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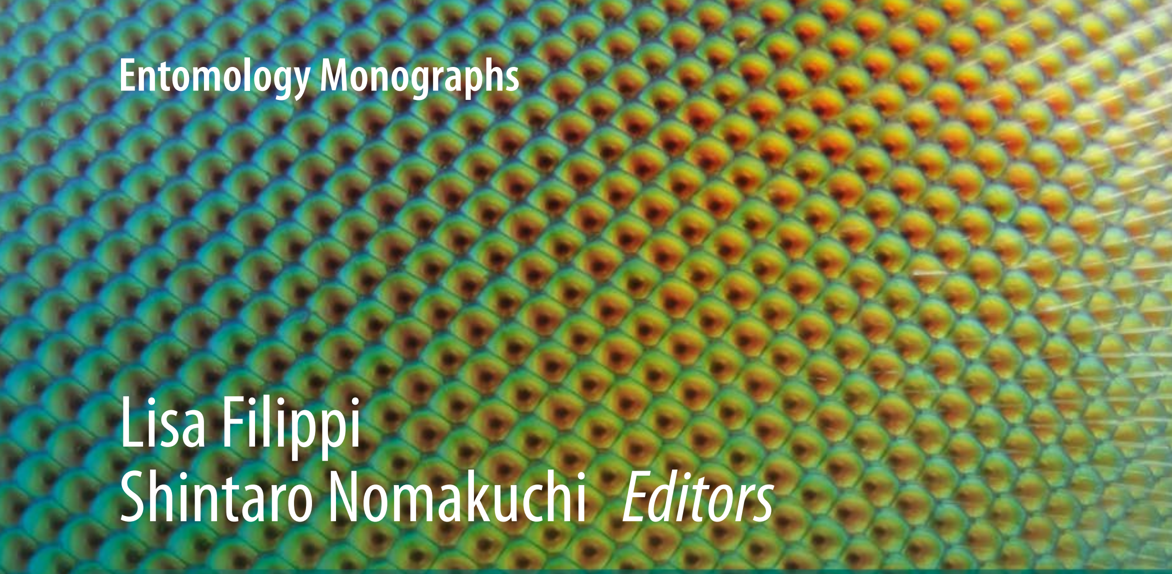

Lisa Filippi
Shintaro Nomakuchi *Editors*

The Life History of the Parental Shield Bug, *Parastrachia japonensis*

Springer

Entomology Monographs

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Lisa Filippi • Shintaro Nomakuchi
Editors

The Life History of the Parental Shield Bug, *Parastrachia japonensis*

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Dedication to Professor Sumio Tojo

Like most entomologists at the time, Tojo Sensei, as he is known by his colleagues and students in Japan, entered the field as an applied scientist with the goal of mitigating the severe economic losses that occur because of insect infestations of monoculture crops. He worked tirelessly over a long career to this end and established a formidable reputation both in Japan and abroad for his physiological and biochemical studies on a variety of pest insects, including the brown planthopper, Nilaparvata lugens*, the tobacco hornworm,* Manduca sexta*, the common cutworm,* Spodoptera litura, *and the silkworm,* Bombyx mori. *However, Tojo Sensei’s interest in insects was not merely to eradicate pests; he had a deep love and passion for these six-legged wonders that began in early childhood. He even wrote an insect field guide when he was still a high school student! Thus, his interest in, and fascination with insects goes far deeper than their negative impact on humans. In fact, this work came to fruition because of the deep passion and dedication of Tojo Sensei, whose love for and fascination* *with* Parastrachia japonensis *infected all members of his lab. Even after he retired, he continued his romps through Mt. Hinokuma Park carrying out field studies on the formation of aggregations in* P. japonensis *until his illness overcame him. Tojo Sensei was a talented researcher and an inspiring, humble, nurturing, and dedicated mentor who challenged and encouraged his students to pursue their interests and guided them enthusiastically in those pursuits. He was loved and respected by all members of his lab, and we became a family, sharing meals and celebrations on a regular basis, with Tojo Sensei as the head of that family. Thank you, Tojo Sensei, for your inspiration, guidance, support and friendship, and the high standard that you set every day by example.*

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At the 43rd Annual Meeting of the Japanese Society of Applied Entomology and Zoology (ODOKON), held at Ryukyu University in

Okinawa. From left to right, Shoh Nomakuchi, Lisa Filippi, Shintaro Nomakuchi, Sumio Tojo, and Mantaro Hironaka

Dinner during the 57th Annual Meeting of the Japanese Society of Applied Entomology and Zoology (ODOKON), held at the Shonan Campus of Nihon University in Kanagawa, Japan, in 2013. From left to right, back row, Toyoshi Yoshiga, Mantaro Hironaka, Tomoki Etoh, Masasuke Ryuda, Shinya Yokoo, Shinji Takii, Fumitaka Kuchiki, Kyoko Iwanaga (Sawabe), Kyo Itoyama; middle row, Tadashi Matsunaga, Hiromi Mukai, Shoko Shiraishi (Motomura); front row, Kentaro Doi, Sumio Tojo, Yasuo Chinzei, Aki Gotoh (Doi)

Tojo Sensei and Cherry Blossoms, circa at the same ODOKON conference in March, 2013. Photo credit Hiromi Mukai

Tojo Sensei at the field site at Hinokuma during his final field season in July, 2013. Photo credit Lisa Filippi

Foreword

Back in the late 1990s, I found myself fully immersed in the insect sociality literature, having taken on, foolishly or otherwise, a book project that seemed straightforward enough but soon took on a life of its own. The project was *The Other Insect Societies*, eventually published in 2006—a review of sociality among the little-known, unsung, and overlooked forms of sociality found across the arthropod world. It was a subject that I thought I would, or could, treat concisely, until the sheer diversity disabused me of that notion. If I got carried away with my subject, my defense is that there's an awful lot of subject to get carried away by! I discovered a richness of social-behavioral ecology I had not fully appreciated before, and a richness of literature both historical and modern to match. I reveled in learning about the natural history of endlessly interesting social species familiar and exotic—caterpillars and sawflies certainly (my own study groups), but also weird and wonderful forms of social behavior in earwigs and booklice, beetles and bugs, cockroaches and thrips, aphids and grasshoppers; it was an epiphany. One day in my literature-based sociality safari, I came across some papers by Lisa Filippi, Shintaro Nomakuchi, and colleagues on a curious bug endemic to Japan—a semelparous progressive provisioner that specializes on the red drupes of *Schoepfia jasminodora* trees. It was my first introduction to *Parastrachia japonensis*, and I was immediately taken with these handsome bugs.

I ended up dedicating about five pages to this "most un-cydnid-like cydnid," as I called it—parastrachiines only just recently having been elevated from a subfamily of the burrower-bug family Cydnidae to full family status. I was fascinated by this insect, delving into the literature beginning with a 1954 note on its feeding habits by the prolific entomologist Syoiti Miyamoto and on through all the papers I could find to date by a remarkable group of researchers mainly at Tokyo University of Agriculture, Saga University, Kagoshima University, and a few others. I was as impressed with the beauty of the entomologists' experimental work as I was with the bugs themselves—shining red and black females, repeatedly running the gauntlet of carabid beetle predators to bring home the *S. jasminodora* bacon to their bright red hungry brood, ensconced in the safety of their well-hidden nest. There is much more to their natural history, of course—trophic eggs to tide the young over until fruits ripen, parent-offspring communication via vibration, navigational cues used in central-place foraging, kleptoparasitism by rival females, and more. I was delighted when Lisa and her colleague Mantaro Hironaka kindly provided beautiful photographs of photogenic *P. japonensis* for the color plates of *The Other Insect Societies*. And delighted and honored when, years later, I was asked to provide the Foreword to a forthcoming book synthesizing a remarkable body of work on this remarkable insect. To appreciate just how remarkable, on both counts, it is worth considering this research in a broader context.

The Heteroptera, or true bugs, have given behavioral ecologists great insights into social evolution. That should come as no surprise, perhaps: sociality in this group has been known for over two centuries, common in some two dozen bug families across several great clades and encompassing a remarkable diversity of social forms. From nymphal herds to maternal care to paternal care, heteropteran social behavior most often entails guarding and tending clutches, and carrying and provisioning young—along with such fascinating interactions as egg dumping, chemical and behavioral defense, trophic egg production, chemical and acoustic communication, social cannibalism, and more. Yet for all that, it is surprising that few heteropteran groups have been the subject of sustained, detailed empirical study—the kind of careful lab and field studies necessary to give full measure of the depth and breadth of the behavioral ecology and evolution of a group. The giant water bugs, Belostomatidae, come to mind, notable for their exclusive paternal care. And the fascinating Parastrachiidae, notably *Parastrachia* spp., are perhaps the only other heteropteran group to be so thoroughly studied, as this fine volume abundantly illustrates.

Even more importantly, perhaps, is what this small but remarkable pentatomoid family teaches us about social-behavioral ecology and evolution in relation to other parental care taxa—such so-called subsocial groups as necrophorine beetles, membracid treehoppers, forficulid earwigs, and others. Such groups long received relatively scant attention, most likely owing to the conceptual framework typically used to categorize social behavior and evolution. That framework began with Harvard myrmecologist William Morton Wheeler, who in the 1920s first postulated a kind of "finely graded series" of social forms—a hierarchical view of insect societies that began with species deemed "social *sensu stricto*" at the top (the category later to become "eusocial") and backed into lesser categories that were defined on the basis of which and how many social attributes of the most complex groups were missing. In his 1928 book *The Social Insects: Their Origin and Evolution*, Wheeler viewed the ladder from the other direction, describing a seven-step social evolutionary series characterized by "constantly increasing intimacy of the mother with her progeny." In this progression, Wheeler identified family groups exhibiting parental care as "incipiently social or subsocial forms." The object of this categorizing at the time was to define attributes thought to be key to the evolution of the most complex forms of sociality, the ant, bee, wasp, and termite groups dubbed eusocial today, and identify those traits in other taxa to envision a social-evolutionary trajectory. An unintended consequence of such hierarchical classification schemes was to direct research interest largely away from groups further down the supposed hierarchy, less complex somehow equating, erroneously, to less interesting. Much has changed, I would argue for the better.

In *The Other Insect Societies* and elsewhere I have argued for the importance of recognizing “pathway pluralism” in social evolution—distinct social-evolutionary pathways reflecting qualitatively different kinds of societies and social structures, from herds to fortress defenders to varied forms of family-societies: exclusive maternal and exclusive paternal care, biparental care, and communal care. To understand a given social system is to understand the interplay of ecological and evolutionary factors shaping it: the dynamic interplay of such factors as life history, predation and parasitism, mate competition, future reproductive options, group effects, resource quality and quantity, relatedness, and so on. While an understanding of the behavioral ecology of extant “subsocial” societies may indeed yield insights into the origin and evolution of sociality in eusocial lineages, there has been a growing appreciation for the importance of studying and understanding such insect societies in their own right, as well.

In that spirit, the wealth of studies of *Parastrachia japonensis* and relatives that Prof. Sumio Tojo inspired over three decades ago is rich fruit indeed. The collective research efforts and insights summarized in this book present a compelling model system where ecology, behavior, life history, and physiology collectively shed light on sociality in a singular group of insects, and in so doing enrich our understanding of the social evolutionary process writ large.

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James T. Costa

Preface

The research of Parastrachia japonensis at Hinokuma Mountain Park in Saga Prefecture, Japan, described in these pages is a culmination of over three decades of work by the many contributors of this book who hailed from the research laboratories of Professors Sumio Tojo, Shintaro Nomakuchi, and Toyoshi Yoshiga at Saga University's Faculty of Agriculture. However, because it was initiated by Lisa Filippi as a Master's student at Saga University some 32 years ago, she has the honor of scribing the Preface.

This journey with *Parastrachia japonensis* began in early April of 1989 with a stroll along a seriously overgrown path at Hinokuma Mountain Park in Kanzaki Town, Saga Prefecture, Japan. My graduate school mentor at Saga University, Professor Sumio Tojo, and I fought our way through the dense vegetation with a sickle, precariously navigating the narrow uphill path through a secondary forest sporting a variety of small and medium height broadleaf evergreen trees and shrubs, and a canopy of taller deciduous trees. The dense ground cover consisted of a variety of ferns and small bushes. Our mission was to observe one of the potential organisms that we were considering for my Master's thesis research. As a mother of three very young children, the field site appealed to my desire to carry out a research project that my very active young children could on occasion participate in. As we plodded along, Professor Tojo cautioned me to watch out for pit vipers (Fig. 1), giant hornets (murder hornets), and poison sumac trees. Because the canopy was dense, it was fairly dark even in the early afternoon on this lovely, sunny day. After about 10 min, a wide grin spread across Professor Tojo's face as his arm flew out, gesturing to the broadleaf evergreen East Asian eurya, *Eurya japonica*, trees bordering the narrow path. While I did not notice anything initially, my eyes gradually became accustomed to the low light, and on closer inspection I saw on the undersides of the waxy leaves something that at once horrified and delighted me: thousands of red and black insects, that I later learned could correctly be called "bugs," were aggregated in dozens of sedate clusters of about 10–100 individuals throughout the lower branches of the *E. japonica* trees. The trees covered with these bizarre aggregations went on for about 15 m along the path, looking so much like lavishly decorated Christmas trees. Professor Tojo gently blew on one of the clusters, and it fell apart, with the

Fig. 1 Pit viper (*Gloydius blomhoffii;* mamushi) at Hinokuma Mountain Park in June, 2015. Photo credit Lisa Filippi

individual insects tumbling artlessly to the ground where they feigned death. After a few minutes, they climbed back up to the branches once again. Although 58 years old at the time, the vision before him brought out the insect-loving child in Professor Tojo, and his face just beamed in delight. I asked him what the bugs were doing, and his response was, “That’s what I am hoping you will want to figure out.” His enthusiasm and curiosity reached right to my heart, and I knew that I had found my research project.

Professor Tojo’s long-term fascination with this bug was shared with his dear friend Shuji Tachikawa of Tokyo University of Agriculture, who was a great source of inspiration and encouragement as I delved headlong into my research. These two dedicated and enthusiastic mentors were keen to explore the life history of what they suspected was a very fascinating insect, but both were unable to take it on themselves. Professor Tachikawa’s position was in Tokyo, too far from Saga to be able to carry out comprehensive, long-term field studies, and the other students who typically joined Professor Tojo’s laboratory of Entomology and Biochemistry at Saga University were primarily interested in working on physiological studies involving crop pests in the laboratory. Being a newbie in the disciplines of entomology and behavioral ecology, I did not yet fully appreciate what a fabulous opportunity I had fallen into. It isn’t often that a student comes upon a research organism with a fascinating life history that is essentially a blank page, and I soon realized how very lucky I was to have been guided to this particular project. Anything I discovered about *P. japonensis* was new, and it turned out that there were several very remarkable aspects to the bug’s life history that were not only newly discovered, but highly unusual among insects. We begin this book with a recap of the earliest studies by those on whose shoulders we stand, the reports through 1991. We then introduce the many fascinating aspects of the life history, neurobiology, physiology, and behavior of *P. japonensis* that have been newly discovered since, and those aspects that have been confirmed through experimentation over the past 30 years by a small army of dedicated and talented researchers that began on that lovely spring day some 32 years ago.

Hempstead, NY, USA Lisa Filippi
December 24th, 2021

Acknowledgments

The authors owe a debt of gratitude to the host of mentors and colleagues encountered over the years for their encouragement, support, and valuable advice. Many thanks to Y. Chinzei, J. Costa, R.F. Denno, A. Doi, K. Fujisaki, A.G. Gatehouse, J. Hardie, the late W.D. Hamilton, T. Hariyama, Y. Hayakawa, K. Higashi, H. Horiguchi, N. Ishibashi, O. Iwahashi, the late Y. Ito, E. Kondo, S. Kudo, S.R. Leather, G. Machado, S. Morooka, R. Nishida, H. Noda, H. Numata, G. Requeña, the late C.W. Schaefer, T. Sunahara, D.W. Tallamy, S. Tokunaga, and N. Yamamura. This research could not have taken place without the enthusiastic and tireless assistance of a parade of undergraduate and graduate students from the Laboratories of Entomology, Animal Behavioral Ecology and Nematology at the Faculty of Agriculture at Saga University, Saga, Japan. In particular, the earliest work required much help in the field. LF recalls quite vividly the image of Shunichi Nishimura, as he plowed forward when the site was nothing short of a jungle, blazing that initial trail with a sickle the very first season of the study. Thank you, Nishimura san! Tojo Sensei exceled at recruiting students, most of whom were researching other organisms to help out in the field when numbers and muscle were needed! When Dr. Nomakuchi came to the Faculty of Agriculture with his expertise in behavior and ecology, those aspects of the research really took off. A steady stream of students from his lab contributed significantly to this work. We attempted to list each of the students, but the list grew too long, and we did not want to take the chance of leaving anyone out! You know who you are, and please know that we gratefully acknowledge your tireless contributions to the success of these studies! For financial support, SN thanks the Japanese Ministry of Science and Culture and LF thanks Hofstra University. Finally, many thanks to Professor Hideharu Numata (Graduate School of Science, Kyoto University and Series Editor of Entomology Monographs) for inviting us to write this book as a contribution to this Series and to Fumiko Yamaguchi, Jayesh Kalleri, Sindhuja Aroumougame and the staff at Springer Nature of Springer for their patience and editing of this manuscript.

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About the Editors

Lisa Filippi began investigating *Parastrachia japonensis* in 1989 for her master's and doctoral degrees in Saga, Japan, where she lived for 20 years. She joined the faculty at Hofstra University upon returning to New York in 2002 and is a Professor of Biology. Dr. Filippi teaches General Biology, Entomology, Organic Evolution, and Behavior. She continues to study *P. japonensis* on visits to Japan and investigates the related species, *Sehirus cinctus*, here in New York. She recently began monitoring the natural expansion of coyotes onto Long Island, the last reaches of the continental U.S. to have an established coyote population.

Shintaro Nomakuchi earned his doctoral degree investigating the reproductive behavior of the damselfly, *Mnais pruinosa*. His *P. japonensis* research began in 1993 when he joined the faculty of Saga University in Japan. In addition to teaching and supervising an army of undergraduate and graduate students investigating *P. japonensis* and four provisioning Japanese cydnids, Dr. Nomakuchi translated into Japanese, either alone or with others, three biostatistics texts, a seminal text on dragonflies, and *An Introduction to Behavioural Ecology* (Davies, Krebs and West, 2012). He is currently Professor Emeritus of Agriculture at Saga University and lives with his family in New York.

Chapter 1
General Biology of *Parastrachia japonensis*

Shuji Tachikawa, Shintaro Nomakuchi, and Lisa Filippi

Abstract A series of studies over the past 32 years has revealed many remarkable parameters of the life history of *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae), the parental shield bug. This chapter briefly reviews the information that had been derived by earlier researchers; discusses the morphology and worldwide distribution of the bug, as well as the specific habitat and resource that it utilizes at our field site in Japan; and presents the overall life cycle of *P. japonensis*. *P. japonensis* inhabits secondary forests in regions from eastern China to southern Japan. It feeds solely on drupes of its host tree, the grey twig tree, *Schoepfia jasminodora* (Schoepfiaceae), an ephemeral, unpredictable, and scarce resource. Early studies suggested that females care for young, and perhaps provision nests with drupes. There were about 59 subpopulations at the field site in Japan, Mt. Hinokuma, in Kanzaki Town, Saga, Japan, and there is a host tree within 10 m of every subpopulation of bugs. Subpopulations range from just a few dozen to around 10,000 individuals, and subpopulation size is correlated with the size of the host tree. Not every host tree has a subpopulation of *P. japonensis*, and the combination of ecological features that must coincide for a subpopulation to exist was clarified. The life history can last 1–2 years. The reproductive period (mating, oviposition, egg, and nymph guarding and progressive provisioning) extends from May through mid-July; the remainder of the year is spent in a long reproductive diapause which includes hibernation below ground from December through February. The bug's utilization of a toxic resource is also discussed.

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Keywords Distribution · Geographic distribution · Habitat · Host tree · Life cycle · Local distribution · Morphology · *Parastrachia japonensis* · *Parastrachia nagaensis* · Phylogeny · Resource

1.1 Introduction

Many early studies on the life history of *Parastrachia japonensis* (Scott) documented findings that suggested the bug had a very interesting life history and unusual behaviors (Esaki 1930a, b; Hiura 1952; Miyamoto 1954, 1956, 1965, 1966; Nakao 1956; Tachikawa 1980, 1984; Gyôtoku and Tachikawa 1980; Tachikawa and Gyotoku 1981; Tachikawa and Schaefer 1985; Schaefer et al. 1988). Most of the studies were done in Kurume City and Fukuoka Prefecture, Japan, and they all indicated a tantalizing need for more in-depth field studies on this fascinating bug. This chapter begins with a review of the historical studies and then describes the morphology and classification of *P. japonensis* and its elusive sister species, *P. nagaensis*. We then introduce the phylogenetic status of the genus based on morphologic, molecular, and behavioral data. The global distribution of the two sister species is described, followed by detailed descriptions of the local distribution of *P. japonensis* at Mt. Hinokuma Park, in Kanzaki town, Saga Prefecture, Japan, where most of the recent studies have taken place. We delve into the particular features of the local habitat that support subpopulations of the bug and the distribution in the local habitat of the sole resource it feeds on, drupes of the grey twig tree, *Schoepfia jasminodora* (Schoepfiaceae), its phenology, and its toxic nature. Finally, we describe the details of the life cycle that have been filled in over the years.

1.2 Historical Perspective of Studies on the Life History of *P. japonensis*

Prior to the beginning of L.F.'s graduate studies, there had been no intensive, long-term studies done on this fascinating bug. Several authors had reported on its existence, but for the most part, the early literature on *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) primarily represented findings from short-term studies and, more often, snapshot impressions of the bug's life history. Many observations were anecdotal, made in isolation of a larger life history context, while others involved data collected several times over a few hours of observations during different seasons. Clearly all of the studies contributed to our understanding of this fascinating species; however, because of the irregular and short-term nature of these early studies, several of the findings incorrectly represented the bug's behaviors. In a book on subsocial heteropterans of Japan, Tachikawa (1991) reported findings of the earliest studies on *P. japonensis*, and we summarize some of that information here.

The earliest reports on *P. japonensis* (Esaki 1930a, b) are based on observations made at Koura Mountain in Kurume City, on Kyushu Island in Japan. Esaki observed the bugs, which he identified as a genus in a subfamily of the predatory Pentatomidae, on *Camellia japonica* and *Lyonia ovalifolia* and incorrectly assumed that was their food source. Hiura (1952) made ten observations of a large aggregation at the same site from April to July and estimated the life history sequence without actually observing all the stages, erroneously stating that mating occurred in July. He reported the habitat as a fairly dark, densely forested area of primarily broadleaf evergreen trees such as *Castanopsis* and *Camellia*, which is consistent with other reports. Hiura observed a large aggregation of post-hibernation adults comprising more than 4000 individuals on leaves of ferns and other ground cover. Because of evidence of penetration by the proboscis on the back of leaves, he also incorrectly concluded that the evergreen trees were the food source of *P. japonensis*. He observed that, while they are attracted to and fly to lights at night, the aggregations are primarily sedate, with individuals displaying very little movement. Hiura also was the first to describe the mechanism for the stridulatory chattering sound emitted by *P. japonensis* adults.

In July of 1953, Miyamoto (1954) observed third and fourth instar nymphs feeding as aggregations on apparently rotting unidentified drupes and also observed five incidents of cannibalism. Miyamoto (1956) reported that he had been unable to observe mating and oviposition under rearing conditions in the laboratory, but on June 8–9, he collected an egg mass under leaf litter in the field, where he assumed the female had deposited it. The eggs were similar in appearance to those of familiar cydnids.

In 1956, Nakao reported a hibernating aggregation under the leaf litter and concluded that the tendency to aggregate after hibernation was just a continuation of the overwintering structure.

Oviposition was finally observed by Miyamoto (1965) in early June; he noted that females guarded a round egg mass and that nymphs remained in the leaf litter on the ground and tended to gather on still unidentified drupes. He later observed that oviposition occurred during the rainy season, which begins in June and goes on until mid- to late July, under the leaf litter (Miyamoto 1966). He noted that the pattern of oviposition of a round egg mass and guarding of the egg mass by females resembled the ecology of *Adomerus triguttulus* (Heteroptera: Cydnidae). Finally, Miyamoto (1966) also observed that while eggs were very inconspicuous under the leaf litter, the bright red color of nymphs as they roam on the forest floor while foraging rendered them highly conspicuous.

Tachikawa carried out a series of field and laboratory observational studies between 1978 and 1982 and presented his findings at several Japanese conferences and at an International Entomology Congress in 1984 (Tachikawa 1984). Gyôtoku and Tachikawa (1980) and Tachikawa and Schaefer (1985) described more details of the life history of *P. japonensis* that had been gleaned through 1982, and the earlier report finally correctly identified the sole food of this bug as drupes of the gray twig tree, *Schoepfia jasminodora* Sieb. & Zucc. Thereafter follows a blank in the scientific literature, though the studies continued and were presented at several conferences in Japan, until several papers between 1985 and 1991 that described the general biology and detailed anatomy and also attempted to sort out the confusing taxonomy (Tachikawa and Schaefer 1985; Schaefer et al. 1988; Tachikawa 1991). The following information, based on field and laboratory observations from June 1981 to July 1982 and summarized in Tachikawa (1991), essentially represents what was known at the time our research began in 1989.

> *P. japonensis* is a monophagous insect that feeds on the endosperm of *Schoepfia jasminodora* Sieb. & Zucc. (Schoepfiaceae Rosidae: Santalales), a host plant that invariably occurs wherever the bug is found and one that requires a stable secondary forest. The bug has one generation per year, and its life cycle coincides with the phenology of the host tree. Adults are strongly gregarious; they remain aggregated on plants and trees in the understory and herbaceous layer that are sheltered from direct sunlight; the aggregations remain largely sedentary. Throughout most of the year, the bugs don't eat, and the ovaries remain undeveloped. In fact, males and females apparently remain in a state of reproductive diapause throughout much of the year. When an aggregation is disturbed, individuals crawl into and over each other and make a chattering sound; they also release a noxious odor. Individuals fall from the tree to the ground and feign death (thanatosis); they later climb back onto the plants and reform into aggregations.
>
> When *S. jasminodora* is fruiting, a portion of the males and females mate near the aggregations; females leave the aggregations and relocate to the *S. jasminodora* tree. Mated females feed on the drupes to develop their eggs. Mothers climb down the host tree and excavate a shallow burrow under the leaf litter where they deposit about 60–100 eggs in a round mass. Moreover, Tachikawa (1991) observed females dragging drupes under the leaf litter, and suggested this might be for young, but never actually observed provisioning.

1.3 General Morphology

P. japonensis is a large bug of 16–19 mm in length (Fig. 1.1). The coloration comprises five black patches on a red background: large black diamond-shaped patches on the forewings, black patches on the bottom of the hindwings forming a diamond when closed, a triangular black patch on the pronotum, and a horizontal rectangular black patch on the dorsal thorax (Tachikawa 1991). The morphological

Fig. 1.1 *Parastrachia japonensis* female and aggregation on *Eurya japonica*

peculiarity of this species placed it in the Cydnidae; however, it was suggested that this genus should actually be assigned to a separate subfamily, Parastrachiinae (Schaefer et al. 1988) *(it has since been elevated to family level, Parastrachiidae;* Sweet and Schaefer 2002).

1.4 Classification

1.4.1 Phylogenetic Status Based on Morphology

There are only two species within the genus *Parastrachia*, *P. japonensis* Scott (Scott 1880), and *P. nagaensis* Distant (Distant 1908). Although distinct morphological features clearly indicated that the genus belonged in the Pentatomoidea, its classification beyond that had a very ambiguous beginning. The history of its earliest classification is detailed in Schaefer et al. (1988) and Tachikawa (1991). The genus had been variously assigned to the Pentatomidae: Asopinae or Pentatominae, Cydnidae: Sehirinae, Tessaratomidae, and there was also a suggestion of Urostylidae. Schaefer et al. (1988) concluded that none of the arguments for those groupings were convincing enough to merit the argued placement and, while recognizing that it was still not a perfect fit, assigned *Parastrachia* to a new monogeneric subfamily, Parastrachiinae, within the Cydnidae. Although there is considerable discrepancy between *Parastrachia* and other members of the Cydnidae with regard to size and color, with the cydnids being small and mostly drab, dark coloring, compared to the much larger and aposematically colored *Parastrachia*, similarities in the pattern of oviposition, the shape of the eggs, and the maternal parental care behaviors suggest that these behaviors arose in a common ancestor of *Parastrachia* and Cydnidae (Schaefer et al. 1988). Sweet and Schaefer (2002) describe 13 morphological features they considered sufficient to exclude *Parastrachia* from Pentatomidae and Tessaratomidae. In addition, the difference in the eggs (round, cohesive egg mass in the former and barrel-shaped or round eggs deposited individually or in a monolayer on a substrate in the latter two families) also deviated from those families.

Sweet and Schaefer (2002) also described 12 morphological features that excluded *Parastrachia* from the Cydnidae, including the striking aposematic color of the former, compared to the dark color of the latter. The authors concluded that most of the morphological features described for *Parastrachia* that excluded them from the other families were likely plesiomorphic. Finally, they described 13 largely apomorphic morphological features, some unique, that unite *Parastrachia* and ultimately concluded the most reasonable course, one that helped maintain the integrity of the Cydnidae, was to elevate the genus to family status (Parastrachiidae, Oshanin 1922) (Sweet and Schaefer 2002).

Parastrachia japonensis and *P. nagaensis* can be distinguished by genitalia and some color or somatic features (Schaefer et al. 1991), though these features are less reliable. Differences in the male aedeagus, particularly the vesicular process, the proximal conjunctival appendages, and the conjunctiva's midventral sclerotization, are notable (Schaefer et al. 1991). On the other hand, similarities in the aedeagus between the two species, such as the large vesicular process, the two pairs of well-sclerotized laterodorsal conjunctival appendages, and the small gonoporal process, unite them (Schaefer et al. 1988, 1991).

1.4.2 Phylogenetic Status Based on Morphology and Molecular Data

Grazia et al. (2008) carried out a phylogenetic analysis of the Pentatomoidea using both morphological and DNA sequence data. A character matrix employing 57 morphological features, particularly similarities in the spermatheca (Pluot-Sigwalt and Lis 2008) and pretarsal structures (Lis 2010), supported the monophyly of *Parastrachia* + the six species in the African genus *Dismegistus* with Sehirinae within the Cydnidae, considered to be the closest relatives to both genera. A POY of a 92-taxon dataset using 1:2 and 2:2 indel/transition-transversion cost ratios also supported the monophyly. The findings suggested that *Parastrachia* + *Dismegistus* formed a monophyletic group, which placed *Dismegistus* within the Parastrachiidae (Pluot-Sigwalt and Lis 2008; Lis 2010). However, two Bayesian analysis trees of combined 28S + 18S rDNA datasets using three different substitution models did not support the monophyly of these two genera (Lis et al. 2017). Rather, in that study *Parastrachia japonensis* was never identified as a clade with *Dismegistus*. Instead, *Parastrachia japonensis* formed a sister taxon to *Ochetostethomorpha secunda* in the Cydnidae subfamily Sehirinae, with both species forming a sister clade to the monophyletic group consisting of the four remaining species of Sehirinae examined. In their analyses, *Dismegistus* always branched off as an outgroup to the Sehirinae + *Parastrachia* (Lis et al. 2017). Most recently, Pluot-Sigwalt and Lis (2018) reported that uradenia, paired exocrine glands located ventrally in the abdomen and uniting several superfamilies in the infraorder Pentatomomorpha, were observed in male *Dismegistus*. However, these glands, which until this report were considered to be absent in the Pentatomoidea, did not occur in either males or females of *Parastrachia* (Pluot-Sigwalt and Lis 2018), again muddying the waters regarding the monophyletic status of these two genera. To be sure, the rounded body shape of *Dismegistus* is more similar to Thyreocoridae, Thaumastellidae, or Pentatomidae than Parastrachiidae. It does appear that the puzzling relationship between *Dismegistus*, *Parastrachia*, and the other Sehirinae is not quite resolved, as yet.

1.4.3 Phylogenetic Status Based on Unique Behavioral Features

Sadly, there is virtually no information available on the biology of *Dismegistus*, so it is impossible at this time to say whether in the context of its biology it belongs in the group comprising the five Sehirinae species that share several features related to parental care with *Parastrachia japonensis.* All six species progressively provision seeds to their nests, and the five species that are not *Parastrachia* are all in the Cydnidae subfamily Sehirinae: *Sehirus cinctus* Palisot (Sites and McPherson 1982; Kight 1997; Agrawal et al. 2001, 2005; Kolliker et al. 2006); *Adomerus triguttulus* Motschulsky (Nakahira 1992, 1994; Kudo and Nakahira 2005; Nakahira and Kudo 2008; Nakahira et al. 2013); *Canthophorus niveimarginatus* Scott (Filippi et al. 2009); *Adomerus variegatus* Signoret (Mukai et al. 2010); and *Adomerus rotundus* Hsaio (Mukai et al. 2012; Inadomi et al. 2014).

Cervantes et al. (2013) provided compelling evidence that females of yet another cydnid, *Melanaethus crenatus* Signoret, also progressively provision young. Interestingly, this species belongs to the subfamily Cydninae and, thus, represents the only non-Sehirinae cydnid to progressively provision its nest. We explore the variation in egg production and parental care among these different species in a later chapter.

1.5 Geographic Distribution of *P. japonensis* and *P. nagaensis*

The most recent and detailed report of the distribution of *P. japonensis* (Fig. 1.2; Zhu et al. 2013) shows a continuous distribution extending from Guangxi in the Hengduan region of southeastern China, apparently the southernmost limit to its range, north to western Mt. Qinling. Moving east, in South Korea, it has been recorded in Cheju Island. In Japan, *P. japonensis* occurs in Okinawa, throughout Kyushu, north to Yamaguchi Prefecture in southern Honshu, which is apparently the northern limit to its range, and Ehime Prefecture in western Shikoku, the eastern limit of its range. The long-term studies reported here took place at Mt. Hinokuma Park, in Kanzaki City, Saga Prefecture, Japan. Thus, the distribution of *P. japonensis* ranges in latitude from 23° 43′ 29.28″ N to 33° 36′ 9″ N and in longitude from 107°37′05″ E to 132° 47′ 8.88″ E (Table 1.1).

