

Behavioral ecology of nest building:
construction of nest defensive structure in
paper wasps *Polistes chinensis antennalis*

古市, 生

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**Behavioral ecology of nest building:
construction of nest defensive structure
in paper wasps *Polistes chinensis antennalis***

Sho Furuichi

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Preface

Animals across a wide range of taxa build nests with various structures to protect their offspring from predation. Previous studies have demonstrated the anti-predator function of the nest structures in various animals, and these have suggested that the interspecific variation in nest architectures is adaptation to predators. On the other hand, nest architecture often varies in size and shape even within species. However, the intraspecific variation in nest architecture has been overlooked, and our understanding of adaptive adjustment of nest construction remains poor. In this thesis, I investigated whether animals adjusted the construction of the nest adaptively according to conditions. I focused on nest building in foundress (queen) of a paper wasp, *Polistes chinensis antennalis*.

A foundress of the paper wasp starts a colony in spring and maintains her nest alone until the emergence of workers. At this stage, pupae in the nests are sometimes preyed on by conspecifics of other nests. When a foundress is absent from her nest, a conspecific intruder fly to the nest, break a cocoon cap, extract the pupa from the nest cell and depredate it. Against pupa predation by conspecifics, foundresses often construct a structure on cocoon caps using nest material (pulp). The Pulp structure on cocoons is expected to function to prevent predation on pupae and to be adjusted according to costs and benefits of construction of it.

First, I examined the benefits and costs of the construction of the pulp structure (Chapter 1). Then, I investigated whether foundresses adjusted the construction of the pulp structure adaptively according to conditions (Chapter 1 and 2). I focused on predation risk and offspring value, which are general and important factors in reproductive success for nesting animals.

Chapter 1

Costs, benefits, and plasticity of construction of nest defensive structures in paper wasps

Abstract

Various animals build nests with defensive structures to deter predation on offspring. Construction of nest defensive structures can reduce the probability of predation but will involve various costs. Here I examined both the costs and benefits of the construction of a nest defensive structure in a paper wasp, *Polistes chinensis antennalis*, and clarify whether the paper wasp changes the level of defensive structures of nests depending on predation risk. A foundress (queen) of the paper wasp starts a colony in spring and maintains her nest alone until the emergence of workers. At this stage, pupae in the nests are sometimes preyed on by conspecifics of other nests. The intruder needs to break the cocoon, which seals the entrance of the cell, to extract the pupa from the cell. Foundresses often apply nest material (pulp) to the surface of cocoons in their nests. I found that pulp on a cocoon increased the time an intruder required to break the cocoon. This result shows that the pulp structure on cocoons helps to prevent predation on pupae. On the other hand, pulp on cocoons involved costs, including time required to collect pulp and being a potential obstacle to emergence of workers from the cocoon. Additionally, I found that the amount of pulp on cocoons was greater in nests under higher predation risk than nests under lower predation risk. These results suggest that pulp on cocoons is a nest defensive structure, and foundresses adjusted the construction of the defensive structure depending on predation risk.

Introduction

Many animal species build nests using surrounding and/or self-secreted materials and rear their offspring in the nests (Hansell 2005). Nests function to protect offspring from the adverse effects of the biotic and abiotic environment (Hansell 2005). In particular, nest predation is one of the greatest causes of reproductive failure (e.g., Ricklefs 1969; Fowler 1979; Strassmann et al. 1988). Therefore, animals build nests with various ornaments and structures (hereafter collectively referred to as defensive structures) to deter predation on offspring. For example, many birds (e.g., long-tailed tits, *Aegithalos caudatus*, and blue-gray gnatcatchers, *Polioptila caerulea*) cover nest exteriors with lichen, moss, and spider silk. This helps to camouflage nests from visually oriented predators (Collias & Collias 1984; Hansell 1996). Common waxbills, *Estrilda astrild*, and great crested flycatchers, *Myiarchus crinitus*, place carnivore scat and snake skins respectively, in, on, and around their nests, which act as an olfactory camouflage (Schuetz 2004; Medlin & Risch 2006). The mud wasp, *Paralastor* sp., builds a nest with a funnel-shaped entrance, which prevents predators entering the nest (Smith 1978).

On the other hand, building behavior involves costs in time and energy to collect, process, and assemble nest materials (Withers 1977; Collias 1986; Stanley 2002). In addition, defensive structures could harm the development of the offspring (Mayer et al. 2009; Prokop & Trnka 2011). Therefore, animals should avoid excessively constructing defensive structures.

Generally, predation risk varies spatially and temporally (Lima & Bednekoff 1999; Sih 2005; Creel & Winnie 2005), and therefore, animals are predicted to adjust the construction of defensive structures depending on predation risk. Many previous studies have demonstrated

the anti-predator function (benefits) of nest defensive structures in various animals (Martin 1992; Hansell 1996; Schuetz 2004; Medlin & Risch 2006) and suggested that the interspecific variation of nest architectures is adaptation to predators (Jeanne 1975). However, those studies have overlooked the plasticity in the construction of nest defensive structures, and therefore, it is not known whether individuals change the construction of defensive structures of nest depending on predation risk. Because plasticity in anti-predator traits plays an important role in predator-prey interactions (Lima et al. 2003; Sih 2005; Caro 2005), we need to investigate not only the defensive function of nest structures but also whether the structures are adjusted to predation risk to understand the interactions between nest predators and nesting animals.

The purpose of this study was to examine both the costs and benefits of the construction of a nest defensive structure, and then clarify whether construction of the defensive structure was adjusted to predation risk. I focused on a nest structure of a paper wasp, *Polistes chinensis antennalis*, whose immatures are often preyed on by conspecifics from other nests (Kasuya et al. 1980; Furuichi & Kasuya 2013a).

A foundress (queen) of *P. chinensis* initiates a new colony solitarily in spring (Miyano 1980). The foundress builds a nest with pulp, which is a mixture of plant fiber and oral secretion of the foundress (Kudô et al 1998; Kudô 2000). The foundress maintains a nest alone until the first workers emerge (Kasuya 1983a, b). At this stage, nests were often attacked by conspecific foundresses of other nests (Kasuya et al. 1980). The intruder flies to a nest whose owner is absent, extracts a larva from the cell, takes it back to her nest, and feeds her larvae with it (Kasuya et al. 1980). The owner can repel the attack by the intruder if she is on her nest (Kasuya 1983a). However, the foundress needs to leave the nest to gather resources such as water, pulp, and food for her larvae (Kasuya 1983a, b; Furuichi & Kasuya

2013b). Therefore, the foundress cannot completely prevent predation by intruders.

Intruders mainly prey on larvae, but recently I found that sometimes they also prey on pupae. Predation on pupae will delay the emergence of the first workers and extend the most vulnerable period when a solitary foundress maintains the nest (Miyano 1980). Therefore, predation on pupae has a severe negative effect on the nesting success of the foundresses. Foundresses should use additional ways to surely prevent predation on pupae.

The larva of a paper wasp spins a cocoon to seal the entrance of its cell before pupation (Figure 1.1). Therefore, intruders need to break the cocoon to extract the pupa from the cell. In *P. chinensis*, foundresses often apply pulp to the surface of their cocoons (Figure 1.1). They will reinforce the cocoon by the application of pulp, and pulp on the cocoon will increase the time that intruders require to break the cocoon. If a foundress returns to the nest when the intruder is still breaking a cocoon, the foundress can chase off the intruder before the intruder preys on the pupa (Kasuya 1983a). That is, increasing the time required for breaking the cocoon increases the probability of preventing predation on pupae and is an effective means of defense. Pulp that is applied to cocoons is predicted to function to prevent predation on pupae.

