected when severe disturbance was already caused to the queen tree. Colony H is also excluded from the analysis as only two nests were housed in the queen tree, viz., the queen nest and a small non-queen nest in its direct vicinity.

The three colonies housed indoors provide additional data to the fieldwork data. In the three colonies housed indoors, the number of major workers positioned on the queen nest exterior (median = 31.75) is nearly significantly higher than on the exterior of a non-queen nest (median = 8.00, $z = -1.604$, $p = 0.055$; Table 4.3). The queen nest opening in these three colonies (median = 1.18) is also nearly significantly larger than a non-queen nest opening (median = 0.79, $z = -1.604$, $p = 0.055$). The significance values are likely affected by the small sample size.

Table 4.3. Number of major workers positioned on the nest exterior and nest opening size (cm²) in three colonies housed indoors.

	Total # of Nests ^a	Number of Major Workers on Nest ^a Exterior				Nest Opening (cm ²) ^b			
		Non-Queen			Queen	Non-Queen			Queen
		Mean ± SD	Min	Max		Mean ± SD	Min	Max	
Colony F	19	2.22 ± 2.59	0.00	8.00	16.75	0.38 ± 0.07	0.29	0.52	1.18
Colony	6	5.02 ± 2.67	2.25	9.25	66.25	0.47 ± 0.29	0.20	0.79	1.18
Colony I	11	2.83 ± 2.00	0.75	6.50	31.75	0.50 ± 0.23	0.31	1.08	2.36

^a Nests are glass test tubes.^b Calculated as $E = \pi * a / 2 * b / 2$ where E is the nest opening size, a is the longest diameter, and b is the shortest diameter.

In addition, the queen nest could be distinguished from small non-queen nests by the effects of nest disturbance, i.e., tapping/shaking either the nest, or the supporting branch, or the entire tree when small. First, such form of disturbance caused many major workers to exit the nest and position themselves on the nest exterior. This number of major workers was much higher at the queen nest than at a small non-queen nest. Possibly, major worker abundance in the queen nest was higher than in a small non-queen nest. Second, the disturbance elicited queen evacuation. An evacuating queen was recognized by the tight cluster of ants completely covering her (a retinue). When no retinue appeared and brood was evacuated, the nest was not the queen nest.

Discussion

Ants employ an array of individual and group defence strategies against invertebrate and vertebrate predators including morphological, chemical, behavioural, and structural defences (Ayasse and Paxton 2002, Dornhaus and Powell 2010). *Oecophylla smaragdina* major workers have strong mandibles and spray formic acid; they are rapidly recruited, aggressive, and selfless ants; and they build peripheral “barrack” nests that play a major role in territorial defence (Hölldobler and Wilson 1990). The location and external characteristics of the queen nest in a mature *O. smaragdina* colony described here and in Peng et al. (1998a) most probably involve specific structural and behavioural defences to safeguard the queen. Peng et al. (1998a) found multiple queens in Australia but since in a single nest, the following discussion most likely applies.

It can be expected to be highly beneficial for a *O. smaragdina* colony to house the queen, the “vital organ” in monogynous ant species (Franks and Sendova-Franks 1999), as far as possible from any potential source of threat. Our results on the location of the queen nest in the horizontal and vertical plane in the queen tree, queen tree height, and queen tree location within the territory, then suggest that potential threats to the *O. smaragdina* queen primarily originate from the ground level and from neighbouring tree crowns. For example, even though *O. smaragdina* is a territorially dominant ant, its nests are attacked from the tree base up by *Crematogaster (Physocrema) aurita* (Karavaiev, 1935) (Joost Van Itterbeeck personal observations).

The ability of ants to subdue an intruder, whether or not conspecific, is suggested to be highly dependent on individual prowess and numerical superiority (Dornhaus and Powell 2010). This likely explains the high number of non-queen nests housed in the queen tree, which can indicate the presence of a high number of major workers. A high number of nests distributed

in the queen tree crown probably also benefits rapid detection of a threat to the queen (and brood) and prompt defensive action rapidly. Yet, the queen tree does not obligatorily house most nests within the colony (Table 4.1); a certain minimal nest and worker ant presence probably suffices. A tall and dense non-queen evergreen tree may thus also house many nests, for the benefit of the colony. Peng et al. (1998a) show that the queen tree is most connected by ant trails in Australia. We could not verify this as we did not determine the daily peak activity time of *O. smaragdina* in Laos which varies with changes in humidity and temperature (Peng et al. 2012). A high number of ant trails probably facilitates detecting, communicating, and combating a threat. Despite our relatively small sample size, our results suggest that ant trails do not need to be taken into account to identify the queen tree. The advantage is that quick and reliable queen tree identification is possible using two variables that are not climate sensitive during the course of one day. The need to take both tree height and number of nests in a tree into account is due to tree crown size, leaf density, and leaf condition, and therefore, to some extent, tree species. Large and dense tree crowns allow for the construction of a large number of nests (and large-sized nests) while some tree species lose their leaves during the dry season, the time of investigation. Thus, a tall tree with few, and old, leaves does not house the queen, and can be ignored. Evergreen species seem to be favoured as queen tree. This is probably because these species can be expected to provide better internal nest climate, and higher prey and trophobiont availability during the dry season (the time of investigation) than other tree species. Tree characteristics should be taken into account in subsequent research to further facilitate the identification of the queen tree.

In addition, nest site selection in ants is a process in which worker ants that locate an appropriate site attract fellow worker ants to the site, which in turn, upon “approval” of the site, attract more workers to it, so that finally the site that attracts most ants is selected (a stigmergic response). In *O. smaragdina*, the ability of a worker ant to commence pulling down or rolling a leaf in position attracts other workers in the vicinity to join the effort (Hölldobler and Wilson 2009). Our results indicate that queen nest site selection must also involve the ability of major workers to assess tree height and communicate this assessment. Worker ants may fail though to select the optimal site available and select an acceptable one (Hölldobler and Wilson 2009). Therefore, the *O. smaragdina* queen tree may not be, for example, the taller of two further equally appropriate trees. Yet, there may be factors that were not considered in our study that play an important role in queen nest site selection.

The high number of major workers positioned on the queen nest exterior, and non-queen nests in its direct vicinity, can be expected to primarily serve a defensive purpose. The small size of the queen nest benefits rapid queen evacuation with retinue when under threat. A non-queen nest in the queen nests’ direct vicinity with similar characteristics may be a former queen nest. The queen nest can be reliably identified by the characteristics and method described here, which complement the findings of Peng et al. (1998a). We also observed that the queen nest opening is larger than a non-queen nest opening but did not collect sufficient data. A large nest opening is required for the queen to exit with retinue (Joost Van Itterbeeck personal observations). This is an additional identification characteristic. However, the nest opening is often not clearly visible.

The small size of the queen nest may also follow from the queen’s function in the colony as principal egg-layer (Hölldobler and Wilson 1990). Eggs do not take up much space and thus

a large queen nest is not required. Spherical shaped egg clusters, up to approximately three cm in diameter, were observed in the investigated queen nests (infrequently, small amounts of larvae and pupae of any caste are also present). The distribution of brood to nests throughout the colony's territory may be facilitated by housing many nests in the queen tree and by connecting this tree directly to several other occupied trees with a high number of ant trails. The observed differences with Peng et al. (1998a, 1998b), e.g., the occurrence of polygyny and the medium size of the queen nest, may indicate geographical differences.

Our findings further explain why the gravid queen is rarely encountered by ant brood collectors in the Vientiane Plain, Lao PDR, and unknown to most as indicated by Van Itterbeeck et al. (2014). First, this is because of the small size of the queen nest: such nests are ignored by collectors (Van Itterbeeck et al. 2014). Second, as the queen nest is located near the top and generally in the centre of the queen tree crown, it may be difficult to detect and reach. This occurs especially in tall trees and in trees with dense crown. In the latter, the queen nest may then appear to be concealed. The ant brood can be expected to have been used as human food for centuries. Whether, and to what extent, this human activity has influenced the observed queen nest location and external characteristics remains unanswered.

Conclusions

Our findings make a valuable contribution to further developments in the use of *O. smaragdina* as a biological control agent and as a source of human food. There is however ample scope to improve the identification of the queen nest. Future efforts should take into account, e.g., the number of ant trails connecting the queen tree to other trees, tree species and their characteristics, and the process of nest site selection.

Chapter 5

The organisation of queen defence in *Oecophylla smaragdina*: Warning, evacuation, and retinue function

In preparation as Van Itterbeeck J, Sivongxay N, Praxaysombath B, van Huis A (2014) The organisation of queen defence in *Oecophylla smaragdina*: Warning, evacuation, and retinue function.

Abstract

The arboreal weaver ants of the genus *Oecophylla*, which comprises the Asian *O. smaragdina* and the African *O. longinoda*, employ various mechanisms to defend their polydomous colonies. The queen is the most important member of the *O. smaragdina* colony and specific queen defence mechanisms can be expected. We hypothesise the existence of a specific behavioural mechanism in queen defence which includes: (1) feedback of warning to the queen nest, and (2) well-timed queen evacuation with retinue. An experiment was conducted where nests were disturbed and ant density of the ant trail to and from the queen nest and number of ants on the queen nest exterior were monitored. The results suggest that a threat within the colony's territory elicits a feedback of warning to the queen. An alarm response at the queen nest is only elicited when a threat is near the queen nest. Queen evacuation does not seem to be influenced by the feedback. Queen evacuation in *O. smaragdina* seems to be an unfavoured response and only occurs when the queen nest is under direct threat. The significance of retinue formation during *O. smaragdina* queen evacuation and emigration to the survival of the queen is demonstrated.

Keywords: weaver ants, Formicidae, social organisation, superorganism, protection

Introduction

Colonies of social insects are attacked by various organisms, ranging from viruses to vertebrate predators. These particularly target the brood (i.e., eggs, larvae, and pupae) and food stores of the colonies. Social insects use a variety of defence mechanisms against these organisms including morphological, chemical, behavioural, and structural adaptations (Ayasse and Paxton 2002, Dornhaus and Powell 2010). The destruction of the nest, the loss of brood, and the loss of food stores can be, however, fixed: the nest can be repaired or a new one can be built; new eggs can be laid and raised; and new food can be collected. Things get more difficult when during an attack the queen is killed. The loss of the queen may not be easily fixed. The queen is said to be the “vital organ” of the colony in monogynous ant species (Franks and Sendova-Franks 1999): the survival of the colony is determined by the survival of the queen. Despite this central role of the queen, very little work has been done on behavioural interactions between the queen and the workers. Defence mechanisms that are specifically adapted to protect the queen can be expected to exist. These defences should be particularly important in monogynous (i.e., single queen) species, and in species where queen turnover (i.e., the acceptance of a new queen [Heinze and Keller 2000]) does not occur. A frequently observed mechanism associated with queen defence is the formation of a retinue, i.e., “a group of workers, not necessarily permanent or even long lasting in composition, who closely attend the queen” (Hölldobler and Wilson 1990). The largest retinue formation occurs in ant species that move aboveground and are thus exposed to predators, e.g., *Dorylinae* spp. and *Ecitoninae* spp. army ants, and *Pheidologeton* spp. marauder ants. In addition, Franks and Sendova-Franks (1999) show that the *Leptothorax albipennis* queen moves in the middle of an emigration to a new nest site. This is a group-level adaptation whereby the queen is transferred from the protection of half the colony at the old nest site to the protection of the other half of the colony at the new nest site.

The arboreal weaver ants of the genus *Oecophylla*, which comprises the Asian *O. smaragdina* and the African *O. longinoda*, are considered a pinnacle of social evolution (Hölldobler and Wilson 1990). Weaver ants employ various mechanisms to defend their polydomous colonies. Hölldobler (1983) showed the role of barrack nests in territorial defence. These nests are located at the periphery of the colony and near the tree trunks. The *Oecophylla* genus is well-known for its advanced recruitment system of nest mates to subdue intruders at the branches, the trunk, and the base of a tree (Hölldobler and Wilson 1978; Hölldobler 1983). *Oecophylla* spp. workers are highly aggressive and selfless. The major workers are equipped with strong mandibles and spray venom from their abdomen (Bradshaw et al. 1979). They also deposit anal spots throughout their territory which are believed to function as deterrent (Offenberg 2007). In Chapter 4 we postulate that the location of the queen nest within the colony’s territory and the size of the queen nest serve the protection of the queen. The *O. smaragdina* queen nest is also well guarded by worker ants (Peng et al. 1998, Chapter 4) and the queen is tightly covered by worker ants (retinue) during emigration (Joost Van Itterbeeck personal observations; see also Hölldobler and Wilson 1983) and evacuation (Chapter 4). Eliciting queen evacuation is recommended to facilitate her capture (Chapter 4). The queen evacuation and formation of a retinue are clearly defence mechanisms and the current chapter builds on the observations made during the study reported in Chapter 4. These observations

suggest that the *O. smaragdina* queen defence includes: (1) warning of the queen nest by worker ants that detect a danger in the colony's territory, and (2) the well-timed queen evacuation with retinue. The organization of these behavioural queen defence mechanisms has been unexplored. Specifically, we ask the following questions.