Parastrachia nagaensis occurs further west and south, compared to *P. japonensis*, and tends to occur at higher altitudes (Fig. 1.2). The westernmost record of *P. nagaensis* is from the Naga Region of Assam, India. Moving east from there, it has been recorded at several sites in China, from Yunnan north to Ya'an and Mt. Qingcheng. *P. nagaensis* has also been recorded in Vietnam, the easternmost record, and Laos, the southernmost and most recent record. The distribution of *P. nagaensis* thus ranges from a latitude of 20° 13′ 12″ N to 30° 53′ 60″ N and a

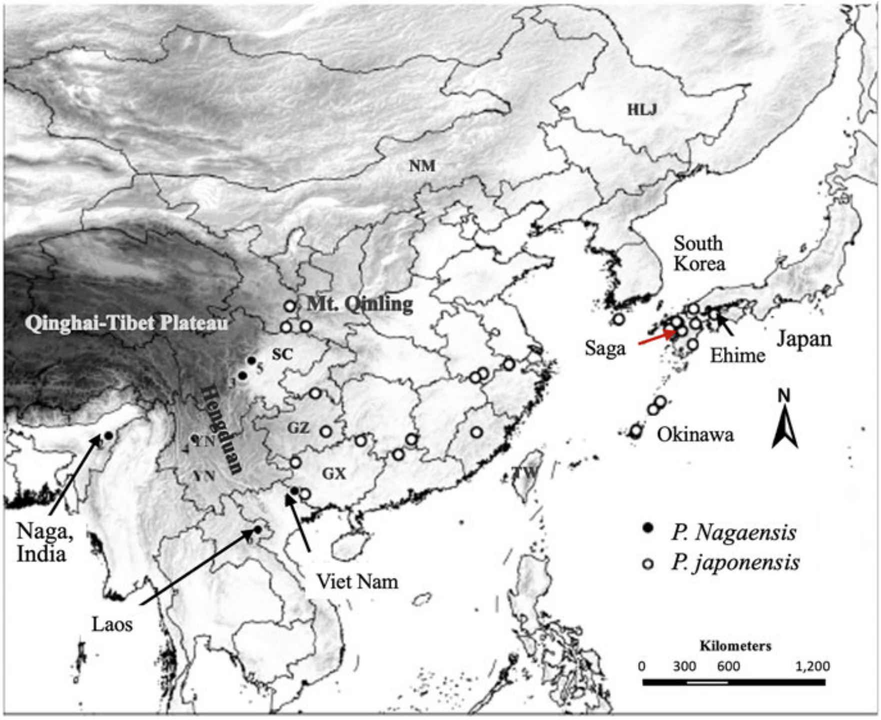

Fig. 1.2 Geographic distribution of *Parastrachia*. Low to high altitudes are shown as a gradient from white to black. The Chinese province names in the text were simplified on the map (NM, Inner Mongolia; HLJ, Heilongjiang; SC, Sichuan; GZ, Guizhou; GX, Guangxi; YN, Yunnan). Numbers beside black dots indicate the *P. nagaensis* records (1, Vietnam, New country record; 2, India; 3, Ya'an, China; 4, Yunnan, China; 5, Mt. Qingcheng, China; 6, Laos). Adapted from Zhu et al. (2013). Gengping Zhu, Guoqing Liu, Wenjun Bu, Jerzy A. Lis, Geographic distribution and niche divergence of two stinkbugs, *Parastrachia japonensis* and *Parastrachia nagaensis*, *Journal of Insect Science*, Volume 13, Issue 1, 2013, 102, https://doi.org/10.1673/031.013.10201

longitude of 94° 33′ 36″ E to 106° 17′ 60″ E (Schaefer et al. 1988; Schaefer and Kikuhara 2007; Zhu et al. 2013).

The host tree of *P. japonensis* was identified by Gyotoku & Tachikawa (1980) as the Jasminodora grey twig (*Schoepfia jasminodora*: Schoepfiaceae). Several related species of *Schoepfia* occur throughout the range of the two *Parastrachia* species; however, the food resource of *P. nagaensis* has not yet been identified. It has been suggested by Schaefer et al. (1988, 1991) that *P. japonensis* is the plesiomorphic species that more closely resembles the ancestor of the two species and spread to the east and that *P. nagaensis* diverged to the west and likely uses another *Schoepfia* species as its host tree. In support of this hypothesis, Zhu et al. (2013) have described distinct niche differences between the two species, likely related, in part, to differences in altitude. Moreover, some samples of *Parastrachia* collected in the overlap region have features in between the easternmost populations of *P. japonensis* and

Table 1.1 Occurrence records for *P. japonensis* and *P. nagaensis* demonstrating the extent of their ranges. Not all occurrences for *P. japonensis* represented in Fig. 1.1 are included in the table.

Species	Location	Latitude	Longitude	Reference
P. japonensis	Guangxi, China	23° 43′ 29.28″N	108° 48′ 27.36″ E	Zhu et al. (2013)
P. japonensis	Mt. Qingling	33°57′48″N	107°37′05″ E	Yang (1934)
P. japonensis	Cheju Island	33° 29′ 20.4″ N	126° 29′ 53.88″ E	Schaefer et al. (1991)
P. japonensis	Okinawa, Japan	26° 7′ 12.72″ N	127° 42′ 9″ E	Gyôtoku and Tachikawa (1980)
P. japonensis	Saga, Japan	33° 15′ 47.16″ N	130° 18′ 3.24″ E	Gyôtoku and Tachikawa (1980)
P. japonensis	Yamaguchi, Japan	34° 16′ 46.92″ N	131° 31′ 16.68″ E	Tachikawa (1991)
P. japonensis	Ehime, Japan	33° 36′ 9″ N	132° 47′ 8.88″ E	Gyôtoku and Tachikawa (1980)
P. nagaensis	Nagaland, India	26° 9′ 36″ N	94° 33′ 36″ E	Schaefer et al. (1991)
P. nagaensis	Yunnan, China	26° N	100° E	Schaefer et al. (1991)
P. nagaensis	Ya'an, China	29° 58′ 48″ N	103° 0′ 36″ E	Schaefer et al. (1991)
P. nagaensis	Mt. Qingcheng, China	30° 53′ 60″ N	103° 34′ 12″ E	Zhu et al. (2013)
P. nagaensis	Viet Nam	22° 40′ 48″ N	106° 17′ 60″ E	Zhu et al. (2013)
P. nagaensis	Laos	20° 13′ 12″ N	103° 59′ 24″ E	Schaefer and Kikuhara (2007)

P. nagaensis but were mostly designated as *P. japonensis* (Zhu et al. 2013). These findings and data from various niche divergence models described in that report also support the divergence hypothesis of Schaefer et al. (1988, 1991).

1.6 General Characteristics of the Habitat

Early descriptions of the habitat of *P. japonensis* (Miyamoto 1954, 1956; Hiura 1977; Gyôtoku and Tachikawa 1980; Tachikawa and Schaefer 1985; Tachikawa 1991) are very consistent with the field site at Mt. Hinokuma Park in Kanzaki City, Saga Prefecture, Japan, where we carried out studies for nearly 30 years (Fig. 1.3a-e). Like the sites identified in those earlier studies, Hinokuma is a dense secondary laurel forest on a small mountain encompassing a series of valleys and hilly areas throughout. Vegetation includes a variety of young broadleaf evergreen trees such as *Eurya japonica* Thunb. and *Castanopsis* and ferns such as *Woodwardia* and *Gleichenia*. Several species of tall deciduous trees, including *Ilex chinensis*, *Toxicodendron*, several species of *Quercus*, *Viburnum japonicum* Thunb., *Rhus sylvestris* Sieb. & Zucc., and *Laurus camphora* L., form a dense canopy that essentially blocks the sun, creating a fairly dark habitat. Host trees, *Schoepfia*

Fig. 1.3 Mt. Hinokuma, Kanzaki City, Saga, Japan. Clockwise from top left: Mountain range including Mt. Hinokuma viewed from the north. Arrow indicates Mt. Hinokuma; Mt. Hinokuma viewed from the west; access point to initial field site; Pond II, along the trail at the field site; Mt. Hinokuma viewed from the south where we approached the main access point to our field sites

jasminodora Sieb. & Zucc., in various stages of maturity, are scattered throughout the site (Tsukamoto and Tojo 1992; Filippi-Tsukamoto et al. 1995b).

1.7 Climate in Southern Japan

We present here a detailed description of the climate throughout the year because the constraints imposed on *P. japonensis* by the extreme climatic conditions it has experienced over evolutionary time have contributed greatly to molding the life history of this bug. The climate in Southern Japan is mild temperate, with distinct and dramatic seasonal features throughout the year. The winter, from December through February, is cold, damp, and gray, with heavy clouds; temperatures range from about 4–13° C (Japan Meteorological Agency n.d.) and reach freezing. The spring, from March through May, brings many days of fine weather with temperatures ranging from about 12 to 24 °C. The latter half of spring is fairly sunny.

There is a relentless rainy season throughout most of June and July, which coincides with the reproductive phase of *P. japonensis*' life history. Temperatures during the rainy season range from 20° to 30 °C. The rain can be quite heavy, resulting in intense flooding and landslides in the early days of our studies that often created a river of rainwater flowing down the path we used to get to the local field site. At that stage in our research, instead of a maintained park, as its name implied, Mt. Hinokuma was essentially a hilly forest in a natural state of secondary succession. As such, when we first began our studies, the path leading up the small mountain had not been maintained, and for the entire duration of our first field season, because the torrential rains kept the vegetation growing at a feverish rate, we literally had to fight our way through the brush with a sickle every day to get to our site.

The rainy season ends abruptly in mid-July. There will be rain one day, and then it literally ends the next day; the sky dramatically turns a deep cerulean blue and is decorated with huge billowing white clouds. Prior to this day, the persistent rain makes for uncomfortably chilly and wet days, but once the rains stop, the days immediately become unbearably hot! The temperatures are not particularly high for summer, ranging from 24 to 33 °C, but the humidity, often 100%, and only going down to 70% indoors with the air conditioner blasting, renders it uncomfortably hot even in the shade. The intense heat continues through August and much of September, when the next dramatic shift in weather, the typhoon season, arrives.

Typhoon season, from late August through the months of September and October, can be severe and typically brings with it lots of rain, which again leads to significant flooding and landslides. Temperatures during the typhoon season range from 15 to 30 °C. One of the severest typhoon seasons occurred in the fall of 1989, just after our first field season. Very bad flooding then caused a major landslide at our field site, tragically burying a subpopulation of about 8000 bugs and rearranging the landscape of the mountain. It was a terrible loss, but following that devastating event, the town invested in considerable infrastructure work, building and maintaining a real path up the mountain, which made our work a bit easier, and a true park area at the base of the mountain. November makes up the rest of the fall, with temperatures ranging from 10 to 18 °C; it is another gray, chilly month.

Thus, the life history of *P. japonensis* has adapted to frequent major flooding; extended periods of intense heat and no rain, though it is very humid then; and frequent severe damage to the habitat when typhoons occur.

1.8 Local Field Site at Mt. Hinokuma Park

The field site features several inherent dangers that include giant hornets (because of recent introduction, now known in North America as murder hornets) and pit vipers, so it was a rule that no one was allowed to go to the field site alone. Alas, L.F. found that rule hard to abide by and in fact never enjoyed field visits so much as when alone. There is a different level of attention one pays to one's surroundings when alone, free of any conversation and distraction. On one such solitary visit, L.F. noticed something peculiar. We had noted that most of the nests made by females were not within the area where drupes from the host tree were available. Moreover, they were not randomly scattered about but clustered in one particular area (see Fig. 4.1; Tsukamoto and Tojo 1992). This seemed counterintuitive. Why wouldn't the females nest in the middle of the fallen drupe area, where investment in foraging would be at a minimum and independent older nymphs could more easily access food on their own? In the quiet of the forest, L.F. looked around the area for clues. While there was leaf litter throughout the site, it was admittedly sparse under the host tree. That was a clue. However, it did not explain the clustering of nests in one place, while leaf litter was scattered throughout much of the site. It was immediately apparent that most nests were confined to the area of the field site that was closest to the host tree but also were still within the area covered by the crown of a tall *Ilex chinensis* tree. It suddenly made sense. That tree had leaves on it in March, dead, brown leaves, but they were still attached to the tree. The leaves fell in early spring and so were not as degraded as the leaf litter from most of the deciduous trees that had dropped to the ground in the fall. This tree and other deciduous trees that drop their leaves in the spring apparently represent an important feature of the local habitat because of the high-quality, airy leaf litter they provide during the nesting season.

1.9 Local Habitat Required to Support a Subpopulation of *P. japonensis*

We were also aware that, despite there being many *S. jasminodora* trees scattered throughout the forested mountain, many of which produced abundant drupes, we did not find a subpopulation of *P. japonensis* associated with every host tree. What was it about the locations of these seemingly fine host trees that was not conducive to supporting a subpopulation of bugs? To answer this question, and to assess the number and size of subpopulations inhabiting the area, we undertook an exhaustive study from April 4 to 29 of 1994 to identify all subpopulations at the larger, more inclusive, field site (Fig. 1.4) and clarify the ecological conditions that favored establishment of a subpopulation of *P. japonensis*. We surveyed and mapped a roughly rectangular area that comprised approximately ten hectares and included two small ponds. The survey was done during the flowering season of the host tree. We assumed that, as a minimum requirement, only those trees that were mature enough to flower could produce drupes and therefore support a subpopulation of bugs, so trees that were too young to be flowering were excluded from the survey. We measured the circumference of each tree at its base, noted the presence or absence of any subpopulations associated with each tree, and counted or estimated, when very large, the number of individuals in each subpopulation (Fig. 1.4; Filippi-Tsukamoto et al. 1995a).

We also assessed in a 10 meter radius around each host tree the quality of six habitat attributes we considered relevant to the life history of *P. japonensis*. The attributes assessed were sunlight on the host tree, degree of slope, quality of surrounding bush for aggregating, intensity of sunlight on that surrounding bush, quantity of leaf litter, and quality of leaf litter.

Finally, to assess the correlation between tree size and abundance of drupes produced, we set a triangular seed trap (43 cm^2) made of black netting and supported

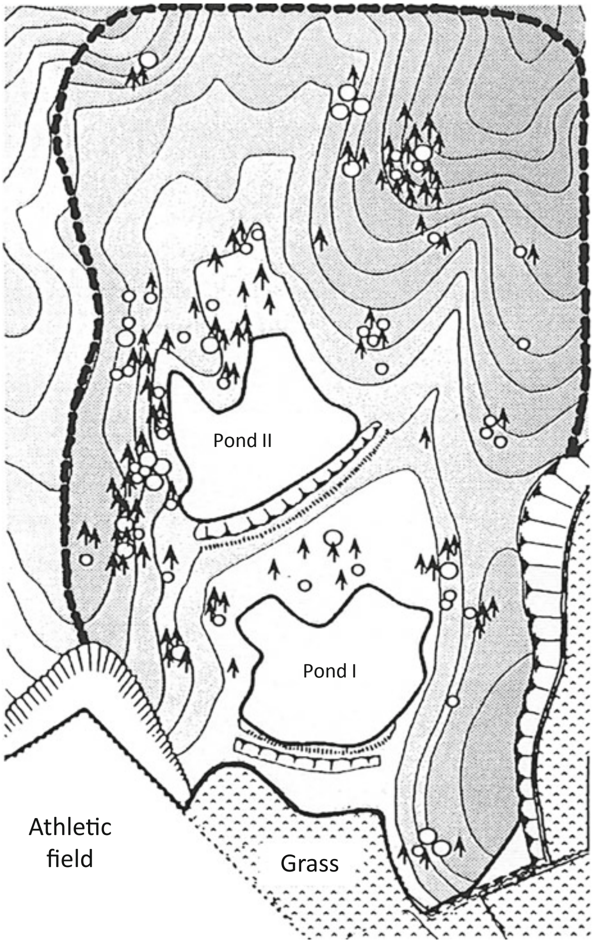

Fig. 1.4 Survey site at Mt. Hinokuma Park. Small and large arrows represent host trees of 50–69 cm and 70–129 cm circumference, respectively. Only trees with a circumference larger than 50 cm are shown (91/342 trees). Small and large arrows represent subpopulations of ≤500 or 500–10,000 individuals, respectively. The size of the survey site was approximately 10 ha. Adapted from Filippi-Tsukamoto et al. (1995b)

with three garden poles under three trees for each of three different size categories: small (30–40 cm circumference), medium (45–55 cm circumference), and large (70–95 cm circumference). The bottom of the nets of the nine seed traps were set about 50 cm from the ground, midway between the trunk and the edge of the crown. Drupes were collected weekly from May 20 to September 21, 1994 and counted.

1.10 Distribution of Host Trees and *P. japonensis* and Abundance of Drupes

As indicated in the map (Fig. 1.4; Filippi-Tsukamoto et al. 1995a), the survey site, and indeed all of Hinokuma Mountain, is mostly sloping terrain, gently sloping in places, but steep in others. In addition to the two ponds indicated in the map, several small streams also flowed through the site. Most of Hinokuma Mountain was a fairly early stage secondary forest at the start of our studies, but even early on, the survey site included some older areas, as well. Earlier stage areas were characterized by having denser understory growth, while later stage areas had taller deciduous trees that formed denser canopies, less sunlight, and less understory growth. In the approximately 10-hectare site surveyed, flowering host trees ranged from 15 to 129 cm in diameter, and we identified 342 *S. jasminodora* trees of flowering maturity. About 60% (207) of the host trees were associated with a subpopulation of *P. japonensis* (Fig. 1.5; Filippi-Tsukamoto et al. 1995b), and no subpopulation occurred where the largest host tree was smaller than 30 cm diameter; thus we concluded that 30 cm was the minimum size necessary to sustain even a small subpopulation. Nearly 40% of all host trees had a diameter smaller than 30 cm. On average, 55% of trees with a diameter smaller than 50 cm were associated with a subpopulation of *P. japonensis*, while an average of 72% of trees larger than 50 cm were associated with a subpopulation. Consistent with this finding, large trees produced nearly 4 times as many drupes (350) as medium trees (84) and over 18 times as many drupes as small trees (19) and were clearly able to support more bugs. Host trees tended to occur in clusters, typically, but not always, with one older tree, likely the parent, and several younger ones nearby. We identified 59 subpopulations of *P. japonensis*, and at least one host tree larger than 30 cm in diameter was invariably located within 10 m of each subpopulation. The subpopulations ranged in

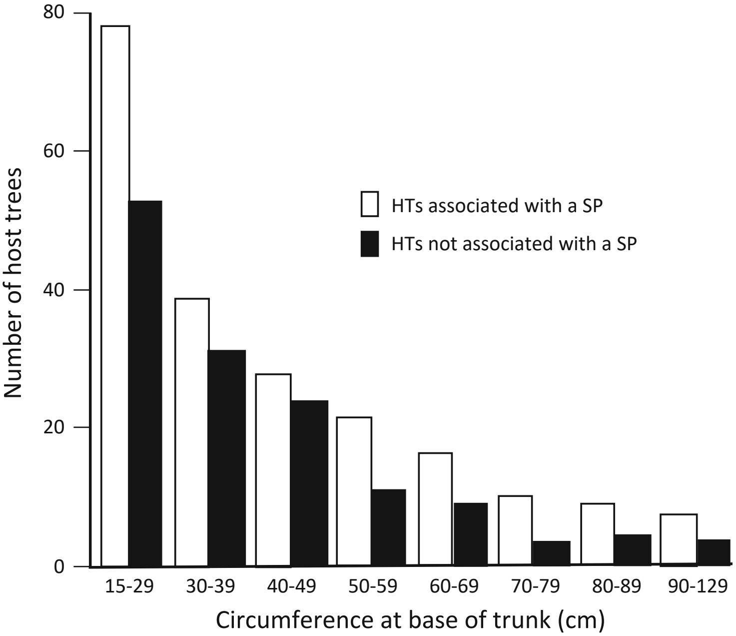

Fig. 1.5 Size distribution of host trees at Hinokuma. *HT* host tree; *SP* subpopulation. Filippi-Tsukamoto et al. (1995b)

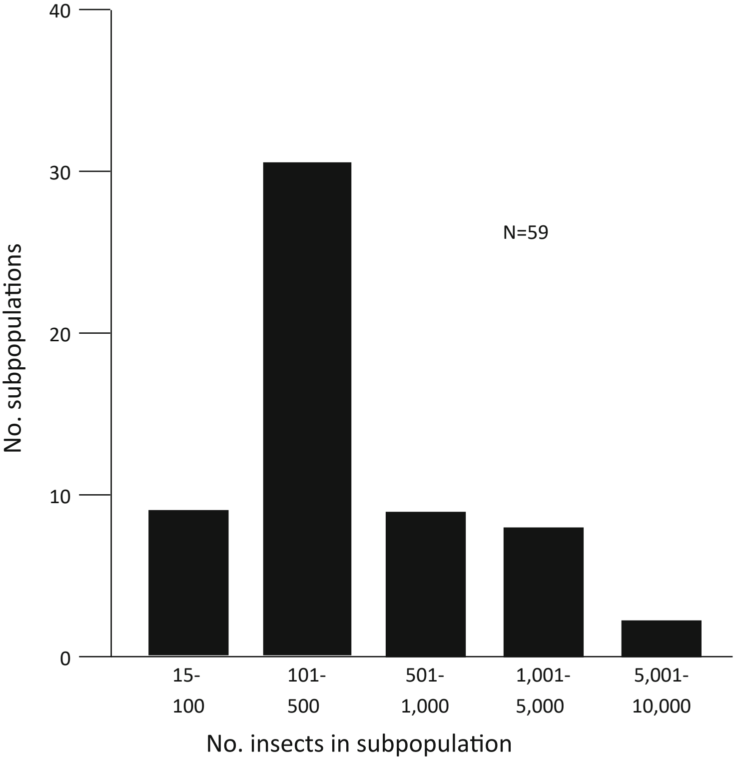

Fig. 1.6 Distribution of *P. japonensis* subpopulation size at Hinokuma. Adapted from Filippi-Tsukamoto et al. (1995b)

size from just 15 individuals to about 10,000, but the vast majority of subpopulations contained between 100 and 500 individuals (Fig. 1.6; Filippi-Tsukamoto et al. 1995b). Not surprisingly, subpopulation size was positively correlated with the cumulative diameter of the flowering host trees within 10 m of the subpopulation, and that correlation was particularly strong with subpopulations greater than 1000 individuals (Fig. 1.7; Filippi-Tsukamoto et al. 1995b).

1.11 Non-food Resource Qualities Essential to the Microhabitat

Clearly, access to the single food resource is of utmost importance in determining whether *P. japonensis* will be found in a given area, but when food is available, what other features of the microhabitat are critical? We assigned a value of 1–5 for each parameter assessed based on its suitability to the bug's life history: 1 was poor, 2 was

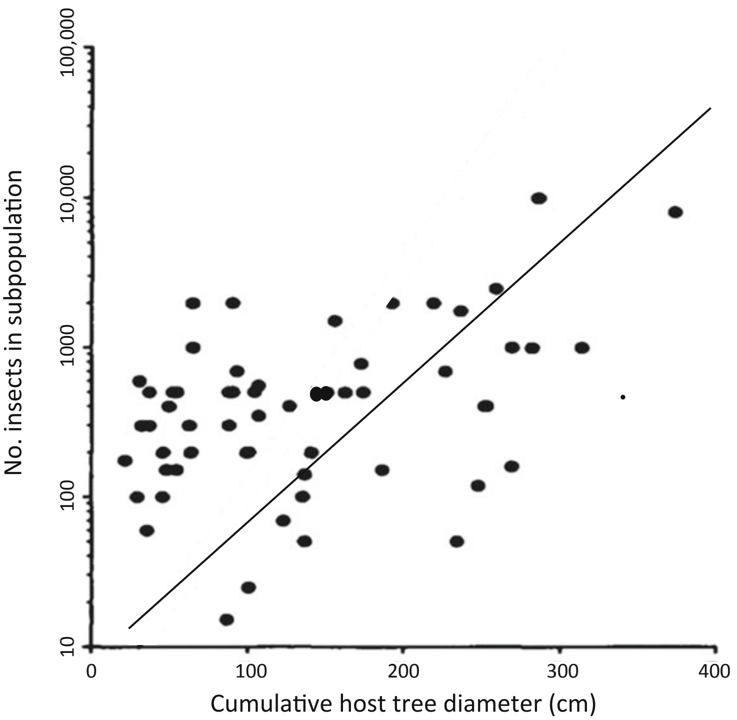

Fig. 1.7 Correlation between cumulative host tree diameter and *P. japonensis* subpopulation size. $P < 0.01$. Adapted from Filippi-Tsukamoto et al. (1995b)

fair, 3 was adequate/good, 4 was very good, and 5 was excellent. Light intensity on the host tree was considered important because the host tree is a pioneer species and is adapted to flourish, in other words produce more drupes, in direct sunlight. It was considered that a fair degree of slope in the nesting area would be important to keep the nests from becoming flooded during the rainy season. Surrounding vegetation, referred to as bush, comprised of small broadleaf evergreen trees, shrubs, and plants such as fern is essential to support aggregations because *P. japonensis* does not enter hibernation until mid-December, well after the deciduous trees have dropped their leaves. The intensity of light on the aggregation bush should be extremely low because the bugs only aggregate in well-shaded and dark locations. As with other insects, this preference for darkness is likely related to conservation of moisture. The quantity of leaf litter should be fairly dense because females nest under the leaf litter. Finally, the quality of leaf litter was considered high if it was primarily composed of the freshly fallen leaf litter that females apparently prefer for nesting.

The biggest contradictory feature of the bug's habitat is the need of the host tree to have access to direct sunlight, but for the bugs' aggregations to have good shade. The host tree, being a pioneer species, apparently emerges as one of the first trees in the secondary forest. It grows slowly but flourishes in the open areas exposed to full sunlight, flowering densely and producing abundant drupes. However, *S. jasminodora* never grows very tall, reaching heights of only about 30 feet, and other deciduous trees, *Quercus*, *Ilex*, *Viburnum*, *Rhus*, and *Laurus*, gradually overtake it. These trees eventually form a tall and thick canopy over *S. jasminodora*, blocking much of *S. jasminodora*'s access to sunlight, and we have noted a marked decrease in drupe production of host trees cast in the deep shade of older parts of the forest. The main pollinator of *S. jasminodora* at Mt. Hinokuma is the honeybee, which relies on sunlight to detect the flowers it collects pollen from. We suspect that the darkened habitat interferes with the honeybee's ability to find the flowers, thus leading to reduced pollination and drupe production. At any rate, it remains a conundrum that the only food source *P. jasminodora* utilizes requires a condition that is in direct conflict with a key environmental criterion of the bugs, deep shade. Reflecting this conundrum, we found that the optimal level of sunlight on the host tree for predicting whether a subpopulation would be found was a compromise, the middle level, which was apparently bright enough for the tree to produce an adequate supply of drupes, yet not too bright for the bugs (Fig. 1.8; Filippi-Tsukamoto et al. 1995b). Thirty three percent more of the host trees in this category were associated with subpopulations, than those without subpopulations, but for light intensities greater or less, the numbers with and without subpopulations were identical.

For each of the other five parameters assessed, more subpopulations were invariably associated with conditions that were assessed as 3 (good/adequate) and above. Of course, it would be rare to find excellent quality for all parameters at any given locale, and the data indicate that sites with a minimum requirement of good/adequate or better for each of the criteria were much more likely to be associated with a subpopulation of *P. japonensis* than those with parameters of lower quality.

1.12 Do *P. japonensis* Discriminate Habitat Based on Collective Optimality of Essential Environmental Features?

We were interested in understanding whether *P. japonensis* only chose habitats where all of the criteria were optimal for their life history. We considered an optimal habitat to be one that was assessed as level three or above for all attributes except light intensity on the host tree. Light intensity on the host tree was not included in this because that attribute showed no difference between presence and absence of a subpopulation (Fig. 1.8), likely because most trees were inhabiting the optimal area to begin with. In fact, a significant proportion of *P. japonensis* subpopulations (60%) were associated with host trees found in a habitat where all of the other five environmental parameters met optimal levels, and a significant portion of host trees with optimal conditions (69%) were associated with a subpopulation (Table 1.2; $X^2 = 11.72$, $p = 0.0006$).

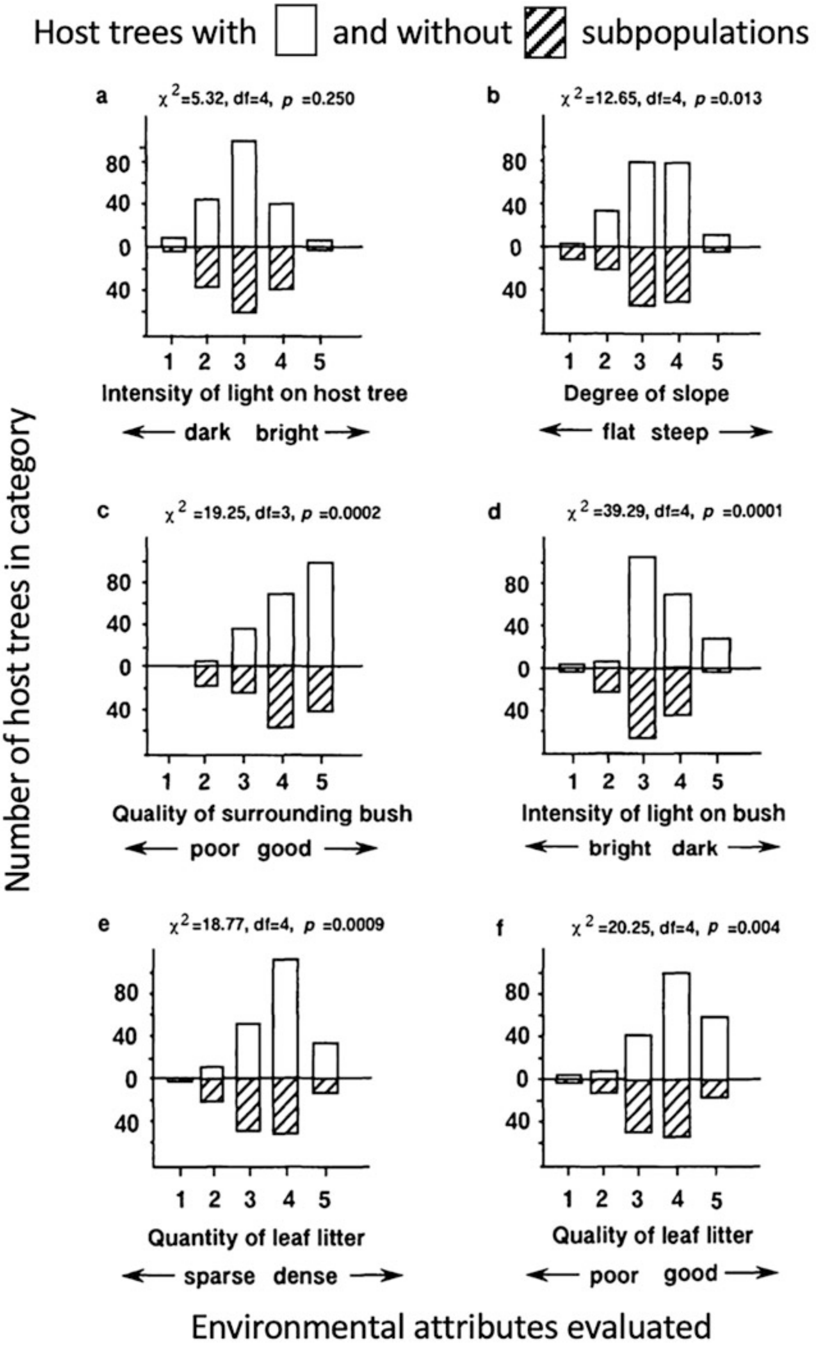

Fig. 1.8 Assessment of six relevant environmental parameters around host trees with and without subpopulations of *P. japonensis*. Assessments were determined based on the condition of the parameter that was deemed to be most appropriate for the life history of *P. japonensis* using a scale of 1–5, where 1 was the poorest condition and 5 was the best condition. Adapted from Filippi-Tsukamoto et al. (1995b)

Table 1.2 The relationship between host trees (HT) surrounded by optimal environmental conditions and association with a subpopulation (SP)

HT type	Optimal conditions[a]	Suboptimal conditions	Total
HTs with SP	125	82	207
HTs without SP	56	79	135
Total	181	161	342

[a] See text for description of optimal conditions. X^2, $p = 0.0006$. Adapted from Filippi-Tsukamoto et al. (1995b)

1.12.1 Most Determinant Environmental Features

Finally, we were curious to know which of the environmental parameters had the greatest impact on determining subpopulation size. Because we anticipated that total resource available to a given subpopulation would be an important determinant, for this assessment, we added a seventh environmental factor, cumulative host tree size at a given subpopulation, and carried out a multivariate regression analysis. Subpopulation size and cumulative host tree size were log transformed for normalization. The stepwise method (Table 1.3) revealed that Cumulative Host Tree size (CumHT), Leaf litter Quality (LQlt), and, surprisingly, Slope (Slp) were the three variables that best explained the variation in subpopulation size (SP size) using this multiple regression model:

$$\mathrm{Log(SP\ size)} = 0.1032 + 0.6456\ \mathrm{log(Cum\ HT)} + 0.4176\ \mathrm{LQlt} + 0.3484\ \mathrm{Slp}$$

Food resource availability, as represented by cumulative host tree size, was, predictably, determined to be the single most important of the three variables in determining subpopulation size. The multiple correlation coefficient of the model was significant ($p = 0.0006$), and the model accounted for 27% of the total variance in subpopulation size. Slope was likely important to prevent flooding of the nest.

Table 1.3 Correlation coefficient between variables used in model

Attribute	$\mathrm{Log_e}$(SP size)	$\mathrm{Log_e}$ (Cum HT)	LQlt	Slp
$\mathrm{Log_e}$ (SP size)	1.00			
$\mathrm{Log_e}$ (Cum HT)	0.354	1.000		
LQlt	0.322	0.061	1.000	
Slp	0.248	−0.092	0.183	1.000
$\mathrm{Log_e}$ (Cum HT), LQlt	.454[a]			
$\mathrm{Log_e}$ (Cum HT), Slp	.452[a]			
$\mathrm{Log_e}$ (Cum HT), LQlt, Slp	.518[a]			

SP subpopulation; *Cum HT* cumulative host tree size (>30 cm); *LQlt* leaf litter quality; *Slp* degree of slope

[a] Multiple correlation coefficient. Adapted from Filippi-Tsukamoto et al. (1995b)

1.13 Phenology of the Host Tree and Instability of the Resource

Like *P. japonensis, Schoepfia jasminodora* Sieb. & Zucc. (Schoepfiaceae), or gray twig, is the single-genus, single-species representative of its family, Schoepfiaceae, in Japan. It is a flowering deciduous tree of medium height, bearing fragile branches and very thin and delicate leaves (Fig. 1.9a). In fact, the thinner branches are so fragile that they break off under minimal pressure, and new growth continually pops out where the branches have broken off giving the tree a very disorderly appearance. For this reason, the common name for *S. jasminodora* in Japan is "tattered tree." The leaves are a very lovely Kelly green, and the branches and leaves become quite dense. At the field site in Saga, Japan, *S. jasminodora* leaves emerge in early April, and flowers begin to bloom in mid-April. The delicate, clustered, bell-shaped flowers, 5–7 mm in length, are cream to pale yellow, sometimes with pale green borders (Fig. 1.9b). In early May, tiny green drupes begin to form on the tree. The oval drupes mature in a staggered fashion over the course of about a month as the color changes from green and yellow to pink, then deep red, and finally purple, reaching a final length of about 5–9 mm (Fig. 1.9c). Probably owing to the fragile nature of the tree, many drupes fall to the ground before they are ripe; color does not necessarily coincide with the degree of endosperm development, and drupes of any color might have a well-developed endosperm or very little endosperm, the part of

Fig. 1.9 (**a**) Host tree of *P. japonensis*, *Schoepfia jasminodora*, with (**b**) flowers and (**c**) drupes

the drupe that *P. japonensis* feeds on. Drupes fall to the ground for about 1 month, coinciding with the early reproductive phase of the bug (Tachikawa and Schaefer 1985). The delicate leaves begin to turn brown in late August, well before other deciduous trees, and fall to the ground in September/October. Because the leaves are so thin, they deteriorate very quickly once they fall from the tree.

1.13.1 Variation in Drupe Quality

The habitat study was done fairly early on in our research, but considering the decline in population size that has happened at the original site where early studies took place at the later succession stage Koura Mountain in Kurume City, Fukuoka (LF personal observation), and what we have seen over the years since this study began, we have come to understand a good bit more about habitat suitability for *P. japonensis*. Firstly, the host tree clearly becomes a poorer producer of the valued drupes as the canopy around it grows denser, blocking the sunlight. An otherwise healthy and large tree will produce relatively few drupes under such conditions, so a once excellent site with abundant drupes and a large subpopulation of *P. japonensis* will gradually become poor, and the subpopulation will disperse and/or die off. Secondly, we quickly came to realize that the resource is not only ephemeral but the abundance of drupes produced and the proportion of drupes with adequate/good endosperm fluctuate dramatically from year to year for a given host tree, even when the tree is located in a suitably sunny site. Over 4 years, three of them consecutive (1995, 1997, 1998, and 1999), the total number of drupes and the proportion of adequate drupes collected in seed traps varied considerably (36, 26, 14, 167 adequate drupes, respectively; Fig. 1.10; Filippi et al. 2002). In fact, over 10 years of observations, only 1 year had a high proportion of adequate drupes (Filippi et al. 2002). Thus, the availability of the food resource is generally poor, ranging from typically scarce to rarely abundant, and, as such, constitutes an unpredictable and scarce resource.