On the other hand, the application of pulp to cocoons involves time and energy costs. In addition, there are two other possible costs. First, workers that have eclosed from pupae need to break the cocoon. The body of a worker that has just eclosed is still soft, and therefore pulp on the cocoon is a possible obstacle to emergence from it. Second, pulp contains a significant amount of protein derived from the oral secretion of the foundress, a valuable and limited resource derived from ingested prey (Kudô 2000; Kudô 2002). Therefore, applying a large amount of pulp to cocoons could decrease the amount of pulp available for the construction of the body of a nest. For these reasons, foundresses should not apply an excess of pulp to

cocoons. Foundresses, therefore, are predicted to adjust the amount of pulp on cocoons depending on predation risk.

In this study, first, to clarify the defensive function of pulp on cocoons, I examined whether pulp on the cocoon increased the time intruders required to break the cocoon. Second, to clarify whether there were costs of pulp application to cocoons in addition to the expenditure of time and energy, I examined whether pulp on a cocoon was an obstacle to the emergence of workers from the cocoons, and whether the investment of pulp applied to cocoons decreased the amount of pulp available for the construction of the body of the nest. Third, to clarify whether foundresses adjusted the construction of the nest structure to predation risk, I examined whether foundresses applied a greater amount of pulp to cocoons under a higher risk of nest predation.

Methods

Study species and site

P. chinensis is a eusocial wasp with an annual life cycle. Overwintering foundresses emerge in spring and begin nest construction and brood rearing alone (Kasuya 1983a,b). Workers emerge from late spring to summer (Miyano 1980). Foundresses mainly prey on lepidopteran larvae (Suzuki 1978; Kasuya 1980), but often attack conspecific nests (Kasuya et al 1980; Kasuya 1983a).

This study was conducted in a grassland area (ca. 0.54 ha) of Kashiihama in Fukuoka, Japan (33° 39' 27" N, 130° 25' 37" E), from May 9 to June 15, 2011. Forty-seven nests of *P.*

chinensis were found. I marked foundresses individually with enamel paint (Opaque Color, Teranishi Chemical Industry, Osaka, Japan), and measured their head widths with a digital caliper (CD-15B, Mitutoyo Corporation, Kanagawa, Japan) to the nearest 0.01 mm.

Measurement method of the amount of pulp on a cocoon

I photographed each cocoon using a digital camera (Optio W80, Pentax Ricoh imaging Company, Tokyo, Japan). The amount of pulp on a cocoon was calculated as the ratio of the area of pulp on a cocoon to the frontal area of the cocoon, both determined using the image analysis software imageJ ver.1.46r (Abramoff et al. 2004). Because the dry weight of pulp on a cocoon was strongly positively correlated with the ratio of the pulp area ($r = 0.94$, $t = 11.1$, $df = 17$, $P < 0.0001$, $N = 19$; S. Furuichi, unpublished data), I used the ratio of the pulp area as an index of the amount of pulp.

Field observations

At the study site, I started the observation of each nest when only eggs and/or small larvae (the first and second instar larvae) were present in the nest, and continued it until emergence of the first worker from the nest. I photographed each nest at 17:00 hours every day using a digital camera to record the states of the nest. From the images, I recoded the number and arrangement of cells in a nest, the contents of cells (empty, egg, 1-5 instar larva, and pupa), and the amount of pulp on each cocoon. I calculated two indices of the amount of pulp on cocoons in a nest; (1) the total amount of pulp on cocoons in a nest, which was the sum of the amount of pulp on a cocoon of all the cocoons in a nest, and (2) the mean amount

of pulp on a cocoon in a nest. I recorded the number of predation events by conspecifics as an index of predation risk from the daily data on the states of nests. I defined a sudden disappearance of larvae or pupae as predation by conspecifics because it never arose unless predation by conspecifics occurred. Nineteen nests were abandoned by the foundresses before the emergence of workers, and I was able to observe 13 nests until the emergence of workers. The data from these 13 nests were used in analyses.

Predation experiment

To observe predation on pupae by conspecifics, I conducted a predation experiment from May 25 to June 5. I collected 10 nests with cocoons from the study site and recorded the amount of pulp on each cocoon. I removed the foundress, eggs, and larvae from the nest, and then returned the nest to where it had been at the study site. Nests were placed and monitored from 0800 to 1600 hours. Nests were monitored by video cameras (DCR-SR87, Sony, Tokyo, Japan), which were placed approximately 1 m from the nest. From the recorded images of predation by conspecifics, I recorded the identity of the intruder, the identity of the pupa preyed on, and the time required for breaking a cocoon (in seconds). The time required for breaking a cocoon was defined as the time from when the intruder bit the cocoon to when she completely pulled the pupa out of the cell.

Emergence-from-cocoon experiment

I collected five nests with cocoons from the study site on June 5 and recorded the amount of pulp on each cocoon. To observe the emergence of workers from the cocoons, I

removed the foundress, eggs and larvae from the nest, and then placed the nest in the laboratory at 25°C and 60% relative humidity. Nests were constantly monitored by the video cameras, which were placed approximately 1 m from the nest. For monitoring, I kept the laboratory lit. From the recorded images of the emergence of workers from the cocoons, I recorded the identity of the emerging worker, and the time required to emerge from a cocoon (in seconds). The time required to emerge from a cocoon was defined as the time from the appearance of a part of the mandibles of the emerging worker to the complete appearance of its body from the cell. In addition, I measured the head width of workers with a digital caliper to the nearest 0.01 mm.

Data analyses

First, I investigated whether pulp on cocoons functions to prevent predation on pupae. I examined whether the amount of pulp on a cocoon affected the time required for breaking a cocoon, using the data of the predation experiment, and generalized linear mixed models (GLMMs) with a Gaussian error structure and an identity link. In the model, the identity of the attacked nest and the identity of the attacking conspecific were incorporated as random effects. Because the body size of the intruders (confounding factor) possibly affects the time required for breaking a cocoon, it was also incorporated as an explanatory variable in the model to adjust for the confounding effect.

Second, I investigated the costs of pulp application. To investigate whether pulp on a cocoon was an obstacle to the emergence of a worker from the cocoon, I examined whether pulp on a cocoon increased the time required for emergence from a cocoon, using the data of the emergence-from-cocoon experiment, and using GLMMs with a Gaussian error structure

and the identity link. In the model, the identity of the nest was incorporated as a random effect. Because the body size of the worker (confounding factor) possibly affects the time required for emerging from a cocoon, it was also incorporated as an explanatory variable in the model to adjust for the confounding effect. To investigate whether the investment of pulp in application to cocoons decreases the amount of pulp available for the construction of the body of the nest, I examined whether the total amount of pulp on cocoons affected the number of cells in the nest. In this analysis, I used the data of the field observation, and used generalized linear models (GLMs) with a Poisson error structure and the log link. I used the total amount of pulp on cocoons and the number of cells in the nest on the seventh day after the first cocoon was spun in the nest. Because predation risk possibly affects the number of cells, the index of predation risk (confounding factor) was also incorporated as an explanatory variable in the model to adjust for the confounding effect.

Third, I investigated whether foundresses adjusted the amount of pulp on cocoons depending on predation risk. I examined whether predation risk affected the total amount of pulp on cocoons and the mean amount of pulp on a cocoon in a nest using field observation data. In these analyses, we used the amounts of pulp in a nest on the seventh day after the first cocoon was spun in the nest. For the total amount of pulp, I used GLMs with a Gaussian error structure and the identity link. For the mean amount of pulp on a cocoon, I used GLMs with quasiliikelihood with the logit link and setting the variance proportional to $\text{mean} \times (1 - \text{mean})$ because the amount of pulp on a cocoon was a ratio of the pulp area and ranged from 0 to 1. Because the number of old larvae (fourth- and fifth-instar larvae) possibly affected the amount of pulp on cocoons, the number of old larvae (confounding factor) was also incorporated as an explanatory variable in the models to adjust for the confounding effect. When many old larvae, which consume a large amount of food, are present in a nest, the foundress of the nest will

need to spend more time foraging and not spend enough time collecting pulp, possibly resulting in a decrease in the amount of pulp on cocoons.