Does a threat within the *O. smaragdina* colony's territory, e.g., an antagonistic ant species attacking nests, elicit a warning to the queen nest? If so, an effect should be visible at the queen nest. I hypothesise that:

- the ant trail leading into the queen nest increases in worker ant density; and
- the warning elicits many major workers to exit the queen nest, stand guard on its exterior, and display aggressive behaviour (i.e., raised gaster, opened mandibles, jerking body movements, and others).

The evacuation of the *O. smaragdina* queen from her nest can be expected to be an unfavoured response and to occur as a last resort. I hypothesise that:

- only when the queen nest is under direct attack, the queen, tightly covered by workers (retinue), evacuates her nest.

If queen evacuation is indeed a last resort, what then is the role of the warning in the organization of queen defence? I hypothesize that:

- queen evacuation is faster when the queen nest is warned than without a warning.

As a retinue covers the queen when she is out of her nest, I also ask what the role of this retinue is. I hypothesise that the retinue plays a key role in her protection:

- the retinue prevents the queen to get injured if she falls to the ground.

I expect that only in absence of a retinue a fall inflict injuries to the queen, notably to the gaster. This is probably due to the physogastricity of the queen: a large amount of soft membranous tissue (intersegmental membrane) of the gaster is exposed.

Lastly, the queen's evacuation destination remains uncertain. What is a safe location for the queen when her nest is attacked?

Methods

The experiments were conducted in Sanghouabor village, Xaythani district, Vientiane Municipality, Lao PDR, from February to May 2013.

Warning

Five colonies ($n = 5$) were randomly sampled along the edges of dry dipterocarp forest and in isolated clusters of trees amid paddy fields. The colonies were of small size and produced sexual forms. The queen nests were identified by using the findings of Chapter 4. In each colony, all the nests, except the queen nest, were disturbed and in predetermined order, viz., starting with the nest farthest from the queen nest (based on visual assessment of ant trail lengths). Medium and large nests were disturbed by penetrating the nests with a stick and shaking the stick for two minutes; small nests were disturbed by tapping the exterior of the nests with a stick for two minutes. These methods of nest disturbance cause an alarm response at the disturbed nest: many major workers exit the nest, stand guard on its exterior, and display aggressive behaviour (e.g., raised gaster, opened mandibles, and jerking body movements). The methods of nest disturbance, i.e., shaking and tapping, never caused an alarm response

at an undisturbed nest. Prior to each nest disturbance, records were made of (1) the number of ants located on the queen nest exterior (rapid count) and (2) the density of ants in the ant trail connecting the queen nest to the rest of the colony (measured as the number of ants crossing a reference point at ± 10 cm from the queen nest opening during one minute). In none of the colonies did the ant trail cross the queen nest exterior. Ants that arrived at the queen nest did not position themselves on the nest exterior but immediately entered the queen nest. There was an unavoidable time lag of 20-40 seconds between disturbing an individual nest and making measurements because the observers needed to change equipment and position.

The ant trail densities and the number of ants on the queen nest exterior are analysed with the paired-samples t-test (before-and-after design). Though these variables are measured prior to each nest disturbance, the analyses are conducted with ant trail densities and number of ants on the queen nest exterior measured at four instances: (1) no disturbance (no nest disturbed), (2) low disturbance (half of all non-queen nests disturbed), (3) medium disturbance (all non-queen nests disturbed except those in the queen nest's direct vicinity, i.e., on the same supporting branch), and (4) high disturbance (all non-queen nests disturbed).

Queen evacuation

In the above experiment where all the nests were disturbed but not the queen nest, the queen never evacuated. Queen evacuation only occurred when the queen nest itself was disturbed. The queen was considered to have evacuated when the retinue tightly covering her had clearly exited the queen nest (the queen cannot be observed directly). The retinue may extend several centimetres in front of and behind the queen but a ball-shape of tightly packed ants around the queen is clearly recognizable. To elucidate the effect of a warning on queen evacuation behaviour, I compare queen evacuation time, retinue size, and queen return time with and without prior disturbance of all non-queen nests. Queen evacuation time (in seconds) is the time between the start of disturbing the queen nest and queen evacuation. Retinue size was estimated by visual assessment (small – medium – large). Queen return time is the time between queen evacuation and the return of the queen inside her nest (observations were stopped after five minutes and return time noted as five minutes when the queen had not yet returned). The queen was considered to have returned when the retinue tightly covering her had clearly re-entered the queen nest. Eliciting queen evacuation with prior disturbance was conducted in conjunction with the warning experiment above. Eliciting queen evacuation without prior disturbance was conducted by using the same five colonies as above ($n = 5$). Queen evacuation was elicited either at least two weeks before or at least two weeks after the investigation of the warning. Immediately after queen evacuation, the nest disturbance was stopped.

I subsequently made observations on the queen's evacuation destination under continued disturbance both with and without prior disturbance of all non-queen nests. I disturbed the queen nest for ten minutes and noted her location.

The effect of prior nest disturbance on evacuation time and return time is tested with the paired-samples t-test (repeated measures design). The effect on retinue size is tested with the two-sample Wilcoxon's signed ranks test.

Protective function of the retinue

Five colonies ($n = 5$), sampled as described above, were used. In each colony, queen evacuation was elicited by tapping the queen nest exterior with a stick. An evacuating queen was forced to fall to the ground by severely shaking tree branches. The queen was then isolated from worker ants, her body inspected for injuries, and the height, width, and length of the gaster were measured. Because none of the queens had external injuries and preliminary observations suggested that a fall in absence of a retinue causes the queen's gaster to burst a little, thereby exposing ovarioles, the gaster of each queen was subsequently experimentally damaged. Queen survival and gaster volume were then investigated in a before-and-after experimental design. A horizontal incision (2 – 3 mm) was made in the intersegmental membrane centrally on the right hand side with sharp scissors (Swiss army knife, disinfected by holding the scissors in 90% alcohol and left for one minute to dry in ambient air). The queens were then placed in the shadow and at the base of their respective queen tree. Worker ants are able to quickly locate their queen (Peng et al. 1998; Joost Van Itterbeeck personal observations). Three weeks after the incision was made, queen survival was noted and the height, width, and length of a queen's gaster were measured again. The effect of the experimental incision on gaster volume is tested with the paired-samples t-test (before-and-after design). All experiments were conducted in the morning (8:30 – 12:00 AM) and afternoon (3:00 – 5:00 PM) when worker ants are most active (experiment time randomly assigned to the sampled colonies).

All the given significance values are one-tailed. Statistical tests are performed with the software package SPSS 20.

Results

Warning

Alarm responses at disturbed nests that are located far from the queen nest did not have any visible effect on the queen nest. The density of ants in the trail (number of ants per minute) after low disturbance (mean = 4.40, $SE = 1.50$) is not significantly higher than with no disturbance (mean = 4.00, $SE = 1.38$, t-test, $t(4) = -0.459$, $p = 0.335$). Similarly, the number of ants on the queen nest exterior after low disturbance (mean = 14.80, $SE = 9.53$) is not significantly higher than with no disturbance (mean = 14.80, $SE = 9.11$, t-test, $t(4) = 0.000$, $p = 0.500$) (Figure 5.1).

A further decrease of the distance between the source of threat and the queen nest, and invariably with a higher total number of disturbed nests in a colony, did have an effect on the queen nest. The ant density in the ant trail (number of ants per minute) after medium disturbance (mean = 19.40, $SE = 4.78$) is significantly higher than after low disturbance (mean = 4.40, $SE = 1.50$, t-test, $t(4) = -3.649$, $p = 0.011$). However, the number of ants on the queen nest exterior after medium disturbance (mean = 47.20, $SE = 38.59$) is not significantly higher than after low disturbance (mean = 14.80, $SE = 9.53$, t-test, $t(4) = -1.099$, $p = 0.167$). The non-significance probably follows from the high SE . The difference in means may suggest some warning to the queen nest.

Alarm at the queen nest occurs when a threat is in the queen nest's direct vicinity. The number of ants on the queen nest exterior after high disturbance is significantly higher (mean = 70.40,

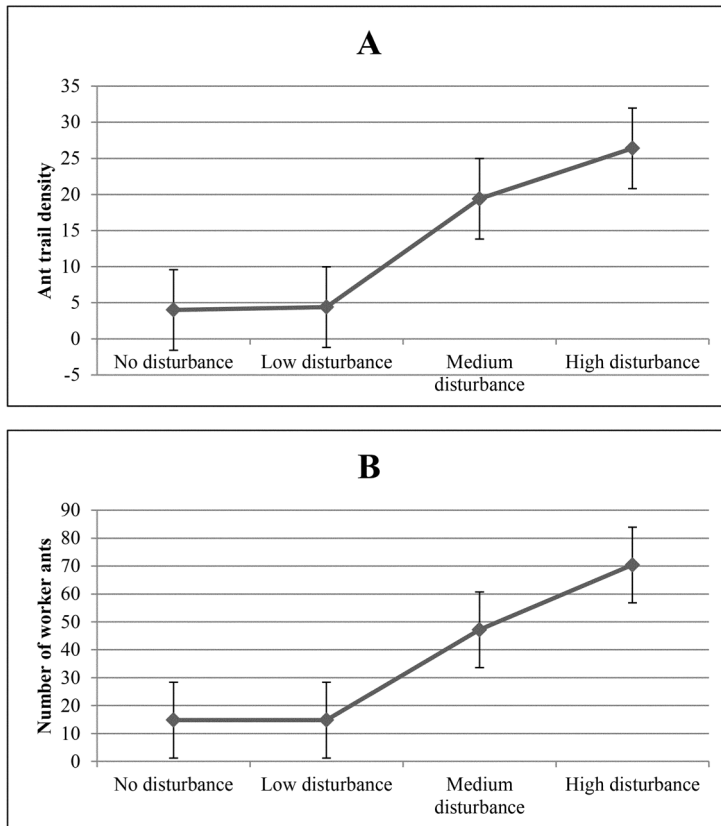


Figure 5.1. Average ant densities of the ant trail leading into the queen nest (A) and average number of ants on queen nest exterior (B) after increasing the number of nests disturbed with decreasing distance to the queen nest. Five colonies ($n = 5$) were used.

$SE = 34.30$) than after medium disturbance (mean = 47.20, $SE = 38.59$, t-test, $t(4) = -2.356$, $p = 0.039$). The alarm response involves a defensive posture by major worker ants, e.g., a raised gaster and opened mandibles, which did not occur previously. This exit response of worker ants and the aggressive display occur without a further increase in ant trail density. The ant density of the ant trail (number of ants per minute) leading into the queen nest after high disturbance (mean = 26.40, $SE = 6.85$) is not significantly higher than after medium disturbance (mean = 19.40, $SE = 4.78$, t-test, $t(4) = -1.190$, $p = 0.150$).

Yet, the alarm response at the queen nest does not elicit queen evacuation. Queen evacuation occurs only when the queen nest is under direct threat thus when the queen nest is disturbed.

Queen evacuation

We elucidate the effect of a warning on queen evacuation behaviour, and compare queen evacuation time, retinue size, and queen return time with and without prior disturbance of all non-queen nests. The warning does not make queen evacuation more swift. The evacuation time (in seconds) of the queen with prior disturbance of non-queen nests (mean = 47.00, SE

= 18.61) is not significantly faster than without prior disturbance of non-queen nests (mean = 45.00, $SE = 11.40$, t-test, $t(4) = 0.260$, $p = 0.404$). The average evacuation time of both treatments together is $46.00 \pm SE 10.30$ seconds. The large standard deviation is due to one colony (Colony #5) where evacuation time was 120 seconds with prior disturbance of non-queen nests and 90 seconds without prior disturbance of non-queen nests. This is possibly due to cloudy and cool climatic circumstances on the days of experimentation which reduces ant activity. Excluding this colony gives an average evacuation time of $31.25 \pm SE 2.63$ seconds. The retinue is not larger with prior disturbance of non-queen nests than without prior disturbance of non-queen nests (Wilcoxon signed ranks test, $z = -1.000$, $p = 0.159$).