1.14 Toxic Drupes and Toxic Bugs

On a positive note, despite the scarce, ephemeral nature of their food resource, the bugs have little competition for the endosperm of the precious drupes that they are wholly dependent on because they are toxic. We had long suspected that the very delicious looking deep red drupes were toxic because we never saw any birds or other vertebrates eating them. One very enthusiastic graduate student, and a coauthor of this book, Mantaro Hironaka, keen to verify whether the drupes were, indeed, toxic decided to taste one. He immediately spit it out and described the taste as nasty and strongly astringent. To our great surprise, and well above and beyond the call of duty, he then very bravely put a bug in his mouth and chewed it! The bug was spat

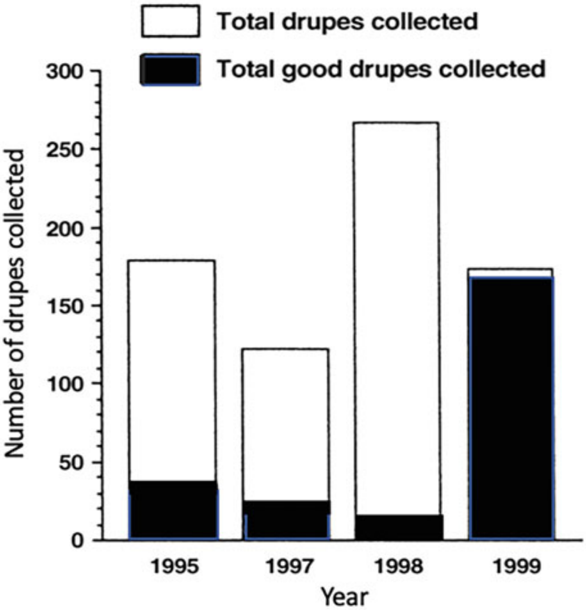

Fig. 1.10 Total yearly number of drupes collected in seed traps over 4 years. Good drupes have >80% endosperm. Adapted from Filippi et al. (2002)

out as quickly as the drupe and apparently had the same nasty, astringent taste. A rather primitive and possibly dangerous experiment, but the results were clear. The bug tastes nasty, probably from the toxins in the drupe that it feeds on, and the red and black color warns all would-be color-sighted visual predators that they attack the bug at their own peril.

In 2004, Professor Tojo decided a more scientific approach to the matter of plant and insect toxins was in order. He contacted a colleague at Kyoto University, Professor Ritsuo Nishida, who specializes in insect toxins. The findings of the studies supported Mantaro's anecdotal bioassay. *Schoepfia jasminodora* drupes contain a large quantity of toxic lipids that the plant no doubt uses for defense. Acetylenic triglycerides such as 9, 11, 13-octadecatriynoic acid and 9, 11-octadecadiynoic acid occur in the drupes along with other typical fatty acids. When orally administered to mice, synthetic esters of the fatty acids were lethal (R. Nishida, unpublished data). The bug sequesters these triglycerides, making it a very unpalatable prey, which explains the beautiful aposematic coloration of *P. japonensis* (Kamata et al., 2004, 2005). When hungry quails were given the opportunity to feed on the bugs and nothing else, they ate them reluctantly but always vomited afterwards. When given a choice between *P. japonensis* and the southern green stink bug, *Nezara viridula*, which is certainly an unappetizing prey item in its own right, the quails always chose the southern green stink bug (S.N., unpublished data). In thousands of hours of observations in the field, with the exception of one unlucky frog who leaped in and stole a bug we had just marked with nail polish, we have never seen birds or any other vertebrate actively feeding on *P. japonensis*. Ants eat the legs and wings of adults but leave the toxin-laden body behind. However, to our great dismay, they eagerly and voraciously feed on eggs and early instar nymphs, which frustratingly tended to wreak havoc on our sample sizes. Ground beetles (Carabidae) are also major egg and nymph predators in the nesting area. We once saw a large unidentified centipede feeding on a female bug and one male hanging fly actually presenting a female *P. japonensis* to its mate as a nuptial gift (L.F., unpublished finding). We did not wait to see if the toxic gift proved to be her last meal. These invertebrate predators might not benefit from the aposematic coloration, but vertebrate predators surely would.

Thus, *P. japonensis* uses the toxic triglycerides as both an energy source and a defense mechanism (Kamata et al. 2004, 2005). Clearly, one of the very useful evolutionary innovations that has emerged in *P. japonensis* is the ability to process and utilize these toxins effectively, enabling the bugs to acquire a scarce but rich resource free of competition. Other herbivores such as moth larvae will eat the outer fleshy part of the drupe, but, to the best of our knowledge, the drupes are apparently unappetizing to every other organism, save *P. japonensis*, who has managed to make the best of a rather poor situation.

1.15 Life Cycle of *Parastrachia japonensis*

The life cycle of *P. japonensis* takes either 1 or 2 years to complete (Fig. 1.11). After adults emerge from their subterranean hibernation in early March, they reform aggregations on broadleaf evergreens, such as *Eurya japonica* and ferns. At this point, the reproductive organs of both males and females are undeveloped (Tsukamoto 1991; Tsukamoto et al. 1994). We discovered early on that only a portion of the bugs in the aggregations become reproductively active during the first post-hibernation spring; the rest, comprising 5–95% of the subpopulation, remarkably, remain in reproductive diapause until the following spring, when they mate (Tsukamoto et al. 1994). Mating behavior is described in detail in Chap. 3. Briefly, the male testes mature over about 5 weeks and contain abundant sperm by mid-April. Female ovaries develop minimally over the same period. Males become active before females and fly about the aggregations seeking a mate. Females do not fly during mating, apparently conserving their energy for the herculean task of provisioning later on. Males mate with many females over a 2-week period, burning up their remaining fat stores, and then die without ever having fed since they were fifth instars the previous summer (Tsukamoto et al. 1994). Inseminated females relocate to a host tree where they feed on a succession of as yet fairly small and immature drupes to develop their eggs (Gyôtoku and Tachikawa 1980; Tachikawa and Schaefer 1985; Tsukamoto and Tojo 1992; Tsukamoto et al. 1994; Filippi-Tsukamoto et al. 1995a; Filippi et al. 2001). Eggs are deposited in early June, after which mothers guard the egg mass until hatching. After hatch, females split their time between guarding and provisioning nymphs throughout June, and then they begin to die off. Nymphs become independent from late June to mid-July. They go through five molts before adults eclose from late July to mid-August. The new adults

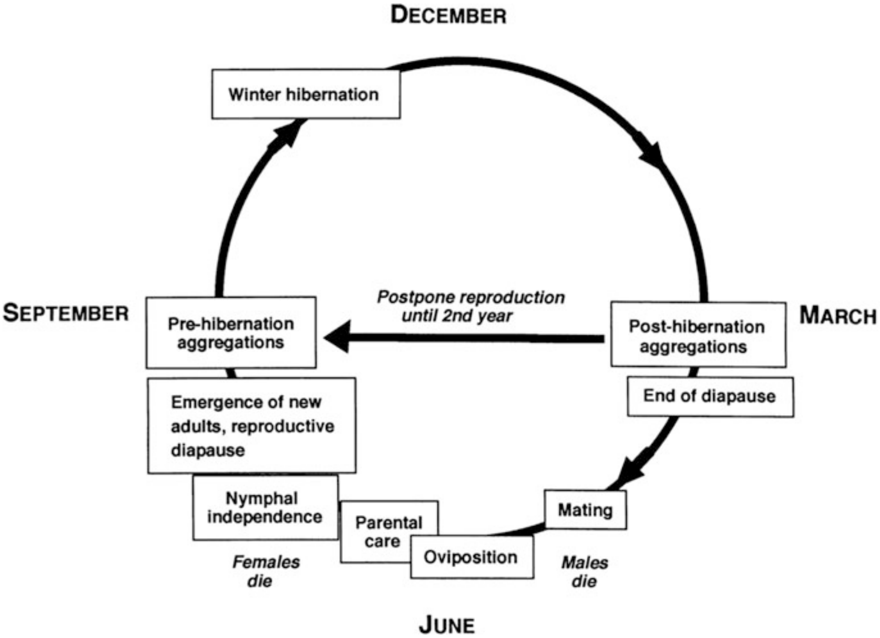

Fig. 1.11 Life cycle of *Parastrachia japonensis.* Adapted from Filippi et al. (2001)

join the aggregations of adults that remained in reproductive diapause. For the remainder of the summer, the estivating aggregations move down into crevasses around the tree roots on particularly hot and dry days and back up the aggregation trees/vegetation again on less stressful days. No more feeding occurs, though they do take water from the veins of leaves. The bugs remain aggregated until December, when they burrow in holes in the ground that were made by other animals such as snakes and moles. They remain hibernating in aggregations until late February, early March when they emerge, and the cycle begins again.

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Shuji Tachikawa an entomologist and Professor Emeritus of Tokyo University of Agriculture, had long been interested in *P. japonensis*, and his early observational studies, presented in several papers and in a chapter in a book that he wrote on subsocial heteropterans in Japan (Tachikawa, 1991), served as a critical springboard into this research. Dr. Tachikawa is retired Associate Professor of Tokyo University, Faculty of Agriculture, and is currently affiliated with the Association for Nature Restoration and Conservation, Tokyo, Japan.

Shintaro Nomakuchi an ecologist focusing on insect behavior, came to Saga University in 1993 as an Associate Professor, and was keen to share his very valuable expertise and join the *P. japonensis* research team, which he eventually headed. The work on clarifying the quality of the habitat, the techniques for monitoring the subpopulations and for carrying out a variety of ecological and behavioral studies, as well as the methods for performing robust data analyses, all got a hefty boost, and we were off and running. He is a Professor Emeritus of the Faculty of Agriculture at Saga University.

Lisa Filippi initiated the work on *Parastrachia japonensis* at Mt. Hinokuma Park in Kanzaki Town, Japan, where she earned her M.S. and doctoral degrees elucidating many of the amazing physiological and behavioral traits of this fascinating insect through field and laboratory studies. She is currently a Professor of Biology at Hofstra University in New York.

Chapter 2
Mechanisms for Prolonged Survival Without Food

Lisa Filippi, Takayuki Kashima, and Takahiro Hosokawa

Abstract Insects have an amazing range of adaptations that allow them to survive in very stressful physical environments and when their food resource is unavailable for prolonged periods of time. *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) depends on a resource that is ephemeral, unpredictable, and scarce when available. To optimize use of the poor resource, *P. japonensis* has incorporated several extreme behavioral and physiological traits into its life history that include remaining in aggregations for much of its life cycle, prolonged reproductive diapause, and suppression of the metabolic rate. Moreover, they can recycle uric acid to generate a continuous supply of nitrogen when they cannot feed. This chapter reviews the studies that uncovered these adaptations and their functions and how *P. japonensis* uses symbiotic bacteria to recycle uric acid.

Keywords Aggregations · Bacterial symbiont · Diapause · Metabolic rate suppression · Transmission of symbiont · Reproductive diapause · Uric acid recycling

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2.1 Introduction

Insects whose life cycle extends beyond the period that their resource is available have adopted a variety of strategies to increase survivorship during the prolonged periods when the food source is unavailable. To dramatically reduce their nutritional needs during such times, many enter diapause (Tauber and Tauber 1976), others reduce their metabolic rate considerably by aggregating (Tanaka et al. 1988), and aggregation can take on different adaptive functions. Still other species recycle metabolic waste products (Sasaki et al. 1996; Sabree et al. 2009). We have found that each of these survival mechanisms is built into the fascinating life history of *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae). We present here the discoveries we have made that allow *P. japonensis* to survive despite the ephemeral and scarce nature of its sole resource, drupes of the host tree, *Schoepfia jasminodora* Sieb. and Zucc. (Schoepfiaceae).

2.2 Pattern of Aggregation Formation

From the outset of our studies, one of the most conspicuous and intriguing behaviors of *Parastrachia japonensis* was its remarkable tendency to form sedate aggregations of various sizes, from just 5 or 10 individuals up to about 1000 (Tsukamoto and Tojo 1992). We were interested in understanding the mechanism of how the aggregations formed.

P. japonensis remains in aggregations throughout much of its life cycle and during all stages of development. Adults are aggregated during the entire reproductive diapause phase, and immatures feed in aggregations and aggregate prior to molting. Male and female adults behave individually during mating and females behave individually during egg and nymph guarding and provisioning (Filippi et al. 2000b). Aggregating is a common behavior in insects and occurs for a variety of reasons outlined below, and it is typically induced by an aggregation pheromone. Not surprisingly, cuticular extracts in ethanol demonstrated that *P. japonensis* does, in fact, produce a contact pheromone that likely serves as an aggregation pheromone (Sumio Tojo, unpublished data). We set out to determine the pattern of aggregation formation. How did they initially come together? We collected 200 insects of mixed sex from the field in mid-October, when they were in reproductive diapause. During this phase of their life history, the bugs are fairly sedentary in the aggregations unless disturbed. We released the bugs on the ground at the base of a small *Eurya japonica* tree (~1 m tall), enclosed them and the tree in black netting, and observed their behavior over 48 h. They immediately started climbing up the tree. By the end of the first hour, 55% of the bugs were still roaming up the tree or perched on a leaf as individuals (Fig. 2.1; Tojo et al. 2005). About 5% were in clusters of two individuals, and the remainder were in clusters of 6–15 individuals. By 4 h, over 80% of the bugs were in clusters of different sizes, and most of the clusters contained 6–25

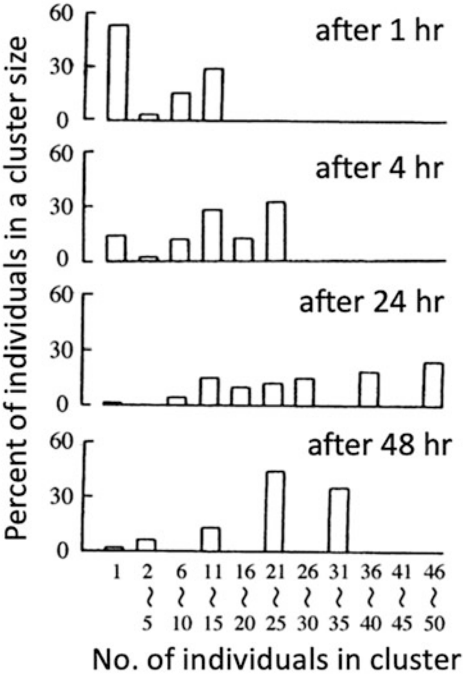


Fig. 2.1 Pattern of aggregation formation in *P. japonensis.* 200 bugs of mixed sex were released at the base of a *Eurya japonica* tree (1 m height). Adapted from Tojo et al. (2005)

individuals. Larger clusters (36–50 individuals) had formed by 24 h, but they were clearly somewhat fluid because by 48 h, the largest clusters had disbanded and joined the smaller ones, which had fused into four aggregations. The aggregation size for this number of bugs (200) on the 1 m tree stabilized at 21–35 individuals. Interestingly, there were always a couple of individuals that were not in an aggregation. Apparently, it is the contact pheromone that promotes settling, with formation of aggregations as the eventual outcome.

Examination of 27 clusters of *P. japonensis* on *Eurya japonica* trees in October during the reproductive diapause phase indicated there was no bias in the sex ratio (Fig. 2.2; Binomial test, $p > 0.05$).

2.3 Functions of Aggregations

Next we sought to understand the functions of the aggregations. The evolutionary ecological role of aggregation pheromones and aggregating in nonsocial insects was reviewed by Wertheim et al. (2005). The benefits of pheromone-induced aggregation were summarized as four general categories: protection against natural enemies, mate acquisition, protection from harmful environmental conditions, and increased efficiency of resource use. We have evidence, some anecdotal, that all of these categories describe the functions of aggregating in *P. japonensis.*

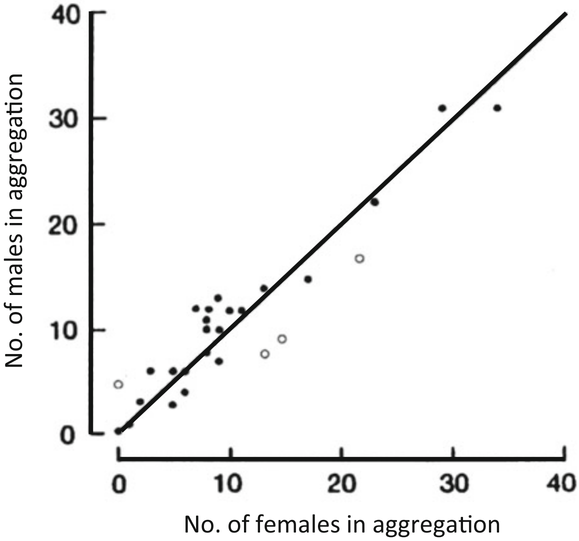


Fig. 2.2 Sex ratio of *P. japonensis* aggregations at Hinokuma Mountain Park. $N = 27$. Regression line represents sex ratio of 1: 1. The four open circles did not statistically fit on the line. Binomial test, $p > 0.05$. Adapted from Tojo et al. (2005)

2.3.1 Protection Against Predators, Mate Acquisition, and Reduction of Water Loss

Beginning with the most obvious, the flamboyant red and black color and the large size of *P. japonensis* (for an insect!) certainly pointed to a likely function. The flashy color of so many clustered individuals, not just in *an* aggregation but in very many aggregations spread over a stretch of forest extending for sometimes 10 or 20 m, suggests aposematism: the superstimulus of the large and abundant aggregations likely serves as an effective warning to would-be predators (Vulinec 1990; Sword 1999). Of course, aposematic coloration would only be effective with predators, presumably vertebrate, that have the potential to see color and to learn (Brock and Riffenburgh 1960). In the habitat at Mt. Hinokuma in Saga, Japan, even after thousands of hours of observation, we saw only one vertebrate attack a *P. japonensis*, the unlucky frog mentioned in Chap. 1. We did not test this, but the reluctance of the quails to eat the bugs and the vomiting after ingesting one in the study described in Chap. 1 strongly suggests that the superstimulus effect of aposematic color in massive aggregations would serve as an effective warning signal.

Of course, should a predator come to prefer them, the dilution effect (Hamilton 1971) would make aggregating adaptive, as well. Finally, the bugs aggregate by hanging from the leaves on trees for most of the year and only spend time on the ground during reproduction and when forming large aggregations to avoid the hottest and driest days of summer during estivation and the coldest months of the winter during hibernation. These patterns with regard to location of the aggregations would allow the bugs to minimize time spent on the ground and in the leaf litter during the active seasons of their most aggressive ground-dwelling invertebrate predators, ground beetles, giant centipedes, and ants.

Mate Acquisition

Because the bugs remain aggregated except during the parental care phase, when mating season comes around, there is no need to invest time and energy in searching for a mate, so this function of aggregating is clearly met.

Reduction of Water Loss

Because of the large surface area to volume ratio of insects, and the ready loss of water across the cuticle via the spiracles, behavioral strategies to minimize water loss are critical. By reducing the exposure of each individual's spiracles to the environment, aggregation in insects can help maintain moisture in stressful dry seasons (Klok and Chown 1999; Broly et al. 2013). Antarctic collembolans aggregate in response to chemical cues and benefit from reduced moisture loss (Benoit et al. 2009). Density of aggregations of first instar cockroach nymphs is inversely correlated with relative humidity (Dambach and Goehlen 1999). Aggregated Emperor moth larvae lose less water per individual than those that are isolated (Klok and Chown 1999). The climate in Saga, Japan, in late summer is hot and dry, and it is dry throughout winter. It is reasonable to assume that bugs in aggregations will suffer less water loss than lone individuals due to reduced surface area to volume ratio. In support of this, in April, when the relative humidity in the field was 15%, that inside an aggregation of ~100 individuals was 45% (Sumio Tojo, unpublished data). However, we were most curious about whether aggregating behavior was a factor in explaining how the bugs could survive for up to 2 years as diapausing adults without feeding.

2.3.2 Increased Efficiency of Resource Use

Aggregation and Reduction of Metabolic Rate

Insects with lengthy life cycles frequently encounter periods when food is unavailable or climate is particularly inhospitable, as in the cold, dry winter or hot, dry summer. They mitigate these stressful periods by entering diapause, a phase that allows insects to minimize nutrient and water depletion until conditions become favorable once again. Insects that enter a prolonged diapause phase must conserve moisture and energy and efficiently utilize the nutrient reserves acquired during the immature stages. Diapause can occur at any stage of development, egg, larva/nymph, pupa, or adult, and the developmental stage of diapause is usually species specific and often occurs in aggregations. Adult or reproductive diapause is most common in Coleoptera, occurring in ~90% of species, while 70% of heteropteran species, including *P. japonensis*, diapause as adults (Danks 1987; Hodek 2012). Most insects adapt to prolonged diapause by reducing their respiration rate compared to the non-diapause phases (Chapman 1971; Wigglesworth 1972; Denlinger 1986; Danks 1987). *Stenotarsus subtilis* (Coleoptera: Endomychidae), a subtropical beetle, forms large aggregations of diapausing adults that maintain a higher level of moisture during dry seasons. Interestingly, the higher moisture level induces reduced metabolic rate (MR) (Wolda and Denlinger 1984; Tanaka et al. 1988; Tanaka 2000). We investigated whether *P. japonensis* might also be reducing its MR during reproductive diapause, which would allow it to make better use of its stored nutrient reserves (Tojo et al. 2005). This is particularly important for *P. japonensis* because the drupes of *S. jasminodora*, its sole food source, are ephemeral and scarce (Tsukamoto and Tojo 1992; Nomakuchi et al. 1998; Filippi et al. 2000a, 2001, 2002). In fact, the only adults that actually feed on drupes are inseminated females, fully 10 months after they become adults (Filippi et al. 2000b). It is, thus, essential that the bugs make the best use of the drupes they fed on as nymphs, as that nutrition might have to sustain them for 2 years.

As a proxy for MR, we measured oxygen consumption using a Model 5B O_2-up Tester connected to a Cl-80 Coolnit water bath regulator (TaiTec, Co. Ltd., Fukuoka). Adults were collected at different stages (different intensities of reproductive diapause and reproductive) and individually weighed. Female wet weight ranged from 160 to 230 mg, and male wet weight ranged from 130 to 160 mg. Bugs were enclosed in a 700 mL airtight respiration chamber containing a piece of water-soaked cotton and 1 mL w/v KOH aqueous solution, separated from the bugs by a wire mesh. The chamber was set in an incubator at 25 °C, and the bugs were allowed to settle for 3–4 h. During that time, they aggregated, and then oxygen consumption was monitored for 5 h. We measured the oxygen consumption of solitary males and females and in separate sex groups of three, five, and ten individuals in the chamber. During diapause, solitary males and females had oxygen consumption rates that were more than twice as high as those in groups of three, five, or ten (Tojo et al. 2005; Fig. 2.3), and male rates were always about 20% higher than those of females, possibly reflecting the much smaller body size/lower body weight of males. After the first hour of measurement, which showed a precipitous increase, the oxygen consumption rate (μL O_2/g wet weight/h) stabilized somewhat. Over the next 4 h, solitary females averaged 316 μL O_2/g wet weight/h, while the O_2 consumption rates for groups of three, five, and ten females were less than half that rate, at 116, 124, and 128 μL O_2/g wet weight/h, respectively. The corresponding rates for males were 276, 111, 125, and 117 μL O_2/g wet weight/h for solitary males and groups of three, five, and ten males, respectively (Tojo et al. 2005).

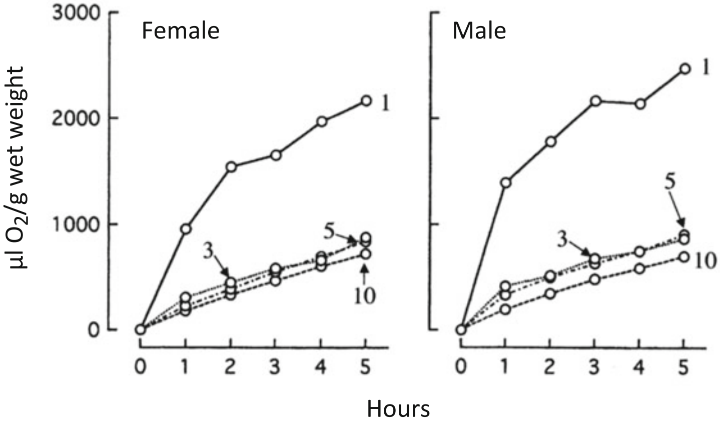


Fig. 2.3 Cumulative oxygen consumption of female and male *P. japonensis* in reproductive diapause over 5 h. Bugs were collected in October, when temperatures are still mild. Measurements were done at 25 °C, 16 L:8D, as solitary individuals or groups of three, five, or ten. Adapted from Tojo et al. (2005)

2.3.3 Effect of Group Size on Metabolic Rate

To determine whether there was a correlation between group size and O_2 consumption rate, we collected males in November, just prior to the winter hibernation, and compared O_2 consumption rates in solitary males and groups of 10, 20, 30, and 40 males. The overall rates were lower than the data for the October collection, probably reflecting the deeper diapause just prior to hibernation. However, there was still no correlation between group size and O_2 consumption rate other than that between solitary individuals and groups of any size (Fig. 2.4; Tojo et al. 2005). In contrast to the case of *S. subtilis*, where group size was negatively correlated with O_2 consumption rate (Tanaka 1988; Tanaka et al. 2000), physical contact with at least one individual was enough to reduce the MR dramatically, and beyond that, larger size had no impact on MR. Isopods also show reduced respiration in aggregations, although the effect might be temporary. Interestingly, they show a similar tendency to that seen in *P. japonensis* in that the effect is most prominent when in contact with just one individual and is not correlated to group size (Allee 1926; Takeda 1984; Broly et al. 2013).

2.3.4 Effect of Physical Contact on Metabolic Rate

Next we attempted to determine whether the MR effect was in response to a chemical cue or the actual physical contact. We put a stainless steel mesh cage inside the chamber with ten compartments. Five adults were individually put into every other compartment, so although there were five individuals in the chamber, and volatile

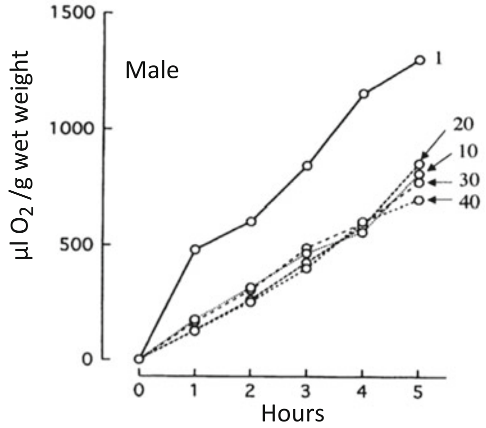


Fig. 2.4 Cumulative oxygen consumption of male *P. japonensis* in reproductive diapause over 5 h. Bugs were collected in November, just prior to the start of the winter hibernation. Measurements were done at 25 °C, 16 L:8D, as solitary individuals or groups of 10, 20, 30, or 40 males. Adapted from Tojo et al. (2005)

chemicals could flow, no physical contact was possible. Although O_2 consumption rates were somewhat lower overall for both males and females, probably because these bugs were collected from the field a month later in their diapause phase than those in Fig. 2.3, the pattern was similar to what was observed under solitary conditions. The O_2 consumption rates of solitary females and males were 260 and 290 µL O_2/g wet weight/h, respectively. However, when they were measured in aggregated groups of five, having direct physical contact, the O_2 consumption rates dropped approximately 50% to 137 and 142 µL O_2/g wet weight/h, respectively.

Interestingly, when physical contact was prohibited, the rates were 257 and 312 µL O_2/g wet weight/h, respectively, essentially not different from those of solitary bugs (Fig. 2.5; Tojo et al. 2005), indicating that the physical contact, and not a volatile chemical cue, was in some way mediating the reduction in MR.

2.3.5 *Combined Effects of Contact Pheromone and Physical Contact*

We then exposed one living *P. japonensis* female to four dead females inside the chamber. The live female approached the dead females and moved a bit on the surface of the dead females. The metabolic rate suppression effect in this condition was still present, though to a lesser degree (Table 2.1). However, when the four dead females were washed in diethyl ether, which would remove any pheromone on the cuticle, the live female never stopped moving in the chamber, and the MR suppression effect was lost. The live female showed the same response, that is, she never stopped moving, when placed with four dead *Erthesina fullo* females. In this case, as well, there was also no MR suppression effect. These findings indicate that the aggregation pheromone on the cuticle of living conspecific females is what brings

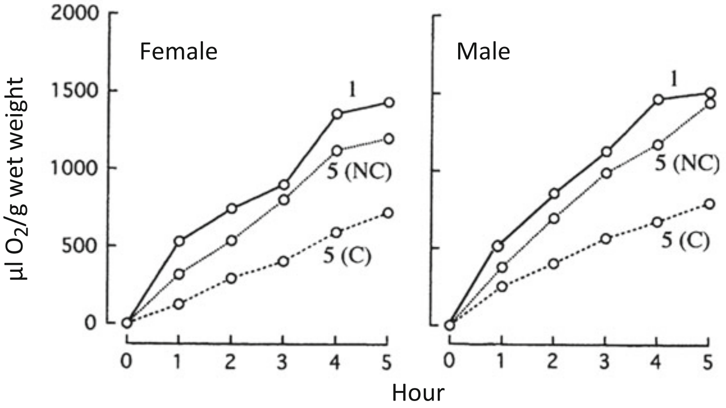


Fig. 2.5 Cumulative oxygen consumption of solitary *P. japonensis* and those in groups of five with (C) and without (NC) physical contact over 5 h. Bugs were in reproductive diapause and were collected in November, just prior to hibernation. Adapted from Tojo et al. (2005)

Table 2.1 Oxygen consumption rates of one live *P. japonensis* female with four other dead individuals

Condition	Oxygen consumption (μL O_2/g wet weight/h)
One live female	395 ± 93^{a}
Five live females	150 ± 25^{b}
One live female + four dead females	$245 \pm 67^{a,b}$
One live female + four dead females washed in diethyl ether	413 ± 92^{a}
One live female + four dead *Erthesina fullo* females	379 ± 135^{a}

Means ± S.D.; $n = 4$ for each condition
Rates followed by different letters were significantly different. Student's *t*-test, $p < 0.05$. Adapted from Tojo et al. (2005)

them together, and it is the sustained physical contact, and possibly vibration, that suppresses the MR.

We also measured the O_2 consumption rates of two pentatomid species that were collected in January, when they were aggregated under the bark of a tree for the winter hibernation to see if there was a similar effect of aggregation on their MR. Neither species showed a suppression of MR rate due to contact with other conspecifics (Fig. 2.6), suggesting the effect on *P. japonensis* is unusual, and we suspect is an evolutionary response to the severe food limitations imposed on this species.

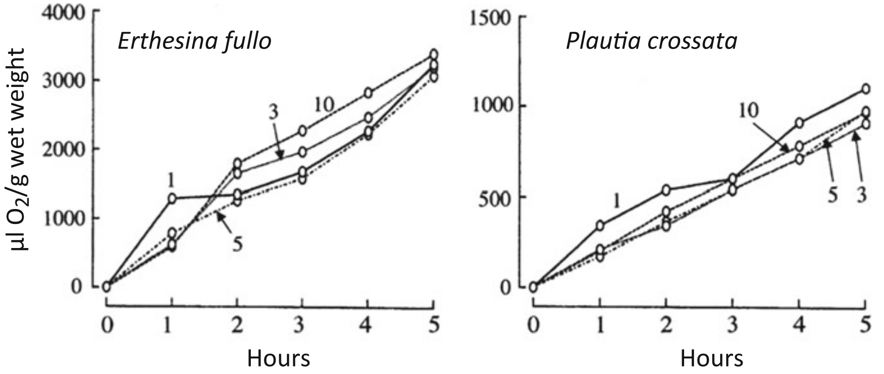


Fig. 2.6 Effect of conspecific contact on cumulative O_2 consumption of two pentatomid species hibernating in aggregations. Adapted from Tojo et al. (2005)

2.3.6 Effect of Humidity Level on Metabolic Rate

We also sought to determine whether the humidity effect on suppression of MR seen in *S. subtilis* (Wolda and Denlinger 1984; Tanaka et al. 1988; Tanaka 2000) was at play in *P. japonensis*. We set up the respiration chamber with 10 mL of one of four different aqueous solutions, $CaCl_2 \cdot 6H_2O$, $Ca(NO_3)_2$, NaCl, or K_2SO_4, and let them sit in an incubator at 25 °C overnight. The resulting relative humidity (RH) levels as measured by a Thermo Recorder TR-72S (T & D Corp, Tokyo) were 9%, 62%, 82%, and 99%, respectively. We then measured the O_2 consumption rates at each RH level using only groups of five individuals. There was no humidity effect on MR at the different RH levels (Fig. 2.7). The O_2 consumption rates/h for females were 85, 101, 93, and 85 µL O_2/g wet weight/h and for males were 127, 128, 124, and 130 µL O_2/g wet weight/h at RH of 29, 62, 82, and 99%, respectively (Tojo et al. 2005).

This finding makes sense when considering the fact that humidity in Saga during the summer is very high (~70%), although after the rainy season ends, there is very little rainfall.

2.3.7 Effect of Reproductive Stage on Metabolic Rate

P. japonensis begin mating in late April, early May (Tsukamoto et al. 1994). We collected bugs from reproductively active aggregations in early May to determine whether the group effect on suppression of MR was still apparent. The females never aggregated in the respiration chamber and showed no suppressive effect based on group size. Males also moved about a good bit but frequently stopped and settled in an aggregation. The lone males had very high MRs, and while there was some effect of group size, we suspect that, rather than simple contact, per se, in males, the effect was likely because of the greater chance of settling into a cluster when there were

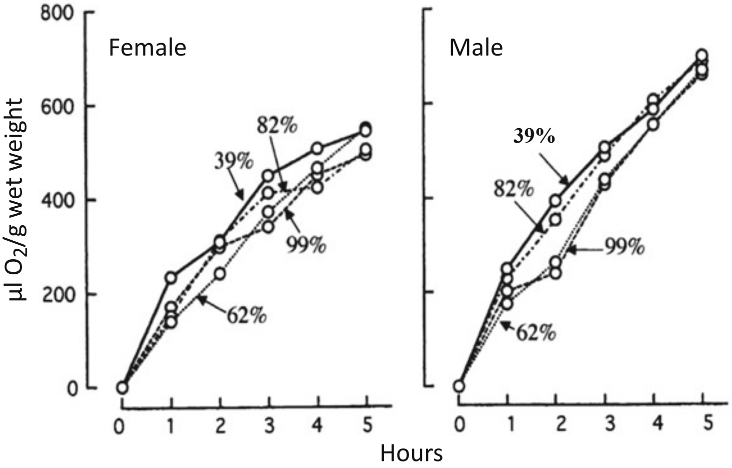


Fig. 2.7 Cumulative O_2 consumption over 5 h of *P. japonensis* at different relative humidity levels. Bugs were collected from diapausing aggregations of 1-year-old adults in June and measured in groups of five individuals. From Tojo et al. (2005)

more individuals in the chamber. The MRs of both males and females were much higher than during diapause or hibernation (Fig. 2.8). The rates were particularly high for males (660, 539, 314, and 447 µL O_2/g wet weight/h) compared to females (285, 239, 281, and 219) in groups of 1, 3 5, and 10, respectively. This stark disparity is reasonable because only males incur the higher metabolic cost of flight muscle development during mating; males fly about the aggregations during mating, while females do not.