To examine the significance of the explanatory variables, I conducted a likelihood ratio test in all the analyses except when GLMs with quasiliikelihood were used. We conducted an *F* test when using GLMs with quasiliikelihood. All the tests were two-tailed, and the level of significance was 0.05. All the analyses were performed with the statistical software R ver.2.15.1 (R Development Core Team 2012).

Results

Field observations

During the field observations, predation by conspecifics occurred 19 times. Of them, 16 were predation on larvae, and three on pupae. The mean number of predation events per nest was 1.47 ± 1.33 (\pm SD), and ranged from 0 to 3. Of all the 13 nests observed, application of pulp to cocoons was found in 12 nests. Pulp was applied to a cocoon to cover it partially rather than entirely.

Defensive function of pulp application

In the predation experiment, I observed 19 predation events by 12 intruders. Intruders required 144.4 ± 119.3 (mean \pm SD) seconds to break a cocoon. The time required to break a cocoon increased significantly ($\beta \pm$ SE = 476.49 ± 64.15 , $\chi^2 = 55.2$, $P < 0.0001$) as the amount

of pulp on the cocoon increased (Figure 1.2). This relationship appeared to be driven by one datum (the rightmost point in Figure 1.2), but removal of this datum still resulted in a significant relationship ($\beta \pm SE = 337.0 \pm 53.0, \chi^2 = 40.5, P < 0.0001$).

Costs of pulp application

In the emergence-from-cocoon experiment, I observed the emergence of 16 workers. No worker was injured or failed to emerge from cocoons owing to pulp on a cocoon. Workers required a significantly longer time ($\beta \pm SE = 4021 \pm 1475, \chi^2 = 7.43, P = 0.006$) to emerge when the amount of pulp on a cocoon was greater (Figure 1.3).

The mean number of cells in a nest was 37.1 ± 5.3 (\pm SD). The total amount of pulp on cocoons did not significantly affect the number of cells (Figure 1.4; $\beta \pm SE = 1.83 \pm 2.81, \chi^2 = 0.43, P = 0.51$).

Adjustment of pulp application to predation risk

The total amount of pulp on cocoons in a nest was 0.93 ± 0.79 (\pm SD) on average, and ranged from 0 to 2.92. The nest without pulp on cocoons did not suffer predation during the observation. The total and mean amounts of pulp were significantly higher in the nests that suffered predation more frequently (Figure 1.5; total amount of pulp: $\beta \pm SE = 0.38 \pm 0.16, \chi^2 = 5.97, P = 0.01$; mean amount of pulp: $\beta \pm SE = 0.71 \pm 0.18, F_{1,10} = 18.4, P = 0.002$).

Discussion

Pulp on a cocoon increased the time that intruders required to break the cocoon (Figure 1.2). This shows that pulp on a cocoon helps to prevent predation on pupae. Increasing the time required for breaking the cocoon increases the likelihood that the owner returns to the nest before the intruder finishes removing the pupa (Kasuya 1983a). On the other hand, to construct the pulp structure on cocoons, foundresses need to spend time collecting pulp. In the nests observed in the field, the amount of pulp applied to cocoons (the total amount of pulp on cocoons in a nest) was 0.46 ± 0.39 mg (\pm SD, $N = 13$) on average, and up to 1.44 mg (the dry weight of the total amount of pulp on cocoons in a nest was estimated by regression analysis; S. Furuichi, unpublished data). The mean amount of pulp that foundresses gathered in one pulp-gathering trip was 0.39 ± 0.09 mg (\pm SD, $N = 4$ foundresses, dry weight; S. Furuichi, personal observation). Therefore, it is estimated that foundresses conducted pulp-gathering trips 1.2 times on average, and up to 3.7 times to construct the pulp structure on cocoons. Because the time of one pulp-gathering trip was 141.1 ± 62.2 seconds (mean \pm SD, $N = 10$ foundresses; S. Furuichi, personal observation), foundresses were estimated to spend 169.3 seconds on average, and up to 522.1 seconds to construct the pulp structure on cocoons.

Pulp on cocoons possibly becomes the obstacle to the emergence of workers from the cocoon. I found that pulp on a cocoon increased the time that workers required for emerging from cocoons (Figure 1.3). This suggests that pulp on cocoons has the potential to injure the emerging workers and lead to failure of the emergence from the cocoon. In the present study, however, no worker failed to emerge or was injured owing to pulp on cocoons. Although pulp on a cocoon is a possible obstacle to the emergence from cocoons, the adverse effect would be small.

There is a possibility that the amount of pulp available is limited because pulp contains a significant amount of protein, a valuable and limited resource (Kudô 2000; Kudô 2002). Under such conditions, if a wasp was to apply a large amount of pulp to cocoons, it would lead to a decreased amount of pulp available for the construction of the body of the nest. However, in the present results, the investment of pulp in application to cocoons did not have a significant effect on the number of cells in the nest (Figure 1.4). The amount of pulp applied to cocoons might only be a small part of pulp available.

This study suggests that application of pulp to cocoons has a benefit of preventing predation on pupae, but also involves costs. In the field observation, I found that foundresses applied a larger amount of pulp to cocoons when predation risk was high, and a smaller amount of pulp to cocoons when predation risk was low (Figure 1.5). This suggests that pulp on cocoons is a nest defensive structure, and foundresses adjust the amount of pulp on cocoons depending on predation risk. When predation risk is high, foundresses will gain a high benefit from applying the large amount of pulp to cocoons, and the benefit will outweigh the cost. In contrast, when predation risk is low, even if foundresses apply the large amount of pulp to cocoons, the benefit from pulp application will be small, and the cost will outweigh the benefit. Foundresses will adjust the amount of pulp on cocoons to maximize the net benefit from pulp application.

In the field observation, I found that predation on larvae was far more common than on pupae. The rarity of predation on pupae in the field could be interpreted in two ways. First, the cocoons and pulp structures on cocoons are highly successful in deterring predation by conspecifics. Second, intruders prefer larvae, which intruders can rob in a shorter time, to pupae. In fact, when attacking nests with larvae and pupae, intruders often contact and ignore the capped brood (S. Furuichi, personal observation). The cocoons and pulp on cocoons

would effectively prevent predation on pupae.

Defense of offspring through nest structures can be divided into two stages (Edmunds 1974); ‘primary defense’ that reduces the likelihood of detection of nests by predators (e.g., camouflage, Collias & Collias 1984; Hansell 1996), and ‘secondary defense’ that reduces the likelihood of successful predation after detection of nests by predators (e.g., mechanical protection, Martin 1992; Smith 1995; Leader & Yom-Tov 1998). Pulp on cocoons is a defensive structure that increases the time required for predation and decreases the probability of successful predation. Therefore, it has the function of a secondary defense. Previous studies reported that the application of pulp to cocoons occurs in many paper wasps that make exposed nests (Jeanne 1972; Kojima 1982; Spradbery & Kojima 1989; Hagiwara & Kojima 1994). These studies interpreted this behavior as camouflaging the nests by making the conspicuous white cocoons less obvious (Jeanne 1972; Kojima 1982; Spradbery & Kojima 1989; Hagiwara & Kojima 1994). That is, these studies inferred that pulp on cocoons has a primary defensive function. In *P. chinensis*, pulp on cocoons may also have such a function in addition to secondary defense.