When nest disturbance is stopped immediately after the queen has evacuated – and she is therefore positioned very near the queen nest entrance – the queen and retinue move back inside the queen nest. The analysis does not show the queen's return time to be significantly faster with prior disturbance of non-queen nests (mean = 173.00, $SE = 43.41$) than without (mean = 226.00, $SE = 45.34$, t-test, $t(4) = -0.691$, $p = 0.264$). This should, however, be taken with caution. With prior disturbance of non-queen nests, only one queen had not returned after five minutes (Colony #5, which also had a slow evacuation time). Without prior disturbance of non-queen nests, three queens had not returned after five minutes (Colony #1, #2, and #4). The analysis probably fails to give a significant result because the chosen maximum observation time of five minutes is too short.

Under continued disturbance however, both with and without prior disturbance of non-queen nests, the queen with retinue evacuates but does not return to her nest. The queen nevertheless stays in the vicinity of her nest. The retinue enlarges during this phase. Near the queen nest, there are usually several leaves and twigs that are not used in nest construction. The queen and retinue position themselves between these. The ants forming the retinue hold on to these leaves and pull them close so that the queen and retinue are covered by these leaves. This behaviour is depicted in Figure 5.2.

Protective function of the retinue

The retinue prevents the queen to fall down. Excessive force is required to make the retinue fall from its position, especially when the ants forming the retinue hold on to nearby leaves and twigs. In many instances, the queen with retinue does not fall directly to the ground but the ants forming the retinue manage to grasp and hold on to leaves, twigs, and branches. None of the queens that were forced to fall to the ground when covered with retinue showed injuries on legs, antennae, or gaster.

All queens survived the experimental incision. The gaster volume is significantly smaller three weeks after the application of the experimental incision (mean = 1.58, $SE = 0.21$) than before making the incision (mean = 1.83, $SE = 0.15$, t-test, $t(4) = 2.215$, $p = 0.046$). Black coloured scar tissue is formed. There is no further apparent change in body colouration, behaviour, or attractiveness to workers.

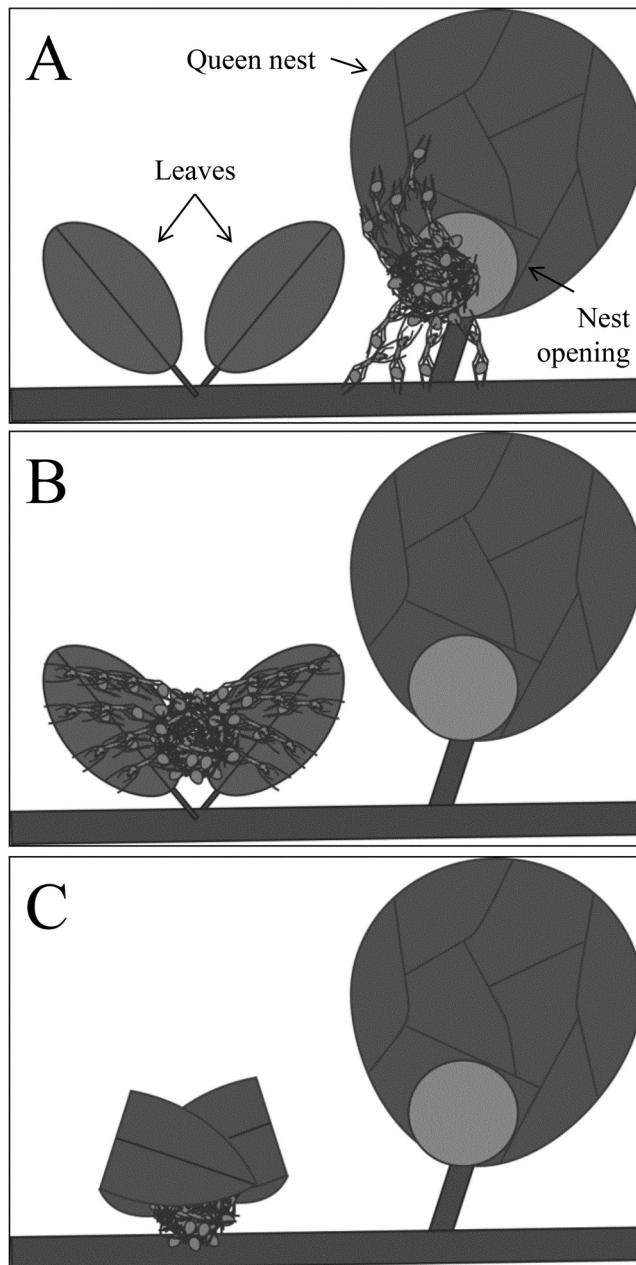


Figure 5.2. Graphic depiction of queen and retinue during evacuation under continued nest disturbance: (A) queen evacuation, (B) worker ants in the retinue hold on to each other and to nearby leaves and twigs, and (C) nearby leaves are pulled to cover the retinue and queen.

Discussion

Feedback of warning

A *O. smaragdina* colony is a superorganism as its organization is analogous to the physiological properties of a single organism (Hölldobler and Wilson 1990). This organization is largely determined by the interaction between individual ants. Notably the interaction rates and thresholds of response are of importance in decision making by individual ants (Gordon 2010).

Despite the lack of detailed investigations of the characteristics of *Oecophylla* spp. interactions, clear examples of interaction driven processes in *Oecophylla* spp. are nest site selection (Hölldobler and Wilson 2009) and the recruitment systems to a food source and an intruder (Hölldobler and Wilson 1978). Hölldobler and Wilson (1978) indicate that alarmed ants spread out around the source of a threat, and use chemical and tactile cues in their recruitment of other ants. The interaction rate between individual ants and the threshold of response of the recruited ants to the chemical and tactile cues can then be expected to determine the overall response.

The queen is a “vital organ” of the superorganism (Franks and Sendova-Franks 1999). Feedback to the queen can be expected. In case of a queen-related response, a specific queen-related chemical may be involved. The queen is highly attractive to worker ants by the chemical cues she emits (Hölldobler and Wilson 1983). Newey et al. (2008) show that cuticular hydrocarbon compositions of *O. smaragdina* worker ants differ between nests within a colony. Possibly, a queen-related cuticular hydrocarbon composition, or ‘scent’, is obtained by worker ants that interact frequently with the queen. This is in line with Hölldobler and Wilson’s (1983) consideration of the existence of ‘messenger’ ants, analogous to ‘messenger’ worker bees which spread out throughout the honey bee hive (Seeley 1979). Furthermore, Gordon (2010) found that the cuticular hydrocarbon composition of harvester ants *Pogonomyrmex barbatus* is task-dependent and shows how interactions with a task-related ‘scent’ can play an important role in decision making by individual ants.

Assuming that there is indeed a queen-related ‘scent’ in *O. smaragdina*, even a low interaction rate of alarmed ants with ants carrying a queen-related ‘scent’ may induce a highly queen-focussed response in the alarmed ants, because of the attractiveness of the queen. Conversely (or simultaneously), workers ants carrying a queen-related ‘scent’ which interact with alarmed ants may themselves respond back to the queen. This could explain an increase in ant trail density at the queen nest even with a relatively large distance between the threat and the queen nest. But most alarmed workers probably do not interact with ants carrying a queen-related ‘scent’: the interaction rate with worker ants carrying a queen-related ‘scent’ decreases with increasing distance from the queen nest. The overall response is therefore directed to the threat itself and a queen-focussed response occurs in only few worker ants. The observed further increase in ant trail density with a further decrease in distance of the threat to the queen nest could then be explained by an increased interaction rate between alarmed worker ants and worker ants carrying a queen-related ‘scent’.

An alarm response at the queen nest only occurs when the threat is near the queen nest. The threshold for an alarm response is thus only reached when the threat is near the queen. This could suggest that the interaction rate of ants inside the queen nest with alarmed ants is kept

low until this point. In Chapter 4 we suggest that the queen nest houses a large number of worker ants. These ants inside the queen nest may act as a buffer (Gordon 2010). The number of alarmed ants arriving at the queen nest must then be very high to cause an alarm response within the queen nest and this is most likely when the source of the alarm is near the queen nest.

Yet, without an understanding of *O. smaragdina* interactions and response thresholds our interpretations remain preliminary.

Queen evacuation and retinue function

Oecophylla smaragdina queen evacuation seems to be unfavoured and occurs as a last resort: the queen only evacuates when her nest is disturbed. If worker ants inside the queen nest act as a buffer, they could assure that the queen only evacuates when her nest is under threat. The benefit of this behaviour may lay in the risk of evacuation, i.e., the difficulty of finding a safer location than the queen nest. This is further supported by the fact that the queen returns to her nest when disturbance is stopped immediately after evacuation and by her evacuation destination, i.e., near the queen nest, when disturbance is continued.

The ant behaviour during evacuation and at the evacuation destination seems directed at preventing the fall of the queen. We observed that the queen is able to walk by herself but she does not seem to be able to climb vertically (e.g., on a tree trunk) in absence of a retinue. This is due to: (1) the weight of the enlarged gaster, and/or (2) the lack of arolia (adhesive pads on the tarsi) or reduction in arolia development (the queen does not partake in nest construction or in foraging and therefore the arolia do not need to be as developed as they are in worker ants). The *O. smaragdina* queen thus probably requires the assistance of worker ants when she is outside her nest to prevent her from falling. Even if the queen falls to hard ground, the retinue, which is a very tight cluster of worker ants, protects the queen from injuries. The queen can only get injured in absence of a retinue.

Conclusions

Our findings suggest that a feedback of warning to the *O. smaragdina* queen exists and that queen evacuation only occurs as a last resort. The interpretations of the results presented here are preliminary. An understanding of *O. smaragdina* worker-worker and worker-queen interactions and thresholds of response is required to elucidate the complex organisation of a *O. smaragdina* colony. The significance of retinue formation to the survival of the *O. smaragdina* queen, however, is demonstrated.

Chapter 6

The resilience of *Oecophylla smaragdina* to its use as human food

Abstract

An important issue in the use of the Asian weaver ant *Oecophylla smaragdina* as a source of human food is the resilience of a colony to harvesting its queen brood, i.e., large-sized larvae and pupae that develop into virgin queens. We conducted an experiment in which we quantified the effect of queen brood removal on subsequent brood production. Queen brood production was significantly reduced. The reduction is however explained by high worker ant mortality. The results suggest that with increasing harvesting pressure, and thus handling time, worker ant mortality increases. Likely, this also negatively affects colony survival and the maintenance of the *O. smaragdina* population. Improvements need to be made on queen brood harvesting techniques and tools to avoid worker ant mortality.

Keywords: weaver ant, Formicidae, natural resource use, non-wood forest product, queen brood

Introduction

Ants (Hymenoptera: Formicidae) are used as human food throughout the world (Del Toro et al. 2012, Rastogi 2011, Van Itterbeeck and Fisher 2013). The Asian weaver ant *Oecophylla smaragdina* (Fabricius) is a particularly popular source of human food in Thailand and Laos (Nonaka, Boulidam et al. 2008, Sribandit et al. 2008). This arboreal ant species is well-known for the leaf-nests it builds from which the brood and adult ants are collected (Chapter 3, Crozier et al. 2009, Sribandit et al. 2008, Van Itterbeeck et al. 2014). The queen brood, i.e., large-sized larvae and pupae that develop into virgin queens, is favoured. Worker ants and virgin queens are used as a condiment because of their sour taste. Worker and male brood, i.e., small-sized larvae and pupae that develop into workers and males, may also be consumed. Adult males are however said to have a foul taste (Chapter 3, Van Itterbeeck et al. 2014). The collecting period is determined by queen brood availability, roughly from February to April (Chapter 3, Sribandit et al. 2008, Van Itterbeeck et al. 2014). *Oecophylla smaragdina* is a Non-Wood Forest Product of particular importance to rural livelihoods. One kilogram of queen brood is sold in the markets for up to 6.2 USD in Thailand (Sribandit et al. 2008) and up to 12 USD in Laos (Chapter 3). These prices are up to three times higher than those of conventional meat, i.e., chicken, pork, and beef (Sribandit et al. 2008, Chapter 3). The brood and adults are good sources of protein and fat (Sungpuag and Puwastien 1983, Rumpold and Schlüter 2013). The queen brood and virgin queens are also canned in Thailand (Joost Van Itterbeeck personal observation).