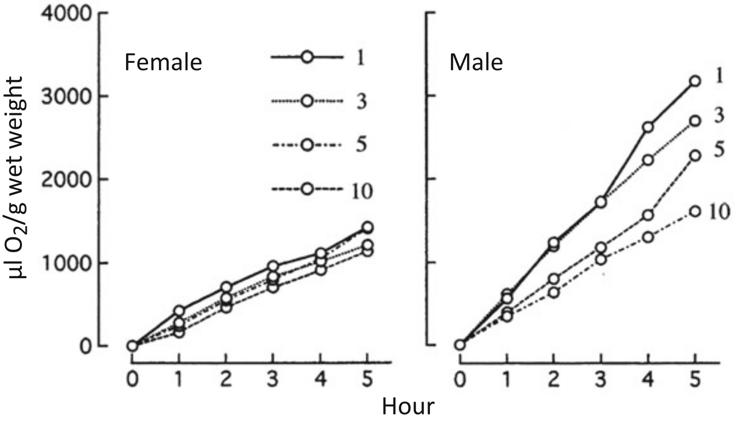


Fig. 2.8 Cumulative O_2 consumption over 5 h in *P. japonensis* collected in May, at the height of the mating season in groups of 1, 3, 5, or 10. Adapted from Tojo et al. (2005)

2.3.8 Monthly Metabolic Rates from the End of Diapause until Hibernation

Metabolic rates of males and females started to rise in April as the reproductive organs were maturing and peaked during the mating phase (Fig. 2.9a). During reproductive maturation, female O_2 consumption increased to a high of ~380 μL O_2/g wet weight/h for solitary females and ~180 μL O_2/g wet weight/h for clustered females. The O_2 consumption for solitary and clustered males in late April was more than twice that for solitary and clustered females, reflecting the heavy investment by males in flight muscle development and flight during mating.

There were differences in the group MR suppressive effect between males and females during the mating phase (Fig. 2.9a). With the exception of associating with their offspring, females that enter the reproductive phase become solitary until they die. On the other hand, during the mating season, males intermittently join aggregations when not mating over a period of about 2 weeks, and then they die (Tsukamoto et al. 1994). Consistent with the data in Fig. 2.8, this behavioral difference between the sexes is apparent in Fig. 2.9. The similar pattern of O_2 consumption for solitary and clustered females collected on May 2 and May 15 suggests that the contact effect on suppression of MR in females is lost during the mating phase but is apparently recovered by late May when females in the field would be in a nest, guarding their egg mass. Consistent with the intermittent aggregating behavior of mating males in the field, males continued to show a group suppression effect on MR throughout the mating phase (Fig. 2.9).

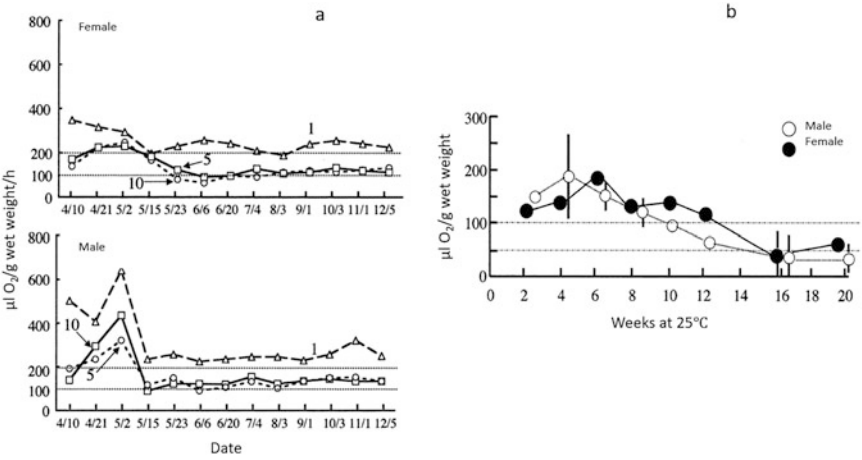


Fig. 2.9 O_2 consumption rate of adults. (**a**) Rates from bugs collected at monthly intervals from early in the reproductive phase until hibernation. Bugs were measured in groups of 1, 5 or 10 individuals. Values plotted are averages. (**b**) Rates from 150 bugs collected in August (new adults) and maintained in the laboratory for 20 weeks. Adapted from Tojo et al. 2005

Reproductive males die off after mating in early May, and females are individually guarding and provisioning their offspring during June, so the adults measured in June and July were those that had put off mating until the following spring and remained in the aggregations (Tsukamoto et al. 1994). The bugs measured from August onward were newly eclosed adults. From June through December, adults are again aggregating in the field; thus the MR for the clusters rather than solitary individuals is a more accurate reflection of what is happening in the field. The O_2 consumption rates of both clustered males and females hovered at a low of between 100 and 110 μL O_2/g wet weight/h (Fig. 2.9a).

2.3.9 Metabolic Rate of New Adults over 20 Weeks

To understand the change in MR of new adults over time as they deepen the diapause, we collected 150 adults in August and maintained them in the laboratory at 25 °C, 16L:8D for 20 weeks on only water. O_2 consumption of groups of 5 individuals was measured weekly. Over the first 6 weeks, the MR of males and females increased to about 150 μL O_2/g wet weight/h and then decreased over the next 10 weeks to a low of <50 μL O_2/g wet weight/h for males and females. Females showed a slight increase at 20 weeks, hovering at about 55, and males were at about 40 μL O_2/g wet weight/h.

Finally, males and females were dissected throughout the 20-week period, and the reproductive organs were confirmed to be undeveloped. Bugs were clearly in reproductive diapause. Remarkably, despite being reared on only water, 100% of the bugs were alive at the end of the 20-week period. We have also confirmed that $>80\%$ of the bugs that forego mating during their first spring as adults can survive to the following spring in the laboratory. Further, between 5 and 95% of bugs put off mating in the field and survive a second winter to reproduce the following spring (LF, unpublished findings).

2.3.10 Conclusions About Formation and Functions of Aggregations in **P. japonensis**

Males and females have a strong tendency to aggregate at all times of year, except for females during nesting. Aggregation formation is apparently mediated by a contact pheromone, and once contact with a conspecific is made, bugs settle. The aggregations are not sex biased or initiated by a particular sex. They form on leaves of broadleaf perennials in mild seasons, and on the ground, or in holes during the hottest and coldest periods.

Aposematism appears to be a function of the sedentary aggregations. The aggregations also function to minimize energy expense in mate-finding and likely maintain higher levels of moisture.

Humidity level apparently does not affect metabolic rate.

Physical contact with just a few individuals leads to a greater than 50% reduction in metabolic rate. The suppressive effect on MR of aggregating varies with the behavioral phase of males and females but is not positively correlated with group size.

Metabolic rate decreases to extremely low levels as bugs enter deeper into diapause, in fact to values that are lower than most other diapausing insects; only *S. subtilis* has a comparable MR during diapause.

Finally, extreme suppression of the metabolic rate is likely an adaptation to mitigate the severe food resource constraints on *P. japonensis*.

2.4 The Roles of Uric Acid and the Bacterial Symbiont During Prolonged Reproductive Diapause

As a diligent graduate student in Professor Tojo's lab, Takayuki Kashima, carried out an excellent series of experiments that uncovered another major way that *P. japonensis* enhances utilization of the scarce resource (Kashima et al. 2006). In terrestrial insects, nitrogenous wastes are typically eliminated as uric acid (Bursell 1967). However, some insects can recycle nitrogenous wastes with the aid of symbiotic microorganisms. For example, the brown planthopper (*Nilaparvata lugens*) stores uric acid in its body, and yeast-like symbionts harbored in the fat bodies produce an enzyme with uricase activity (Urate oxidase; EC 1.7.3.3), which is critical in the recycling of uric acid (Sasaki et al. 1996; Hongoh and Ishikawa 1997; Hongoh et al. 2000). Most phytophagous pentatomomorphan shield bugs, including *P. japonensis,* are associated with bacterial symbionts in caeca (or crypts) of the midgut (Buchner 1965; Miyamoto 1961; Boush and Coppel 1974), and the symbionts may be involved in uric acid recycling in the host shield bugs. Kashima et al. (2006) investigated whether uric acid recycling involving the symbiont could be occurring in *P. japonensis*, providing it with a valuable source of amino acids that might enable it to withstand the long period of starvation during reproductive diapause, which could last up to 1 year and 10 months.

They collected early and late instar nymphs from the field in June, as well as newly emerged adults in July, and 3-month-old adults in October. To assess levels of nitrogenous wastes inside the body, chemical analyses were carried out on nymphs and adults that had been frozen within 1 day of collection. Feces of fresh nymphs and adults were analyzed to assess excreted levels of nitrogenous wastes. Another group of 3-month-old adults in reproductive diapause was used for physiological and biochemical studies. These bugs were reared in the lab on either a cotton pad moistened with distilled water or an antibiotic, aqueous Rifampicin, and survivorship over 10 months, and nitrogenous waste products and total nitrogen levels were monitored.

2.4.1 *Nitrogenous Waste Levels in Active* **P. japonensis** *Nymphs and Reproductive Adults and their Excreta*

In active bugs, uric acid was the most predominant nitrogenous waste inside nymphs metabolizing the nitrogen extracted from drupes and adults of all stages and conditions and in their feces (Fig. 2.10; Kashima et al. 2006). Uric acid was most abundant in reproductive females after oviposition, when the ovaries are undergoing histolysis, and particularly abundant in the excreta of females during ovarian development, where it was 13 times greater than in females before mating. Urea was the next most abundant nitrogenous waste, although it was in much lower abundance than uric acid, ranging from 0 to less than 20% of total nitrogenous wastes inside the body and 0 to a little over 0.02% in excreta. Allantoic acid levels were negligible across the board.

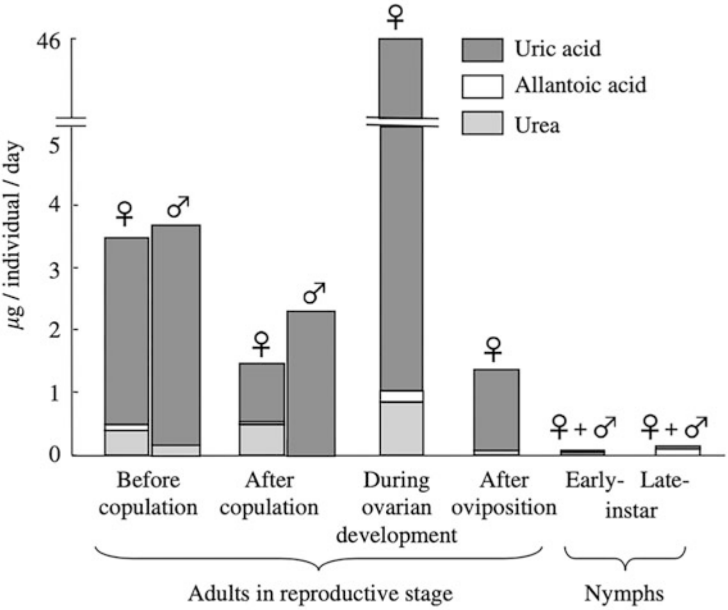


Fig. 2.10 Nitrogenous waste products in *P. japonensis*. Nitrogenous waste contents excreted by *P. japonensis* adults and nymphs at different stages. Five nymphs or five adults of each sex were used per analysis. Average values from two different groups are represented at each respective stage. Adapted from Kashima et al. (2006)

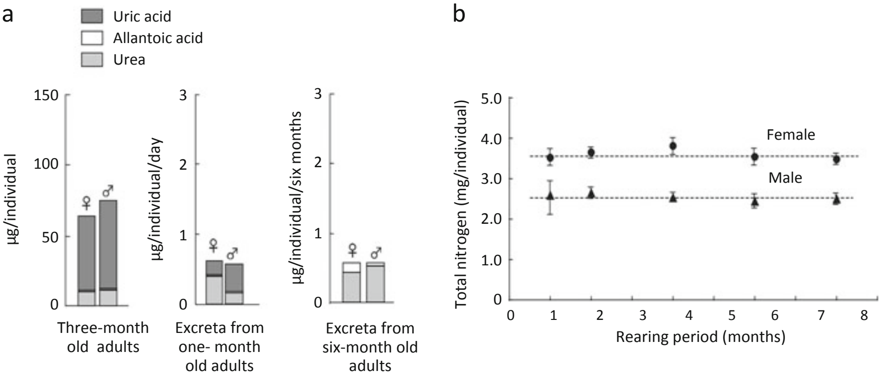


Fig. 2.11 Nitrogenous waste products and total nitrogen in diapausing *P. japonensis* adults over time. (**a**) Nitrogenous wastes in bodies and excreta. Left, $n = 5$ individuals pooled; middle, $n = 5$ individuals; excreta collected after 3 days; right, $n = 100$ individuals; excreta collected after 6 months. (**b**) Total nitrogen in males and females. $n = 5$ individuals averaged for each data point. Adapted from Kashima et al. (2006)

2.4.2 *Nitrogenous Waste Levels and Total Nitrogen in Diapausing* P. japonensis *Adults and their Excreta*

The most abundant nitrogenous waste inside the body of both males and females at 3 months into their reproductive diapause was uric acid, and urea comprised less than one fifth of the nitrogenous wastes inside the body, yet the excreta of 1-month-old diapausing females was over 60% urea (Fig. 2.11a; Kashima et al. 2006). Interestingly, the proportion of excreta that comprised urea in 1-month-old males was only about 25%. Allantoic acid was negligible inside the body of 3-month-old diapausing adults and in the excreta of 1-month-old diapausing adults. By 6 months, uric acid had disappeared from the excreta, and allantoic acid, while still negligible, increased somewhat; at this point, urea was the predominant nitrogenous waste in males and females. Astonishingly, the total nitrogen in both males and females remained at nearly the same level over 8 months of diapause on a diet of water only (Fig. 2.11b), though levels in females were consistently significantly higher (Fig. 2.11b; ANOVA; $p < 0.05$, Kashima et al. 2006). This highly efficient utilization of nitrogen supported the authors' hypothesis that uric acid was being recycled.

2.4.3 *Involvement of the Midgut Symbiont in Uric Acid Metabolism*

A DNA analysis of various body tissues revealed that *P. japonensis* harbored a gammaproteobacterial symbiont in the midgut caeca (Fig. 2.12). When diapausing bugs were reared on water with 0.02% Rifampicin, the symbiont was not detected in

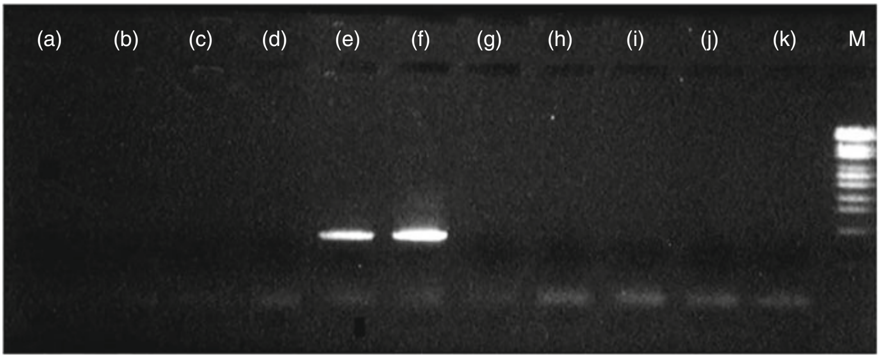
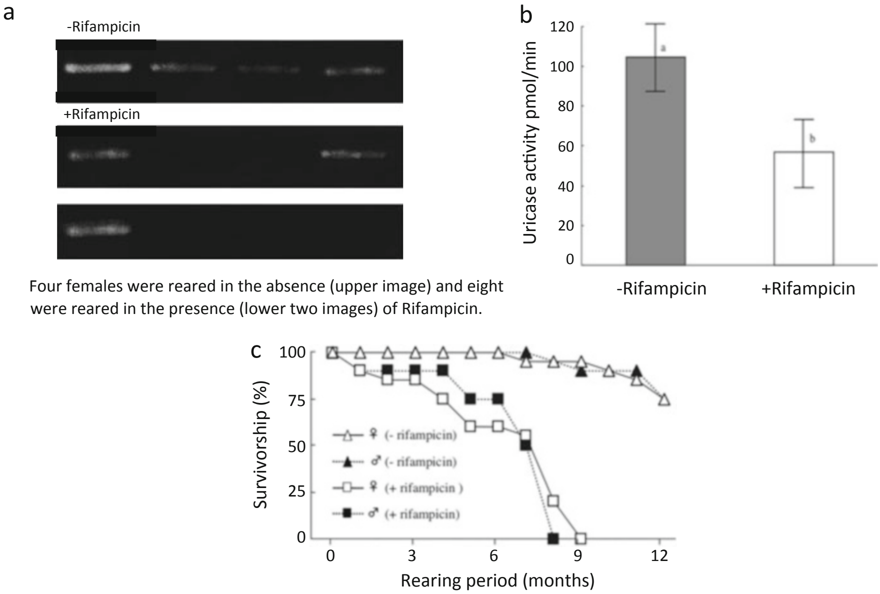


Fig. 2.12 PCR detection of DNA of the bacterial symbionts in *P. japonensis* adults in diapause. (**a**) Principal gland of salivary gland; (**b**) accessory gland of salivary gland; (**c**) rectum; (**d**) esophagus; (**e**) gastric cecum; (**f**) bacterial symbionts; (**g**) Malpighian tubules; (**h**) excretory chamber; (**i**) stomach; (**j**) fat body; (**k**) hemolymph; *M* marker DNA. (Adapted from Kashima et al. 2006)

five out of eight bugs (Fig. 2.13a), and simultaneously, uricase activity decreased by over 40% on average (Fig. 2.13b). The survival rate of both males and females reared on water with antibiotic decreased by 10% after just 1 month and by 50% after 8 months, and all were dead by 9 months (Fig. 2.13c).



Four females were reared in the absence (upper image) and eight were reared in the presence (lower two images) of Rifampicin.

Fig. 2.13 Impact of antibiotic on presence of bacterial symbiont, uricase activity, and survivorship of *P. japonensis*. (**a**) DNA from caeca of *P. japonensis* reared over 4 months in the absence or presence of the antibiotic, Rifampicin. (**b**) Uricase activity in caeca of *P. japonensis* in the absence or presence of Rifampicin over 4 months. (**c**) Survivorship of males and females in the absence or presence of Rifampicin over 12 months. The concentration of Rifampicin was 0.02% for all experiments. Adapted from Kashima et al. (2006)

On the other hand, males and females reared on plain water had 100% survivorship until 8 months. By 12 months, 90% of males and 70% of females were still alive and seemingly healthy. The data strongly suggest that the symbiont contributes to long-term survival of the bugs on water by recycling uric acid through the action of uricase. The uric acid recycling pathway enables recycling of nitrogenous wastes for a continuous supply of nitrogen throughout diapause. In fact, the amount of total free amino acids in the hemolymph had decreased by 52% at 4 months, and the amino acid profile showed that every amino acid except methionine decreased by at least 50% in the presence of 0.02% Rifampicin, further supporting the premise that the symbiont was providing a nitrogen source for *P. japonensis* that contributed to its ability to withstand the prolonged diapause.

Hosokawa et al. (2010) analyzed the symbiont in detail both phylogenetically and with DNA analysis and proposed the name of the symbiont as "*Candidatus* Benitsuchiphilus tojoi." The generic name refers to the Japanese name for *P. japonensis* (Benitsuchi-Kamemushi) and the specific epithet honors Professor Tojo. A recent study (Mondal et al. 2020) sequenced the *Benitsuchiphilus* genome and confirmed that the symbiont has a gene encoding uricase. However, the symbiont genome lacked three genes encoding enzymes involved in the uric acid recycling pathway, suggesting that uric acid recycling in *P. japonensis* may be completed by a combination of enzymes from not only the symbiont but also the insect host and/or other bacterial associates (Mondal et al. 2020). It has been known that uric acid also serves as a reservoir for nitrogen in some cockroaches, and interestingly, the recycling pathway in cockroaches is also chimeric with enzymes being produced by both the insect and its symbiont, *Blattabacterium* (Sabree et al. 2009; Patiño-Navarrete et al. 2014). In both *P. japonensis* and the cockroaches, the host and symbiont metabolic networks likely merged after the symbiotic relationships had already been established.

2.5 Unique Mechanism of Vertical Symbiont Transmission

In general, the midgut bacterial symbionts of pentatomoid stinkbugs are essential for host growth and survival and are maintained through host generations by vertical transmission from mothers to offspring (Buchner 1965). The mechanisms of vertical symbiont transmission are diverse depending on stinkbug taxonomic groups (Salem et al. 2015; Hosokawa and Fukatsu 2020). In the family Plataspidae, for example, when females lay eggs, they always deposit small symbiont-containing capsules under the egg mass, and hatchlings emerging from the eggs orally acquire the symbiont from the capsules (Hosokawa et al. 2006). In the family Urostylididae, females lay eggs covered with voluminous symbiont-containing jelly, and newborn nymphs acquire the symbiont by ingesting the jelly (Kaiwa et al. 2014). In the families Pentatomidae, Acanthosomatidae, and Scutelleridae, females smear symbiont-containing secretions onto the egg surface upon oviposition, and newborn nymphs acquire the symbionts from the secretions (Abe et al. 1995; Kikuchi et al.

2009; Hosokawa et al. 2016, 2019). So, in *P. japonensis* of the family Parastrachiidae, how is the symbiont vertically transmitted? This question led us to a very exciting discovery (Hosokawa et al. 2012).

An old paper, Schorr (1957), reported an exceptional mechanism of vertical symbiont transmission from the subsocial cydnid bug, *Brachypelta atterima* (= *Cydnus atterimus*), where mothers do not excrete any symbiont-containing secretions upon oviposition and newborn nymphs acquire the symbiont from a watery excretion from the mother's anus. We expected that *P. japonensis*, also a subsocial bug where mothers and their offspring remain together in their nests, would transmit the symbiont in the same way as *Brachypelta atterima*. So we first examined whether females provide symbiont-containing excretions or capsules for eggs upon oviposition. PCR analysis did not detect the symbiont from any freshly laid egg masses examined, which was what we expected. Next, we observed behavior of nymphs placed in a rearing container with their mother. However, contrary to our expectation, we could never observe nymphs ingesting the mother's excretion. Based on these results, we suspected that symbiont transmission occurs immediately after egg hatching. When we observed mothers guarding the egg mass just prior to hatch, we saw an unexpected and surprising behavior.

During the egg-guarding period, mothers usually kept the egg mass under their body, suspending it with their proboscis (Fig. 2.14a). Close to the time of hatch, however, mothers suddenly placed the egg mass on the ground, directed the abdominal tip to the egg mass, and excreted a copious amount of white mucus from the anus onto the egg mass (Fig. 2.14b). The excreting behavior continued for approximately 40 min, and when the excretion ended, the mothers resumed the egg-guarding posture (Fig. 2.14c). Several minutes later, strikingly, eggs in the egg mass started to hatch synchronously (Fig. 2.14d, e). The newborn nymphs immediately took up the mucous secretion as well as the contents of the trophic eggs with their proboscis (Fig. 2.14f). The mucous secretion and trophic eggs were completely consumed within 24 h after hatching. Microscopic observation and DNA analysis confirmed that the mucous secretion contains an abundance of symbiont cells (Fig. 2.15a). Dissection of the alimentary canal of females in the egg guarding stage and in the pre-reproductive diapause stage revealed that the midgut of females with eggs was much larger, in particular the M4 section where the symbiont-containing crypts are located, compared to that of the pre-reproductive diapause females (Fig. 2.15b, c).

To verify that nymphs were acquiring the symbiont through the anal secretion, symbiont content was compared between nymphs hatching from eggs with the mother present until they hatched (control, Fig. 2.16a) and when nymphs were experimentally denied access to the mucous secretion either by removing the mother 24 h prior to hatch (Fig. 2.16b) or by sealing the mother's anus with nail polish to prevent excretion (Fig. 2.16c). Nymphs denied access to the mucous secretion could not acquire the symbiont, indicating that the vertical symbiont transmission in *P. japonensis* is mediated by the nymphs' exploitation of the mother-derived mucous secretion (Hosokawa et al. 2012). Moreover, the symbiont had a significant effect on the growth of nymphs. When nymphs hatched under the three conditions described above (access or no access to mother's excretion prior to hatch) were reared to adults

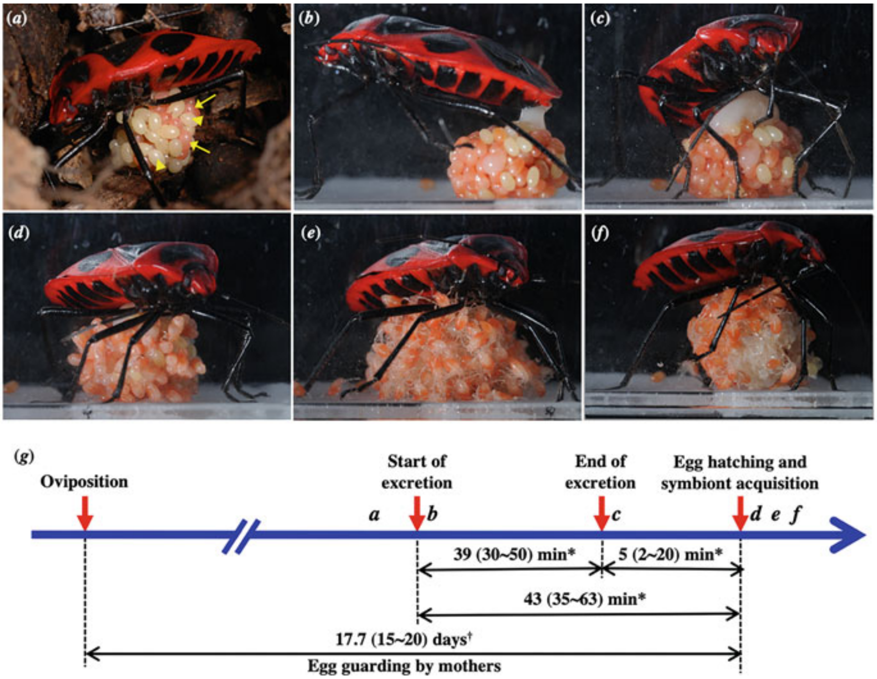


Fig. 2.14 Vertical transmission of the bacterial symbiont in *P. japonensis*. Photos: (**a**) A mother is guarding an egg mass in the nest. Yellow arrows indicate fertile eggs; yellow arrow heads indicate trophic eggs. (**b**) A mother is excreting white mucus from the anus onto the egg mass. (**c**) The mother resumes the egg-guarding posture after finishing the excretion. (**d**, **e**) Soon after that, fertile eggs in the egg mass start hatching synchronously. (**f**) The newborn nymphs immediately start to take up the mucous secretion and feed on the trophic eggs. Timeline of the events in the photos presented below, with letters on the timeline corresponding to the respective images. (From Hosokawa et al. 2012)

with drupes of *S. jasminodora*, the weight of adults in the control group (Fig. 2.17a) was significantly greater than that of adults in either of the two experimental groups (Fig. 2.17b, c; Hosokawa et al. 2012). Further, although there was no significant difference in adult emergence rate (Fig. 2.18a) or duration of the nymphal stage (Fig. 2.18b), symbiont-deprived nymphs had a significantly smaller body size of both males and females as adults (Fig. 2.18c), and the lighter color of the symbiont-deprived bugs (Fig. 2.18d) suggests lesser sclerotization of the cuticle. Clearly the symbiont is essential for proper acquisition of nutrients from the drupes and optimal growth. This mechanism of vertical symbiont transmission in *P. japonensis* is not what we initially expected, but we were very excited to discover an unprecedented phenomenon.

Interestingly, the mothers began to excrete the mucus secretion, never missing the timing just before hatching, which suggests that the mothers are somehow able to know the timing of egg hatching precisely. Considering that the egg stage of *P. japonensis* varies from 15 to 20 days depending on ambient temperature and

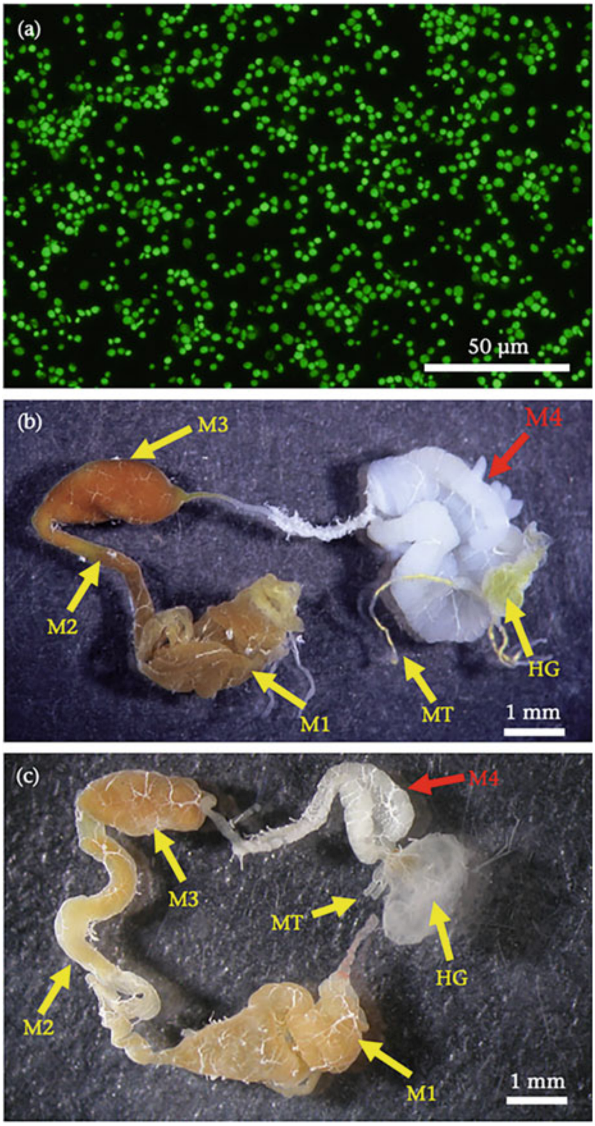


Fig. 2.15 Symbiont contained in mucous secretion. (**a**) Symbiont cells (green) in the mucous secretion. Smeared mucous secretion was stained with undiluted SYTOX green. (**b**) Dissected alimentary tract of an adult female guarding an egg mass. (**c**) Dissected alimentary tract of an adult female in pre-reproductive diapause. *HG* hindgut; *M1* midgut first section; *M2* midgut second section; *M3* midgut third section; *M4* midgut fourth section with crypts (symbiotic organ); *MT* Malpighian tubule. Adapted from Hosokawa et al. (2012)

other environmental factors, it seems unlikely that the mothers are measuring the accumulated time from oviposition. Although speculative, it seems plausible that the mothers know the timing of egg hatching via sensory inputs such as chemical signals and/or nymph movements that are characteristic of eggs about to hatch. The highly synchronous egg hatching was also intriguing and investigated in a later study (Mukai et al. 2014), which showed that mother-derived signals are involved in the hatching synchrony (see Chap. 5).