Previous studies focused only on demonstrating the function of nest structures (Martin 1992; Hansell 1996; Schuetz 2004; Medlin & Risch 2006). Therefore, although many studies have investigated the function of nest structures, none had examined whether individuals changed the construction of nest structures based on predation risk. To my knowledge, this study is the first to suggest that individuals can adjust a nest defensive structure to predation risk. Various animals build nests to protect offspring against predators, and the costs of building nests are common (Hansell 2005). Adjusting nest structures to predation risk could be prevalent in nesting animals. Furthermore, the structures that animals build are not only nests that protect offspring. A number of different animals build structures to protect

themselves against predators (Hansell 2005). For example, the larvae of many caddisfly species build their own portable cases made of organic and/or mineral particles that are cemented with the silk that they secrete (Boyero et al. 2006). The larvae of several species of leaf beetle build shelters using their own waste materials (Eisner and Eisner 2000; Brown and Funk 2010). Many spider species build their own shelter using self-secreted silk (Manicom et al. 2008). Constructing the structures is considered to involve costs (Venner et al. 2003; McKie 2004; Mondy et al. 2011), and the animals will adjust them depending on predation risk. Many theoretical and empirical studies show that the changes in behavior and morphology in response to predation risk play an important role in predator-prey interactions (e.g., Lima et al. 2003; Sih 2005; Caro 2005). Similarly, the changes in architectural structure in response to predation risk will have a significant effect on predator-prey interactions. The present results suggest that to understand the interactions between predators and the animals, we need to investigate not only the defensive function of the architectural structures but also how the structures are adjusted to predation risk.

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Figures Legends

Figure 1.1 **a** A nest of *P. chinensis*, **b** the frontal image of cocoons in the nest, and **c** diagrammatic representation of cocoons of the nest. The solid area represents pulp applied. **d** A diagram showing a longitudinal section of a cell containing a pupa.

Figure 1.2 The relationship between the amount of pulp on a cocoon and the time that an intruder required for breaking the cocoon. An open circle represents a cocoon.

Figure 1.3 The relationship between the amount of pulp on a cocoon and the time that a worker required for emerging from the cocoon. An open circle represents a cocoon.

Figure 1.4 The relationship between the total amount of pulp on cocoons in a nest and the number of cells in the nest. An open circle represents a nest.

Figure 1.5 The relationship between predation risk and, (a) the total amount of pulp on cocoons in a nest, (b) the mean amount of pulp on a cocoon in a nest. An open circle represents a nest.