Weaver ant colonies can be very large. They often consist of numerous nests and occupy several trees (Crozier et al. 2009). A large variety of trees are host to *O. smaragdina*. Some of these have a high economic value, e.g., mango, cashew, citrus, and coffee (Lim 2007). As a generalist predator, *O. smaragdina* can control a number of pest insects of these trees. The use of weaver ants can increase crop yield and/or quality in comparison to the use of conventional chemical control of pests (Peng and Christian 2005, Peng et al. 2004, Van Mele 2008). Offenberg and Wiwatwitaya (2010) suggest that collecting the queen brood for human consumption does not negatively affect the biological control potential of *O. smaragdina*. The development of a sustainable multi-production system of queen brood and tree crops is proposed in which *O. smaragdina* functions both as a biological control agent and a direct source of human food (Offenberg and Wiwatwitaya 2010, Offenberg 2011, Hanboonsong et al. 2013). Moreover, the ability to increase the production, predictability, and availability of queen brood, which we have termed semi-cultivation (Chapter 1, van Huis et al. 2013), could stimulate its use as a biological control agent in Thailand and Laos (Decha Wiwatwitaya personal communication). The development of *O. smaragdina* semi-cultivation is innovative (Chapter 2, Hanboonsong et al. 2013, van Huis et al. 2013, Van Itterbeeck and van Huis 2012). Its potential benefits are apparent: a reduction in the environmental costs and financial inputs associated with non-biological pest control methods; an increase in the agricultural productivity of plantations both in terms of tree crops and queen brood; and a sustainable diversification of agricultural produce, e.g., by designing agroforestry practices with *O. smaragdina* host trees (Offenberg et al. 2013, Van Mele 2008). This development could also reduce the harvesting pressure on wild *O. smaragdina* colonies and counter their reduction in abundance due to deforestation (Chapter 3).

Concerns of decreasing productivity and availability have been expressed by ant brood collectors and scholars (Sribandit et al. 2008, Chapter 3, Hanboonsong et al. 2013, Van Itterbeeck et al. 2014). Similar concerns have been expressed for other edible ant species, e.g., *Liometopum* spp. of which the brood ('escamoles') is consumed and marketed in Mexico (Ramos-Elorduy 2006). These concerns do not only relate to deforestation issues but also to the ant brood harvesting practices (Chapter 3). It is therefore of major importance to investigate the effect of the ant brood harvest on an individual colony. In the current chapter we address a key question in both the development of sustainable *O. smaragdina* semi-cultivation and concerns of decreasing availability and productivity of wild colonies. What is the effect of queen brood removal on subsequent brood production? In other words, we aim to investigate the resilience of a *O. smaragdina* colony in the short-run to collecting its queen brood for human food.

Methods

Colony sampling and experimental treatments

The experiment was conducted in Sanghouabor village and Houaxiang village, Vientiane Municipality, Xaythani District, Lao PDR, from March to April 2013. Six sexual brood producing colonies ($n = 6$) were randomly sampled in home gardens. At least one resident was home during the day at each house for the duration of the experiment. He/she assured the exclusive use of the colonies (ownership does not occur – Chapter 3).

We determined the effect of queen brood removal on subsequent brood production by applying two treatments: (T1) no brood is removed (control; $n = 3$), and (T2) only queen brood is removed ($n = 3$).

First, the brood content of each colony was quantified by weighing it. To access the brood, a destructive harvesting method was inevitable. In both treatments, each nest of a colony was cut from its supporting branches using pruning shears. Nest dimensions were measured (length – width – height). Nests usually contain brood of all stages and sex. Each nest was then emptied of brood on a piece of cloth which was placed in the shadow near the base of a tree occupied by the colony. To completely empty a nest of brood, we opened the nest, gently shook it, and used a soft make-up brush. Large numbers of worker ants were mixed with the brood. After all nests were emptied of brood, we removed worker ants from the brood by gently pouring the brood onto a new piece of cloth: most worker ants held on to the previous piece of cloth. The far majority of workers were removed from the brood after repeating this procedure about four times. Any remaining workers were carefully removed from the brood with tweezers. Queen brood (large-sized) was then separated from worker and male brood (small-sized) by gently sifting the brood using a cotton net (mesh size \varnothing 4 mm). Tweezers were used when necessary. It is assumed that the small-sized brood consisted of worker and male brood only, although it is unknown at which stage in development caste determination takes place (but see Bhattacharya 1943). Both brood sizes were weighed with a scale. The queen nest was excluded from this procedure in order to assure the survival of the queen. The queen nest was identified by using the findings of Chapter 4.

Second, we applied the two treatments. In the colonies assigned to T1, both the small-sized and large-sized brood was returned to the colonies. In the colonies assigned to T2, only the

small-sized brood was returned and queen brood was thus permanently removed from these colonies. Brood was returned to a colony on a piece of cloth which was placed in a plastic basket and hung in a shaded part of the queen tree. This avoided desiccation and predation, e.g., by free-roaming chickens. An additional piece of cloth was used to cover the basket to further assure shade. Worker ants quickly started new nest construction and brood retrieval, i.e., carrying brood from the basket to new nests.

Finally, we determined the brood content of each colony six weeks after treatment application. The brood was weighed using the same procedure as above.

Methodological drawback and adapted experimental design

A drawback was encountered which affected the experimental design and analyses. Two colonies that were assigned to T1, colony #2 and colony #3, retrieved the small-sized brood but not the queen brood. This was noticed when the colonies were monitored two weeks after treatment application. The queen brood was rotting and we removed it. We considered the actual treatment applied to colony #2 and colony #3 to have been T2 (queen brood removed) instead of T1 (no brood removed). This increased the sample size in T2 to five colonies ($n = 5$). With only one colony, colony #1, remaining in the control treatment T1, this treatment was excluded from the analyses.

Statistical analyses are then performed following a before-and-after experimental design (Wilcoxon's signed ranks test; $n = 5$). We analyse (1) queen brood yield, (2) small-sized brood yield, and (3) total brood yield (queen brood yield plus small-sized brood yield). Brood yield is analysed by using absolute values (g) and by using yield per unit nest volume (g/m^3). Yield per unit nest volume is calculated as the brood yield divided by the total nest volume of a colony. Total nest volume of a colony (m^3) was calculated as the sum of the volume of each nest of a colony. We calculated nest volume as $V = 4/3 \cdot \pi \cdot a \cdot b \cdot c$ (formula to calculate the volume of an ellipsoid shape), where V is the nest volume, and a , b , and c are the length, width, and height of the nest. Total nest volume (m^3) is also analysed in a before-and-after experimental design (Wilcoxon's signed ranks test; $n = 5$).

Results

The average total nest volume of the five sampled colonies ($n = 5$) decreased to 40% of the average initial total nest volume (Figure 6.1.a). Total nest volume was significantly smaller after treatment application (median = 0.08) than before (median = 0.16, $z = -2.023$, $p = 0.031$). The decrease in total nest volume was strongest in the colonies with largest initial total nest volume. Notice the steepest decline in colony #2 (■) and colony #3 (▲) in Figure 6.1.b.

Total brood yield was reduced (Figure 6.2.a). It was significantly lower after treatment application (median = 77.80) than before (median = 357.50, $z = -2.023$, $p = 0.031$). Figure 6.2.b shows that there is a positive relation between total nest volume of a colony and total brood yield both before and after treatment application.

Total brood yield consisted of worker and male brood (small-sized brood) and queen brood both before and after the treatment was applied (Figure 6.2.a). Queen brood yield was significantly lower after the treatment (median = 17.50) than before (median = 286.90, $z = -2.023$, p

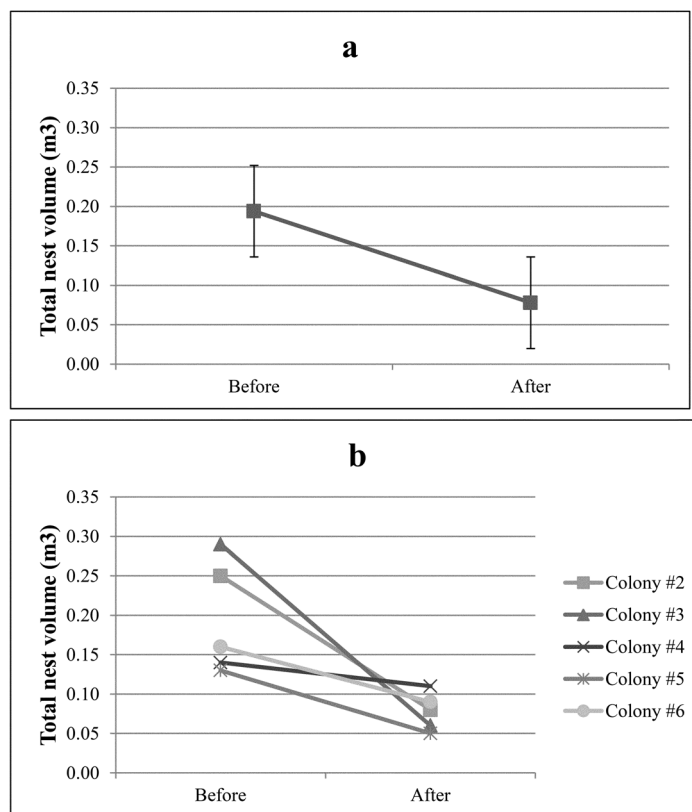


Figure 6.1.a. Average total nest volume (m³) and error bars with standard error before and after treatment application.
Figure 6.1.b. Total nest volume (m³) before and after experimental treatment.

= 0.031). Small-sized brood yield was lower after the treatment (median = 60.30) than before (median = 134.70) but the reduction was not statistically significant ($z = -0.944$, $p = 0.219$). There was a positive relation between total nest volume and queen brood yield both before and after treatment application (Figure 6.2.c). The relation between total nest volume and small-sized brood yield however changed from negative before treatment application to positive after treatment application (Figure 6.2.d). This was because the investment ratio queen brood : small-sized brood had changed. It decreased from 2.35 to 0.46. Before the treatment was applied, the colonies invested more in virgin queen production than in male and worker production: 70% of total brood yield was queen brood (Figure 6.2.a). This supports the findings of Offenberg and Wiwatwitaya (2010) who found queen brood to constitute 86% of all brood in North Eastern Thailand. After the treatment was applied, and the colonies were reduced in total nest volume, queen brood yield had reduced to 32%. Imago virgin queens and males were also present but these were not quantified. This indicates that the larger the *O. smaragdina* colony, the more it invests in virgin queen production; the smaller the *O. smaragdina* colony, the less it invests in virgin queen production.