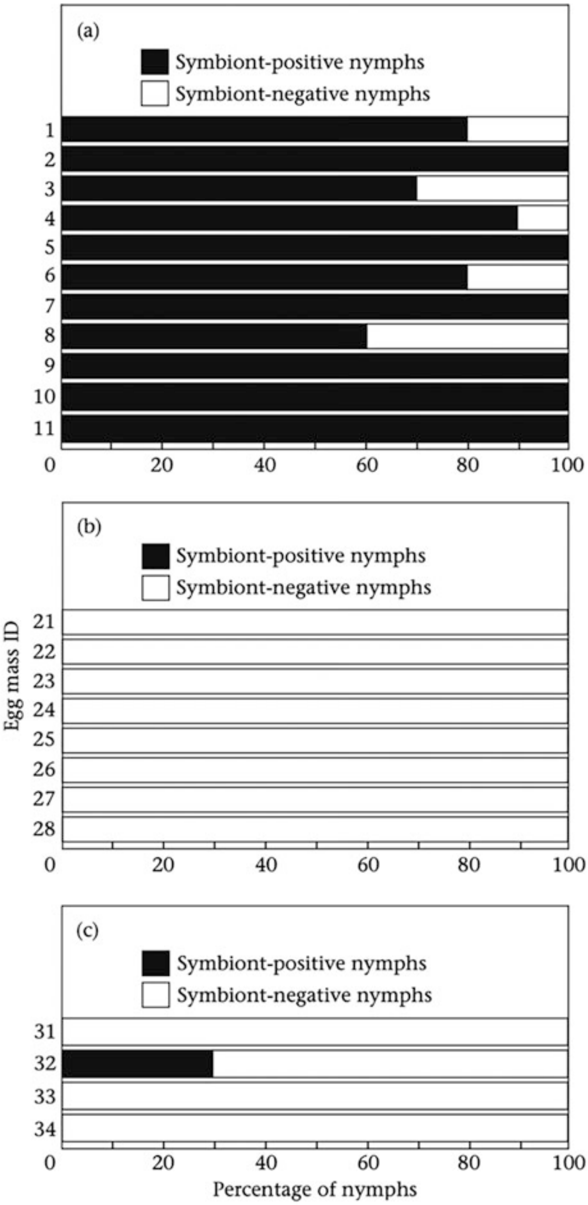


Fig. 2.16 Role of mucous secretion in symbiont transmission to newborn nymphs. (**a**) Control treatment: mother present and nymphs could consume mucous secretion. (**b**) Mother removal treatment: mother removed; nymphs could not ingest mucous secretion. (**c**) Anal-sealing treatment: mother was present but anus was sealed with nail polish; nymphs' access to mucous secretion was prevented. For each of the egg masses, ten nymphs were randomly selected and subjected to PCR detection of the symbiont. Percentages of symbiont-positive nymph, control versus mother removal: $\mathscr{B} \pm \mathrm{SE} = 23.2 \pm 2940$, $t_{17} = 0.008$, $P < 0.0001$. Control versus anal sealing: $\mathscr{B} \pm \mathrm{SE} = 4.61 \pm 1.04$, $t_{13} = 4.42$, $P < 0.0001$. Adapted from Hosokawa et al. (2012)

The mechanism of symbiont transmission in *P. japonensis* is unique. We have also investigated vertical symbiont transmission in two subsocial burrower bugs of the cydnid subfamily Sehirinae, *Adomerus triguttulus* and *Adomerus rotundus*, that share several maternal behaviors, including egg guarding and food provisioning, with *P. japonensis* (see Chap. 1). Surprisingly, the mechanism of vertical symbiont

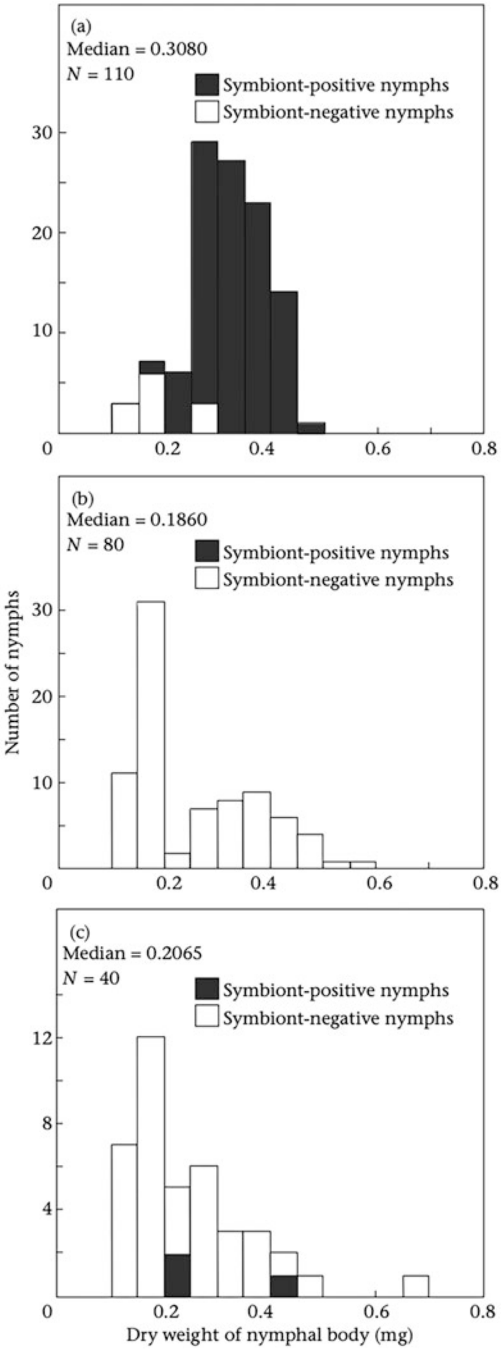


Fig. 2.17 Effect of the mucous secretion on nymph body weight. (**a**) Control treatment, (**b**) mother removal treatment, and (**c**) anal-sealing treatment as in Fig. 2.16. Nymph body weight, control versus mother removal: $\mathscr{B} \pm \text{SE} = -0.0576 \pm 0.0222$, $t_1 = -2.60$, $P < 0.05$. Control versus anal sealing: $\mathscr{B} \pm \text{SE} = -0.0690 \pm 0.0329$, $t_1 = -2.10$, $P = 0.08$. Adapted from Hosokawa et al. (2012)

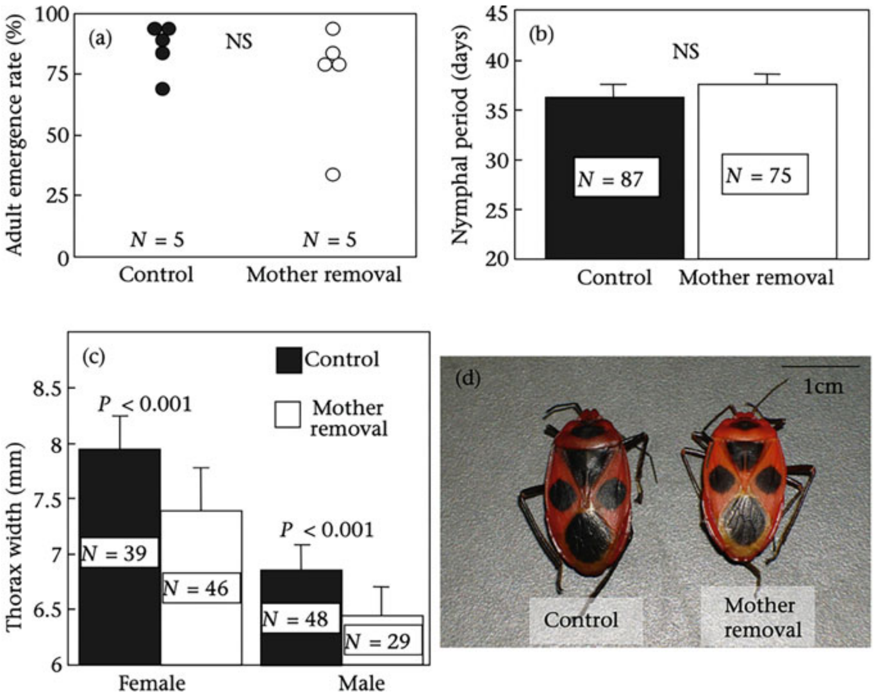


Fig. 2.18 Effect of the symbiont on components of offspring fitness. (**a**) Adult emergence rate; (**b**) duration of nymphal stage (mean + SD; $\mathscr{B} \pm \mathrm{SE} = -0.0375 \pm 0.0260$, $z_1 = -1.44$, $P = 0.15$.) (**c**) Adult thorax width (mean + SD; male: $\mathscr{B} \pm \mathrm{SE} = -43.3 \pm 9.51$, $t_1 = -4.55$, $P < 0.001$; female: $\mathscr{B} \pm \mathrm{SE} = -53.9 \pm 11.4$, $t_1 = -4.72$, $P < 0.001$). (**d**) External appearance of adult females from control (with the symbiont) and mother removal (without the symbiont) treatments. Adapted from Hosokawa et al. (2012)

transmission in these species was quite different from that in *P. japonensis*, and females of *A. triguttulus* and *A. rotundus* smear symbiont-containing secretions onto eggs upon oviposition as many non-social stinkbugs do (Hosokawa et al. 2013). It is currently unclear why only females of *P. japonensis* and *B. atterima* do not excrete symbiont-containing materials upon oviposition but do so at later stages; this warrants future investigation.

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Takahiro Hosokawa at the time a post-doctoral fellow at the Japan National Agriculture and Food Research Organization (NARO) (formerly known as the Japan National Institute of Advanced Industrial Science and Technology Agricultural Techniques Research Center (AIST)) brought his fine microbiology skills to the table to investigate *P. japonensis'* symbiotic gut bacteria, and the mode of its transmission to offspring. He is currently a Research Associate in the Faculty of Science at Kyushu University.

Chapter 3
Mating Behavior

Lisa Filippi, Mantaro Hironaka, and Shintaro Nomakuchi

Abstract The mating behaviors of *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) were observed under both natural field conditions and manipulated field conditions. The mating system is best described as scramble competition polygyny. Males terminate reproductive diapause before females. They fly about the aggregations, land on one, and immediately evert the aedeagus and attempt copulation. Females do not fly and reject all attempts by males to mate. Males and females have both short- (<3 min) and long-term (up to 1 h) copulations, and long-term copulations are much more effective at inseminating females. They mate both end to end (short term) and in a V-pattern (long term). Both males and females mate multiply, but females typically have only one long-term mating. Females that had long-term copulations had slightly more advanced ovarian development and were more likely to be roaming near but not in the aggregations. Males achieving long-term matings were not larger than unsuccessful males. We discuss the possibility of cryptic female choice.

Keywords Cryptic female choice · Fat body · Female resistance · Intrasexual competition · Mating strategies · Mating success · Ovarian development · Promiscuity

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3.1 Introduction

One of the most bizarre observations we made soon after initiating this research was the comical antics that occurred during the mating season. Male *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) would fly erratically about the aggregation site, dropping down to, and rapidly scurrying about on, different sedate clusters, seemingly trying to wake everyone up. And the landing was not at all skillful; the males just appeared to fall out of the sky and happen to artlessly land on a cluster, which was easy to do when the foliage on the trees was covered with the clustered bugs. Once a male had landed, he everted his aedeagus and backed it into the abdomen of any bug he encountered, whether male or female. Once joined end to end, his legs thrusted rhythmically, a movement that appeared to be consistent with ejaculation. The entire time the male was attempting to copulate, and even well after he had succeeded in inserting his aedeagus, his partner, male or female, continued to rock its body from side to side, kicking him with its hindlegs and running away, even after they were attached. Of course, the goal of the male should be to meet with some measure of reproductive success if repeated often enough, and this behavior was repeated numerous times over the remaining 2 weeks of the male's life. Males were clearly polygynous, attempting to maximize their reproductive success by mating with as many females as possible. Nonetheless, we recognized early on that despite living in predominantly sedate aggregations where half of his cluster mates are female should preclude the need to search for a mate, the dogged reluctance of females means that securing a mate is still a costly endeavor for a *P. japonensis* male. So, what was going on here? And why were females so invariably reluctant to mate when the goal of their existence should also be to reproduce? We undertook a series of studies between 1990 and 1995 and reported the results in two papers. The goal of the first paper was to characterize the behavioral and ecological parameters and the physiological conditions that prevail under natural conditions during the mating season of *P. japonensis* (Tsukamoto et al. 1994). The second series of studies also took place in the field, but involved individually marked and measured bugs, and containment such that we could observe every mating a female had (Filippi et al. 2000). In an effort to share our journey of discovery with the reader, we will introduce the findings of the two studies in sequence. As polygyny was the most conspicuous aspect of the mating system we observed, we begin by briefly considering the factors that promote the different types of polygyny.

3.2 Mating Systems That Involve Polygyny

Polygyny is common in insects and other terrestrial arthropods, and their mating systems typically fall into one of three categories: female defense polygyny, resource defense polygyny, or scramble competition polygyny, and polygyny of all types is particularly common in the Heteroptera (Thornhill and Alcock 1983). The specific mating system is determined by the environment that the insects inhabit; the dispersion and abundance of resources, which could be food, nests, territories, oviposition sites, etc.; and the insects' abundance and dispersion in the habitat (Emlen and Oring 1977; Alcock 1980; Dickinson 1992; Choe and Crespi 1997). Because all of these conditions can vary over the course of a season, temporal variation in social and environmental conditions can cause a shift in the mode of polygyny (McLain 1992). For example, males of the neotropical harvestman *Acutisoma proximum* (Arachnida: Opiliones) initially aggressively defend territories from other males (resource defense polygyny). The territories contain particular vegetation that females are attracted to for oviposition sites. However, later in the reproductive season, males stop patrolling and defending territories and sequentially defend individual females that will oviposit in their territory (Buzatto and Machado 2008).

3.2.1 Female Defense Polygyny

Female defense polygyny is predicted when a population is male-biased and/or there is a likelihood of sperm displacement (Parker 1970), as in the aggregating tropical bug, *Dysdercus bimaculatus* Stål (Pyrrhocoridae; Carroll and Loye 1990); the soapberry bug, *Jadera haematoloma* Herrich-Schaeffer (Rhopalidae: Carroll 1991); and the ambush bug, *Phymata fasciata* Gray (Phymatidae; Dodson and Marshall 1984). Post-copulatory guarding of females that mate multiply is an effective means to mitigate sperm competition because sperm precedence patterns in insects indicate that the last male to mate with the female is favored to fertilize a significantly greater proportion of the eggs (Walker 1980). Female defense polygyny is costly to males because they forfeit other mating opportunities while guarding a female. However, in high-density, male-biased aggregations where a female could remate quickly and where sperm displacement is likely to occur, guarding would be more likely to increase the male's reproductive success than searching for another female (Carroll and Loye 1990; Carroll 1991; Schöfl and Taborsky 2002). Males can gain an advantage from guarding a female even when the guarding does not continue until oviposition, as in *Megacopta punctissima* Montandon (Plataspidae; Hibino 1985; Hosokawa 2009), where there is a 24-h cycle for mating, and females have a 7-day oviposition pattern. Most matings occur during the 20 h between 2:00 pm and 10:00 am the following day. Average copulations lasted 10 h, and males remaining with the female for longer than 4 h were considered to be engaging in mate-guarding because sperm transfer occurs between 2 and 4 h of copulation. Thus, any guarding beyond what is needed for sperm transfer should enhance sperm competition.

3.2.2 Resource Defense Polygyny

Resource defense polygyny is predicted when females are attracted to a resource, which could be food, a foraging or nesting territory, or an oviposition site that a male can defend (Emlen and Oring 1977). Limited resources can be defended by high-quality males, and females are attracted to the defended territory, leading to a reproductive skew. The strength of sexual selection and the intensity of contests are increased with reproductive skew, and this leads to the evolution of exaggerated traits (Emlen 2014; Herberstein et al. 2017), such as the large mandibles used by males of the tropical rove beetle, *Leistotrophus versicolor* (Coleoptera: Staphylinidae) (Forsyth and Alcock 1990). Male size varies widely and so does the size of their mandibles, which they use to aggressively displace other males from dung pats. Dung pats are foraging sites that females are attracted to, although the resource is actually the dipterans that are attracted to the dung. In the Heteroptera, nonterritorial polygyny is the norm and occurs in many species, including *Euschistus conspersus* Uhler, Pentatomidae (Alcock 1971), and the infamous *Nezara viridula* Linnaeus, Pentatomidae (McLain 1981), but there are four exceptions. *Acanthocoris sordidus* Thunberg, (Fujisaki 1980, 1981); *Acanthocephala femorata* Fabricius (Mitchell 1980); *Neacoryphus bicrucis* Say (McLain 1984, 1986, 1992); and *Notobitus meleagris* Fabricius (Coreidae; Miyatake 1995) all display resource competition polygyny. In these species, the resource occurs in patches that are defendable by the male.

3.2.3 Scramble Competition Polygyny

On the other hand, a variety of ecological parameters promote the evolution of scramble competition polygyny. Patterns that range from the situation where many potential mates or resources are indefensible because they are widely scattered over time and space to that where there is an abundance of potential mates gathered in a small and accessible area, as is the case with *Parastrachia japonensis*, promote the evolution of scramble competition polygyny (Thornhill and Alcock 1983; Nishida 1989, 1993; Herberstein et al. 2017). Inherent in the premise of scramble competition polygyny is the concept that little or no direct male-to-male aggression or female defense is involved (Thornhill and Alcock 1983; Herberstein et al. 2017). This type of polygyny aligns best with what we observe in *P. japonensis*.

3.3 Mating System in *Parastrachia japonensis*

The bugs emerge from their underground winter hibernation in late February, early March, and relocate to the aggregation site. As described in previous chapters, the aggregation site comprises small perennial trees and plants, primarily *Eurya* *japonica*, and ferns within ten meters of the host trees, and it is at this site, in late April, that the mating behavior begins. We first observed the overall mating behavior at the aggregation site during the reproductive season of 1992 in an attempt to characterize the mating system and gain an understanding as to how males and females are optimizing their reproductive success (Tsukamoto et al. 1994).

3.3.1 Flight by Males and Mating Behaviors in the Field

Just prior to the start of the mating season in late April of 1992, the trees, shrubs, and plants at the aggregation site were laden with the flamboyant red and black clusters of bugs that were still fairly sedate. The focal subpopulation for our observations in 1992 consisted of about 1300 individuals in clusters on a Japanese viburnum bush (*Viburnum japonicum*; ~2m^2). We positioned ourselves such that we could observe the entire aggregation site unobtrusively and logged 34.5 h of mating behavior over the 19-day mating season from April 25th to May 14th, with an average of 2.0 h of observations per day (range 0.5–4.0 h). The temperature during the mating season ranged from 15.5 to 26.5 °C. The initial sex ratio of the clustered individuals was 1:1. By the third week in April, males began to leave the clusters and walk about the aggregations, occasionally stopping to engage in a behavior we have termed "genital grooming" (Fig. 3.6a). They everted the aedeagus and rubbed it with the hindlegs for a few minutes before they moved on. Males did not become active simultaneously; rather, they seemed to gradually awaken from their reproductive diapause, with increasing numbers of males moving about the aggregation site daily, first walking and then flying in a somewhat circular pattern about the aggregation site. We observed over 7600 flights, and all were brief, lasting under 10 s. It is significant that only males flew, probably as a means to gain access to as many females as possible, while females only walked, conserving their energy for the costly parental care behaviors. Males flew from just a few centimeters to 10 m or more but rarely strayed more than 2 m from the aggregations until most of the females had relocated to the host tree. They were certainly not graceful in flight and were even clumsier on landing, appearing to tumble haphazardly out of the sky and fall onto the clusters. The clusters were so densely arranged at the aggregation site that the neurological investment in more skillful flight and landing behaviors was apparently deemed unnecessary by evolutionary forces. The number of flights observed per hour increased dramatically as the season progressed and then dropped off precipitously in early May before increasing again and peaking at 750 flights per hour near the end of the mating season (Fig. 3.1a; Tsukamoto et al. 1994). The dip in the second week of May was likely due to a spell of low temperatures as the frequency of flight behavior during the mating season was clearly temperature dependent (Fig. 3.1b, Tsukamoto et al. 1994). Sixty seven percent of all flights occurred in the temperature range of 22–24 °C, although only 47% of the observations took place in that range.

Further, in 1992, no flights were observed below 19 °C. Light rain, indicated by asterisks in Fig. 3.1a, apparently had no effect on flight behavior. Males alighted on

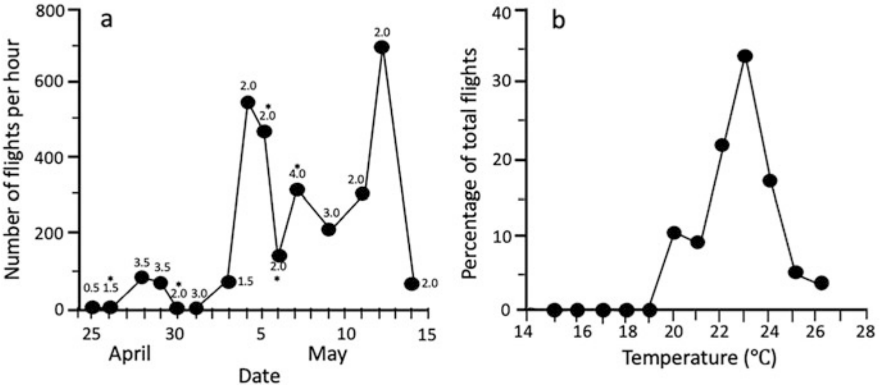

Fig. 3.1 Flight frequency of males (**a**) throughout the mating season and (**b**) as a function of temperature. Numbers above dots represent hours of observation; asterisks indicate rainy days. Adapted from Tsukamoto et al. (1994)

the clusters in an apparent attempt to "wake up" the females. There was no courtship behavior at all. In fact, when a male landed on a cluster of bugs, he rapidly everted his aedeagus and, backing up, immediately attempted to insert it into the abdomen of whatever individual happened to be closest to him, whether male or female. Interestingly, both males and females resisted the male's attempt at intromission, though males resisted more intensely. They kicked out at him with their hindlegs, rocked their bodies, and attempted to run away, as the male feverishly continued to attempt intromission. If successful, the copulation was most often end to end, as is common in stink bugs, but also occurred as a V-pattern, with the male grasping the female across the dorsum with his front and midlegs (Fig. 3.6b). In both postures, the male displayed a rhythmic jerking movement with his legs and abdomen soon after intromission was achieved which suggested ejaculation was taking place. We categorized this mating system as scramble competition polygyny, though, as will be described later, there was also behavior that indicated intrasexual competition by males.

3.3.2 Copulation Frequency and Success Rate by Males in Aggregations and at Isolated Sites

From May 4 to 11, we observed an average of 59 copulations per hour despite that during this period the overall number of bugs at the site had dropped to nearly one fourth the original number (see Sect. 3.6). This suggests that the number of matings per male actually increased over time. Copulation occurred with individuals within aggregations and with those that had walked away from the aggregations and were isolated on nearby branches. Suspecting that bugs remaining in the aggregations were not yet receptive to mating, we compared the rate of successful intromission (copulation) by males attempting to mate within an aggregation and at an isolated site near the aggregations. Among 1281 copulations observed, 45% occurred in aggregations and 55% occurred in isolation. Near the end of the mating season, up to three times as many matings occurred with isolated females (Fig. 3.2a; Tsukamoto et al. 1994). Males had a significantly greater chance (12%) of successfully mating with a female when the attempt was with an isolated female (Fig. 3.2b; Mann-Whitney U test, $U = 81$, $p < 0.001$). Despite the intense rejection males encountered, greater than 70% of their attempts to mate were ultimately successful.

3.3.3 Duration of Copulation in Aggregated and Isolated Conditions

As previously mentioned, copulations were both end to end and in a V-shaped pattern with the male on top, grasping the female across the dorsum. Both males and females mated repeatedly, but females always resisted initially. Of the 1281 copulations observed in 1992, 987 were timed. We noted two types of copulation based on duration, short term (ST) 2–100 s and long term (LT) 3–57 min, and the duration was correlated with mating posture. ST matings tended to be end to end, and LT matings were in the V-shaped pattern. In 1992, we did not observe copulation durations between these two ranges. All copulations that took place within the aggregations and at isolated sites near the aggregations during the observation period on a given day were recorded. 93% of all timed copulations were ST and averaged 14.9 ± 10.3 s (mean ± SEM, $n = 922$; Fig. 3.3a, Table 3.1; Tsukamoto et al. 1994). As with flight, the duration of copulation was temperature dependent (Fig. 3.3b); there was a significant negative correlation between temperature and copulation duration (Pearson correlation coefficient, $r = 0.953$, $p < 0.001$). It is likely that insemination takes longer at lower temperatures because of the greater metabolic requirements of muscles required for ejaculation and insemination. Long-term copulations averaged 22.6 ± 14.7 min (mean ± SEM, $n = 64$; Fig. 3.3c; Table 3.1), and the incidence of LT matings increased markedly after May 5th (Fig. 3.3c) when female numbers at the aggregation/mating site dropped by nearly 50% (Fig. 3.4). This suggested that the LT matings might represent a switch from simple scramble competition polygyny to guarding behavior, as described for *N. viridula* (McLain 1981), once intromission was achieved in response to the reduced operational sex ratio (OSR). While there was no difference between the numbers of ST copulations that occurred within an aggregation and those at isolated sites, there were significantly more LT copulations at isolated sites (Table 3.1; Fisher's exact test, $p < 0.00001$). There was no significant difference between the duration of ST or LT matings that occurred within an aggregation or in isolation, although LT matings occurring in isolation tended to be longer.

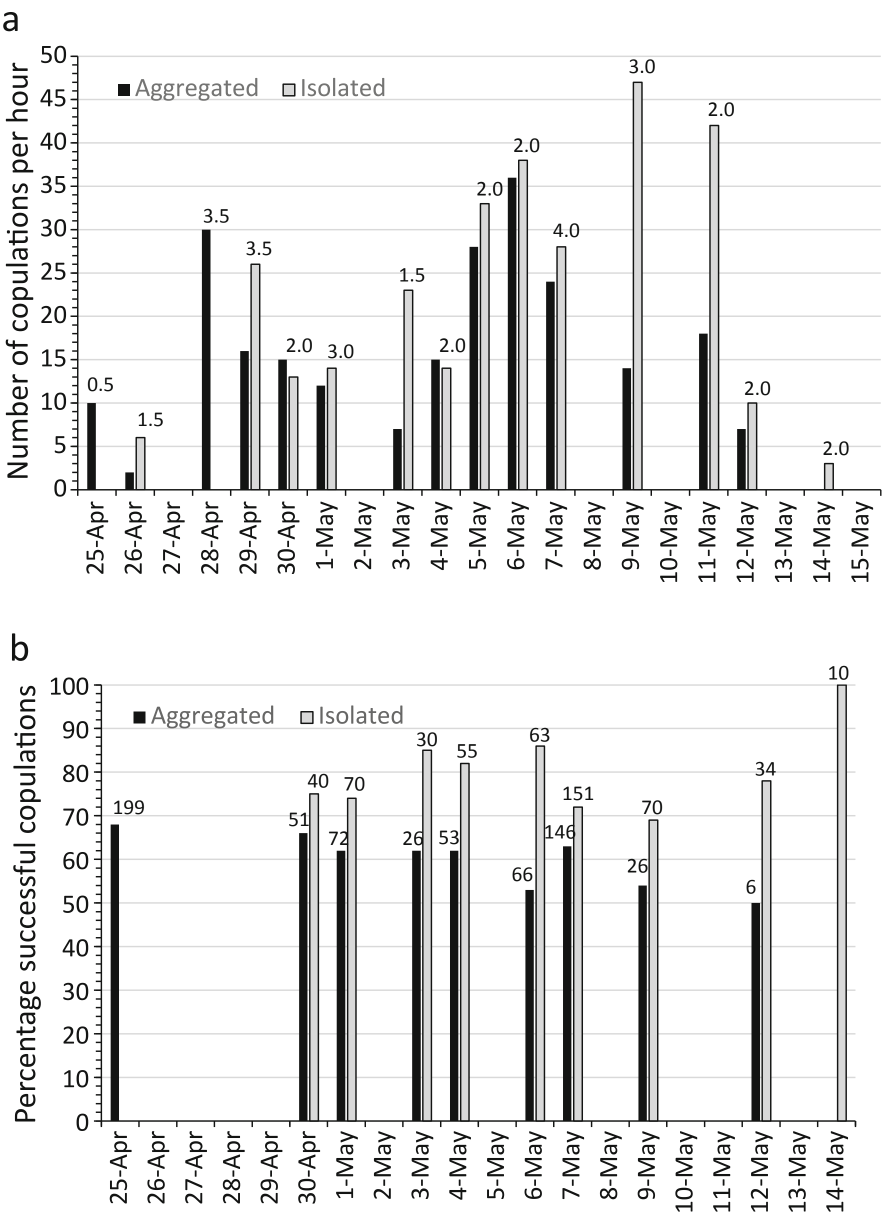

Fig. 3.2 Copulation success and frequency in aggregated and isolated *P. japonensis* throughout the mating season. (**a**) Number of matings observed in aggregations and in isolated conditions per hour. Numbers above columns represent number of hours of observation on that date. (**b**) Percentage of mating attempts by males that were successful in aggregations and isolated conditions on each date. Numbers above bars represent number of mating attempts under each condition. Adapted from Tsukamoto et al. (1994)

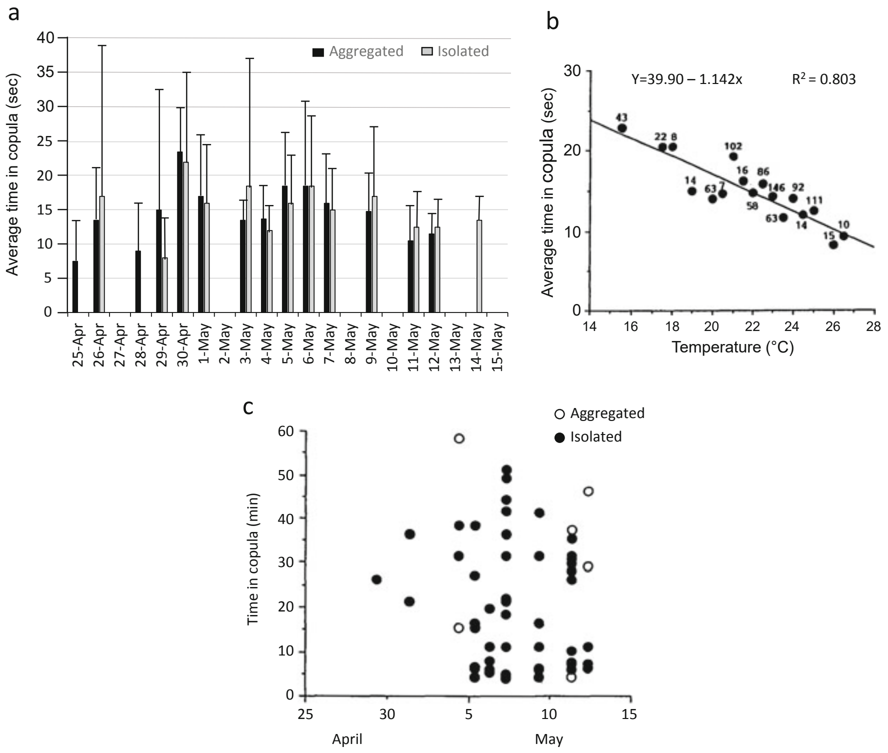

Fig. 3.3 Duration of copulations in *P. japonensis*. (**a**) Duration of short-term copulations (2–100 s) throughout the 19-day mating season. Error bars represent S.E.M. (**b**) Impact of temperature on copulation duration. $P < 0.001$. (**c**) Duration of long-term matings throughout the mating season (3–57 min). Comparison of number of matings in aggregation vs isolated for short-term and long-term matings, Fisher's exact test, $p < 0.00001$. Adapted from Tsukamoto et al. (1994)

Table 3.1 Duration of short- and long-term copulations of *P. japonensis* in aggregations and in isolated conditions in 1992. Adapted from Tsukamoto et al. (1994)

Mating	Parameter	Aggregated	Isolated	Overall	
Short term	*n*	455	467	922	
	Mean duration, s ± SEM	13.6 ± 5.2	13.6 ± 3.6	14.9 ± 10.3	
	Range, s	2–100	2–80	2–100	
Long term	*n*	6	58	64	Fisher Exact test $P < 0.000001$
	Mean duration, min ± SEM	30.5 ± 19.9	21.9 ± 14.0	22.6 ± 14.7	
	Range, min	3–57	3–50	3–57	

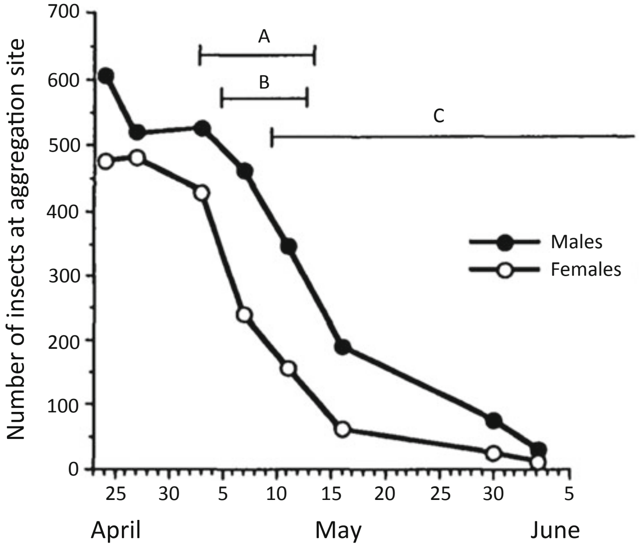

Fig. 3.4 Change in numbers of males and females at the aggregation site during the mating season. Horizontal bars indicate (**a**) period of peak number of male flights; (**b**) peak number of copulations; (c) period that females were relocating to host tree

3.3.4 Change in Numbers of Males and Females at the Aggregation Site over the Mating Season

Although most of the observations for the 1994 paper were from the mating season of 1992, we collected data on the pattern of dispersal from the aggregation site at the same site in 1993. In 1993, the size of the subpopulation at that site was similar to 1992; however, the initial sex ratio differed (57% male, 43% female). As males continued to fly about the aggregation site mating with females, some females seemed to lose their response to the aggregation pheromone and began to relocate to the host tree as lone individuals. At this point (early May), the drupes on the host tree were still very immature, so females did not begin feeding but walked along the branches of the host tree. As the season progressed, the sex ratio at the aggregation site became markedly male biased (Fig. 3.4), although the numbers of both sexes decreased dramatically as more and more females moved on to the host tree and males died from depletion of their fat body reserves (unpublished data). By May 7th, the sex ratio (M:F) was 66:34, and by May 11th, there were half as many bugs as the initial number, and the sex ratio was 70:30. By May 14th, nearly 76% of the remaining individuals at the aggregation site were males. By June 2nd, only 40 individuals remained, 30 of which were males. These 40 individuals did not mate that year and remained in the aggregation until they were joined by the new adults that emerged in late July/early August. Females that had relocated to the host tree were first noted feeding about 10 days after they began moving to the host tree; they fed on the bits of endosperm in the as yet unripe and green drupes. Males, including the few that had followed the females, never fed on the host tree and, thus, never fed after becoming adults the previous summer or two summers earlier.

3.4 Ovarian Development and Fat Body Content Throughout the Mating Season

Because *P. japonensis* enter reproductive diapause after emerging as adults, we were curious to understand at what point their reproductive organs become sexually mature and also how their fat body stores are used. In 1990, we collected females from different sites, aggregated and isolated, at different points during and soon after the mating season and dissected them to assess their reproductive development. We also examined fat body content in individuals at different stages of ovarian development. As the dates of the mating season are fairly consistent from year to year, we attempted to correlate that data with the behavioral data we collected in 1992. The data indicate that ovarian development was staggered among the females and did not progress beyond the earliest stage of previtellogenesis unless the female was inseminated (Fig. 3.5, Tsukamoto et al. 1994). This suggests that insemination might be triggering the initiation of ovarian development. One isolated female on June 7 still had undeveloped ovarioles, although she was inseminated. Since this date was well after all males had already died, that particular female's undeveloped yet inseminated state was a curious anomaly. Remarkably, the data for fat body content revealed that even after 9 months, and in many cases 1 year and 9 months, 74% of females still maintained the maximum level of fat body content. Fat body content decreased with progression of ovarian development. While 12% of females with early previtellogenesis had depleted fat body content, the majority had a fair amount of fat bodies remaining, nearly 90% of females in ovarian stage 2 (late previtellogenesis) had depleted their fat bodies to Factor 1 or 2, but fully 41% of those in the latest stage of ovarian development (vitellogenesis and beyond) had a fat body Factor of 3. This suggests that females utilized their abundant fat body reserves stored during the nymphal stage in the previous spring (or two springs prior!) for the earlier stages of ovarian development over ~10 days that they remained on the host tree without feeding and then fed as their stores dwindled in order to achieve full egg production. After about 10 days of actually feeding on the host tree, females descended the tree and oviposited under the leaf litter typically several meters beyond the crown of the host tree (Tsukamoto and Tojo 1992; Filippi-Tsukamoto et al. 1995).

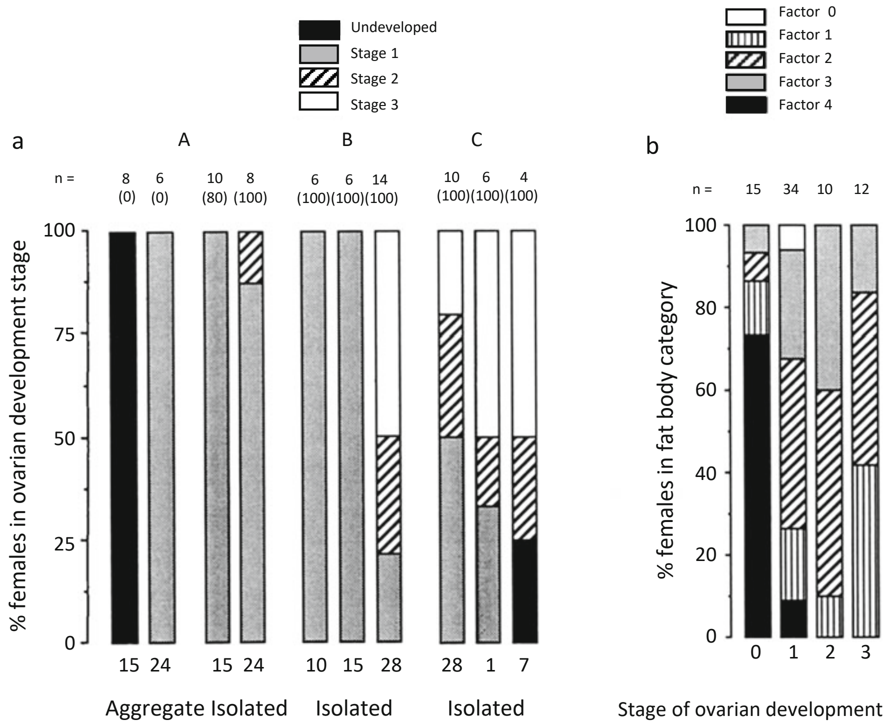

Fig. 3.5 Ovarian development and nutritional status of *P. japonensis* females during the mating season. (**a**) Ovarian development at three different sites during the mating season as determined by the state of the largest of eight unilateral ovarioles. Stage 0, undeveloped; stage 1, early/mid-previtellogenesis; stage 2, late previtellogenesis; stage 3, vitellogenesis to mature eggs. Numbers in parentheses indicate percentage of inseminated females. Aggregated and isolated females indicated. (**b**) Fat body content and stage of ovarian development. Subjects were lone females collected from host tree from May 15 to June 7 (mating season ended May 15). Adapted from Tsukamoto et al. (1994)

3.5 Male Intrasexual Competition

While the predominant mating system is clearly scramble competition polygyny, with males competing indirectly to gain access to as many females as possible, several other behaviors suggestive of more direct male intrasexual competition were observed. We considered the possible advantage of the V-posture as opposed to the end-to-end posture. This posture was more common in the LT matings, and we considered whether it might be a form of mate guarding (Parker 1970). However, because females do not oviposit until about 2 weeks after mating and have ample time to mate with other males in the interim, we concluded that in the absence of evidence that insemination after the mating was impossible, guarding as a function of the LT mating was unlikely.