Figure 1.1

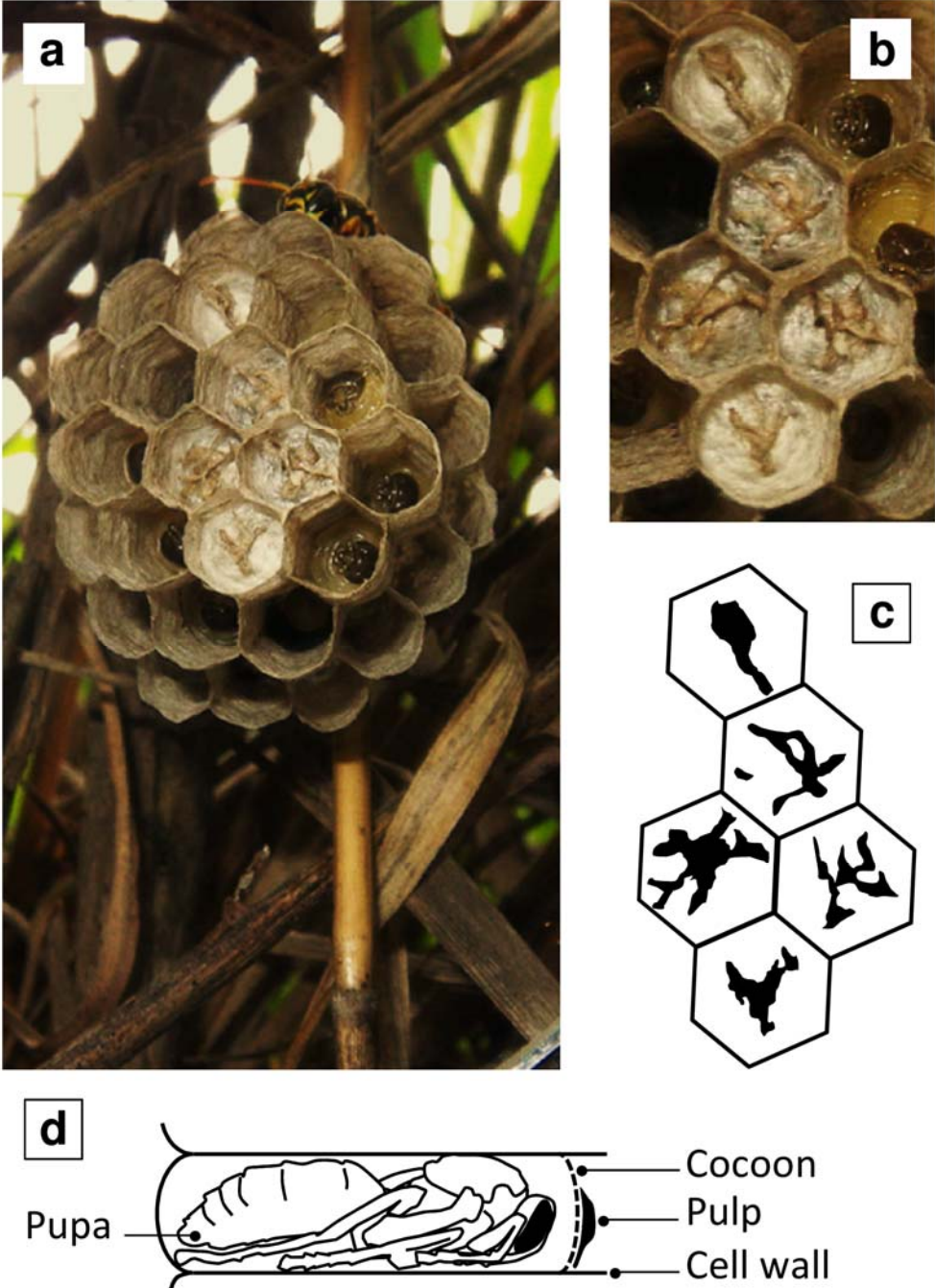


Figure 1.2

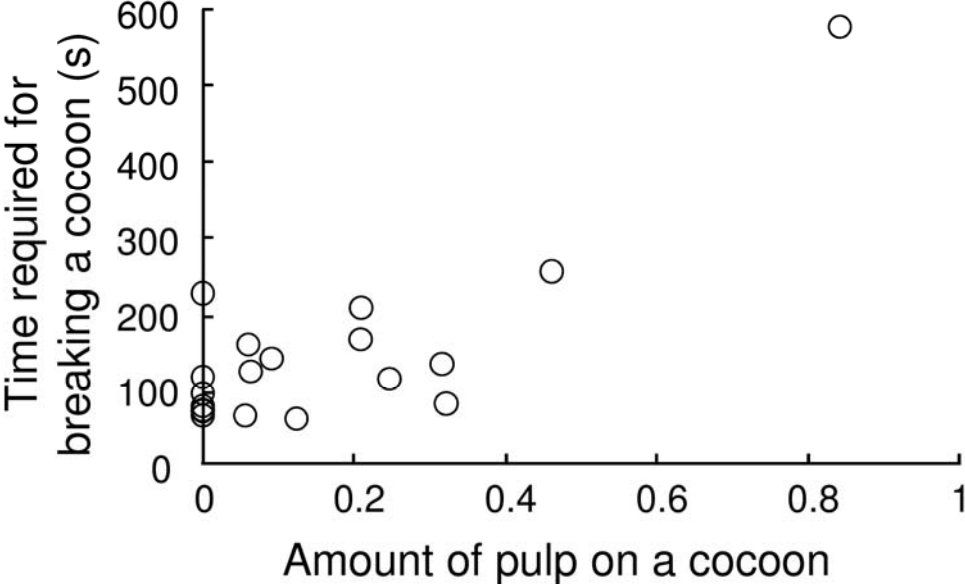


Figure 1.3

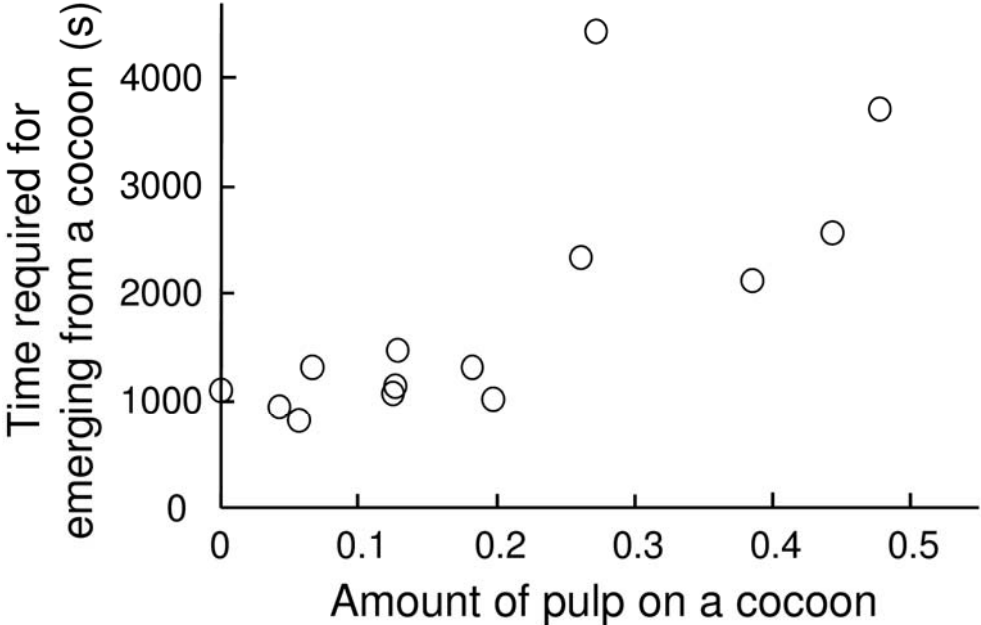


Figure 1.4

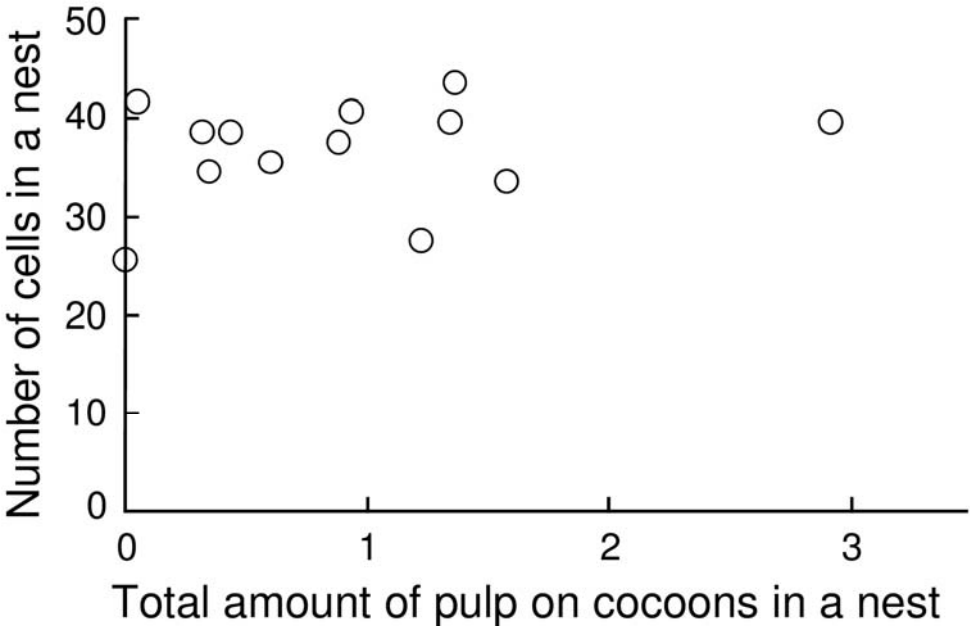
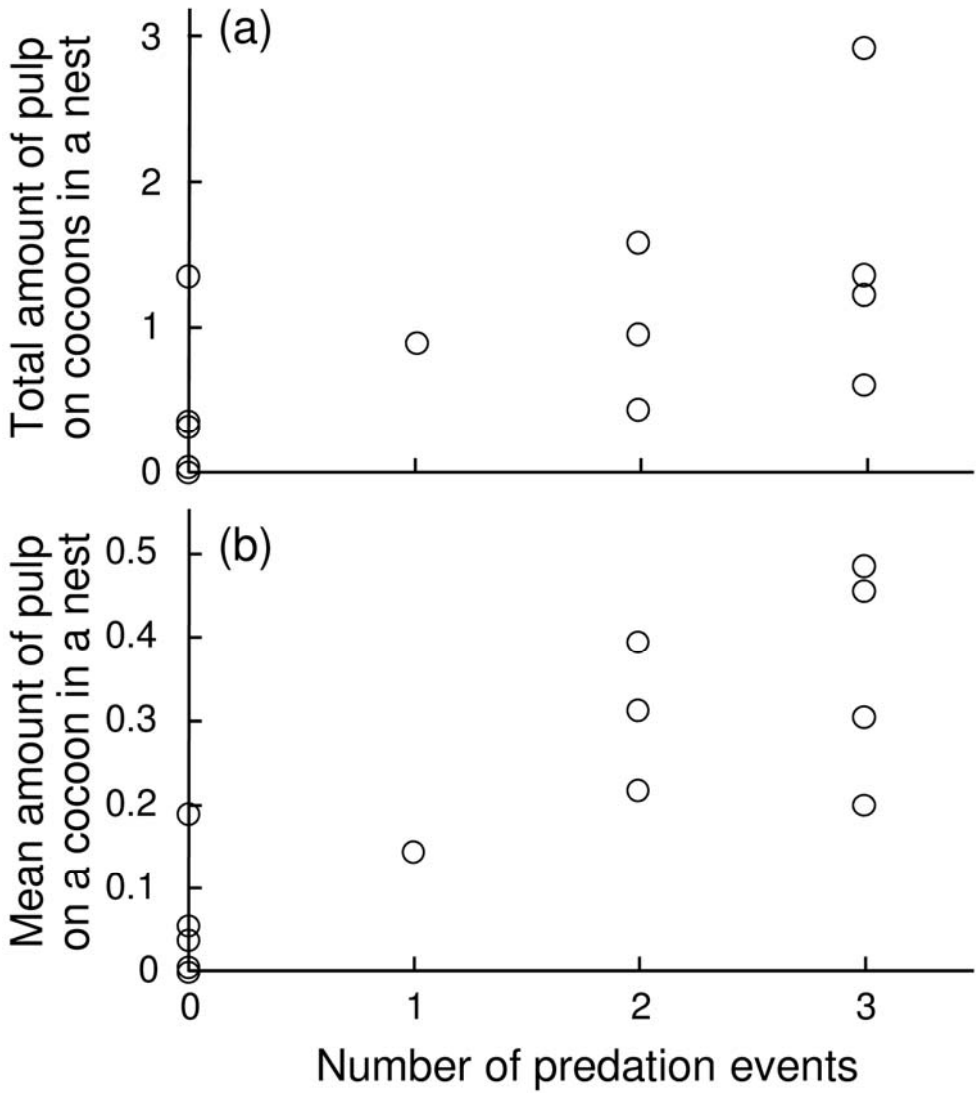