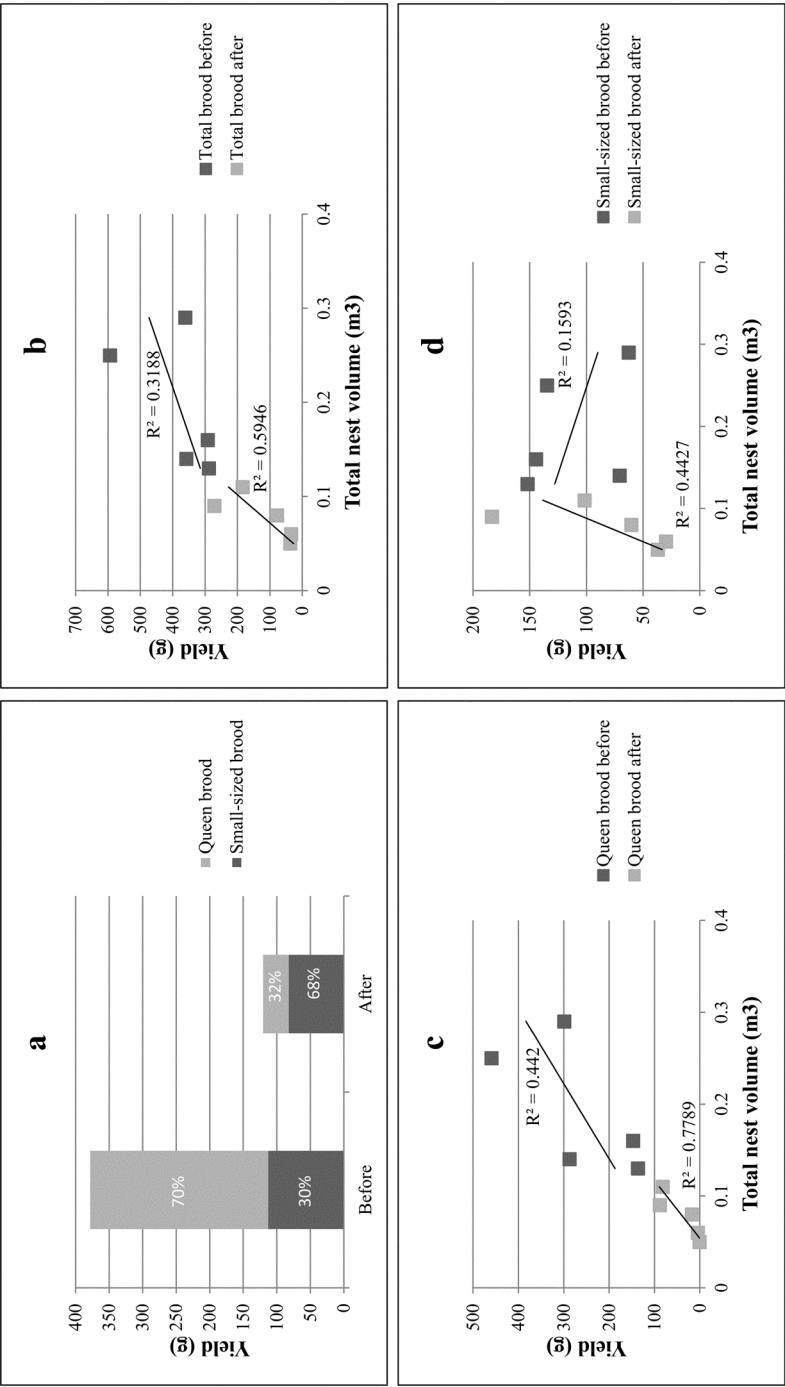


Figure 6.2.a. Average brood yield (g) before and after treatment application.
Figure 6.2.b. Total brood yield (g), i.e., queen brood and worker ant and male brood, per total nest volume (m³) before and after treatment application.
Figure 6.2.c. Queen brood yield (g) per total nest volume (m³) before and after treatment application.
Figure 6.2.d. Small-sized brood yield (g) per total nest volume (m³) before and after treatment application.

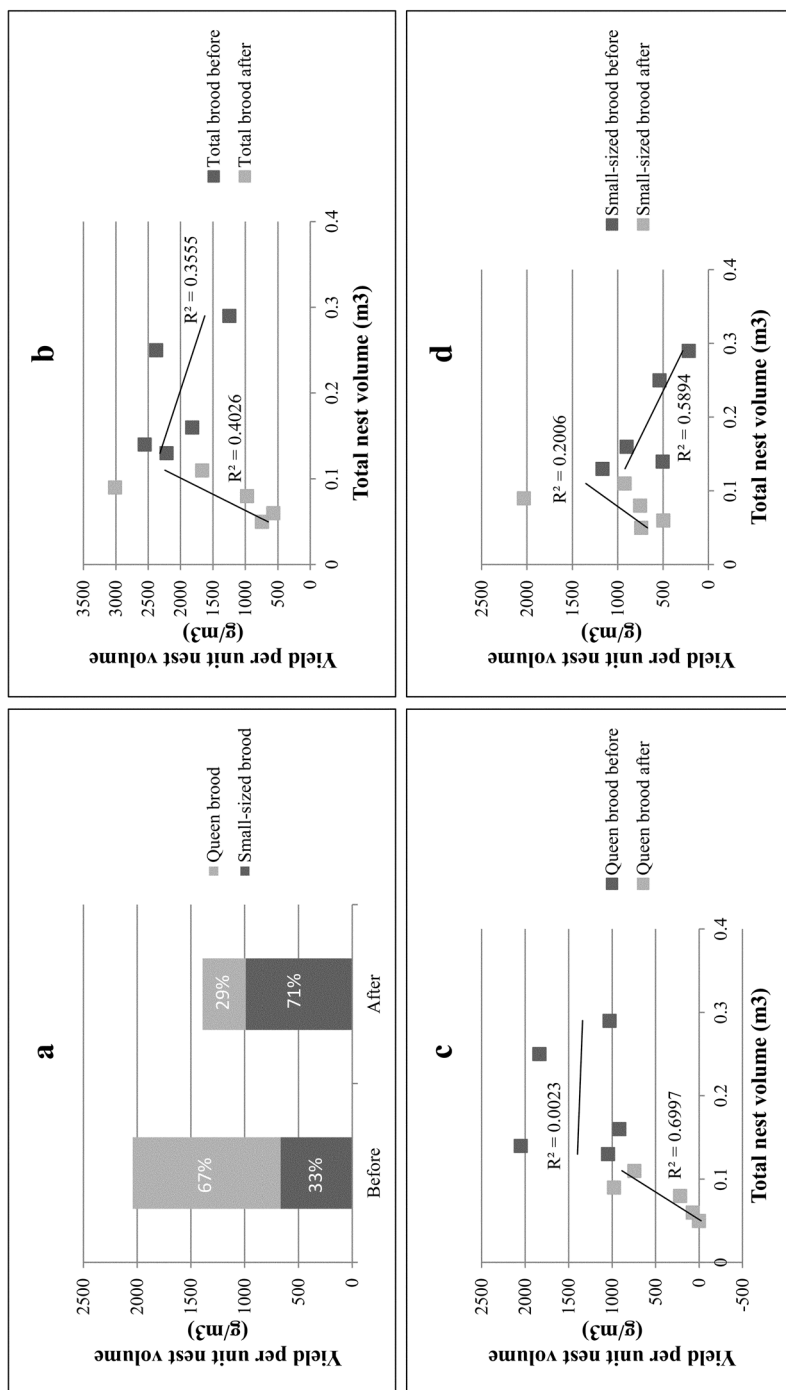


Figure 6.2.a. Average brood yield (g) before and after treatment application.
 Figure 6.2.b. Total brood yield (g), i.e., queen brood and worker ant and male brood, per total nest volume (m³) before and after treatment application.
 Figure 6.2.c. Queen brood yield (g) per total nest volume (m³) before and after treatment application.
 Figure 6.2.d. Small-sized brood yield (g) per total nest volume (m³) before and after treatment application.

The effect of a decrease in total nest volume on brood production can be further elucidated by comparing brood yields per unit of nest volume. Total brood yield per unit nest volume had decreased but it was not significantly lower after treatment application (median = 972.5) than before (median = 2215.4, $z = -1.214$, $p = 0.313$). Queen brood yield per unit nest volume had decreased but it was also not significantly lower after treatment application (median = 218.1) than before (median = 1047.7, $z = -1.753$, $p = 0.125$). Small-sized brood yield per unit nest volume, however, had increased after treatment application (median = 753.8) but it was not significantly higher than before (median = 538.8, $z = -0.944$, $p = 0.438$).

The investment ratio queen brood : small-sized brood per unit nest volume changed in a similar way as when comparing absolute values. It decreased from 2.07 to 0.41. Before the treatment was applied, the colonies invested more in virgin queen production per unit nest volume than in worker and male production: 67% of total brood yield per unit nest volume was queen brood. After the treatment was applied, queen brood yield per unit nest volume had decreased to 29% (Figure 6.3.a). However, the relation between total nest volume and (1) total brood yield per unit nest volume, (2) queen brood yield per unit nest volume, and (3) small-sized brood yield per unit nest volume, changed from negative (total brood yield and small-sized brood yield) and neutral (queen brood yield) before treatment application to positive after treatment application (Figures 6.3.b, 6.3.c, and 6.3.d). This indicates that the larger the colony, the less it invests in brood production per unit nest volume; and the smaller the colony, the more it invests in brood production per unit nest volume.

Discussion

There are important implications to the method that we used. First, in our experimental method we accurately collected all the brood from all the nests of a colony. We thus applied a high harvesting pressure. This increased our handling time in comparison to local ant brood collectors who focus on large nests and who do not necessarily empty the nests of brood (Chapter 3, Van Itterbeeck et al. 2014). Second, worker ants were unintentionally killed. Ant brood could not quickly nor easily be harvested and separated from worker ants due to the highly aggressive and selfless behaviour of *O. smaragdina* worker ants. For example, *O. smaragdina* worker ants employ a “death grip”, similar to, e.g., wasps (Shorter and Rueppell 2012). The ants bite the protective clothes of the experimenter until their death. Measures were taken to reduce worker ant mortality, e.g., the use of starch barriers (Chapter 3, Van Itterbeeck et al. 2014, Chapter 4) around the wrists and ankles of the experimenter. The measures taken were not sufficiently effective. The traditional ant brood collecting practices (Chapter 3, Van Itterbeeck et al. 2014, Offenberg and Wiwatwitaya 2010) were not used because prior testing showed that they do not assure the capture of all brood from a nest. The traditional ant brood separation methods, viz., the wet method and the use of starch in the dry method (Chapter 3), were neither used because prior testing showed that they negatively affect brood retrieval and do not decrease worker ant mortality.

The long handling time in combination with the inefficiency of the worker ant mortality-reducing measures negatively affected colony size, i.e., number of worker ants, immediately. The decrease in colony size is shown by the significant decrease in total nest volume (Figure 6.1.a): there is a positive relation between the volume of a nest and the number of worker ants

housed in it (Gupta 1968). The decrease in colony size was strongest in colony #2 and colony #3 (Figure 6.1.b). These colonies were the largest in the sample and consequently required the longest handling time. Cooperation between worker ants is necessary to retrieve the large-sized queen brood (Joost Van Itterbeeck personal observations). Changes in group size likely affect collective organization (Dornhaus et al. 2012). It is thus likely that the decrease in colony size caused a negative effect on queen brood retrieval behaviour of the worker ants in these two colonies: queen brood was not retrieved. However, the exact mechanisms in *O. smaragdina* cooperative transport (Czaczkes and Ratnieks 2013, McCreery and Breed 2013). This drawback affected the experimental design (see Methods section).

The decrease in colony size had a major and negative impact on the resilience of the colonies. It outweighed the removal of queen brood as the cause for the observed effects, viz.: (1) a decrease in total brood yield, and (2) a decrease in the ratio queen brood : small-sized brood. The decrease in total brood yield follows from the positive relation between the *O. smaragdina* colony size and the amount of queen brood that is produced by a colony (Cole 2009, Gupta 1968). The decreased ratio queen brood : small-sized brood suggests that there is a trade-off between the production of sexual forms and the production of worker ants in *O. smaragdina*. This trade-off is driven by colony size (Cole 2009, Hölldobler and Wilson 1990). Colonies that have been severely reduced in size can be expected to invest their resources in the maintenance and growth of the colony, i.e., in producing worker ants. We could not fully ascertain this in *O. smaragdina* for three reasons. First, a thorough and comparative study of immature body characteristics of the different *O. smaragdina* castes is not available (see Bharti and Kaur 2011, Bhattacharya 1943). This implies, for example, that worker larvae could not be distinguished from male larvae. Second, development time and caste determination mechanisms in mature *O. smaragdina* colonies are unknown. Thus, eggs could not be designated to caste. Third, the seasonal cycle of colonies of varying sizes and ages is unknown. We had made an earlier attempt to investigate the seasonal variation in brood production. Despite a variety of measures taken with the local community we quickly encountered difficulties in assuring the exclusive use of the weaver ant colonies. The nests that we did collect were unfortunately destroyed due to human error (deep freezers were unplugged). No such difficulties were encountered during the experiment reported here.

The collection of queen brood for human food is not detrimental to a colony because sexual forms, i.e., virgin queens and males, do not contribute to the maintenance or growth of the colony (Hölldobler and Wilson 1990, Offenberg and Wiwatwitaya 2010). However, the removal of queen brood implies the removal of a fraction of worker ants. This fraction increases with handling time and harvesting pressure. The workers are necessary for the maintenance and growth of the colony, and the production of queen brood. It is thus of major importance that colony size is not affected by the queen brood harvest.

Whether weaver ant colonies are resilient to queen brood loss when colony size is unaffected could not be tested. However, worker ants are also used as a condiment in Thailand and Laos (Chapter 3, Sribandit et al. 2008, Van Itterbeeck et al. 2014), and worker ant brood is collected year round in Indonesia to serve as bird feed (Césard 2004). Our results show that the intensive use of weaver ants for human food is a potential threat to the maintenance of an *O. smaragdina* population. Colonies that are decreased in size (i.e., decreased in worker numbers) are negatively affected in their production of sexual forms, and thus possibly in the

founding of daughter colonies. It would be most valuable to follow up colonies in Indonesia where worker brood is collected year round.