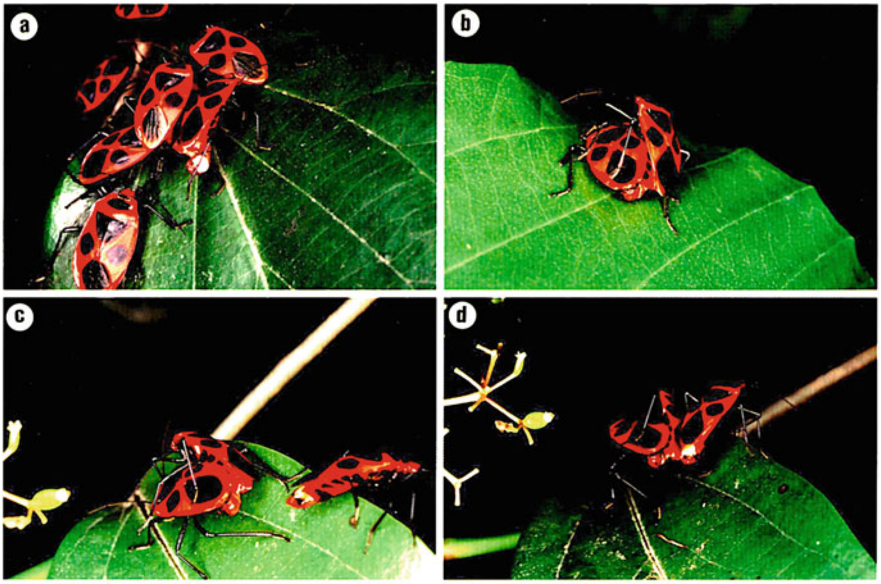

Fig. 3.6 Copulation behavior in *Parastrachia japonensis*. (**a**) Male everting the aedeagus and engaging in "genital grooming"; (**b**) V-pattern copulation with male on top, grasping female across the dorsum; (**c**) male intruding on a mating couple; (**d**) successful displacement of original male and intromission by intruding male. Adapted from Tsukamoto et al. (1994)

Another male behavior that indicated direct competition was that of males intruding on V-pattern mating couples in an attempt to dislodge the engaged male. Intrusions were observed on 121 occasions (9.4% of all mating attempts; Fig. 3.6c). The intruder would back into the joined abdomens, kicking at the male in an attempt to dislodge him and simultaneously attempting intromission with the female. 16 (13.2%) of these intrusions resulted in the original male being displaced and the intruder successfully mating with the female (Fig. 3.6d). Males were also seen attempting to mate with other males on 84 occasions and met with success 12 times (14.3%). The couplings were invariably end to end, complete with the thrusting phase suggestive of ejaculation (Tsukamoto et al. 1994). As no studies on sperm precedence have been done on this species, the impact of these behaviors on male reproductive success remains unclear, but they certainly warrant further scrutiny. In particular, information on how the change in OSR at the aggregation site impacts these male behaviors would contribute to our understanding of their significance in male reproductive success. OSR changes considerably as the mating season progresses because, as described above, females eventually relocate to the host tree after mating; however, most males remain at the aggregation site trying to mate with the remaining females. This skew in OSR suggests that competition between males likely becomes intense (Emlen and Oring 1977; Shuster 2009). Moreover, females already engaged in an LT copulation (V-pattern) might be more receptive to mating and emitting more pheromones and thus be more attractive to males who then try to dislodge and replace an already engaged male.

With regard to same sex mating between males, which is quite common in insects (Monk et al. 2019), Lerch and Servedio (2021) present a model that supports the hypothesis that same-sex mating can evolve if conditions favor indiscriminate mating as the optimal strategy. Further, Monk et al. (2019) present a convincing argument that indiscriminate mating might not be the conundrum it appears to be and is in fact the ancestral state. The authors suggest that it had not been selected out because the cost of possible missed different sex copulations is greater than the cost of indiscriminately engaging in same-sex mating. The densely aggregated arrangement of *P. japonensis* at the aggregation/mating site, and the likelihood that pheromones from one sex end up on the other, confusing the males further, suggests that the same-sex mating that occurs between males in this species likely represents a classic case of mistaken identity in the optimal strategy of indiscriminate mating (Scharf and Martin 2013; Monk et al. 2019).

3.6 Conclusions of Initial Mating Study

Through solely observing behavior in the field, without individual identification, we found that males become active first, in late April, early May; they wander around the aggregations, occasionally stopping to engage in “genital grooming.” Only males engage in flight, and this seems to facilitate males gaining access to as many females as possible. Males mate with females in clusters at the aggregation site and with isolated individuals near the aggregations, as well. The mating system can best be described as scramble competition polygyny (Thornhill and Alcock 1983). Females resist all attempts by males to mate, even after intromission has occurred, and copulations are in both the end-to-end posture, and in the V-posture, with the male on top, grasping the female across her dorsum. Both postures are accompanied by a rhythmic thrusting motion by the male as soon as intromission is achieved. Mating with regard to the sex of the partner appears to be indiscriminate, probably because for males the strategy of occasionally mating with a male during the chaotic scramble is less costly than the missed mating opportunities that might occur otherwise. Both males and females mated repeatedly. Both short-term and long-term matings were observed, the long term being more associated with the V-posture. Short-term matings are much more prevalent, and long-term matings are more common later in the mating season. As females mate, they gradually move over to the host tree, and the sex ratio at the aggregation site becomes decidedly male biased, likely intensifying competition between males. Ovarian development does not begin in earnest until after insemination has taken place, and the earliest stages of ovarian development are achieved with the fat body reserves from the nymphal stage, before the females actually begin feeding.

3.7 Observations of Manipulated Mating Behavior in the Field

Our goal of the next series of studies was to sort out the significance of the different mating durations, ST and LT. The “thrusting” behavior by males as soon as intromission had been achieved appeared to be consistent with ejaculation, so we assumed sperm was being transferred in both ST and LT matings. However, in 1993, LF did a preliminary investigation into this, dissecting several females that had just had either an ST or an LT copulation in the field. Of course, it was unclear whether the females had mated previously; however, among the females examined, sperm were only found in females that had had a long-term copulation. This was curious and led us to undertake a semi-natural study in the field. In both 1994 and 1995, 1 month before males and females became active, females were individually marked with liquid paint markers, their weight was recorded with a digital balance, and their pronotum width was measured with a digital calipers. The virgin females were enclosed in mesh bags (50 cm × 80 cm) on branches of a *Eurya japonica* tree, which prevented any unobserved mating behavior. We attempted to also enclose males, but because they expended so much energy trying very hard to fly in the bags, they did not survive to mate, so thereafter we collected males on the date that they were used for the mating trials. Mating trials were carried out in the field in three-tiered mesh cages suspended on a pole between two trees. Each of the three compartments was 50 cm × 30 cm × 25 cm (L × W × H). To avoid physical contact across the mesh between the bugs in adjacent compartments, either only the top and bottom compartments were used, or contact was prevented by separating adjacent chambers with cardboard dividers. Males were extremely fragile when it came to being manipulated in any way once they had initiated mating behaviors for the season. Recall that they do not feed, and with all the flight activity, they burned through their fat reserves fairly quickly and seemed to die off faster in the mesh cages than they did in the field. Our objective was to determine whether there was a discrepancy in insemination rate between ST and LT matings, so we just kept adjusting the sex ratio (M:F) to ensure that there were enough males that were vigorous enough to mate with the females in the cages. Thus, the sex ratios ranged from 1:2 to 2:1 in 1994 and, to increase the sample size of mated females, from 2:1 to 3:1 in 1995. Though there might have been some impact of sex ratio, for the purposes of this study, we assumed that there was no impact of sex ratio on insemination success of ST and LT matings and pooled data for all sex ratios.

In 1994 we carried out observations for a total of 23.3 h on 8 days throughout the mating period from May 8 to May 27, averaging ~174 ± 36 min per day, and in 1995 observations totaled 34.6 h on 14 days from May 5 to May 30, averaging 148.2 ± 36 min per day. Males and females were introduced to the chambers at the start of each observation session, and, after one or several copulations, females only were either returned to their bags and set back on the *Eurya* branches; transferred to the laboratory for dissection to determine stage of ovarian development, fat body abundance, and insemination rate; or enclosed in mesh bags on the host tree so they could feed. Those females were then dissected after feeding for 11–58 days. We documented the sequence and duration of each female's copulations and recorded their post-copulatory weight.

3.8 Patterns of Short- and Long-Term Matings over the Mating Season

In 1994, 160 copulations were observed for 95 females. In 1994 we did not record the number of females that did not mate. In 1995, among 138 females, 85 females engaged in 154 copulations, and 53 females did not mate at all. Although male's flight was constrained in the mesh cages, and we suspect that this "frustration" contributed to their early demise, the actual copulations proceeded as described for the bugs in the natural aggregation in the field. Males everted the aedeagus, backed into the female's abdomen, and attempted intromission. Females rejected males by rocking the body, kicking them with their hindlegs and running away, even after intromission had been achieved. The rhythmic thrusting behavior by males began 4–5 s after intromission and continued until about 10 s, while in the end-to-end position (stage 1). The male then tried to grasp the female across the dorsum with his front and midlegs, rotating his head dorsolaterally. Only if this maneuver was successful did the mating proceed to the V-pattern posture (stage 2) and become a long-term copulation.

Even while in the V-pattern posture the linked couple would intermittently walk about the cage, always with the female dragging the male along and occasionally rocking her body in an attempt to dislodge the male. This behavior was more prevalent after about 15 min in copula. ST matings were terminated by the female, but LT matings appeared to be terminated by the male. He disengaged the aedeagus, engaged in genital grooming for a few seconds, and then went off to seek another mating. Stage 1 and stage 2 postures are illustrated in Fig. 3.8.

In 1995, individual females were given multiple opportunities to mate, but only on one day, during one observation session. The seasonal changes in the frequency of ST and LT matings in 1995 demonstrate that the frequency of ST matings decreased over the season and that of LT matings increased; there were females who did not mate at all at every stage in the mating season. Short-term matings comprised 75% of all matings in the early phase of the mating season (Fig. 3.7a). Less than 10% of the females had LT matings, and about 23% of the females did not mate early in the season. By the middle of the mating season, the percentage of females experiencing ST matings dropped by about 30% to about 45%, and the percentage of females that had LT matings increased to about 35%, while the percentage of females who did not mate at all increased by 17% to about 40%. Late in the season only about 25% of females had ST matings, fully 50% of females experienced an LT mating, and the same proportion (40%) did not mate at all. The data suggest that as the mating season progressed, females were more willing to accept the LT mating. The data for 1994 followed a similar seasonal pattern.

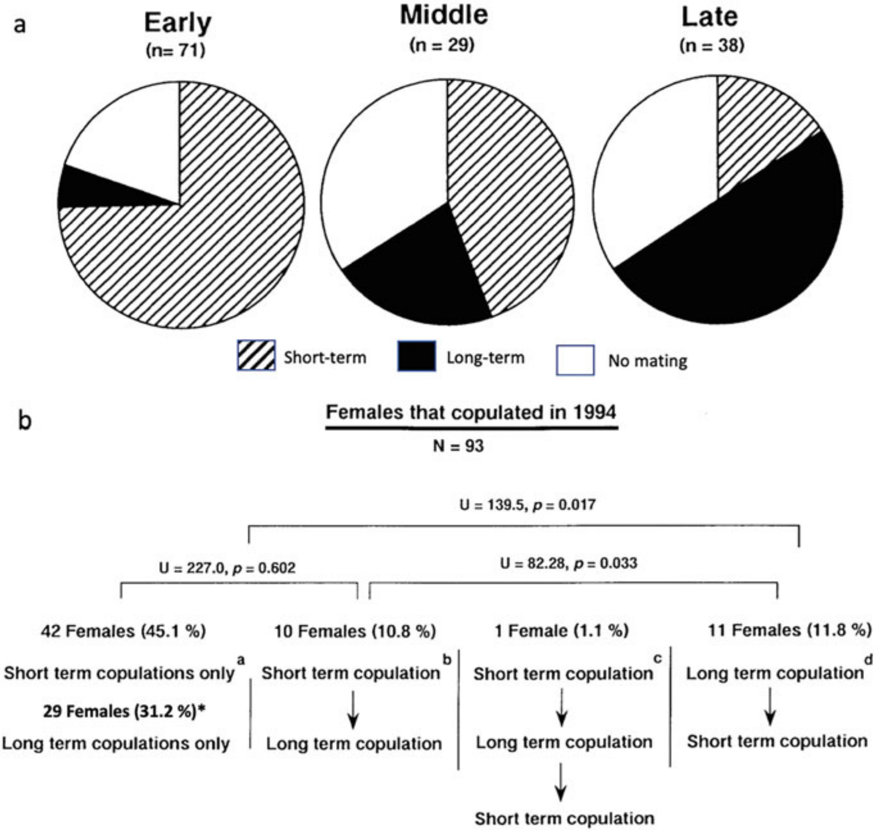

Fig. 3.7 Frequency and pattern of short- and long-term matings during the mating season. (**a**) Seasonal changes in numbers of females that experienced short-term, long-term, or no matings over the course of the mating season in 1995. Females were allowed multiple opportunities to mate, but only on one day of observation. (**b**) Pattern of short- and long-term matings in females who were given multiple opportunities to mate on multiple days during the mating season of 1994. Females that did not copulate are not included in the figure. (*) One of these females had two long-term copulations. (a) 1–7 short-term copulations (ST) only; mean 1.5 ± 1.7 (S.D.); (b) 1–2 ST before long-term copulation (LT); mean 1.2 ± 0.42; (c) 2 ST before LT, 1 ST after LT; (d) 1–7 ST after LT; mean 2.5 ± 2.1. Mann-Whitney U test for significance of number of ST among the three groups that experienced ST. Adapted from Filippi et al. (2000)

3.9 Frequency, Duration, and Insemination Success Rate of Short- and Long-Term Matings

Because many females experienced multiple matings, we examined whether there might be a pattern with regard to the sequence of ST and LT matings. While nearly half of the females only ever experienced ST matings despite having multiple opportunities to mate during the mating season, just over one third of females had

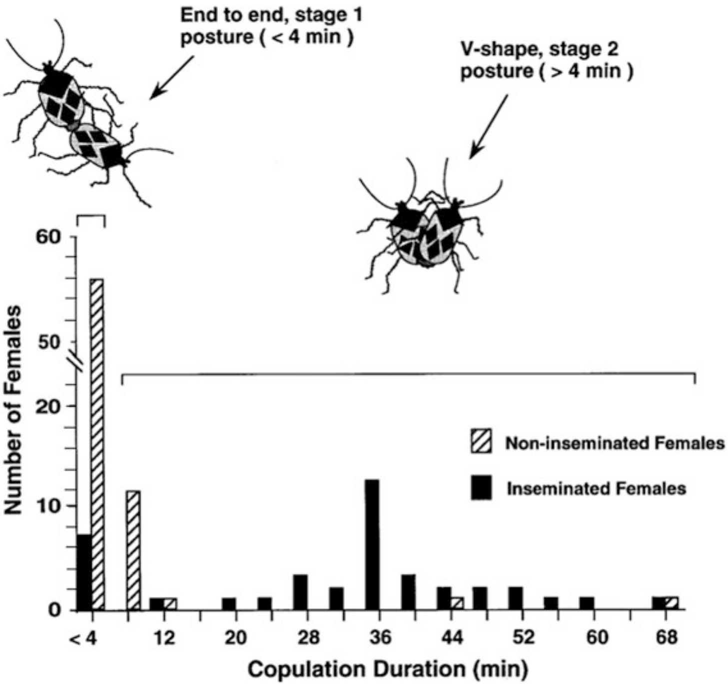

Fig. 3.8 Frequency distribution of duration of long-term-only and short-term-only matings in 1994 and 1995. Stage 1 posture occurred in all ST matings and at the beginning of all LT matings, which then invariably proceeded to stage 2 posture. Adapted from Filippi et al. (2000)

LT matings only (Fig. 3.7b). Among these, one female had two LT matings, and the remainder had only one. Among females that had both ST and LT, roughly equal numbers of females had one or two ST matings before or after the LT mating. Only one female had an ST mating followed by an LT mating and then another ST mating. Among the four groups of females that experienced ST mating at any point, those that had them after experiencing an LT mating had significantly more ST matings. This suggests that LT females might be more attractive to males, who then attempted many more matings that ended up as ST matings.

We examined the duration of mating and insemination rate of females experiencing only ST or LT matings in 1994 and 1995. The frequency of durations was bimodal, with an ST peak at 4 min and an LT peak at 36 min. Only 12% of females that had a copulation duration of less than 12 min had evidence of sperm transfer to their spermatheca, while 94% of females experiencing a copulation of more than 12 min had sperm transfer to their spermatheca (Fig. 3.8). Clearly the LT mating is a far more effective way for males to achieve insemination and is likely to be the actual goal of all males. In many heteropterans, such as lygaeid bugs, the spermathecal duct tends to be long and convoluted, and the prolonged copulations seen in species of this group are apparently related to the time it takes for the male to navigate the intromittent organ to and then through the spermathecal duct to the spermatheca

(Micholitsch and Pass 2000; Himuro and Fujisaki 2015). However, the spermathecal duct in *P. japonensis* is simple and quite short (Pluot-Sigwalt and Lis 2008). Females tend to have only one long-term mating, so the likelihood that males are spending time displacing sperm (Parker 1970; Thornhill and Alcock 1983) also seems unlikely. It might just be that it takes time for the sperm to travel to the spermatheca. Unfortunately, we have not carried out detailed examination of the female reproductive tract at various times during the long-term copulation, which could help answer this question. Another puzzle that has not been resolved is the rhythmic thrusting phase at the beginning of stage 1 in the ST mating. We have stated that there is no courtship behavior prior to intromission, but could the rhythmic thrusting behavior that we assumed was ejaculation actually represent copulatory courtship in the context of cryptic female choice? The behavior certainly meets the criteria of Eberhard (1994, 1997). The jerking motion, similar to what is described for water striders (Arnqvist and Danielsson 1999), is repeated rhythmically, it produces stimulation of the female, it is mechanically "irrelevant" to the physical coupling, and it does not suggest another function. We conclude that this is a strong possibility, but one that requires further scrutiny.

There was considerable variation in the proportion of ST and LT matings from year to year. In 1992, 93% of observed matings were ST and only 7% were LT (Tsukamoto et al. 1994). However, in 1994, among the natural population, 46% were ST and 54% were LT. The respective values for the manipulated matings in 1994 were 67.5% and 32.5%, so the confinement to the mesh cages might have affected the behavior; in 1995 the respective ratios for the manipulated matings were 79% and 21%.

3.10 Effect of Female Body Size, Weight, and Ovarian Development on Mating Duration and Insemination Rate

We examined whether body size, weight, or ovarian development of females impacted the duration of mating and insemination success rate. No correlation was found between female body size and weight and whether she experienced a short- or long-term mating, or no mating at all (Filippi et al. 2000). However, we found evidence that ovarian development affected mating status. We used the criteria for ovarian development described above (Sect. 3.7). In 1995, 45% of control females enclosed on the host tree that did not mate but might have mated if they had been given the opportunity had stage 1 ovarian development (early previtellogenesis). Females that had ST or LT matings were dissected either within 8 h of mating (5% within 30 h) as a proxy for ovarian development at the time of mating or after being allowed to feed for 11–58 days after mating, during which time post-copulation ovarian development could occur. Among the ST individuals soon after mating, 87% had no or the earliest stages of previtellogenesis, and 13% had late previtellogenesis

(Fig. 3.9). None had vitellogenesis. Moreover, among the ST females at the time of mating, only 7% had evidence of sperm transfer. On the other hand, while none of the LT mated females had ovarian development that had advanced to the stage of vitellogenesis at the time of mating, significantly more of them, fully 91% had evidence of sperm transfer. When compared to the control females, there was no significant difference in ovarian development of the ST mated females soon after mating, but the ovarian development of the LT mated females was significantly more advanced than that of the ST mated females (Mann-Whitney U test, $p < 0.03$). Interestingly, after being allowed to feed for a minimum of 11 days after mating, the

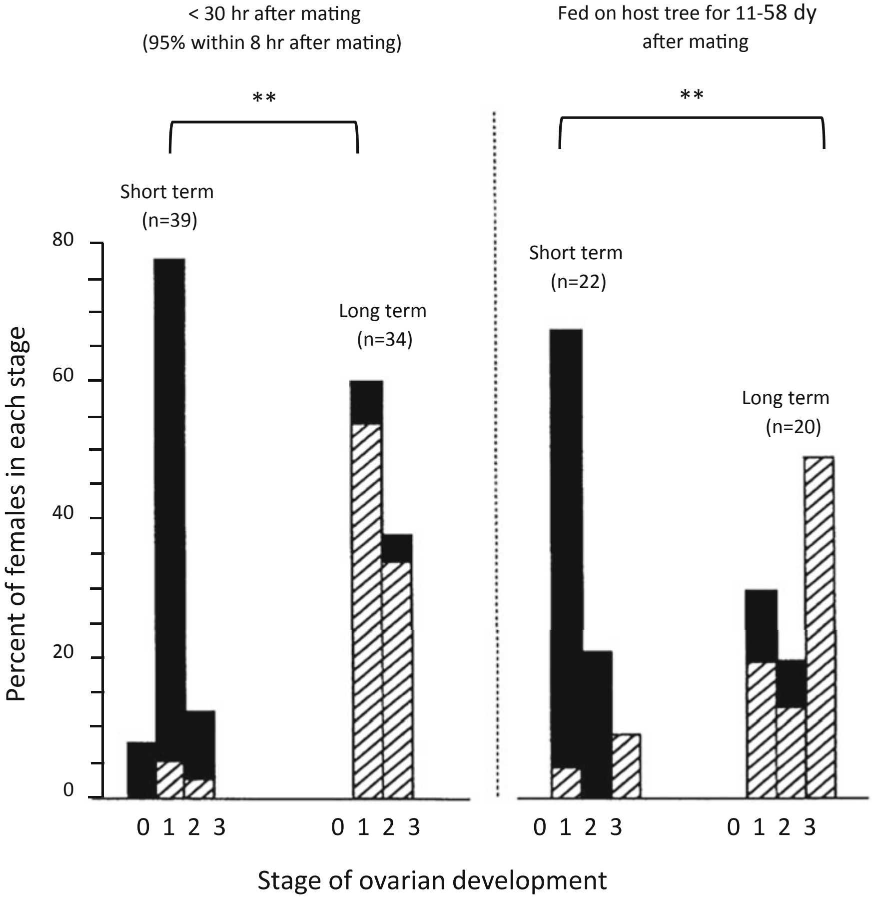

Fig. 3.9 Relationship between stage of ovarian development, mating type (ST, LT), and insemination rate. Hatched areas indicate percent of females that were inseminated. **, Mann-Whitney U test, for differences between ST and LT ovarian development before feeding, $p = 0.005$; for differences between ST and LT ovarian development after feeding, $p = 0.004$. For differences in insemination rate between ST and LT before and after feeding, $p < 0.001$. Adapted from Filippi et al. (2000)

ovarian development of ST mated females increased somewhat, with all females showing some ovarian development and 10% indicating vitellogenesis, but nearly 70% remained in the earliest stage of previtellogenesis. Moreover, only 11% of these females had been inseminated. On the other hand, 62% of females of the LT females had vitellogenesis, and over 85% of these females were inseminated. The findings indicate that females accepting LT matings have greater development of the ovaries at the time of mating and progress to mature ovaries after feeding, while the ovarian development of ST mated females is significantly less. Moreover, LT matings result in significantly greater insemination success. It seems likely that females engaging in LT matings are more receptive because their ovaries are more developed.

3.11 Effect of Male Body Size on Long-Term Mating Success

We also monitored a natural subpopulation of ~3000 individuals in 1994 to determine whether larger males were more successful at achieving LT matings. We individually marked and measured pronotum width and body weight of all males and females 1 month prior to the start of the mating season and returned them to their original site. On each of 7 days during the course of the mating season mating behavior was observed. When a mating was observed, individuals in the mating pairs were noted, and the ratio of female to male body size was calculated. Females are always larger than males, but we predicted that the ratio of female size to male size would be smaller in LT copulations. Interestingly, there were no significant differences in the body size ratios between short- and long-term matings (Filippi et al. 2000). Han et al. (2010, 2018) used the laws of physics, specifically mechanics, to discover that female resistance to mating has led to a size-assortative mating and sexual size dimorphism in water striders. Males of this species use the same grasping pattern across the female dorsum to achieve mating as seen in *P. japonensis*. They found that a particular male-to-female size ratio (0.86) was the optimal ratio for males to effectively grasp the female. Larger and smaller ratios were considerably less successful. We have not examined this possibility in *P. japonensis*, but it would be interesting to explore. Another possibility is that after expending so much energy flying and vigorously repeatedly mating, males might not have the energy to subdue the female. This might be the cue to the female that he has also depleted his sperm on earlier matings, as seen in the stream-dwelling isopod, *Lirceus fontinalis*. Sparkes et al. (2002) found that females resisted all males, but they resisted low-glycogen males more vigorously; successful males had higher levels of glycogen. They also found that females mating with recently mated males had an 18% reduction in fertilization success. With the high rate of matings that male *P. japonensis* engage in and no apparent refractory period in between matings, females would benefit by mating with the most vigorous males, as they would be most likely to have sufficient sperm to fertilize all of their eggs. The data indicate that nearly all females that had long-term matings had only one LT mating, indicating that one LT mating is sufficient to fertilize all of a female's eggs. Thus, females would benefit from mating with a male that is likely to fertilize all of her eggs and refusing all other mating attempts. Females also only lay one egg mass in their lifetime (see Chap. 6), so optimizing fertilization rate of the lone egg mass is critical. Clearly, much work on mating behavior in this species remains to be done!

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Lisa Filippi initiated the work on *Parastrachia japonensis* at Mt. Hinokuma Park in Kanzaki Town, Japan, where she earned her M.S. and doctoral degrees elucidating many of the amazing physiological and behavioral traits of this fascinating insect through field and laboratory studies. She is currently a Professor of Biology at Hofstra University in New York.

Mantaro Hironaka in fact applied to Saga University in order to eventually join the *P. japonensis* research team. He proved to be a brilliant, enthusiastic, energetic, and creative member of the team who contributed significantly to a variety of behavioral studies. Mantaro went on to get his doctoral degree in Dr. Tojo's laboratory from the United Graduate School of Agriculture, Kagoshima University, focusing on the neurological processes involved in the complex navigation that *P. japonensis* uses when provisioning. He is currently an Associate Professor at the Department of Applied Entomology, Faculty of Bioproduction Science, Ishikawa Prefectural University.

Shintaro Nomakuchi an ecologist focusing on insect behavior, came to Saga University in 1993 as an Associate Professor, and was keen to share his very valuable expertise and join the *P. japonensis* research team, which he eventually headed. The work on clarifying the quality of the habitat, the techniques for monitoring the subpopulations and for carrying out a variety of ecological and behavioral studies, as well as the methods for performing robust data analyses, all got a hefty boost, and we were off and running. He is a Professor Emeritus of the Faculty of Agriculture at Saga University.

Chapter 4
Parental Care

Lisa Filippi, Mantaro Hironaka, and Shintaro Nomakuchi

Abstract *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) mothers guard eggs and nymphs and provision nymph-containing nests with drupes of the host tree, *Schoepfia jasminodora* (Schoepfiaceae). We describe our journey of discovery over the years from drawing conclusions about observations of natural behavior in the field to testing of hypotheses through manipulated field and laboratory studies. The journey has clarified the ecological constraints on the life history of *P. japonensis*: poor quality and abundance of drupes, predation, intraspecific competition, and severe weather. We explain how we identified patterns of nesting and the adaptiveness of the parental care behaviors that mitigate these constraints. Provisioning females choose only high-quality drupes to provision their nests because nymphs on their own cannot access sufficient high-quality drupes to sustain development. Female guarding of eggs and nymphs protects against predation by a ground beetle, but, importantly, provisioning also protects young because they remain in the safe confines of the nest longer if sufficient drupes are provided. Females also produce trophic eggs that enhance all measures of offspring success. They use risk-sensitive decision-making to choose when and where to nest and how many fertile and trophic eggs to produce. We explore how risk-sensitive decision-making allows mothers to best respond to the severe ecological conditions experienced during the nesting season that enhance their provisioning success and offspring success.

Keywords Cannibalism · Foraging · Intraspecific competition · Kin recognition · Maternal care · Resource constraints · Nymphal independence · Semelparity

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4.1 Introduction

The evolution of parental care represents an enormous leap in a species' life history. To make that leap, critical challenges had to have been met because, while providing parental care always enhances the success of offspring, it also always involves considerable trade-offs. The parent providing care typically experiences limits to foraging time, opportunities to mate and produce future offspring, ability to maintain its health, and even to its survivorship via increased susceptibility to predation and disease. Thus, when parental care emerges in a species, it can be assumed that the trade-offs always favor providing care as the best way to increase the provider's fitness (Clutton-Brock 1991). E.O. Wilson (1971, 1975) established four prime environmental movers that favor the evolution of parental care: unusually harsh and stressful environmental conditions, stable structured environments, predator pressure, and scarce, and specialized food resource. To be sure, these environmental states are commonly experienced by most insects, yet parental care in the insects is not the norm. In fact, outside the eusocial insects, relative to the number of insect species out there, parental care in the insects is pretty darn rare. Among an average estimated 5.5 million insect species (Stork 2018), subsociality, any care of offspring after oviposition (Michener 1969), has evolved in 50 families among more than 12 of the 25–30 insect orders (Costa 2006). Considering that there are an estimated 1000 insect families (Grimaldi and Engel 2005), only 5% of them have evolved any form of parental care, from the simplest behavior of guarding eggs to guarding immatures, provisioning immatures and the pinnacle of care, progressively provisioning immatures. Roughly 1% of all insect species progressively provision young, and that includes all Eusocial species (Royle et al. 2014). Rather, most insects have found the greatest success by evolving minimal investment in care beyond scattering their eggs over time and space in places where the offspring will have a fair chance of finding food and shelter after they hatch (Eickwort 1981a, b). Tallamy and Wood (1986) have categorized the parental care behaviors seen in insects that provide some form of care for their young as those that physically protect young from predation and other environmental dangers, provide and/or guard a resource vital to the offspring, or prepare a food resource for offspring feeding.

We will discuss the specific factors that have promoted the evolution of parental care in *Parastrachia. japonensis* Scott (Heteroptera: Parastrachiidae) from a theoretical framework as we move through the chapter. However, the field has advanced considerably in recent years, and for eloquent, thorough, and thoughtful coverage of these topics, we direct readers to Trumbo (1996), Costa (2006), Royle et al. (2012, 2014), Wong et al. (2013), and Gilbert and Manica (2015). Our goal is to take the reader on our specific journey, the one that revealed the marvelous and varied manifestations of parental care in *P. japonensis*. Prior to the start of our research at Mt. Hinokuma, it was known that female *P. japonensis* engaged in some parental care behaviors; the mother guarded a round egg mass (Miyamoto 1965) and young nymphs (Tachikawa and Schaefer 1985). The food resource had finally been identified as drupes of the gray twig tree, *Schoepfia jasminodora* (Schoepfiaceae)

(Gyôtoku and Tachikawa 1980; Tachikawa and Schaefer 1985; Tachikawa 1991). Moreover, Tachikawa and Schaefer (1985) suspected but never observed females provisioning drupes to the young. We recognized that if mothers were in fact repeatedly provisioning nests with drupes, it would represent an extremely unusual behavior in an insect, and it had never been reported for a phytophagous heteropteran. So, in 1989 we set out to find evidence of progressive provisioning behavior. Over the first 2 years of field work, we verified that females did in fact provision nests with drupes of the *Schoepfia jasminodora*.

Early studies from 1989 to 1991 focused on documenting and gathering evidence of the behaviors; later studies focused on the behaviors from a behavioral ecology perspective. Which of the ecological factors postulated by Wilson (1971, 1975) and Tallamy and Wood (1986) promoted their evolution? Because so little was known about this insect at the outset of our research, every behavior we documented (aggregating, mating, several parental care behaviors) was new and exciting. The early years in the field were nothing short of thrilling; there were so many avenues of research to pursue, and we felt like children in a candy store. We begin this chapter with a summary of the important findings of our first paper on parental care in *P. japonensis* (Tsukamoto and Tojo 1992).

4.2 Nesting and Maternal Care of Eggs

After feeding on the host tree for about 10 days, during which time egg development took place, inseminated females climbed down the host tree, their abdomens conspicuously laden with eggs, and roamed on the ground in and around the leaf litter searching for a site to nest. There was very little leaf litter under the host tree, and most females walked 5–10 m away from the host tree to nest. In fact, as described in Sect. 1.10, in 1990, only 4 of 24 nests were located within the area directly under the crown of the host tree, *S. jasminodora*, where drupes either landed, or where they had rolled to in the area outside the crown (foraging area). The remaining 20 (83%) nests were under the freshly fallen leaf litter of a large *Ilex chinensis* tree (Fig. 4.1; Tsukamoto and Tojo 1992). Females excavated a shallow burrow under the leaf litter and oviposited a round cream-colored egg mass, which gradually turned yellow, then pale pink, and finally red just prior to hatch. We verified that the mothers not only hovered over the egg mass, guarding it, but kept it suspended on the end of the stylets (Fig. 4.2a; Tsukamoto and Tojo 1992). Mothers remained in the nest until hatch about 10–14 days later, unless disturbed, in which case the female carried the eggs off to a new site.

Unlike other cydnid relatives that bring seeds into the nest and feed during the incubation period (Sites and McPherson 1982), females never brought drupes into the nest before the eggs hatched, and they never left to feed. Eggs that were attended by a female in the field always appeared healthy; however, six egg masses separated from the mother and placed on moist filter paper in individual lidded plastic cups in an incubator failed to hatch. Mites were eating some of the eggs in every mass, and

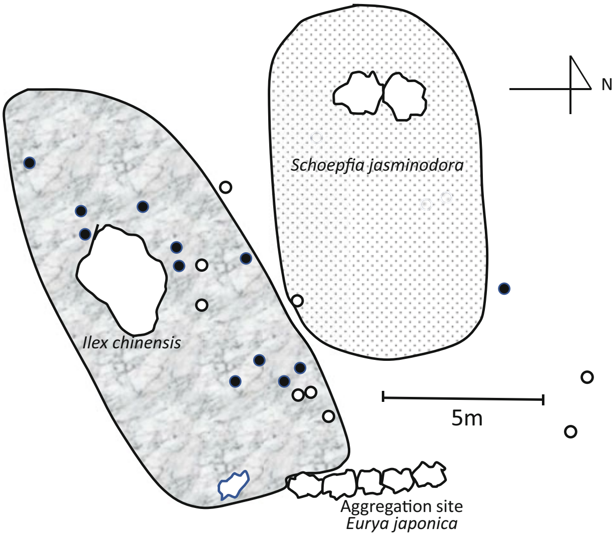


Fig. 4.1 Nesting site studied at Mt. Hinokuma in 1990. White patches represent the trees indicated. The site gradually sloped down from the host tree to the aggregation site. Dark circles, nests that contained drupes; white circles, nests that never contained drupes. Shaded area around *I. chinensis* indicates the area under the canopy of the tree where leaves fell; the dots around the host tree represent the area where drupes fell under the canopy and rolled downhill. (Adapted from Tsukamoto and Tojo 1992)

Fig. 4.2 Nesting behavior of *P. japonensis.* (**a**) Female guarding newly oviposited egg mass and (**b**) newly hatched nymphs in nest. Leaf litter covering was removed to take the photographs. Photo on left is credited to Hiromi Mukai. Adapted from Tsukamoto and Tojo (1992)

fungus was also growing as they gradually turned black and rotted (Tsukamoto and Tojo 1992). Maternal grooming of eggs that prevents fungal and other microbial infections has been reported for a number of insect species including earwigs (Meunier and Kölliker 2012; Boos et al. 2014), termites (Matsuura et al. 2007), and, a close relative of *P. japonensis*, the cydnid *Adomerus triguttulus* (Nakahira and

Kudo 2008), and we strongly suspect that in the field, *P. japonensis* mothers are grooming their eggs during incubation, removing fungus and mites.