Figure 1.5



Chapter 2

Construction of nest defensive structure according to offspring value and its effect on predator's attack decision in paper wasps

Abstract

Various organisms are known to build nests with defensive structures to protect their offspring from predation, but our understanding of plasticity in the nest structure remains poor. In this study, I investigated whether a paper wasp, *Polistes chinensis antennalis*, adjusted the construction of nest defensive structure according to the value of their offspring, and I also analysed the effect of adjusting the construction of the structure on predator's decision to attack. A foundress (queen) of the paper wasp starts a colony and maintains her nest alone until the emergence of workers. During this stage, foundresses often construct a defensive structure on cocoon caps using nest materials (pulp), which prevents predation of cocooned immatures by conspecifics from other nests. The value of cocooned immature to the foundress varies among immatures in a nest, where the value of those that spun the cocoon (and initiated pupation) earlier among immatures in the nest is higher. From field observations, I found that foundresses constructed a larger pulp structure on the cocoons of immatures that cocooned earlier in the order of cocoon spinning, even after considering confounding factors. I also found that the probability of a cocooned immature being attacked by conspecific intruders decreased with the size of pulp structure on the cocoon. This indicates that intruders avoid attacking cocoons with larger pulp structures. The present study indicates that nesting animals adjust the construction of nest defensive structures according to their offspring value, and this allows them to protect the high-value offspring efficiently and effectively.

Introduction

Animals across a wide range of taxa build nests using collected and/or self-secreted materials to rear their offspring (Hansell 2005). These nests have the function of protecting offspring from the adverse effects of the biotic environment (e.g., predation and pathogens) and abiotic environment (e.g., desiccation and extreme temperatures) (Hansell 2005). Nest predation is one of the most serious factors that affect the mortality of offspring (e.g., Ricklefs 1969; Strassmann et al. 1988; Laurenson 1994; Filippi-Tsukamoto et al. 1995). Therefore, animals build nests with various ornaments and structures to prevent the predation of their offspring (which I refer to as defensive structures) (Smith 1978; Martin 1992; Smith 1995; Hansell 1996; Schuetz 2004; Furuichi & Kasuya 2014).

Animals can reduce the probability of nest predation by constructing defensive structures (Schuetz 2004; Medlin & Risch 2006; Furuichi & Kasuya 2014). However, the nest-building behaviour involves costs in time and energy for the collection, processing, and assembly of nest materials (Withers 1977; Collias 1986; Stanley 2002; Furuichi & Kasuya 2014). Therefore, animals should adjust the construction of the defensive structure according to its costs and benefits.

The benefits accrued from the construction of the defensive structures depend on the offspring's value to their parents. The offspring's value varies according to factors such as age and quality of offspring, and thus, the value can differ between offspring (Montgomerie & Weatherhead 1988; Caro 2005). Therefore, parents are expected to adjust the construction of the defensive structures according to their offspring's value.

Previously, many studies have investigated the structures of nests produced by various animals (e.g. Martin 1992; Hansell 1996; Schuetz 2004; Medlin & Risch 2006). However,

these studies often focused only on demonstrating the functions of nest structures, whereas plasticity in nest structures have received less attention, although nest structures often vary even among individuals within a species (e.g., Downing & Jeanne 1986; Karsai & Péntzes 1996; Walsh et al. 2011). Therefore, it is not known whether animals adjust the construction of the nest defensive structures on the basis of their offspring's value.

The aim of this study was to determine whether nesting animals show plasticity in their building behaviour by adjusting the construction of the nest defensive structures according to their offspring's value. In addition, I investigated the effect of adjustments in the construction of the nest defensive structures on the predator's decision to attack. In the present study, I focused on the nest defensive structure produced by a paper wasp, *Polistes chinensis antennalis* (Furuichi & Kasuya 2014).

The paper wasp, *P. chinensis antennalis*, is a eusocial wasp, with an annual life cycle. Mated foundresses (queens) emerge from their overwintering hibernacula in spring, after which they commence solitary nest construction and brood rearing (Kasuya 1983a, 1983b). The nest is built from pulp, which is a composite of the plant fiber and oral secretions of the foundress (Kudô et al. 1998; Kudô et al. 2000). The foundress maintains her nest alone until the emergence of first workers (Miyano 1980). During this stage, the foundress herself needs to leave the nest to gather resources, such as food for her larvae (mainly lepidopteran larvae), nest materials, and water (Suzuki, 1978; Kasuya, 1980). However, when the foundress is absent from the nest, there is a risk that her offspring will be predated by conspecific foundresses from other nests (Kasuya et al. 1980; Furuichi & Kasuya, 2013a, 2013b). These conspecific foundresses visit a nest when the owner is absent to extract an immature from the nest cell, which they take back to their own nest and feed to their larvae (Kasuya et al. 1980). The owner can only repel an attack by an intruder if she is present on her nest (Kasuya

1983a).

Intruders mainly predate exposed larvae, but they sometimes also predate cocooned pupae (Furuichi & Kasuya 2014). The predation of pupae will considerably delay the emergence of first workers, thereby extending the most vulnerable period when a solitary foundress maintains the nest (Miyano 1980). Therefore, the predation of pupae has a severe negative effect on the nesting success of foundresses. To combat the predation of pupae, foundresses often construct a defensive structure on the surface of the cocoon cap using pulp. The pulp structure on cocoons is known to prevent the predation of pupae by increasing the time required by intruders to break into cocoons, thereby decreasing the probability of successful predation (Furuichi & Kasuya 2014). However, the construction of the pulp structure incurs costs, including the time required to collect pulp (Furuichi & Kasuya 2014). Therefore, foundresses need to adjust the construction of the pulp structure according to its costs and benefits.

The value of cocooned immatures to foundresses varies among the individual immatures in a nest. The value of relatively more developed immatures is higher in a nest because colonies of paper wasps are most vulnerable during the period when a solitary foundress maintains the nest alone (Miyano 1980), and foundresses need to produce many workers as early as possible (Reeve 1991; Kudô 2003). Therefore, it is predicted that foundresses construct a larger pulp structure on cocoons of immatures that spun the cocoon (and initiated pupation) earlier among immatures in the nest. In addition, if foundresses adjust the amount of the pulp structure on a cocoon according to the value of the immature, the size of the pulp structure will vary among cocoons in a nest (multiple cocoons are usually present in a nest), and intruders may preferentially avoid attacking cocoons with a larger pulp structure. Adjusting the size of the pulp structure on a cocoon on the basis of the value of the

immature may incur an additional benefit by decreasing the probability that high-value offspring are attacked, as well as decreasing the probability of successful predation on them when they are attacked.

In this study, to test the prediction that foundresses adjust the construction of the pulp structure on a cocoon according to the value of the immature, I investigated whether foundresses construct a larger pulp structure on the cocoons of immatures that spun the cocoon earlier among immatures in the nest. In addition, to clarify the additional benefit of adjusting the construction of the pulp structure according to the value of the immature, I examined whether intruders avoided attacking cocoons with larger pulp structures.

Methods

Study Site

This study was conducted on a grassland (ca 0.54 ha) in Kashiihama, Fukuoka, Japan (33° 39' 27'' N, 130° 25' 37'' E), from May 9 to June 15, 2011. Forty-seven nests of *P. chinensis antennalis* were found, and I marked foundresses individually with enamel paint (Opaque Color, Teranishi Chemical Industry, Osaka, Japan).