Our experiment does not elucidate whether a colony is resilient to a decrease in colony size in the long-run. We did not have the possibility to monitor the colonies for a longer period. The colonies survived because the gravid queen was not killed by the experimental method. Colonies of which the gravid queen was removed died within a few months (Chapter 4). However, a reduction in size makes a colony more vulnerable to attacks from conspecific colonies and antagonistic ant species. On two occasions we had observed a *Crematogaster* spp. killing a *O. smaragdina* colony which was reduced in size after a forest fire.

Conclusions

Problematic to the intensive use of *O. smaragdina* queen brood as a source of human is the associated mortality of worker ants. This reduction follows from the ants' aggressiveness and the inability of the brood harvesting techniques and tools to avoid worker ant mortality. A high worker ant mortality negatively affects the resilience of a colony in the short-run, and probably also negatively affects the *O. smaragdina* population. It is of major importance to preserve a high worker ant number in a *O. smaragdina* colony for the benefit of queen brood production. Improvements need to be made in ant brood harvesting techniques and tools.

Chapter 7

General discussion

Future food production and dietary habits

The human population is estimated to exceed 9 billion by 2050 (UN 2013). This is expected to be accompanied by an economic growth in developing countries that will alter global food demand patterns (Msangi and Rosegrant 2011). The main driving forces to this impending change relating to food production are rising incomes and rapid urbanization, particularly in Asia. This is expected to result in a major increase in meat consumption in Asia (Msangi and Rosegrant 2011). An increased demand for meat (i.e., chicken, pork, and beef) implies an increased demand for feed crops (Tilman et al. 2011, Trostle 2008). Problematic to this increase in meat and feed crop demand is the low efficiency of conventional livestock in converting feed into biomass (Tilman et al. 2011), the scarcity of land available to produce the feed crops and to keep livestock (Godfray et al. 2010), and other environmental issues such as the production of greenhouse gasses (Steinfeld et al. 2006). Sustainable agricultural intensification of underyielding nations is suggested to be of great importance to meet the future food demands (Tilman et al. 2011). In essence, meeting the future global food demands in a sustainable way is said to require drastic global changes in food production and thus an agricultural revolution that cuts across the social and natural sciences (Godfray et al. 2010). The realization that a revolution is required has prompted the scientific community to consider alternative protein sources, e.g., cultured meat (Fayaz Bhat and Fayaz 2011, Post 2012), seaweed (Baweja et al. 2009, Fleurence 1999), vegetables and fungi (Asgar et al. 2010), and minilivestock, i.e., insects, rodents, frogs, and snails (Paoletti 2005). Entomophagy (the consumption of insects) is one alternative that receives a lot of attention. Indeed, why not eat insects? Edible insects compare favourably to conventional meat in regard of environmental issues and nutritional values (Bukkens 2005, Rumpold and Schlüter 2013, Oonincx et al. 2010, Oonincx and de Boer 2012). The nutritional value of edible insects is generally high, while the nutritional composition is variable between species and between developmental stages of a species (Rumpold and Schlüter 2013). This could allow for the fine-tuning of diets by consuming certain insect species. For example, adult crickets (*Acheta domesticus*) provide more protein than palm weevil larvae (e.g., *Rhynchophorus phoenicis*), but these larvae are a better source of fat than the crickets (Rumpold and Schlüter 2013). A highly relevant argument in favour of edible insects is their efficiency in converting feed into edible biomass which far exceeds that of conventional livestock. Crickets (*Acheta domesticus*), for example, are twice as efficient as chickens, almost four times more efficient than pigs, and more than 12 times efficient as cattle (Collavo et al. 2005, van Huis 2013, Nakagaki and DeFoliart 1991). Such large differences in efficiency are expected with many other insect species also because these differences seem to follow from an innate characteristic of insects: they are cold-blooded and they therefore do not require feed to maintain body temperature (van Huis 2013). In addition, insects invest less of their feed into inedible body structures such as bones (van Huis 2013, Nakagaki and DeFoliart 1991, Smil 2002). The higher efficiency in converting feed into biomass implies that less land is required to produce feed while farmed insects use less housing space than conventional livestock to produce the same amount of edible produce (Oonincx and de Boer 2012). Insects are furthermore shown to produce less of the greenhouse gasses and ammonia (Oonincx et al. 2010). Water use is also expected to be less when farming insects because, e.g., the insects do not require (much) additional drinking

water as they get most or all of it from their feed (Dennis Ooninx personal communication). Studies are needed for verification (van Huis 2013). An increased use of edible insects as human food is a highly viable option, especially for countries accustomed to entomophagy. Despite the prospect of edible insects in Southeast Asia, there may be negative attitudes towards entomophagy. From 2010 until 2012, the Food and Agricultural Organization (FAO) contributed an edible insects-based project to the agricultural developments in Laos: *Sustainable insect farming and harvesting for better nutrition, improved food security, and household income generation* (FAO 2011). The project was a technical cooperation with the Lao Ministry of Health and the Faculty of Agriculture, National University of Laos. A nationwide survey conducted by the project indicates the prevalence of entomophagy throughout Laos: more than 95% of Lao people consume insects. The project also notes the scope for further developments in edible insects: an increased availability of edible insects would lead to an increased use. The FAO encouraged insect farming and marketing, and focussed its work on farming cricket (*Acheta domesticus*), palm weevil (*Rhynchophorus ferrugineus*), mealworm (*Tenebrio molitor*), and weaver ant (*Oecophylla smaragdina*) (FAO 2011). However, by the end of FAO's edible insect project, an official of the Ministry of Health declared that edible insects are no priority to the ministry and the project would not be continued (IRIN 2012). This may reflect a low status of entomophagy in Laos' governing bodies. If this is true, why then is there a low status of entomophagy among the governing bodies of a country that has a high prevalence of entomophagy? It is suspected that, at least in part, this is due to (1) negative attitudes towards entomophagy in the developed world, and (2) the westernization of the developing world (DeFoliart 1999, van Huis et al. 2013). The situation seems somewhat different in Thailand. Edible insect farming is widespread, it is encouraged by the Thai government, and research is ongoing to farm more species and improve farming technologies (Hanboonsong et al. 2013, Kayikananta 2000, Yhoung-Aree and Viwatpanich 2005). Thailand could take the lead in developments with edible insects in Southeast Asia in general and the semi-cultivation of weaver ants specifically (Hanboonsong et al. 2013). The efforts by Thailand and its research institutions could be supported by governments and research institutions elsewhere. Positive attitudes towards entomophagy are required to realize the potential of edible insects in agricultural developments. A highly relevant field of study thus concerns the evolution and dynamics of perceptions on insects as food, food choices, and food preferences with regard to an increased westernization (Looy et al. 2014).

Livestock farmers could become minilivestock farmers during the 21st century (Chapter 2, Van Itterbeeck and van Huis 2012) when research institutes and governing bodies invest their resources in developing the semi-cultivation/farming of edible insects and in studying consumer attitudes towards edible insects.

Semi-cultivating weaver ants

This thesis contributes to developing the semi-cultivation of the edible weaver ant *Oecophylla smaragdina*, a potential innovation. The semi-cultivation of *O. smaragdina* could result in an increased productivity of land because it implies a sustainable multi-production system of tree crops and ant brood (Offenberg and Wiwatwitaya 2010). This is a form of agroforestry. Agroforestry practices are diverse and vary in complexity from low (e.g., fruit trees amid graz-

ing land) to very high (e.g., multi-story tree gardens) (Nair 1993). Weaver ants have several lucrative tree crops as host and thus a variety of agroforestry practices can be developed by using the ants and a selection of their host trees. It is relevant to underline that there is a serious economic incentive to develop agroforestry practices that are based on weaver ant host trees and where weaver ants are semi-cultivated. Such systems provide: (1) multiple sources of income (because of multiple produce, i.e., tree crops and ant brood), (2) a reduction in the environmental costs and financial inputs associated with non-biological pest control methods (using weaver ants is cheaper and more sustainable than chemical control methods), and (3) an increased income through an increased yield and/or quality of the tree crops (this is a general effect of weaver ant presence) (Offenberg et al. 2013, Peng et al. 2013). However, weaver ants are not the perfect pest control agents as some of their trophobionts can, e.g., negatively affect flowering and fruit development (Offenberg et al. 2013). Agroforestry practices that are centred on weaver ants could nevertheless be further explored. It has been suggested that the benefits of using the ants outweigh the possible costs (e.g., trophobiont activity) (Way 1954). Research programs should be devoted to the study of key biological and ecological issues to increase the predictability, availability, and productivity of the weaver ant and other edible insects. I thus follow Cohen (2001) who suggests that insect rearing and insect food science and technology need to be formalized as academic disciplines. Multi-production systems can be developed by using other insects also. For example, mechanical pest control (i.e., collecting the edible pest insect) can be beneficial for the environment and livelihoods in a similar way as described above in weaver ant agroforestry. The edible pest insect provides additional income and food, and the use of chemicals to control the pests is reduced. This has been suggested for grasshoppers (*Sphenarium purpurascens*) and palm weevil larvae (e.g., *Rhynchophorus palmarum*) (Cerritos and Cano-Santana 2008, Choo et al. 2009).

Oecophylla smaragdina is used as human food in Laos and Thailand, and as animal feed in Indonesia. There are similarities and differences in *O. smaragdina* harvesting practices between these countries. The harvesting practices in Laos (Chapter 3, Van Itterbeeck et al. 2013) are very similar to the harvesting practices applied in Thailand (Sribandit et al. 2008) and Indonesia (Césard 2004). Major similarities between the three countries are: (1) the used tools and techniques (e.g., a bamboo pole and basket/bag); (2) the ant brood is an open-access resource but permission may need to be obtained from, for example, a plantation owner; (3) the ant brood harvest is an individual activity (each collector harvests brood for his/her own household) but several collectors take the same route together; (4) the majority of the harvest takes place near a collector's own village; (5) the majority of the harvest takes place in open areas (i.e., disturbed forest patches, along forest edges and paths, trees amid paddy fields); and (6) the collectors focus on large nests and small nests are left undisturbed. The biggest difference is that in Indonesia worker brood is favoured while in Laos and Thailand the queen brood is favoured. This implies that the ant harvest is a year-round activity in Indonesia whereas in Laos and Thailand it is seasonal (dry season only). A minor difference between Laos and Thailand involves the application of the wet method (Chapter 3) when separating worker ants from brood and separating small-sized brood from queen brood. In Thailand, the collectors may carry along a bucket of water and apply the wet method separation during the collecting trip (Sribandit et al. 2008). In Laos, the wet method is only applied at home (Chapter 3, Van Itterbeeck et al. 2012).

In Laos, Thailand, and Indonesia, concerns are raised over the sustainability of the ant brood harvest. Key issues in each of these countries are: (1) an increase in the number of collectors; (2) the adoption of non-sustainable harvesting practices such as applying suboptimal rotation schemes (this can be a consequence of the first key issue); and (3) a reduction in colony abundance due to deforestation (Césard 2004, Chapter 3, Sribandit et al. 2008, Van Itterbeeck et al. 2013). However, Offenberg and Wiwatwitaya (2010) suggest that the traditional harvesting practices and methods in Thailand are sustainable (some details were given in the introduction to this thesis). Chapter 6 suggests that when the harvesting pressure is increased, the ant brood harvest is not sustainable. A major constraint to the use of the *O. smaragdina* colony for human food and as a biological control agent is the aggressiveness of the worker ants and the associated high worker ant mortality during the harvesting of queen brood. Technical solutions can be developed that minimize worker ant mortality during the harvesting of queen brood (e.g., an artificial nest that allows the queen brood to be harvested in a non-destructive way and thus with minimal or no worker mortality, see below). Particularly in Indonesia, where worker brood is collected year-round, the future sustainability of the practice should be questioned.

The semi-cultivation of *O. smaragdina* has potential in all three countries. However, the resilience of colonies in Indonesia will be lower than in Thailand and Laos because of the harvesting of worker brood. Therefore, different harvesting practices must be applied to ensure sustainability (e.g., rotation schemes, harvested quantity).

There is little, or no, management of individual colonies and host trees with respect to the ant brood harvest in Laos, Thailand, and Indonesia. In the research areas of this thesis, there are very limited agroforestry practices and no plantations. Chapter 3 was limited to weaver ant biology. Ant colonies in home gardens are not managed in the research areas. The management of colonies is expected to be an important aspect of the future of the ant brood harvest. One key means to this end is the easy identification of the central element of the ant colony, viz., the gravid queen(s). Chapter 4 describes an easy way to identify the queen nest. These findings complement the findings of Peng et al. (1998a). This facilitates the translocation of mature colonies to designated areas. The queen tree is however highly attractive to the ant brood harvest because of the high number of nests located in this tree (Chapter 4, Van Itterbeeck et al. submitted). Chapter 5 shows that the disturbance caused by harvesting ant brood can cause an alarm in the queen nest. Frequent disturbance could induce the queen to migrate to a nearby tree, usually already incorporated in the territory of the respective colony (Joost Van Itterbeeck personal observations). To what extent this influences territory size and territorial competition between colonies (irrespective of food availability) is unknown. Does this put additional pressure on the competition between neighbouring colonies?