We wondered whether females could distinguish their own eggs from those of their neighbors. While mothers of the reduviid bug *Pisilus tipuliformis* can distinguish their own eggs from those of other conspecific females (Parker 1965), *P. japonensis* mothers apparently lack that ability. We brought six females with their egg mass back to the laboratory and placed them in an incubator in individual cups. Each female was placed in the center of three egg masses, one of which was her own. The following morning two of the six females had claimed their own egg mass, three chose another female's eggs, and one female hovered over two of the three egg masses simultaneously, neither of which was her own (Tsukamoto and Tojo 1992)! In the field, females rarely leave their eggs unattended, so we expect that there really was no need for egg discrimination to evolve.

4.3 Provisioning Behavior

4.3.1 Method of Provisioning

In 1990, we observed the 24 nests several times a week from soon after oviposition until the third instar, when nymphs typically left the nest. We did not mark the females for this study, but at each observation, there was only ever one female in the nest, holding her eggs or guarding nymphs. We assumed it was the mother of the eggs in the nest. Females did not leave the nest until the eggs hatched. Within hours after hatch, females left the nest to forage for drupes. A female typically spent hours roaming about the foraging area, inspecting many drupes of different color and ripeness. When a suitable drupe was finally found, she snatched it up and quickly returned to the nest where she deposited it; the nymphs gathered on the drupe to feed (Fig. 4.3, Tsukamoto and Tojo 1992). Females transporting drupes moved with surprising speed and agility over the rough terrain in the field, despite the heavy and cumbersome burden. The average weight of a drupe was 265.5 ± 49.0 (S.D.) mg ($n = 10$). On the other hand, the average weight of a female soon after oviposition was 159.5 ± 28.6 (S.D.) mg ($n = 6$), but the weight of a provisioning female that hasn't fed for up to 2 weeks is likely to be considerably less. Moreover, females were frequently seen transporting two and occasionally three drupes together that were attached on the same bit of stem. If we do the math, females are dragging drupes that weigh an average of at least 1.7 to more than 5 times their weight at a formidable speed of 33.3 cm/min ($n = 5$; range = 17.5–61.4 cm/min) over an average distance of 254 cm (range 160–430 cm)! *Why all the haste?* Read on to Chap. 6 for the answer to that.

At the time of most observations (three to five times per week), the female was in the nest with the young. On two occasions, the female was away from the nest but was there at the next observation, along with several drupes. Occasionally a female that had been gone from the nest, presumably out foraging, was present in the nest at

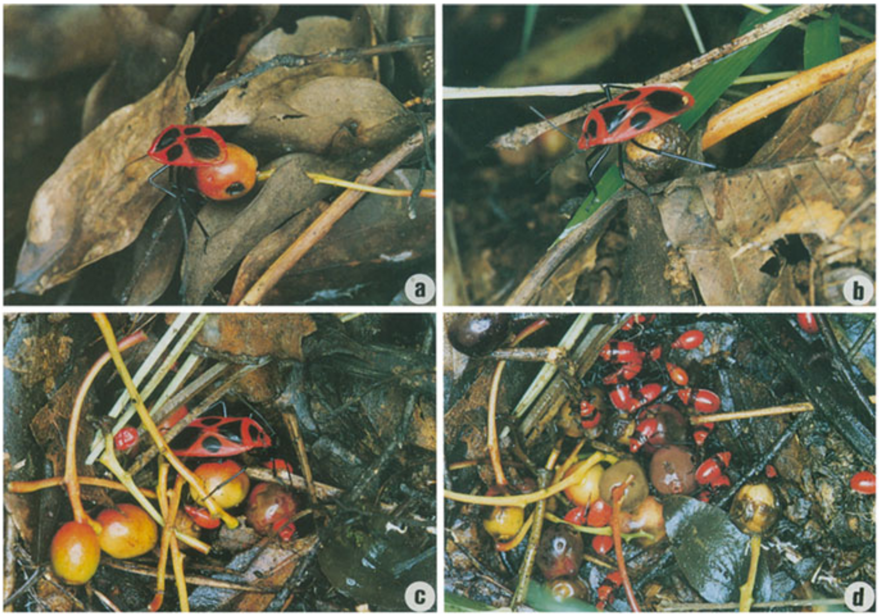


Fig. 4.3 Nest provisioning behavior in *P. japonensis.* (**a** and **b**) Female transporting drupes to nest. Drupe is suspended on the end of the stylets. (**c**) Female in nest with young and drupe stockpile immediately after depositing a drupe in the nest. Two drupes attached by the same stem can be seen in the lower left of the photo. (**d**) Third instar nymphs gathering on drupe stockpile to feed. Adapted from Tsukamoto and Tojo (1992)

the next observation; however, there were no drupes in the nest. Apparently unable to find a suitable drupe, the female would return to the nest for a time before heading out to the foraging site again. Clearly finding a suitable drupe was no easy task.

4.3.2 Relationship Between Nest Distance and Provisioning Rate

We verified that females nesting both inside the foraging area and those nesting well outside the foraging area provisioned their nests with drupes (Fig. 4.1; Tsukamoto and Tojo 1992). Interestingly, but perhaps not surprisingly, in 1990, while 12/20 (60%) of the nests outside the foraging range eventually contained drupes, 3/4 (75%) nests within the foraging area eventually contained drupes. We did not want to disturb the nymphs in the nest, so rather than count the drupes at each observation, we counted them after the nests had been abandoned. However, because the entire provisioning period coincided with the rainy season, many of the nests were degraded at that point. Combining data from 1990 and 1991, we were able to

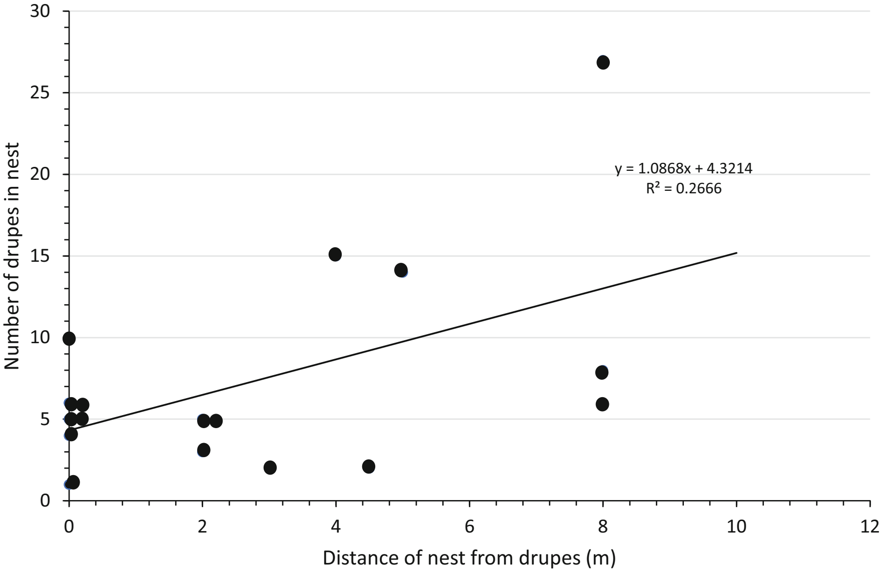


Fig. 4.4 Relationship between distance of nest from fallen drupe area and number of drupes provisioned. Only nests that had drupes provisioned are included. Data is from 1990 to 1991. $n = 17$. Mean number of drupes provisioned ± S.D. = 7.2 ± 6.2. Mean distance from the fallen drupe zone ± S.D. = 2.7 ± 3.0. Pearson correlation coefficient, $r = 0.5163$, $p = 0.034$. Data from 1990 and 1991 combined; adapted from Tsukamoto and Tojo (1992)

count the final number of drupes in 17 nests and plotted the number of drupes provisioned against the distance from the foraging area. Interestingly, there was a significant positive correlation between the distance of the nests from the fallen drupe area and the number of drupes that females provisioned (Fig. 4.4; modified from Tsukamoto and Tojo 1992). It seemed likely that the frail and vulnerable nymphs in nests that were farther away would have a difficult time accessing the foraging site, so females provisioned more drupes. The number of drupes provisioned ranged from 2 to 27, but 25% of all nests (1990 and 1991) never had any drupes. This percentage varies, though, because in 1991, 100% of 12 nests located and monitored from the egg stage eventually contained drupes (data for 1991 not specifically presented in Tsukamoto and Tojo 1992 but inferred).

Females, constrained by obligate semelparity, began dying off when their nymphs were in the third instar, though occasionally they lived and provisioned through the fifth; none ever had a second egg mass, and we have never seen a female lay a second egg mass no matter how much food we provided (unpublished observation, LF). As the mothers died off, nymphs gradually moved to the foraging area where they aggregated as they fed on the drupes.

4.3.3 *Conclusions of Our Earliest Study on Parental Care in* **P. japonensis**

Most females nested well outside the foraging area, where the leaf litter provides better cover and likely more sanitary conditions (Fig. 4.1). We verified that females do not leave their eggs or the nest unattended until hatch (Fig. 4.2); if disturbed, they move to a new spot with the eggs. They cannot distinguish their eggs from other conspecific eggs, likely because over the course of their natural evolutionary history the need rarely arose. We verified that most females do, indeed, progressively provision their nests with drupes (Figs. 4.1 and 4.3), and this was the first report of a female phytophagous heteropteran progressively provisioning a nest; however, this report came out simultaneously with that of a related cydnid, *Adomerus triguttulus*, that also progressively provisions its nest (Nakahira 1992). While more females nesting within the foraging area provisioned their nests, those nesting farther away that did provision the nest stockpiled more drupes (Fig. 4.4). Nymphs typically remain feeding in the nest until the third instar, the time when females typically begin to die off, and nymphs gradually relocate to the foraging area.

So why the enormous effort of progressive provisioning? Because the females seemed to reject most of the drupes they inspected, we suspected that the resource, while seemingly abundant, was generally of poor quality, and screening by females would provide quicker access to higher quality food. We also concluded that the distance between most nests was prohibitive to young and vulnerable nymphs. However, these and other ecological factors were not clarified in this study. The findings of subsequent studies gradually revealed some fascinating answers!

4.4 Thinking About the Big “Whys”

Now that the parental care behaviors, guarding of eggs and nymphs and progressive provisioning, were confirmed, the next series of studies set out to gather information that could help us answer some of the big *why* questions of behavioral ecology (Tinbergen 1963; Davies et al. 2012): the proximate developmental and mechanistic causes and the ultimate adaptive and evolutionary causes. Our first goal was to explore two of the ecological factors proposed by Wilson (1971, 1975) and Tallamy and Wood (1986) that might reveal an adaptive value of the behaviors, predation pressure, and resource availability. Because parental care will always reduce future reproductive potential, when it evolves evolutionary forces will have arrived at a pattern that results in optimizing the trade-offs in favor of care over no care. When predation risk is low and resources are abundant and available for a prolonged period, iteroparity should be favored, with females producing offspring either singly or in batches repeatedly over space and time, and providing no care, for as long as the resource lasts. However, if eggs and/or early-stage immatures suffer from heavy predation, it would pay for females to reduce predation risk by investing in guarding for a time before producing additional offspring (Tallamy and Denno 1982). On the other hand, if the resource is ephemeral or scarce, rendering production of additional eggs unprofitable, *and* predation pressure is high, evolution should favor producing a limited number of offspring and investing in extensive parental care (Kudo 1990). This seems to be what is going on with *P. japonensis*.

4.5 Nesting Pattern and Longevity of Nests in 1994

This was year five of our research (1994), and we came to recognize that, although the location of the aggregations in the trees around the host trees was fairly fluid from year to year, and the numbers of nesting females varied considerably, the general pattern of the nests at this site, where females preferred to make them, remained largely the same (Fig. 4.5; Filippi-Tsukamoto et al. 1995).

Because we recognized that collecting large numbers of bugs for experiments in the laboratory and manipulating them in the field like we did for the mating experiments was sure to affect the outcome of any collection site's reproductive success, we had one area at Mt. Hinokuma that we left completely intact, never collecting or manipulating bugs in the subpopulation in any way. We limited our

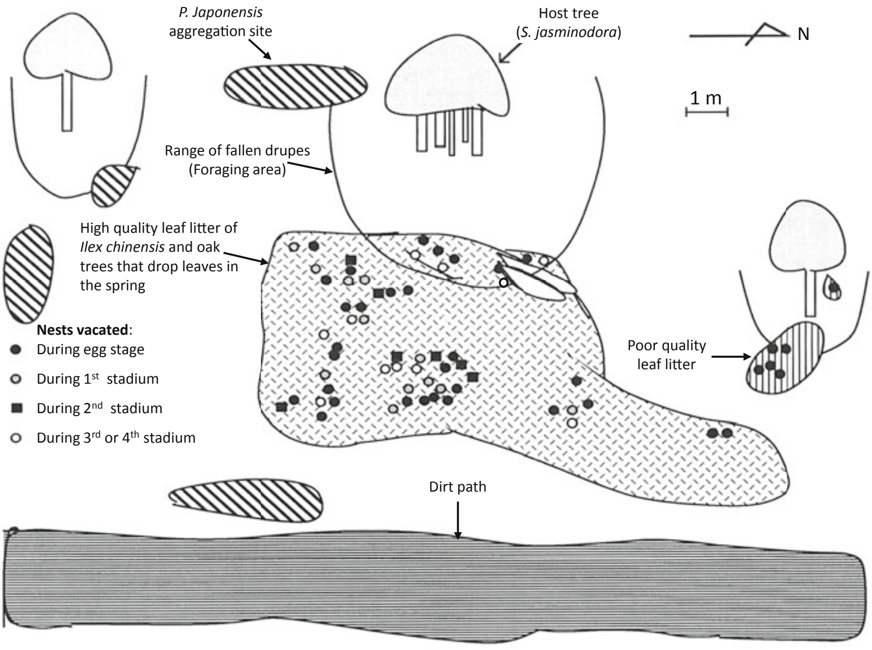


Fig. 4.5 Pattern of nesting and nest persistence at an unmanipulated field site at Mt. Hinokuma in 1994. Adapted from Filippi-Tsukamoto et al. (1995)

work at that site to observing the natural condition, monitoring the natural pattern of nesting each year, and the changes in the size and movement of the subpopulation. Although lifting the leaf litter to observe might have had some effect on the success of the nest, we considered that the disturbance was minimal.

As before, in 1994 females tended to nest at some distance from the host tree and clearly preferred to nest under the leaf litter of trees that dropped their leaves in the spring (*Ilex chinensis* and some oaks). In 1994, we found 66 nests soon after females had oviposited and monitored them regularly throughout the season to gain a better understanding of nesting success in the field. In 1994, the distance of nests from the foraging area ranged from 0 to 5.2 m, mean ± SD = 2.3 ± 1.5 m (Fig. 4.5; Filippi-Tsukamoto et al. 1995). Of course, it is possible we missed some nests, but these distances were considerably shorter than those from the previous study at the same site. 16.7% of the nests were located within the foraging area, and the remaining 83.3% were outside the foraging area. Only 45% (30) of the nests persisted to the first stadium. It is possible that some of the females relocated their nests, but we searched for new nests daily and did not find any. We had come to realize that predation was a big selective pressure for nesting *P. japonensis.* 16 (24%) of the original 66 nests (53% of the nests that survived to the first stadium) persisted to the second stadium, and only 11 (<17%) of the 66 nests (69% of the nests that persisted to the second stadium) persisted to the third or fourth stadium, which is when we typically see nymphs moving over to the foraging area. We were fairly certain these numbers varied from year to year, but the great variation stressed the fact that successful nesting is clearly a challenge for *P. japonensis* mothers. We suspected that the high rate of nest failure was due to predation (see the next section) and to the stressful environment experienced during the heavy rains and flooding of the rainy season, which coincides with the nesting season in Kyushu, Japan.

Second instars rarely relocate to the foraging area, and most nymphs seem to relocate to the foraging area at the third stadium or fourth stadium. Thus, we considered that the 17% of nests that persisted until that point were successful nests and those that vacated before that likely failed. We played with the data presented from the 1994 nesting site in Filippi-Tsukamoto et al. (1995) to see if there was a correlation between distance from the foraging area and success of the nests. Surprisingly, no significant difference between the distance to the foraging area of nests that succeeded to the third instar, and those that failed before the third stadium was found (distance for successful nests, 1.9 ± 1.68 m, and for failed nests, 2.38 ± 1.49 m <mean ± S.D.>). However, interestingly, in 1994, 36% of nests within the foraging area were successful, while only 17% of those outside the foraging area were. Later studies show that these percentages vary dramatically with the conditions prevailing each year.

4.6 Maternal Egg Guarding Behavior

Protection of eggs from parasitism (Odhiambo 1959), predators (Nafus and Schreiner 1988), and desiccation (Nalepa and Bell 1997; Nalepa et al. 2008) is the simplest form of parental care in the insects. The eggs are usually located in a stationary site that can be defended by covering them with the body (Kudo et al. 1989) or in a nest and may be carried off to safety. The next level would be guarding of young larvae or nymphs from predators, either passively or with aggressive displays (Tallamy and Denno 1981, 1982; Tallamy and Wood 1986; Nafus and Schreiner 1988; Choe 1989) or sometimes by following them around, as with the extended care seen in *Elasmucha dorsalis* (Kudo et al. 1989). By 1991, we had identified the first known predators of *P. japonensis*. On 13 occasions, adults and larvae of the carabid ground beetle (*Apotomopterus dehaanii*, ~30 cm length) were observed feeding on eggs, nymphs, and adults of *P. japonensis*. They appeared to be attracted to the high density of *P. japonensis* on the ground during the nesting season (*P. japonensis* are typically aggregated on shrubs and trees over a meter above the ground), as they were commonly observed roaming specifically throughout the nesting and foraging area during that time. We also frequently observed ants carrying off eggs and nymphs one by one from their nests after immobilizing the mother by feeding on her legs, antennae, and wings but leaving the toxic body (Sect. 1.14) behind. This relentless predation interfered with many an experiment!

We assessed whether females guarding eggs ($n = 17$) and nymphs ($n = 8$) displayed a defensive response against a simulated predator. A female was exposed to one of three levels of stimulation that simulated the disturbance of a predator: the mildest stimulation was rustling the litter close to the nest (approach of a predator) with a disposable chopstick (one of our favorite field tools!); the next intense level of stimulation was gentle probing of the female (predator in the nest) with the chopstick; and the most intense level of stimulation was more forceful probing (attack by the predator). We used a different female for each trial, and the subjects were naturally nesting females. Females guarding an egg mass displayed four behaviors in response to the threat, or no reaction at all. From mildest response to most aggressive, females would flagellate the probe with the antennae; feign death (thanatosis); turn about on the egg mass, presenting the dorsum to the disturbance; and finally lift the egg mass and carry it off in escape. Interestingly, several females did not respond at all to any level of stimulation, but the most frequent behavior was to flee with the egg mass (33% of females with the two mildest stimulations, and over 70% of females with the most intense stimulation; Table 4.1; Filippi-Tsukamoto et al. 1995). Only 2/28 (7%) females displayed a milder response before finally escaping. In other words, most females did not seem to display progressively intense responses but had a particular response that they displayed. Notably, no female ever left her egg mass behind. However, with females guarding nymphs, none failed to respond, and at the level of probing, most females escaped the nest leaving the nymphs behind. While none of the females guarding eggs made the typical chattering sound heard when aggregations are disturbed, two females

Table 4.1 Defensive behaviors of egg and nymph guarding females

Behavior mild ↓ Intense	Stimulation mild → intense				
	Eggs			Nymphs	
	Rustling litter	Gentle probing	Strong probing	Rustling litter	Gentle probing
No reaction	2	2	1	2	0
Flagellate antenna	4	1	0	0	0
Feign death	4	2	1	0	0
Chatter	0	0	0	2	0
Present dorsum	2	3	0	2	1
Escape	6	4	5	4	4
Total responses	18	12	7	8	4
Total females	17	11	7	8	4

Adapted from Filippi-Tsukamoto et al. (1995)

guarding nymphs did. We suspect this alarm sound evolved for an aggregation to deter vertebrate predators, and one female chattering would not be a very effective deterrent. There is no way for a female to guard nymphs, which scattered into the crevices of the nest upon disturbance, so we concluded that by running away from the nest, the female was making her larger body the target of the threat.

We repeated the trials on females with eggs, but this time stimulated them with live ground beetle larvae ($n = 11$) or adults ($n = 13$) tethered to a string. The responses were similar, but the females tended to present their dorsum to the intruder more often, keeping the eggs under the abdomen, suspended on the stylets. When the disturbance became too intense, they fled with the eggs. The broad and hard dorsum of the female's body is apparently a good defense against predation, and when that fails, escape with the eggs, or running off to distract and attract the predator when nymphs are in the nest were the most prevalent responses.

4.7 Kin Recognition and Nest Fidelity

We verified the earlier finding that females cannot distinguish their own eggs (Table 4.2; Filippi-Tsukamoto et al. 1995). Females exposed to three egg masses, one of which was her own, chose the one that was nearest. We also removed the egg

Table 4.2 Female discrimination of eggs

	Number of times that female chose own egg mass			
	0	1	2	3
Observed	2	4	3	1
Expected	2.96	4.44	2.22	0.37

$n = 10; \chi^2 = 1702, p > 0.05$
Adapted from Filippi-Tsukamoto et al. (1995)

masses from three females in the field, and each of the three tried to steal the egg masses from other nesting females, clearly not caring whether they were her own, just that she knew she was supposed to be guarding an egg mass. Again, it is reasonable that the ability to distinguish their own eggs from those of conspecifics has likely not evolved because females rarely leave the egg mass unattended.

We know that females cannot distinguish their own eggs, but they progressively provision nests, which means they must repeatedly leave and return to the nest. Therefore, we needed to verify that females were not simply entering any nest but that they could distinguish their own nymphs or nest. We observed the behavior of 17 provisioning females to verify whether they displayed nest fidelity; in other words, were females only provisioning their own offspring? Thus, in this study, nests were labeled, and the females in each nest were individually marked with liquid paint markers. Because we wanted to manipulate some nests to ascertain whether females possibly use chemical cues to find their nest, ten gravid females were allowed to nest on black mesh plastic gardening trays (56 cm L × 36 cm W × 8 cm H) that had been piled with soil and leaf litter similar to the conditions of the surrounding area. The trays were set in the field about one meter distant from each other. We also labeled nests and females in seven naturally occurring nests in the field. We observed the nests nearly daily throughout the reproductive season and documented the presence or absence of the female in the nest and, when a female was there, whether she was in her own nest. Females were sometimes absent from the nest, probably out foraging, and then were back in the nest at a later observation (Fig. 4.6; Filippi-Tsukamoto et al. 1995). In this study, we never observed a female in a nest that was not her own; thus, females have at least nest fidelity. However, in 1993, on a check of 18 natural nests being observed, 2 females were in a nest. The owner of the nest was one of the two. The other female appeared to be feeding on a drupe in the nest. See Sect. 6.4 for more about what we learned regarding this behavior.

To determine whether females were using chemical cues to locate their nest, rather than only other cues, such as celestial and magnetic, we also removed four females from their nests and set them on the ground about a meter away from the nest. While they were out of their nests, we switched the nests around, each one about one meter away from the original site, by moving the entire tray that the nests were in. By the next morning, three of the four females were in the correct nest at the new location, and the fourth one was never seen again (Filippi-Tsukamoto et al. 1995). It seems there is a chemical cue that the females use to recognize their own nest or nymphs that is effective from at least one meter away. See Chap. 6 for exciting information on how females successfully navigate the very long and rambling routes they take while provisioning.

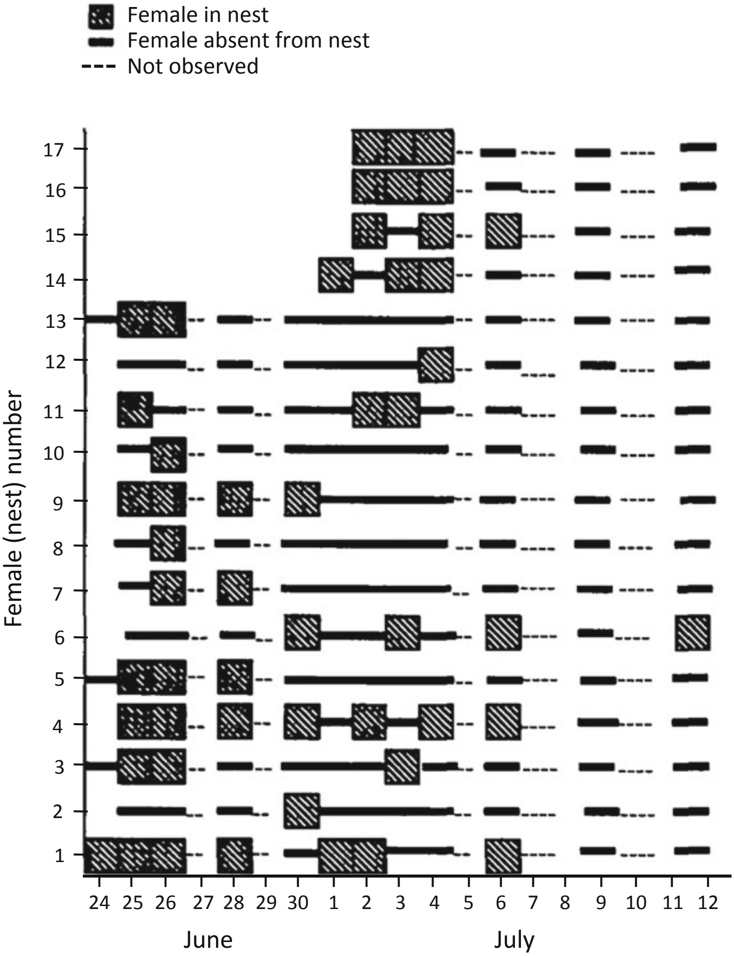


Fig. 4.6 Female nest fidelity. Females frequently left the nests to forage for drupes. Nests were observed for the presence (shaded squares) or absence (bold lines) of a female, and verification that when a female was present, it was the owner of the nest. Observations of a nest began on the date that the first icon is seen. Dotted lines indicate days of no observation. Adapted from Filippi-Tsukamoto et al. (1995)

4.8 Provisioning Behavior

Provisioning a food resource that the offspring could access soon after hatch (Wood 1976), either by preparing a substrate for feeding, such as mass provisioning a dung ball and laying eggs in it for the hatchlings to feed on in a single effort is the next level of care beyond guarding (Brussard and Visser 1987; Hunt and Simmons 1998).

A parent might also excrete substances as food (Becqueart 1935) or regurgitate substances that facilitate feeding for altricial young.

Progressive provisioning is a much rarer behavior in the insects. Burying beetle parents prepare carrion as a single mass for hatchlings to feed on, but they also repeatedly express partially digested carrion into the mouths of offspring (Eggert and Sakaluk 1995; Trumbo 1996, 2012). In the Reduviidae, males repeatedly transport prey items to their young (Ralston 1977; Machado and Trumbo 2018). Among phytophagous heteropterans, progressive provisioning occurs in *P. japonensis* and members of a related cluster of genera within the Sehirinae, a subfamily of the Cydnidae. Seeds or fruits are transported repeatedly to the nest either during the egg guarding phase and after hatch (*Adomerus triguttulus*, Nakahira 1992, 1994; *Sehirus cinctus*, Sites and McPherson 1982; Kight 1997) or after hatch only (*Adomerus rotundus*, Inadomi et al. 2014; *Canthophorus niveimarginatus*, Filippi et al. 2009; *Adomerus variegatus*, Mukai et al. 2010; and *Parastrachia japonensis*).

Of 43 nests with females tending an egg mass that were initially found and marked in 1993, only 18 could be observed through the early nymphal stage. Three of the females that had disappeared were later seen foraging for drupes in the foraging area, so we assumed they had relocated their nests during the egg stage. We never saw any of the other 22 females again. Provisioning females set out on foraging forays soon after hatch. It appeared that first instars were feeding on the eggshells, but, *more on that* in Sect. 4.17. Females left the nest and roamed for hours at a time inspecting drupes, and rejecting most, before returning to the nest. Frequently a female would return without a drupe, stay in the nest for a short time, and then head out again in search of a drupe. Nymphs often climbed onto the mother's dorsum and probed it with their proboscis, apparently in an attempt to feed on her. Females responded by rocking their bodies from side to side to shake them off. As we will see later in this section, at some point the females lost the ability to shake them off!

One foraging female was tracked for 2 h, during which time she covered 30 meters while inspecting ten drupes before finally accepting one for transport back to the nest. Another female that we observed for 2 h on each of 3 consecutive days spent between 15 min and 2 h before finding a drupe that was acceptable. Like all other females, once the drupe was taken up on the stylets, she zipped in a straight beeline directly back to her nest. As soon as the female deposited the drupe in the nest, the young gathered on it and fed, while the mother stood guard. She invariably stayed only about 5 min before taking off on another foraging foray. Often, nymphs would climb on her dorsum and attempt to feed on her. What was particularly interesting was that, while we observed her behavior for 3 days, on days 2 and 3 she continued to provision the nest, although the nymphs had already departed! In fact, in 5 of the 18 nests that had been observed up to the third stadium, drupes continued to increase in number for 1–3 days after the nymphs had abandoned the nest (Filippi-Tsukamoto et al. 1995)! In this obligate semelparous species, it appears that a switch is turned on at hatch taking the mother out of egg guarding mode and bringing her into provisioning mode. There apparently is no turn off switch to the provisioning mode. We frequently also saw mothers that had died either near their nest or somewhere along the path of their arduous provisioning task being fed on by aggregates of nymphs. We surmised that females just keep provisioning until they die because there is no question of conserving resources for future reproductive efforts. If they die near their nest, their body will continue to provide sustenance to their offspring, but we expect that many females die far from the nest, after giving their all to the provisioning effort, and are fed on by the offspring of other females.

4.9 Variation in Number of Provisioned Drupes and Nymphal Nesting Stage

One other interesting finding that surely could have some evolutionary import is that some females are better provisioners than others. There was considerable variation in the final number of drupes that females provisioned and this was positively correlated with the duration of the nymphal nesting stage (Fig. 4.7; Filippi-Tsukamoto et al. 1995). In 1993, among the 18 nests monitored until the nymphs left, the final number of drupes provisioned to nests ranged from 0 to 40 (mean ± S.D.,

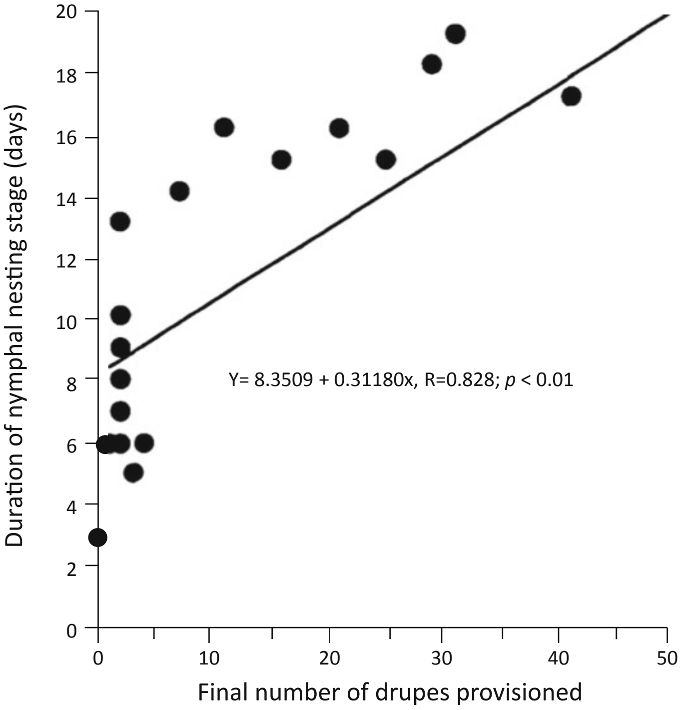


Fig. 4.7 Impact of the number of drupes provisioned on the duration of the nymphal nesting stage. Adapted from Filippi-Tsukamoto et al. (1995)

10.3 ± 12.7). Good drupes seem to fall in patches under the host tree, and finding a good patch was like striking gold. The female would return to the site repeatedly and bring lots of good drupes back to the nest. Some females apparently did not come across a good patch or might just have been bad foragers. We witnessed just such a situation. As diligent at searching as the poor provider was, she was only able to secure a few drupes. On the other hand, the female in a nest about 30 cm away (most nests are not this close together) was a much better provider. The poor provider returned to her nest one day to find that her offspring had moved over to the neighbor's nest. The neighbor ended up feeding twice as many young, and the original mother kept provisioning, albeit poorly, a nest with no young!

The variation in the number of drupes provided is even more stark among years. In 1994, the year represented in Fig. 4.5, only 8 of 11 nests contained any drupes at all; 7 of the 8 nests contained only 1 or 2 drupes, while the remaining nest contained 28! On the other hand, in 1992, many nests contained nearly 100 drupes, and 1 held the record thus far at a remarkable 155 drupes! As shown in Fig. 1.10, there is considerable variation in the abundance of drupes overall from year to year and, importantly, in the abundance of drupes that are acceptable to provisioning females. As will be demonstrated in Sect. 4.19, there is also considerable variation in the number of nesting females from year to year, and the intensity of inclement weather, and these factors also contribute to the success of provisioning females.

The duration of the nymphal nesting stage of the 18 nests monitored in 1993 also showed considerable variation, ranging from 5 to 19 days (mean ± S.D., 11.6 ± 4.8), and there was a positive correlation between the number of drupes provisioned and the duration that nymphs remained in the nest (Fig. 4.7; Filippi-Tsukamoto et al. 1995). Like the variation seen for provisioned drupe numbers over the years, variation in the average duration of the nesting stage was also great, and with the third stadium, which occurs at about 10 days after hatch, being the one that is correlated with young safely reaching the foraging area and feeding with nymphs from other nests, we considered nesting durations shorter than 10 days as being unsuccessful. In 1993, 10 out of the original 43 (23%) nests contained nymphs up to that point. In 1994, only 11/66 (16.7%) nests did so. On the other hand, in 1992, when so many drupes were provisioned, there were nymphs in nests that had reached the fifth instar. Within years and between years, variations seen in provisioning abundance and nesting duration are stark and suggest that several factors, including resource abundance, predator pressure, intraspecific competition, and weather extremes, were at play. We explore these possibilities in Sect. 4.19.