Measurement of Pulp Structure Size

I photographed each cocoon using a digital camera (Optio W80, Pentax Ricoh imaging Company, Tokyo, Japan). The size of the pulp structure on a cocoon was calculated as the

ratio of the area of pulp on a cocoon relative to the frontal area of the cocoon, both of which were determined using the image analysis software imageJ ver.1.46r (Schneider et al. 2012). The dry weight of the pulp structure on a cocoon had a strong positive correlation with the ratio of the pulp area (Furuichi & Kasuya 2014); thus, I used the ratio of the pulp area as an index of the size of the pulp structure.

Field Observations

At the study site, I started the field observation of each nest when only eggs and/or small larvae (first- and second-instar larvae) were present in the nest, and I continued the observations until the emergence of the first worker from the nest. I photographed each nest at 17:00 h each day using a digital camera to record the state of each cell in nests. Using the images, I recorded the number and arrangement of cells in a nest, the contents of cells (empty, egg, larva in instars 1–5, or pupa), and the size of the pulp structure on each cocoon. I also recorded the date when an immature spun a cocoon. Some nests were abandoned by foundresses before the emergence of workers, but I was able to observe 13 nests until the emergence of workers. The data from these 13 nests were used in the analyses.

Predation Observation

I observed the predation of cocooned immatures by conspecifics from May 25 to June 5, 2011. I collected 10 nests with cocoons (5.3 ± 0.8 [mean \pm SE] cocoons per nest) from the study site and recorded the size of the pulp structure on each cocoon. I removed the foundress, eggs, and larvae from the nest and then returned the nest to its original location at the study

site. The nests were then monitored from 08:00 to 16:00 h using video cameras (DCR-SR87, Sony, Tokyo, Japan), which were placed approximately 1 m from the nest. On the basis of the recorded images of predation by conspecifics, I recorded the identity of the intruder and the cocooned immature predated.

Data Analyses

First, I investigated whether foundresses adjusted the construction of the pulp structure according to the value of an immature. The value of cocooned immatures to foundresses should be higher in those that spun the cocoon (and initiated pupation) earlier among immatures in a nest because foundresses need to produce many workers as early as possible. Thus, I examined whether the order of cocoon spinning in a nest affected the size of the pulp structure on a cocoon based on field observational data. In this analysis, I used generalized linear models with quasiliikelihood with the logit link, where I set the variance as proportional to the mean \times (1 – mean) because the size of the pulp structure on a cocoon was the ratio of the pulp area, which ranged from 0 to 1. I incorporated the size of the pulp structure on a cocoon in the model as a response variable. I used the size of the pulp structure on a cocoon on the third day after the cocoon was spun because the pulp structure was constructed on a cocoon within two days after the cocoon was spun, and it did not change with time after it was constructed (S. Furuichi, own data). As explanatory variables, in addition to the order of cocoon spinning, I incorporated the number of old (fourth- and fifth- instar) larvae that were present in a nest when the cocoon was spun in the model. The existence of old larvae, which consume a large amount of food, will increase the time that foundresses spend foraging away from the nest, possibly leading to an increase in the need to construct the pulp structure. In

addition, I also incorporated the date of cocoon spinning (the day when the first cocoon was spun in a nest was assigned as day 1 in each nest) in the model as an explanatory variable. After the first worker emerges, predation by conspecifics can no longer occur; thus, immatures that spin the cocoon later in this stage are subject to the predation threat during a shorter period after cocoon spinning. Therefore, foundresses may adjust the construction of the pulp structure on a cocoon based on the number of days from when the first immature spun a cocoon in the nest. The identity of the nest was also incorporated in the model as an explanatory variable and it was treated as a block. Because the explanatory variables were intercorrelated, I used variance inflation factors (VIF) to assess multicollinearity. A VIF greater than 10 is used to indicate potentially severe multicollinearity (Neter et al. 1996; Chatterjee et al. 2000). In the present model, no VIF exceeded 10 (the maximum VIF was 4.31), and multicollinearity was not a substantial problem. I conducted an F test to determine the significance of the explanatory variables.

Second, I investigated whether intruders avoided attacking cocoons with a larger pulp structure. Using the predation observation data, I examined whether the size of the pulp structure on a cocoon affected the intruder's decision to attack the cocoon. In this analysis, I used generalized linear mixed models, with a binomial error structure and a logit link. The response variable was binary data of whether a cocoon was attacked (1) or not (0), and the explanatory variable was the size of the pulp structure on a cocoon. In addition, the pupal age in days (the number of days after the cocoon was spun) was also incorporated in the model as an explanatory variable. This is because cocooned immatures become hard and inedible near the time of eclosion (S. Furuichi, pers. obs.), and intruders may avoid attacking the cocoons of older immatures, irrespective of the size of the pulp structure on the cocoon. The identity of the attacked nests, intruders, and predation events were incorporated in the model as random

effects. In this analysis, I also calculated VIFs for each explanatory variable and confirmed that there was no marked multicollinearity (the maximum VIF was 1.15). To determine the significance of the explanatory variables, I conducted a likelihood ratio test.

All tests were two-tailed and the level of significance was $p < 0.05$. All analyses were performed using the statistical software R ver.2.15.1 (R Development Core Team 2012).

Results

Adjustment of Pulp Structure Construction

The size of the pulp structure on a cocoon ranged from 0 to 0.89, and it differed substantially among cocoons in a nest. The size of the pulp structure on the cocoon of immatures that spun the cocoon earlier in a nest was larger than that on the cocoon of immatures that cocooned later. The order of cocoon spinning had a significant negative effect on the size of the pulp structure on the cocoon (Figure 2.1a; $\beta \pm SE = -0.78 \pm 0.26$, $F_{1,51} = 8.86$, $P = 0.004$). The date of cocoon spinning had a marginally negative effect on the size of the pulp structure on a cocoon (Figure 2.1b; $\beta \pm SE = -0.16 \pm 0.07$, $F_{1,51} = 3.52$, $P = 0.066$). On the other hand, the number of larvae present in a nest when the cocoon was spun had no significant effect on the size of the pulp structure on the cocoon ($\beta \pm SE = 0.06 \pm 0.09$, $F_{1,51} = 0.51$, $P = 0.48$).

Predators' Choice of Cocoons

I observed 19 predation events by 12 conspecific intruders. The size of the pulp structure on a cocoon attacked by intruders was smaller than that on a cocoon that was not attacked (Figure 2.2, mean difference \pm SE = 0.15 ± 0.05). The size of the pulp structure on a cocoon had a significant negative effect on the intruder's decision about whether to attack the cocoon ($\beta \pm$ SE = -7.03 ± 2.41 , $\chi^2 = 8.53$, $P = 0.003$). On the other hand, the pupal age had no significant effect on the intruder's choice of cocooned immatures (pupal age of attacked immature: 10.3 ± 0.52 [mean \pm SE], pupal age of non-attacked immature: 10.7 ± 0.36 , $\beta \pm$ SE = -0.16 ± 0.14 , $\chi^2 = 1.18$, $P = 0.28$).

Discussion

I found that foundresses constructed the larger pulp structures on the cocoons of immatures that cocooned earlier in the order of cocoon spinning in a nest (Figure 2.1a). The value of cocooned immatures to foundresses is higher in those that spun the cocoon (and initiated pupation) earlier among immatures in a nest because foundresses need to produce many workers as soon as possible (Reeve 1991; Kudô 2003). Therefore, the present result supports the prediction that foundresses adjusted the construction of the pulp structure according to the value of the immatures (hereafter, Hypothesis 1).

However, there are two other possible explanations for this pattern of pulp structure construction (hereafter, Hypotheses 2 & 3), which are not mutually exclusive from Hypothesis 1. Hypothesis 2 is that foundresses may construct the larger pulp structures earlier in this stage (from the cocoon spinning of first immature until the emergence of the first worker) because of the necessity for off-nest activities and the consequent higher risk of predation.