In addition, the semi-cultivation relies on an in-depth understanding of the organization of a colony. Given that the queen is vital to the survival of the colony (only the queen produces workers and virgin queens), she should be the focus of future research. This can only happen when her nest is easily located in natural settings.

Way forward

Fundamental research, innovation, and development are intertwined (ICSU 2004). The

semi-cultivation of *O. smaragdina* is an innovation; it has not been attempted nor achieved before. *Oecophylla smaragdina* can only contribute to social and economic development with fundamental research of *O. smaragdina* biology and ecology. I highlight two issues of major relevance to increasing the predictability, availability, and productivity of *O. smaragdina*. I also propose an *O. smaragdina* development analogous to wasp keeping and beekeeping, viz., artificial weaver ant nests.

First, the mechanisms that regulate queen brood production need to be understood. This involves a study of the seasonal cycle of brood production in mature colonies in relation to abiotic factors, and complementary studies of caste determination mechanisms and queen-worker interactions. Worker brood is produced year round while sexual brood is produced seasonally. The triggers of the onset and conclusion of the reproductive period are only roughly known (Chapter 3, Van Itterbeeck and van Huis 2012). Caste determination can involve a variety of factors which also includes queen pheromones (Hölldobler and Wilson 1990). The exact mechanisms of caste determination in *O. smaragdina* are, however, unknown (Bhattacharya 1943). A study of queen-worker interactions can reveal the exact role of the queen. The moment a colony peaks in its production of queen brood also needs to be identified. It can be beneficial for the queen brood harvest that this peak is correctly predicted for individual colonies. Based on these findings, harvesting experiments can be conducted to determine how much and how frequently queen brood can be harvested, and how queen brood yield can be increased without causing negative effects on the colonies and their biological control capability. From a practical viewpoint, these studies need to attempt to relate queen brood production to abiotic factors (e.g., temperature, relative humidity, photoperiod) and easily measured external features of a colony (e.g., ant density of ant trails, territory size). When such a relation is identified, predictions can be made in a non-destructive manner, i.e., it eliminates the need to open the nests to verify queen brood production.

Second, as land is scarce, can we increase the agricultural output per unit of land, e.g., in plantations and home gardens? We aim for a multi-production system of tree crops and queen brood which already implies an increase in agricultural output. However, the natural *O. smaragdina* nest distribution can be expected to be suboptimal from an economic viewpoint. The pest control capability of *O. smaragdina* is probably lower in trees that house few nests as opposed to trees that house a large number of nests. A more uniform nest distribution can be expected to increase tree crop yield and queen brood yield. Thus, the seasonal dynamics of the *O. smaragdina* territory size and nest distribution need to be understood. The mechanisms that regulate territory size and nest site selection need to be unravelled. Resource availability, and thus foraging range, has been considered a key issue in territory size dynamics. However, the dynamics appear to be more complex and other factors also play key roles, e.g., inter- and intraspecific competition that is not simply explained by resource availability (Parr and Gibb 2010). Nest site selection in *O. smaragdina* is a stigmergic response, which is a form of self-organization that does not necessarily require direct communication between individual ants (Hölldobler and Wilson 2009). However, the mechanisms that favour one location over another, both within a tree crown and between different crowns, is not yet understood. An understanding of the mechanisms that regulate territory size and nest site selection can lead to the ability to manipulate these.

The study of these issues requires the long-term exclusive use of colonies (at least one year).

I had attempted to investigate the seasonal cycle of brood production but encountered difficulties despite a range of measures taken and negotiated agreements made with the local communities. Queen brood had been harvested from all the colonies. This is probably because the queen brood is a highly lucrative trade item and a main source of income for many households.

In order to further facilitate the research and use of weaver ants, I draw on the history of wasp keeping and beekeeping. Wasps and bees have been kept in artificial nests for centuries (Bennet 1964, Villaneuva-G et al. 2005). This was notably for honey but also brood was, and still is, consumed (Bodenheimer 1951, Chen et al. 1998, Joost Van Itterbeeck personal observations). For example, the keeping of wasps for their brood is a traditional custom that persists in present day Japan (Nonaka 2007). The artificial nests that are used today allow the nest interior to be observed in a non-destructive way. Beekeepers, for example, can easily check the quantity of honey inside the nest without destroying it and causing only minimal disturbance to the honeybees. The use of artificial nests has also made major advances possible in our understanding of honeybee social organization (Seeley 2010). Ants are kept indoors by research institutions, museums, zoos, and hobbyists in formicaries. Artificial nests have also been used to benefit ant presence in pest control programs (Way and Khoo 1992). There is scope to be as innovative as wasp keepers and beekeepers once were. Analogous to the artificial honeybee and wasp nests, artificial weaver ant nests could be developed that facilitate the queen brood harvest. Weaver ants have been kept in the laboratory in glass and plastic tubes and in plastic bottles (Chapter 4, Hölldobler and Wilson 1994, Krag et al. 2010, Peng et al. 2013). Offenberg (2014) has shown that weaver ants also make use of plastic bottles in trees. This matches my own observations. I had hung plastic bottles in a number of trees at the end of the dry season in Laos. The ants make readily use of these plastic bottles (Figure 7.1). Additional support for developing artificial weaver ant nests that can be used outdoors comes from observations of a colony that made use of a wooden closet and the plastic bottles inside it. The closet was standing against the outside wall of my house in Laos. The ants made use of the plastic bottles first and later constructed silken chambers in the space between the bottles and the wooden walls of the closet (Figure 7.2.a and 7.2.b). Its dimensions were $\pm 40\text{-}40\text{-}40$ cm (this is a large nest). The ants inhabited this for two months during the dry season in 2012 (queen brood was raised to adulthood in this nest). I assume they abandoned this location because of frequent human disturbance (e.g., walking, talking). Whether weaver ants make use of artificial nests year round remains to be investigated (Offenberg 2014). There are multiple potential advantages to the use of artificial weaver ant nests, and these are similar to the advantages of using artificial nests for honeybees and wasps. The nest interior can be easily observed and, when designed well, queen brood could be harvested in a non-destructive manner and with minimal or no worker mortality. These nests can potentially be used both in natural settings (semi-cultivation) and in indoor weaver ant farming (as defined by van Huis et al. 2013). An in-depth understanding of the weaver ant is required to accomplish its semi-cultivation. Myrmecologists should strive for a weaver ant equivalent of Thomas Seeley's book *Honeybee Democracy* (2010). This will be facilitated with the development of artificial weaver ant nests. The development of artificial nests that can be used outdoors can accelerate and facilitate research of the regulation of brood production, and of the organization of a weaver ant colony.



Figure 7.1. Weaver ants inhabiting a plastic bottle (left) near a leaf nest (right). The bottle was hung tight against the branch so that it does not shake much when it is windy. The bottle opening points down so that, when it rains, water cannot get inside the bottle. (Picture: Joost Van Itterbeeck)

In addition, as Lynegaard et al. (2013) suggest that insect traps can be used to increase prey capture by *O. longinoda* in Africa, this can also be done in Southeast Asia. Moreover, the use of light traps to catch insects for human food and animal (e.g., chicken) feed is widespread in Laos and Thailand (van Huis et al. 2013, Joost Van Itterbeeck personal observations). Perhaps these light traps could be used to improve prey capture by *O. smaragdina*. Instead of catching the insects as feed for livestock (e.g., chicken), they can be caught as feed for minilivestock (weaver ants). However, the use of light traps, with which large amounts of insects can be caught in a single night, demands for the monitoring of insect populations because of the danger of overexploitation.

Finally, how can the widespread and successful adoption of the semi-cultivation of *O. smaragdina* be realized in the Lao PDR? The indigenous knowledge of *O. smaragdina* is possibly sufficient for a sustainable use of *O. smaragdina* as a Non-Wood Forest Product. However, key biological and ecological aspects must first be disseminated to and adopted by locals in order to semi-cultivate *O. smaragdina*. These include the concept of individual colonies and the role of worker ants and the gravid queen. For a widespread adoption, nation-wide development programs are required that are actively supported by the national government (e.g., a Farmer Field Schools approach that is organized and conducted by the relevant national institutions such as ministries). A centrally governed or imposed way towards community-based discovery learning on agroecological principles may be the best approach for the widespread adoption of the semi-cultivation of weaver ants in the Lao PDR. It is possible that by focussing on economic factors, the semi-cultivation of *O. smaragdina* is successfully adopted. However, the successful adoption of the semi-cultivation/farming of any species of edible insect is more than likely determined by the perception and role of insects as food. To encourage the semi-cultivation/farming of edible insects is an issue of promoting the use of insects as human food generally.

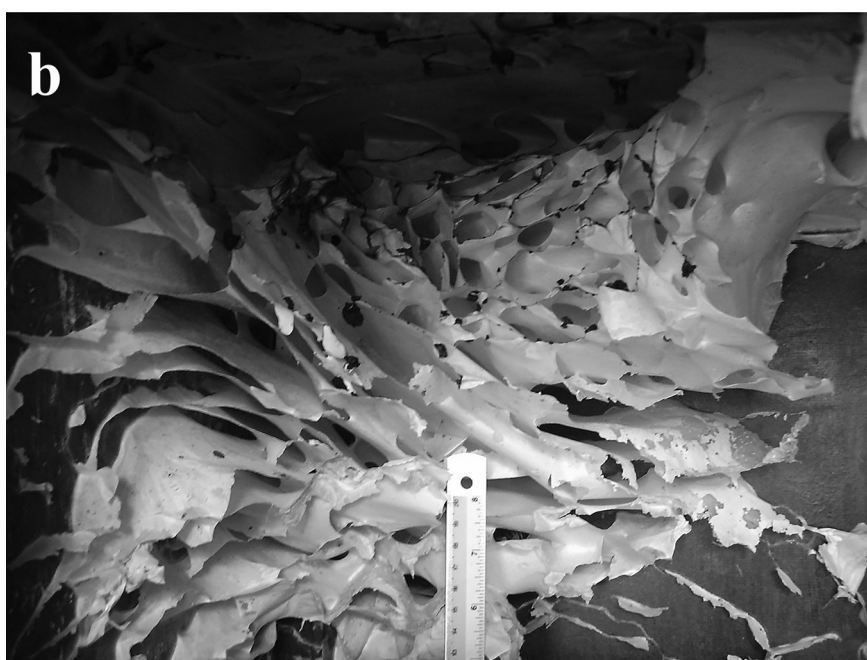


Figure 7.2.a Weaver ants nesting inside a wooden closet. (Picture: Joost Van Itterbeeck)

Figure 7.2.b The silken chambers in the space between the plastic bottles and the wooden walls of the closet. (Picture: Joost Van Itterbeeck)

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Summary

An agricultural revolution is necessary to feed the growing human population and to tackle sustainability issues of the food production systems. We have advocated that using insects for human food and animal feed (entomophagy) is a highly viable and sustainable option. One key issue in realizing the potential of edible insects is assuring an adequate supply in a sustainable way. This can be achieved by semi-cultivating and farming edible insects.

Chapter 2 reviews the semi-cultivation of edible insects in a historical perspective. The chapter focusses on three cases: (1) eggs of aquatic Hemiptera in Mexico which are semi-cultivated by water management and by providing egg laying sites; (2) palm weevil larvae in the Amazon Basin, tropical Africa, and New Guinea of which the collection is facilitated by manipulating host tree distribution and abundance and which are semi-cultivated by deliberately cutting palm trees at a chosen time at a chosen location; and (3) arboreal, foliage consuming caterpillars in sub-Saharan Africa for which the collection is facilitated by manipulating host tree distribution and abundance, shifting cultivation, fire regimes, host tree preservation, and manually introducing caterpillars to a designated area. This chapter also highlights the importance of indigenous knowledge to developments with edible insects.