Early in this stage, it is likely that the number of cocooned immatures is still small, and many larvae are present in the nest, requiring the foundress to spend more time off the nest to forage and feed them. Therefore, early in this stage, cocooned immatures will be subject to higher predation threat, and foundresses may construct a larger pulp structure. Hypothesis 3 is that foundresses may construct smaller pulp structures later in this stage because of the shorter period when the cocooned immatures are subjected to predation threats. Immatures that spun the cocoon later in this stage are subject to the predation threat for a shorter period after cocoon spinning because predation by conspecifics can no longer occur after the first worker emerges. Therefore, foundresses may construct a smaller pulp structure on the cocoons of immatures that spun the cocoon later in this stage, irrespective of the order of cocoon spinning.

In the present results, the number of larvae in a nest had no significant effect on the size of the pulp structure on a cocoon. Therefore, the Hypothesis 2 is unlikely. On the other hand, the present analyses showed that the date when a cocoon was spun had a negative (albeit marginal) effect on the size of the pulp structure on the cocoon (Figure 2.1b). This suggests that foundresses decreased the construction of the pulp structure as days elapsed from when the first immature spun a cocoon in the nest, thereby supporting Hypothesis 3. However, a substantial pulp structure was constructed on the cocoon of an immature that spun the cocoon late in this stage but early in the cocoon-spinning order, and I found that even after controlling the effect of the date of cocoon spinning, the order of cocoon spinning had a significant effect on the size of the pulp structure on a cocoon. This suggests that Hypothesis 1 was supported even after considering Hypothesis 3. Therefore, the present results strongly suggest that foundresses adjust the construction of the pulp structure according to the value of each cocooned immature.

A previous study has suggested that foundresses adjust the total amount of the pulp structure on cocoons in a nest according to the predation risk (Furuichi & Kasuya 2014). In the present study, I found that foundresses adjusted the size of the pulp structure on a cocoon according to the value of each immature in a nest. These suggest that foundresses very efficiently construct the pulp structure by adjusting the total amount of the pulp structure in a nest according to the predation risk for the nest and also the amount of the pulp structure on each cocoon on the basis of the value of individual immatures.

The date of cocoon spinning negatively (but marginally) affected the size of the pulp structure on the cocoon (Figure 2.1b). In particular, foundresses greatly reduced the size of the pulp structure on the cocoons of immature that pupated much later (after day 11). Foundresses of *P. chinensis antennalis* decrease the time spent on off-nest activities and increase the time spent on the nest close to the time of the emergence of the first worker (Kasuya 1983a; S. Furuichi, own data; the pupal period of the first worker was 16.3 ± 0.38 days [mean \pm SE, $N = 13$ nests] in the present study). Thus, near the time of the emergence of the first worker, immatures were protected sufficiently via nest attendance (nest guarding), and therefore, the construction of the pulp structure was possibly stopped. Foundresses may adjust the allocation of behavioural defence (nest guarding) and nest structural defence (pulp structure) depending on the situation. In future studies, I need to consider the relationship between the construction of the pulp structure and the level of nest guarding.

There is a growing recognition that full understanding of the effectiveness of antipredator strategies is unlikely to be achieved without studying the predators as well as the prey (Lima 2002). In the present study, I focused also on decision-making by predators and found that the size of the pulp structure on a cocoon had a negative effect on the intruder's decision about whether to attack the cocoon (Figure 2.2), whereas the pupal age did not affect

the predator's decision. This demonstrates that the predators avoided attacking cocoons with larger pulp structures. Therefore, the present results indicate that adjusting the size of the pulp structure on a cocoon according to the value of individual immatures will allow foundresses to effectively prevent predation on the high-value (older) cocooned immatures by not only decreasing the probability of successful predation but also preventing the occurrence of the attack itself.

Many previous studies have investigated the plasticity in parental investment according to the value of their offspring, and the results suggest that animals change several parental care behaviours (e.g., incubation, nest defence, and provisioning) according to factors such as the age, quality, and number of offspring (Jones 1987; Caro 2005; de Ayala et al. 2007; Lima 2009). However, nest-building behaviour has often received less attention, although this is an important parental care behaviour. This is probably because the costs of nest building have often been viewed as negligible compared with the costs of incubation and brood rearing, although they are substantial (Colias & Colias 1984; Mainwaring et al. 2013). The present study indicates that parents of *P. chinensis antennalis* adjust the construction of the nest defensive structures according to the value of their offspring. Investigating the plasticity in nest construction according to the offspring's value may help understanding the nest building behaviours and parental investment of nest builders more generally.

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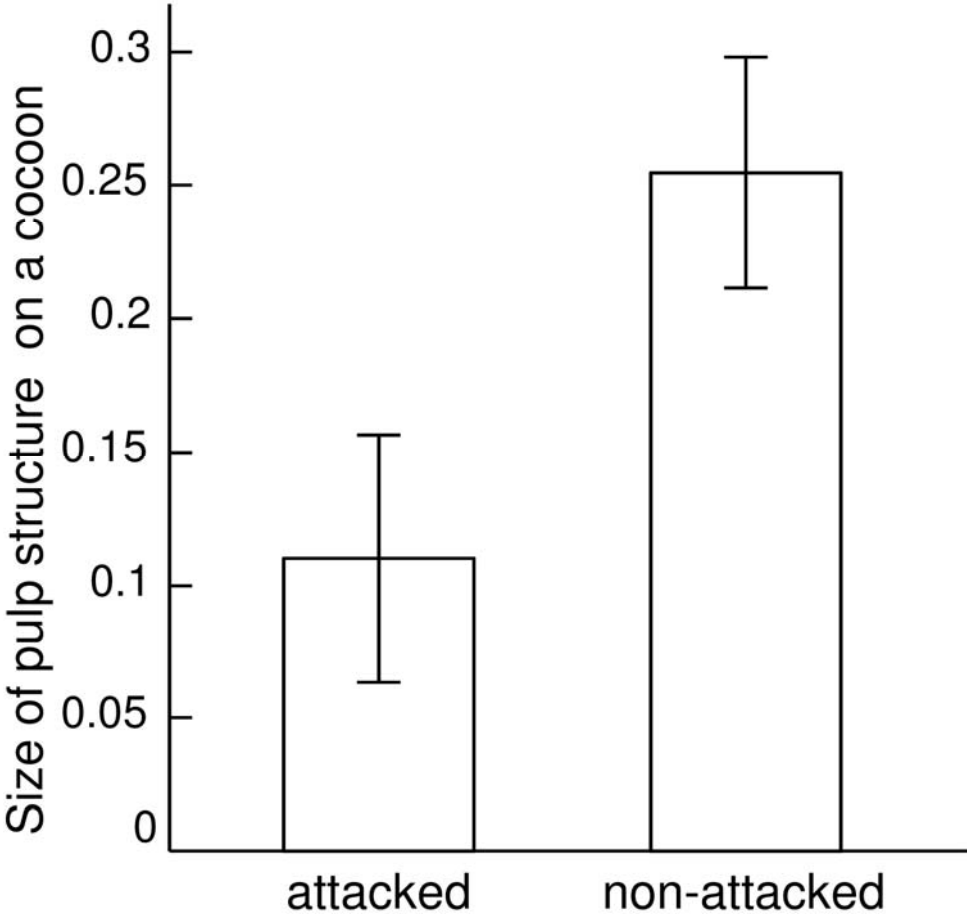
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Figure Legends

Figure 2.1 Relationship between (a) the order of cocoon spinning, (b) the date of cocoon spinning and the size of the pulp structure on a cocoon ($N = 13$ nests). In the date of cocoon spinning, the day when the first cocoon was spun in a nest was assigned as day 1 in each nest.

Figure 2.2 Mean (\pm SE) size of the pulp structure on cocoons that were attacked and not attacked by predators.

Figure 2.2



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