Chapter 3 provides a detailed account of the indigenous knowledge of *Oecophylla smaragdina* and the ant brood collection practices from the Vientiane Plain, Lao PDR. This was compiled through focus group discussions and participant observations. Of major relevance is that the queen is unknown and that collectors refrain from removing large numbers of worker ants. I use these findings to reflect on sustainability and conservation issues, and on (semi-)cultivating constraints and possibilities embedded in indigenous knowledge and ant brood collection practices.

Chapter 4 investigates that location and external characteristics of the queen nest in a mature *O. smaragdina* colony. The chapter provides an easy way to identify the queen nest. This facilitates the introduction of the queen into plantations. This chapter suggests that the queen is unknown to the collectors because her nest is small and inconspicuous. Such nests are ignored by the collectors because they only expect a small yield. The chapter postulates that the location and external characteristics of the queen nest are a form of structural defence that the ants employ to protect the queen.

Chapter 5 shows a number of behavioural mechanisms that protect the queen from a predator. These mechanisms include a warning by worker ants that detect a danger, the evacuation of the queen from her nest (but only as a last resort), and the protective function of the retinue that is formed around the queen during evacuation.

Chapter 6 investigates the resilience of a colony of *O. smaragdina* to harvesting queen brood. This is done by using a high harvesting pressure, i.e., by removing all queen brood from all nests of a colony. The chapter reveals the need to improve the harvesting techniques in order to reduce worker ant mortality. High worker ant mortality negatively affects subsequent queen brood production, and may negatively affect the maintenance of a population of *O. smaragdina*.

Chapter 7 provides a general discussion and conclusion of the thesis and highlights priorities of future research in *O. smaragdina* semi-cultivation. The chapter calls for an investment in

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fundamental research of *O. smaragdina*, it proposes an *O. smaragdina* development analogous to wasp- and beekeeping, viz., an artificial nest, and it advocates that research in the evolution and dynamics of the perceptions of insects as human food are vital to realize the potential benefits of entomophagy to humankind.

Samenvatting

Een revolutie in onze manier van landbouw bedrijven is noodzakelijk om de groeiende wereldbevolking te voeden en om een duurzame vorm van voedselproductie te garanderen. Wij hebben voorgesteld dat het gebruik van insecten als voedsel voor mens en dier (entomofagie) een goede en duurzame mogelijkheid is. Een relevant aspect om het potentieel van eetbare insecten te realiseren, is het verzekeren van een voldoende grote voorraad van eetbare insecten op een duurzame manier. Dit kan verwezenlijkt worden door het semi-cultiveren en kweken van insecten.

Hoofdstuk 2 reviseert de semi-cultivatatie van eetbare insecten in een historisch perspectief. Het hoofdstuk focust op drie voorbeelden: (1) eitjes van aquatische Hemiptera in Mexico die semi-gecultiveerd worden door water management en door het voorzien van plekken waar de insecten eitjes kunnen leggen; (2) palmkever larven in de Amazone, tropisch Africa, en Nieuw Guinea waarvan het verzamelen vergemakkelijkt wordt door het manipuleren van de distributie en voorkomen van de palmbomen waarin de larven leven en die semi-gecultiveerd worden door het intentioneel kappen van palmbomen op een uitgekozen plaats en op een uitgekozen tijdstip; en (3) rupsen in sub-Sahara Africa die de bladeren van bomen eten en waarvan het verzamelen vergemakkelijkt wordt door het manipuleren van de distributie en voorkomen van de bomen, zwerfandbouw (shifting cultivation), bomen preservatie, en het manueel introduceren van rupsen in een uitgekozen plaats. Dit hoofdstuk verduidelijkt ook het belang van locale kennis (indigenous knowledge) voor ontwikkelingen met eetbare insecten.

Hoofdstuk 3 geeft een gedetailleerde weergave van de locale kennis van *Oecophylla smaragdina* en de wijze waarop deze mierensoort verzameld wordt op de Vientiane Plain, Lao PDR. Dit onderzoek was uitgevoerd door het houden van focus groep discussies en door expert verzamelaars te volgen. Het is een belangrijke vaststelling dat de verzamelaars de mierenkoningin niet kennen en dat zij vermijden dat grote aantallen werkster mieren verwijderd worden. Ik gebruik deze bevindingen om te reflecteren op de duurzaamheid van de mierenvangst, en op de mogelijkheden en restricties met betrekking tot het semi-cultiveren van de wevermier. Hoofdstuk 4 onderzoekt de locatie en de externe karakteristieken van de koninginnennest in een volwassen *O. smaragdina* kolonie. Het hoofdstuk geeft een handige manier weer om de koningin te vinden. Dit vergemakkelijkt de introductie van een kolonie in een plantage. Dit hoofdstuk suggereert dat de mierenverzamelaars de koningin niet kennen omdat de nest klein is en onopvallend. Dit type nesten wordt genegeerd door verzamelaars omdat zij slechts een kleine oogst verwachten van deze nesten. Het hoofdstuk suggereert dat de locatie en externe karakteristieken van de koninginnennest een vorm zijn van structurele verdedigingsmechanismen dat de mieren gebruiken om hun koningin te beschermen.

Hoofdstuk 5 toont een aantal gedragsmechanismen die de koningin beschermen tegen predatoren. Deze mechanismen omvatten een waarschuwing van de werkster mieren die een gevaar detecteren, het evacueren van de koningin uit haar nest (maar enkel als laatste oplossing), en de beschermingsfunctie van het retinue dat gevormd wordt rond de koningin tijdens haar evacuatie.

Hoofdstuk 6 onderzoekt de weerstand van een *O. smaragdina* kolonie tegen het verzamelen

van de koninginnenlarven en –poppen. We hebben dit gedaan aan de hand van een hoge verzameldruk: door het verwijderen van al de koninginnenlarven en –poppen van al de nesten van een kolonie. Het hoofdstuk geeft de noodzaak aan van een verbetering van de verzamelt-technieken om de mortaliteit van werkster mieren te reduceren. Hoge werkster mortaliteit heeft een negatief effect op de productie van koninginnenlarven en –poppen, en het kan een negatief effect hebben op het in stand houden van een populatie van *O. smaragdina* kolonies. Hoofdstuk 7 geeft een algemene discussie en conclusie van de thesis weer en benadrukt prioriteiten in verder onderzoek naar het semi-cultiveren van *O. smaragdina*. Het hoofdstuk roept op tot het investeren in fundamenteel onderzoek van *O. smaragdina*, het stelt verdere ontwikkelingen voor analoog met het houden van wespen en bijen, namelijk een artificeel nest, en het benadrukt de nood om verder onderzoek te verrichten in de evolutie en dynamiek van de percepties van insecten als menselijk voedsel om het potentieel van eetbare insecten voor de mens te realiseren.

Publications

Published

Van Itterbeeck J, Sivongxay N, Praxaysombath B, van Huis A (2014) Indigenous knowledge of the edible weaver ant *Oecophylla smaragdina* (Hymenoptera: Formicidae) from the Vientiane Plain, Lao PDR. *Ethnobiology Letters* 5:4-12

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In press

Van Itterbeeck J (2014) Insects. In Beaudry MC, Metheny K (eds) *The Archaeology of Food: An Encyclopedia*.

Van Itterbeeck J (2014) De wevermier: Een eetbare plaagbestrijder. *DIERPLAGEN Informatie* 3:16-18

Submitted

Van Itterbeeck J, Sivongxay N, Praxaysombath B, van Huis A (2014) Location and external characteristics of the *Oecophylla smaragdina* queen nest.

In preparation

Van Itterbeeck J, Sivongxay N, Praxaysombath B, van Huis A (2014) The organisation of queen defence in *Oecophylla smaragdina*: Warning, evacuation, and retinue function

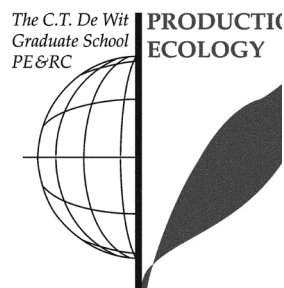
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[<http://www.antweb.org/page.do?name=edible>]

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (4.5 ECTS)

- Environmental manipulation for edible insect procurement: a historical perspective (2012)

Writing of project proposal (4.5 ECTS)

- Sustainability of harvesting *Oecophylla smaragdina* (Fabricius) (Hymenoptera: Formicidae) larvae and pupae for human food in Xaythani District, Vientiane Municipality, Lao PDR (2010)

Post-graduate courses (7.5 ECTS)

- Introduction to Bio-economic modelling; MG3S (2009)
- Advanced statistics course: design of experiments; PE&RC (2010)
- Ant course; California Academy of Science (2013)
- Hunger defeated? Long-term dynamics of global food security; PE&RC (2013)

Laboratory training and working visits (2.5 ECTS)

- Semi-cultivating / farming and captive breeding of the Asian weaver ant *Oecophylla smaragdina*; locating the gravid queen; Kasetsart University, Thailand (2012 / 2013)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Ethnobiology Letters: defoliato pest to caboclos and gourmet food to the Suruí Indians: contrasting Amazonian perspectives of *Lusura sp.* caterpillars (2012)
- British Journal of Applied Science & Technology: nutrient composition and potential contribution of winged termites (*Marcrotermes bellicosus*) to micronutrient intake of consumers (2013)
- Food Reviews International: reducing the impact on global warming livestock production: the minilivestock option (2013)
- International Journal of Nursing and Midwifery: a preliminary study on the estimation of nutrients and anti-nutrients in *Oedaleus abruptus* (Thunberg 1815) (Orthoptera: Acrididae) as a possible alternative food source for human and livestock (2013)
- Journal of Ethnobiology: perceptions of termites Isoptera, Insect in urban communities in semiarid northeastern Brazil (2013)

- Ecology of Food and Nutrition: exploring consumer acceptance of entomophagy: a survey and experiment Australia and the Netherlands (2013)

Deficiency, refresh, brush-up courses (3 ECTS)

- The ecological detective; confronting models with data (2009)
- Methods, techniques and data analysis for field research B (2010)

Competence strengthening / skills courses (2.8 ECTS)

- FAO, Rome, Organizing and maintaining an entomophagy network, and interaction with FAO employees on entomophagy and entomophagy related issues; FAO-WUR (2010)
- PhD Competence assessment; WGS (2010)
- Data management; WGS (2013)
- Career assessment; WGS (2013)

PE&RC Annual meetings, seminars and the PE&RC weekend (1 ECTS)

- How to write a world-class paper (2013)
- Global nutrient cycles and food security (2013)
- The search for tipping points in heterogeneous landscapes (2013)
- Wageningen PhD symposium (2013)
- Whole genome duplications as drivers of evolutionary innovations and radiations? (2013)

Discussion groups / local seminars / other scientific meetings (4.9 ECTS)

- Entomophagy PhD students (2013)
- FAO-Laos Edible insect seminar (2011)
- Biofuel workshop; Vientiane (2013)
- Nam Ngum River Basin Development workshop; Vientiane (2013)
- Stakeholder participation in research (2009-2010)
- Research Institute for Humanity and Nature projects workshops; Vientiane (2010 / 2012)
- PhD Lunch entomology (2009-2013)

International symposia, workshops and conferences (6.9 ECTS)

- Third International NHF workshop (2010)
- XXIV International Congress of entomology (2012)
- 9th ANeT International conference (2013)

Lecturing / supervision of practical's / tutorials (3 ECTS)

- General ant biology and ecology (2010, 2013)
- Supervision of bachelor students (2010)

Supervision of a MSc student

- The organization of queen defense in *Oecophylla smaragdina*: warning, evacuation, and retinue function

Curriculum vitae



Joost was born in Belgium on June 20, 1983. He moved to the Netherlands where he studied biology at Wageningen University. After attending a lecture of Professor Arnold van Huis, Joost got interested in entomophagy and its future prospects. He focussed his master studies on this topic and approached it from different angles. In his master thesis, he explored environmental, psychological, cultural, and ethical aspects that are relevant to promoting edible insects as an alternative source of food in the developed world. He wrote an additional master thesis on the emission of greenhouse gasses and ammonia by edible insects.

He conducted an internship with Professor Kenichi Nonaka in Laos where he explored the collection, consumption, and trade of a variety of edible insects. For his PhD project, Joost returned to Laos and lived there for two years and eight months. He chose the edible weaver ant *Oecophylla smaragdina* as his study subject. The PhD project reinforced Joost's broad interest in entomophagy and it triggered an interest in the biology and ecology of ants generally. This resulted in a contribution on edible ants to AntWeb, an online database of the known ant species of the world. Joost continues to work on the weaver ant *O. smaragdina* and is pursuing its semi-cultivation for the benefit of biological pest control and the *O. smaragdina* use as a source of human food.