4.10 Effects of Drupes and Female Presence

The females were clearly providing food for the young, but we were interested in seeing separately how the presence of drupes and a tending female affected the behavior and survivorship of nymphs and also how the female's tending behavior and survivorship might change with the presence or absence of drupes. We could not

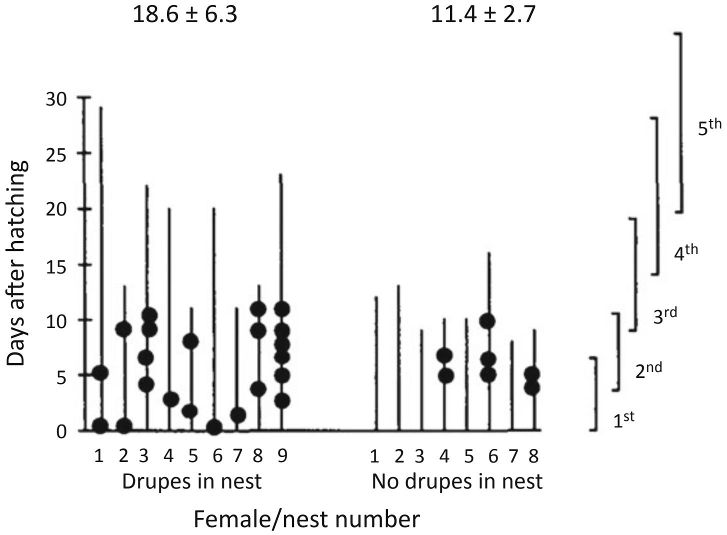


Fig. 4.8 The effect of drupe presence or absence in the nest on female behavior and life span. Life span of each female is represented by the vertical lines; black dots indicate female was in the nest at the time of observation. For comparison of female attendance in nest, Mann-Whitney U test, $p < 0.01$; for life span, Student t-test, $p < 0.05$. Adapted from Filippi-Tsukamoto et al. (1995)

control for predation effect with natural nests, so we carried out a semi-manipulated study by enclosing 40 gravid females in cylindrical chicken wire cages (40 h × 30 D cm) that were then covered with black netting and fastened at the top. The enclosures were set 8 cm into the soil at Mt. Hinokuma, which effectively excluded the worst predators, and were layered with the soil and litter that the females typically nest in. Females oviposited in the cages (no food provided, as they do not feed at this stage in the field), and the nest/cages were divided into four conditions upon hatch: female + nymphs + drupes in the nest, nymphs + drupes in the nest, female + nymphs, no drupes in the nest, and nymphs without a female or drupes. We observed the nests daily to see if the female was guarding her eggs/nymphs and monitored survivorship of the females and the nymphs. Some of the nests failed very early on and were not used in the study. In the two groups where females were present, females were observed in the nest 20.3% of the times checked in the group that had drupes ($n = 9$), but only 7.3% of the times checked when no drupes were in the nest ($n = 8$) (Fig. 4.8; Filippi-Tsukamoto et al. 1995). This suggests that females are more inclined to invest time guarding their nymphs when ample drupes are available but likely spend more time out searching for drupes when they are scarce, which would leave the nymphs vulnerable to predators for longer periods of time. Extrapolating this finding to the natural field, the data also suggest that nymphs would be more vulnerable to predation in years when drupes are scarce because females would be away from the nest for longer periods of time as they searched for drupes. Female life span was also significantly longer when drupes were

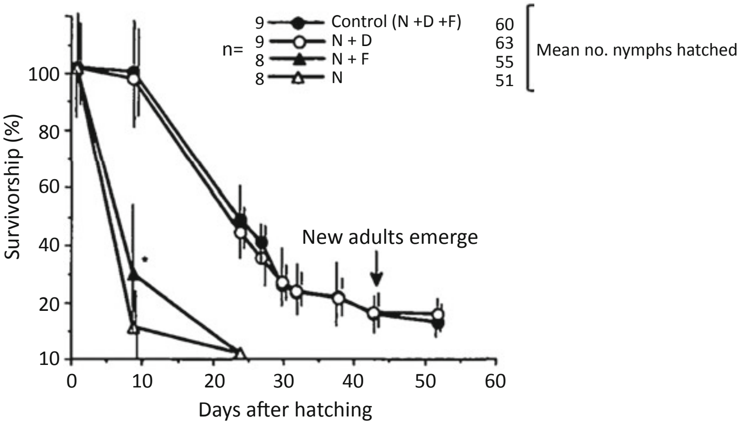


Fig. 4.9 Survival rate of nymphs under different nesting conditions. *N* nymphs; *D* drupes present; *F* female present. *Significant difference at day 10 between nymphs with and without the presence of mother, in the absence of drupes; Student's t-test, $p < 0.05$. Adapted from Filippi-Tsukamoto et al. (1995)

present in the nest, indicating that provisioning has a cost in reduced survivorship (Fig. 4.8; mean days ± S.D., 18.6 ± 6.3 with drupes, 11.4 ± 2.7 without drupes; Filippi-Tsukamoto et al. 1995). Though we have never seen a female feeding in the nest, the shorter female life span in the no-drupe group suggests that they do feed while out foraging for drupes.

Nymphs also had a greater tendency to remain aggregated when drupes or a female were present (70% of observations), and this tendency was enhanced when both drupes and a female were present (82% of observations) (Filippi-Tsukamoto et al. 1995). Feeding is more efficient in aggregations, desiccation is less of a problem, and certainly aggregations keep one safer from predators (dilution effect). Not surprisingly, survivorship of nymphs was also affected by the presence or absence of drupes in the nest (Fig. 4.9).

There was no difference in the survival rate of nymphs in the presence and absence of a tending female as long as food was provided (Fig. 4.9), and the body length of both males and females was the same in both groups (Filippi-Tsukamoto et al. 1995), suggesting that the female is not treating the drupes to facilitate feeding by the nymphs. However, these nymphs were protected from predators. This finding indicated that when nymphs had ready access to food and predators were excluded, they could manage to feed and survive. Interestingly, at day 10, nymphs with a tending female had significantly greater survivorship than those without a tending female (Fig. 4.9; Filippi-Tsukamoto et al. 1995). In the foraging area, cannibalism is rampant, and we suspect that females were mitigating cannibalism among the hungry nymphs.

4.11 Conclusions from This Study

So, what did we glean from these studies? We confirmed that females cannot distinguish their eggs from those of conspecific females (Table 4.2) but distinguish their nymph-containing nest from other nests (Fig. 4.6). We confirmed that females do not feed while incubating their eggs and found that they display several defensive behaviors to would-be predators (carabid ground beetle) (Table 4.1). Once nymphs hatch, females trade-off between guarding and provisioning behaviors. They repeatedly leave the nest to search for drupes but cannot always find an acceptable one, verifying that the resource is constrained. Provisioning provides nymphs with access to high-quality drupes that would be difficult to access (Figs. 4.8 and 4.9), particularly because most nests are far from the foraging area. Females never provision other nests but sometimes unwittingly provision nymphs that intrude into their nest. There is great variation in the number of drupes provisioned among females and among years, likely due to resource availability, competition with other females, and inclement weather. Duration of the nymphal nesting period is positively correlated with the number of drupes a female provides (Fig. 4.7). In this way, progressive provisioning is likely to protect nymphs from predation. Nymphs will abandon the nest if provisioning is too low (Fig. 4.8). Females continue to provision until they die, even after the nymphs have left the nest. Females invest more in guarding at the nest when drupes are abundant, and nymphs remain aggregated more when a female and drupes are present in the nest. Finally, females apparently mitigate cannibalism in the nest. The next set of studies sought to test some of these conclusions.

4.12 Testing the Speculated Functions of Progressive Provisioning

We will take a detour from the chronological presentation of our journey of discovery to maintain the continuity of this part of the story: the adaptive value of progressive provisioning. In Chap. 6, when we get into answering the proximal mechanistic and developmental "whys," we present a detailed description of how we came to understand why females spend so much time inspecting a variety of drupes before selecting one (Nomakuchi et al. 1998), but in order to maintain the flow of our story, suffice it to say here that we discovered most drupes on the ground, regardless of how enticing they appear (bright red, purple) do not contain much endosperm at all, which is the part of the drupe that *P. japonensis* feeds on, and provisioning females were choosing those drupes that contained a hefty amount of endosperm. However, knowing this does not necessarily mean that nymphs really require females to bring them good food, or even that it enhances nymphal success. In fact, as demonstrated in the last series of studies, a number of females, albeit a small percentage, actually nest where the drupes are scattered on the ground and are presumably accessible to nymphs after a bit of hunting. Yet, females in those nests also provisioned them. So, we tested the effect of provisioning from the resource constraint hypothesis. We also knew that ground beetles were predators and concluded that the mother's defensive behaviors and provisioning behavior, which kept the vulnerable nymphs in the safety of the nest longer, were adaptive but had not tested it. In the reproductive seasons of 1995 and 1996, we set out to test these hypotheses.

4.12.1 Testing the Resource Constraint Hypothesis

We tested the hypothesis that, because the resource is of such poor quality, progressive provisioning is essential for nymphs to acquire sufficient nutrients, even when the nest is situated among the scattered drupes of the average (typical) quality in the field. In 1995, there were very few nesting females to be found, but we managed to collect 13 females with an egg mass. To exclude the potential effect of predators, we prepared square cages made of a PVC pipe frame (50 cm^2 × 20 cm h), covered on all sides except the bottom with black mesh. A square opening (10 cm^2) at the top, fastened with Velcro, allowed access to the inside of the cage. The cages were set ~7 cm into the ground in a gently sloped area in the forest at Mt. Hinokuma that had been cleared of groundcover (~10 m^2) (Filippi et al. 2000). We monitored the number and quality of drupes that were falling from the host tree that year using a seed trap made by forming a 1 m^2 section of black mesh into a triangle and suspending it on three 1.2 m garden poles. The seed trap was set under the crown of the main host tree for that area, and drupes that fell into it were counted and their quality assessed on a weekly basis. We used this information to determine the number and type of drupes to seed the cages with. We divided the cages into two groups, one that was provided with typical drupes that were falling in the area and one that was provided with only high-quality drupes. Neither of the groups had the drupes placed inside the nest. Both had them scattered about outside the nest, as if the nest were situated under the host tree, in the same density that was occurring under the host tree. To eliminate the possibility that the female is facilitating feeding or providing some other assistance in the nest, females were removed at hatch. Based on the data from the seed traps, we gradually increased the number of drupes in each cage to 25 by day 15 after hatch. We monitored the development rate and the survivorship of offspring in the two groups.

Drupe Availability

In 1995, the date that good drupes began falling was much later than the date that females were seen out in the foraging area searching for drupes to provision their nests with (Fig. 4.10; extrapolated from Filippi et al. 2002). There were large fluctuations in the numbers of drupes collected, likely due to heavy rains that occurred during the rainy season, which typically lasts throughout most of the

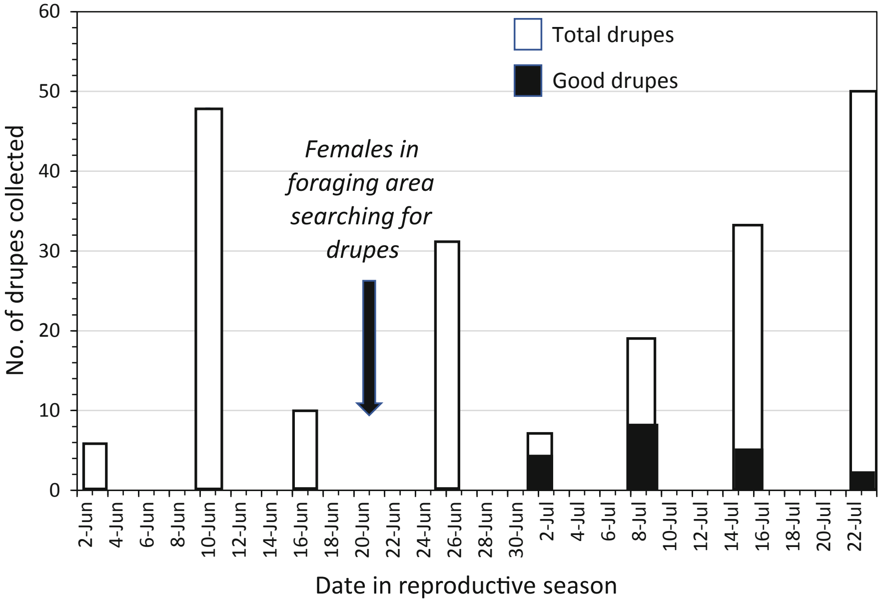


Fig. 4.10 Drupe abundance at the experimental site in 1995. Based on data presented in Filippi et al. (2002)

reproductive season, and a very low percentage of those drupes were good, ranging from 0 to 57%. Clearly in 1995, resource was poor (see Fig. 1.10 for comparison of drupe quality over 4 years).

Development Rate

The behavior of nymphs in the two groups differed considerably. Nymphs in the good drupe groups were seen outside the nest feeding on drupes in aggregations from day 2 after hatch, while those in the typical drupe group were scattered about the cage on day 2, with a few individuals on each of several different drupes. Successful feeding by nymphs requires that they aggregate in large numbers on a drupe and simultaneously inject the enzyme that liquefies the stony endosperm (Tachikawa and Schaefer 1985; Tsukamoto and Tojo 1992), so it is unlikely that feeding in that dispersed way would be successful. There was a significant difference in the development rate between the two groups. The duration of the first and second stadia was shorter for nymphs in the good drupe group ($n = 7$), and they molted to the second stadium significantly earlier than those in the typical drupe group ($n = 6$) (Fig. 4.11a; Mann-Whitney U test, $p = 0.001$; Filippi et al. 2000).

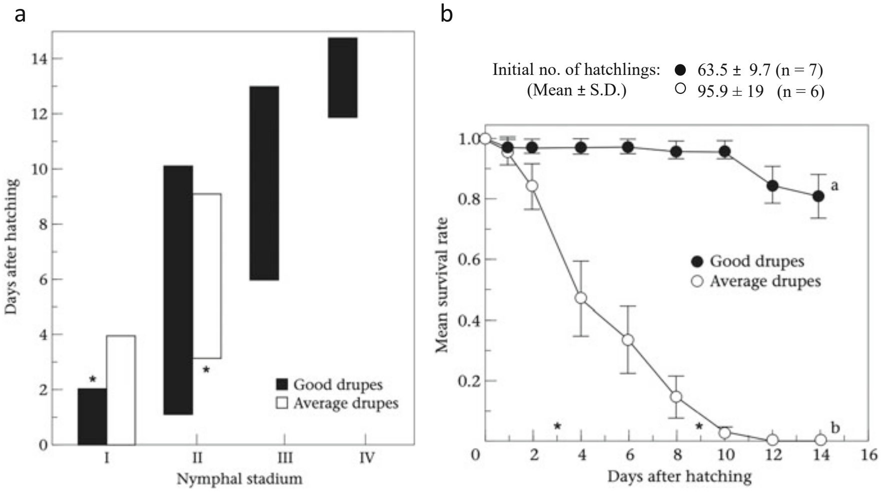


Fig. 4.11 Impact of drupe quality on success of *P. japonensis* offspring. (**a**) Development rate. *, Mann-Whitney U test, $p = 0.001$; (**b**) survivorship. *, Significant difference from days 2–4 and 8–10, $p = 0.012$ and 0.047, respectively. Different letters indicate significant difference, Mann-Whitney U test, $p = 0.003$. Adapted from Filippi et al. (2000)

Survivorship

There was also a significant difference in survivorship between the two groups between days 2–4 and 8–10 (Mann-Whitney U test, $p = 0.012$ and 0.047, respectively), and at day 14, by which time all nymphs in the average drupe group had died ($p = 0.003$; Fig. 4.11b; Filippi et al. 2000). Clearly, the data support the resource constraint hypothesis. The quality of most drupes is typically so poor that provisioning not only enhances offspring success but also is essential, *at least* when drupe quality and abundance are similar to that encountered in 1995. We predict that the data would look quite different in years when the proportion of good drupes is very high and their availability coincides with the presence of foraging females.

4.12.2 Testing the Predation Hypothesis

Using cages like the ones used in the previous experiments, in 1996 we tested the hypothesis that provisioning mitigates predation risk to nymphs. We set up 25 cages at the same site that was used in 1995. The cages were divided into five groups ($n = 5$ per group) based on whether a female was present, whether a predator (ground beetle, *Apotomopterus dehaanii*) was present, and whether the drupes, *all good drupes*, were placed inside the nest or scattered around the outside of the nest, as done for the previous experiment (Table 4.3; Filippi et al. 2000). One cohort from each group except Group D failed very early on from death of the female or loss to

Table 4.3 Experimental design for test of Predation Hypothesis

Group (n)	No. of nymphs Mean ± S.D.	Female	Predator	Drupes
A (4)	63.3 ± 3.3	Present	Absent	Outside nest
B (4)	33.0 ± 10.5	Present	Present	Outside nest
C (4)	55.4 ± 15.3	Absent	Present	Inside nest
D (5)	45.0 ± 17.1	Absent	Present	Outside nest
E (4)	45.3 ± 7.0	Absent	Absent	Outside nest

Adapted from Filippi et al. (2000)

flooding, so all groups but D had four cohorts; D had five cohorts. Survivorship of nymphs was assessed over the first 10 days of the nesting period, after which young are typically in the much less vulnerable third stadium and begin moving on to the foraging area.

The survival curves of Groups A, B, and C were similar until day 6 with a survival rate of 70% (Fig. 4.12a; Filippi et al. 2000). Groups A and B had a female present, but group B also had a predator. Good drupes were scattered outside the nest in both groups. There was no difference in survivorship between these two groups throughout, indicating that female presence effectively mitigates predator threat beyond the provisioning of drupes, which keeps the young in the nest. Group C was provisioned by the researchers, which kept the nymphs safely aggregated in the nest, but in the absence of a tending female, the predator, enclosed in the cage, was eventually able to find the nymphs. By day 10, the survivorship in Group C was significantly lower than that of Groups A and B, reduced to 20%, which was about 30% lower than Groups A and B (comparison between Groups A and C, ANOVA, $p = 0.0005$, Bonferroni $p = 0.01$, and between Groups B and C, ANOVA, $p = 0.0005$, Bonferroni $p = 0.006$).

We examined the effect on survivorship of the mother's presence (by excluding her) in terms of food access and protection from predators. We compared Groups D and E, neither of which had a mother present, both of which had drupes outside the nest, but Group D had a predator, and Group E did not. By day 1, the survivorship of Group D was significantly lower (20%) than that of Group E (ANOVA, $p = 0.013$, Bonferroni, $p = 0.009$; Fig. 4.12b). By day 10, the difference in survivorship between the two groups was 42% (ANOVA, $p = 0.0005$, Bonferroni, $p = 0.0001$).

Finally, to confirm the mother's role in protecting young from predation, we compared the effect on survivorship of young in nests without a female but with a predator present when drupes were inside the nest (provisioned, Group C) or scattered outside (Group D). By day 2, the group with drupes outside the nest had significantly (33%) lower survivorship than the group that had been artificially provisioned. However, by day 6, the ground beetle had clearly found the nest, and the survivorship began to decrease markedly, with no difference in survivorship between the two groups by day 10. Clearly, the role of the female in protecting young from predators is significant, even when they do not have to leave the nest to feed.

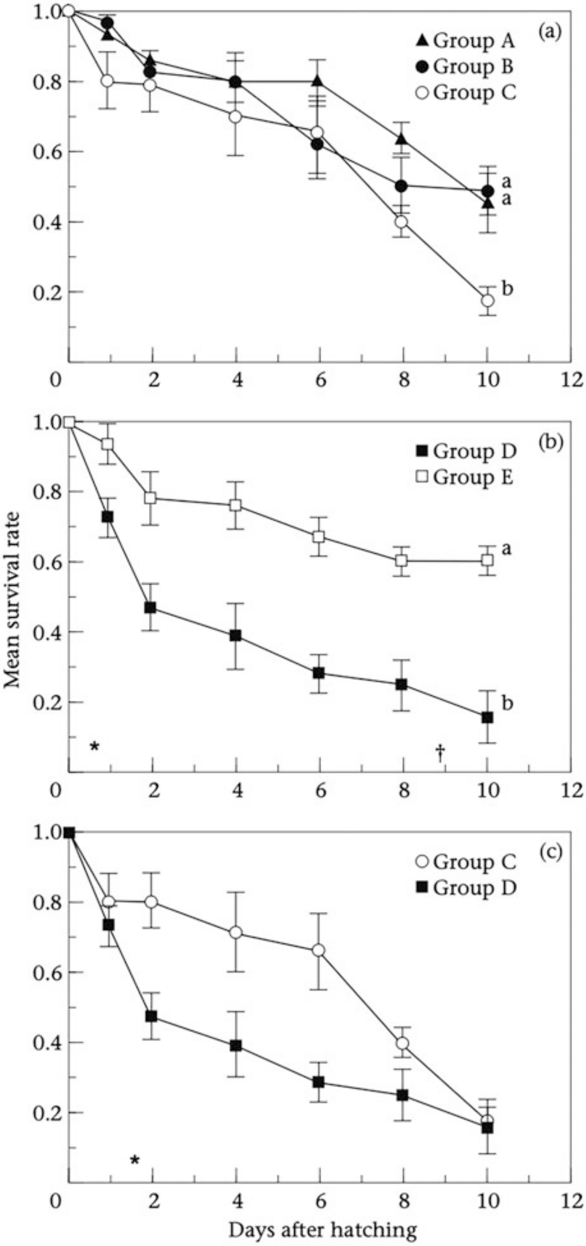


Fig. 4.12 Survival curves of provisioned and unprovisioned nymphs in the presence or absence of a predator and/or a female. (**a**) Comparison of groups A, B, and C. Different letters indicate significant differences in final survival rate at $P < 0.01$ in pairwise comparisons by a one-way ANOVA with a post hoc Bonferroni adjustment for multiple comparisons. (**b**) Comparison of groups D and E. *Mortality rate for the interval showed significant difference at $P = 0.008$; †marginally significant difference at $P = 0.019$ by pairwise comparisons using a one-way ANOVA with a post hoc Bonferroni adjustment for multiple comparisons. Different letters indicate significant difference in final survival rate at $P = 0.0001$. (**c**) Comparison of groups C. *Significant difference in mortality rate for the interval at $P = 0.002$ by a one-way ANOVA with a post hoc Bonferroni adjustment for multiple comparisons. Error bars indicate SE. Adapted from Filippi et al. (2000)

4.13 Conclusions from These Studies

Two assumptions about the adaptive significance of progressive provisioning were tested and resolved. The hypothesis that females provision because the resource is constrained was supported. Drupe availability is ephemeral, and, when available, it is scarce (Fig. 4.10). Moreover, as was the situation in 1995, the availability of resource does not always coincide with the timing of need. These resource constraints should strongly select for subsocial behavior, such as progressive provisioning, so that young might receive better access to the poor food resource (Tallamy and Wood 1986; Eggert et al. 1998). In the condition simulating a nest situated right in the middle of the foraging area, development and survivorship of the nymphs were high when only good drupes were scattered outside the nest but decreased significantly when nymphs were forced to find good drupes on their own from among the many drupes scattered on the ground (Fig. 4.11), verifying the essential role of a progressively provisioning mother to give young access to the scarce good drupes.

The predation hypothesis was also supported. Even with good drupes in the nest, in the absence of a tending female, young eventually succumbed to predation (Fig. 4.12). As long as a provisioning female was present, survivorship in the presence or absence of a predator was the same but decreased significantly when the female was absent (Fig. 4.12a). Further, as long as no predator was present, survivorship was high even if the *good* drupes were only scattered about outside the nest (Fig. 4.12b), but with a predator present and no female to protect the young even when drupes were placed inside the nest, the predator eventually found the young (Fig. 4.12c). The findings that parental care mitigates predator pressure in *P. japonensis* are consistent with findings in numerous other species (Wilson 1971; Tallamy and Wood 1986; Kudo et al. 1989; Wyatt and Foster 1989; Diesel 1992; Royle et al. 2012). These studies demonstrated that progressive provisioning provides both essential access to the food resource and vital protection from predation.

4.14 Predation Risk to Nymphs of Different Stages

Knowing that *P. japonensis* is preyed upon by the ground beetle and that there is variation in the stadium that nymphs vacate the nest left us with another question that begged to be answered. The assumption was that younger nymphs would be more vulnerable to predation, and we know that staying in the nest with mom keeps them safe, yet they do emerge at all different stages and head to the foraging area. In 1998 we tested whether nymphs of different stages had different levels of vulnerability to predators. To understand the natural pattern of when nymphs relocate to the foraging area, we counted the numbers of nymphs in each stadium in a 42 m^2 quadrat of 1 m^2 plots in the foraging area daily from June 15 to July 9. Although, as previously noted (Filippi-Tsukamoto et al. 1995), the third stadium seemed to be the time that nymphs

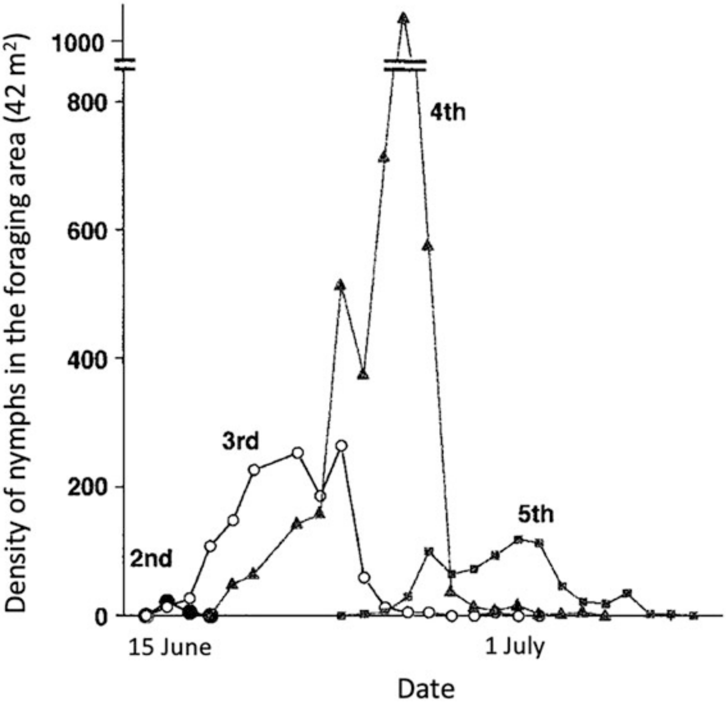


Fig. 4.13 Nymphal occurrence pattern in the foraging area. Adapted from Nomakuchi et al. (2001)

started leaving the nest, 3.6 times as many of the nymphs on the ground were fourth stadium compared to third (Fig. 4.13; Nomakuchi et al. 2001) indicating that most stay in the nest until the end of third stadium. We were surprised to note that even second instars were seen in the foraging area, though in very small numbers. We suspect these are the offspring of poor provisioners and that the variation in stadia that relocate to the foraging area is also related to the provisioning success of their mothers.

The very low number of fifth instars represented in the graph is likely related to the fact that we only counted individuals in the quadrat, and fifth instars tended to spend a considerable amount of time (~ 10 days or so) in aggregations on the lower trunks/branches of trees adjacent to the foraging area and were not counted.

We anticipated that offspring would leave the nest and relocate to the foraging area at a stadium that is less vulnerable to predation. Therefore, we tested the hypothesis that stadia occurring in high abundance in the foraging area would be less vulnerable to predation. We set up 12 square cages (50 × 50 × 25 cm^2) covered in fine black mesh in the field as described for the previous studies. To simulate the conditions of the foraging area where leaf litter and ground cover is sparse, a small pile of leaves was set in one corner of the cage where nymphs could hide, and the rest of the cage was left largely bare. Drupes were scattered on the bottom of the cage in the density that occurred under the host tree; however, to minimize the confounding

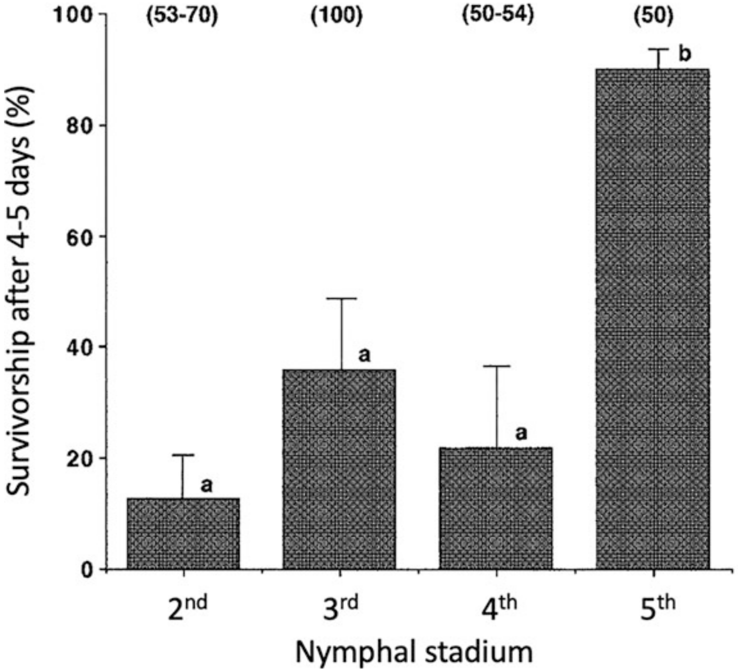


Fig. 4.14 Survivorship of *P. japonensis* nymphs at different nymphal stadia in the presence of a predator, the carabid ground beetle, *Apotomopterus dehaanii*. Adapted from Nomakuchi et al. (2001)

variable that typical (poor) drupes would have on survivorship, only good drupes were used. A nest containing cohorts of second, third, or fourth stadium nymphs was placed under the leaf litter in the corner of each cage, and one ground beetle (*Apotomopterus dehaanii*) was released into each cage. Three replicates were done for each nymphal stadium, and survivorship was assessed after 4–5 days. Although fourth stadium nymphs were most abundant on the ground in the foraging area, there was no difference in survivorship between second, third, and fourth stadium nymphs; only fifth stadium nymphs had significantly higher survivorship (Fig. 4.14; ANOVA, $p = 0.004$, Bonferroni, $p < 0.05$; Nomakuchi et al. 2001).

The optimal duration of the nesting stage of immature insects with regard to predation will vary with the degree of mobility of the young at different stages, in other words, their ability to evade predators. The young of several subsocial holometabolous species with parental care, such as the burying beetle, *Necrophorus* (Eggert and Müller 1997), and the dung beetles, *Copris*, *Synapsis*, and *Heliocopris* (Halffter 1997), often leave the nest just before pupation or after eclosing as adults because the larval stage has far less mobility than the adult stage, and so is much more vulnerable to predation.

The pattern of nymphal occurrence in the foraging area is a manifestation of both direct and indirect effects of predation (Nomakuchi et al. 2001). In other words, there is predation pressure acting directly on the independent nymphs which results in the observed distribution pattern of nymphs in the different nymphal stadia. Because the data showed that second–fourth stadium nymphs have equal predation pressure, the earlier a nymph leaves the nest, the longer it is exposed to the predation pressure and the less likely it is to survive. In fact, the actual number of nymphs that we were able to count might be far less than the numbers that emerged, because many more of the youngest, most vulnerable ones could have been eaten.

The indirect predation effect would occur over evolutionary time and would form the distribution of nymphal stadia at the time of independence. This effect would work primarily on female fitness traits, specifically provisioning behavior in this case, because if predation pressure on younger nymphs outside of the nest is higher than that inside the nest, selection should favor a pattern of parental care that results in nymphs becoming independent at a later stadium or even at the adult stage. In fact, the survival rate of *P. japonensis* nymphs *is* higher inside a nest than outside (Filippi et al. 2000), and there is a positive correlation between the number of drupes provisioned and the duration of the nymphal nesting stage (Filippi-Tsukamoto et al. 1995), so we would expect selection to favor prolonged provisioning in this semelparous species. However, the intense constraints on resource availability (Fig. 1.10; Filippi et al. 2002) confound female efforts and success at provisioning.

4.15 Impact of Female Provisioning Capacity on Duration of Nymphal Stage

While the interactions between parents and offspring have long been a focus of parental care studies (Clutton-Brock 1991; Mock and Parker 1997), interest in the evolutionary basis of parental care has shifted from a static concept focusing on optimal behaviors from the parent's perspective (Lack 1947) to a more dynamic understanding that a conflict must exist between parents and offspring and that conflict will determine the manner and duration of parental care (Godfray 1995). Subsocial behavior in invertebrates has been the focus of numerous studies (Choe and Crespi 1997), but most often from the perspective of parental optimality. Few studies have examined the conflict from the perspective of offspring response to the parental care they receive (Wyatt and Foster 1989; Agrawal et al. 2001).

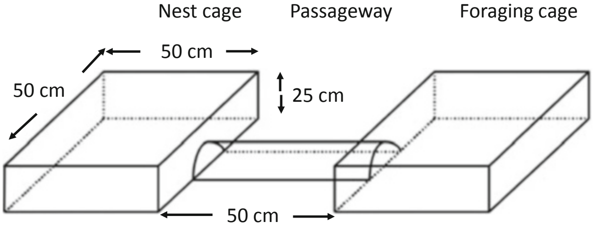
Typically, when a parent provides care, in particular, supplies food, to offspring, a conflict arises between parent and offspring because for the parent the optimal timing for offspring transition to feeding independence comes earlier than that of offspring, who want to stay in the nest and receive food longer (Trivers 1974; Leigh and Smiseth 2012). However, in the case of *P. japonensis*, one can almost consider that the conflict runs in the reverse direction. Because early departure from the nest leads to dramatically increased predation risk (Filippi et al. 2000; Nomakuchi et al. 2001), and, importantly, females have no option of future reproductive success, female fitness would be optimized by provisioning enough food to keep the young in the nest until the adult stage as the nymphs should leave the nest when food becomes insufficient. Some hungry young insects like larvae of the burying beetle (Eggert and Müller 1997) and dung beetles (Halffter 1997; Sato 1997) remain in the "nest" until the adult stage, completely dependent on food being provisioned by the parent, and larval begging results in delivery of more food (Smiseth and Moore 2002). The behavior described earlier of *P.* japonensis offspring attempting to feed on the mother in the nest (Filippi et al. 2000) effectively acts as begging behavior because the female aggressively rocks her body to shake the young off and makes a chattering alarm call and then quickly leaves the nest to forage once again. However, as we have discussed (and will explain in detail in Chap. 6), good drupes are scarce and hard to find, and foraging mothers, notwithstanding their diligent efforts, aren't always successful. So how does provisioning capacity by *P. japonensis* mothers relate to duration of the nymphal nesting period?

Although we observed in an earlier study that there was a positive correlation between the number of drupes provisioned and the duration of the nymphal nesting period, a correlation, of course, is not cause and effect. Other factors, for example, safety against predators, could be keeping the nymphs in the nest. To sort this out, we decided to test the hypothesis that the provisioning capacity of females was directly responsible for the longer nymphal nesting period. Between 1996 and 2000, we carried out three field studies from mid-June through late July to (1) determine the beginning of nest departure (1996); (2) examine the relationship between the duration of the nesting period, i.e., from hatching to departure from the nest and the provisioning effort of the mother (1998); and (3) examine the rate of nymphal accession to the foraging site after departing from the nest (2000). All studies were carried out at our original field site in Mt. Hinokuma Prefectural Park in Kanzaki town, Saga, Japan.

4.15.1 Experiment 1: Determining the Timing of Nymphal Independence

To determine the point at which departure from the nest ensues, in 1996 we constructed a 50 cm^2, open-bottom mesh-covered nest cage as described in the earlier studies and set it into the ground. For each nest cage, we constructed an identical cage and set it into the ground 50 cm away from the nest cage. This was the foraging cage, meant to simulate the foraging site, where drupes were supplied. We connected the two cages with a mesh-covered passageway so that nymphs could leave their nest, which had been set in the nest cage, and freely move to the foraging cage (Fig. 4.15; Nomakuchi et al. 2005). The bottom of the nesting cage was layered firmly with soil and then with leaf litter, similar to the nest sites in the field. The base of the passageway was a wooden board, and the bottom of the foraging cage was a white Styrofoam board to facilitate observation of the drupes. A female and her egg mass were placed into each of ten nesting cages. Inspection of the cages was made

Fig. 4.15 Experimental field cage to assess timing of nymphal departure. Adapted from Nomakuchi et al. (2005)



daily. After hatch, the female could leave her nest and enter the foraging cage to provision her nest. Throughout the second stadium, nine drupes were evenly placed in the foraging cage. Each drupe that a female removed from the foraging cage was replaced at the time of daily inspection. Of note, larger nymphs within a cohort seemed to access the drupes females provisioned more easily, excluding the smaller nymphs. This apparently led to the large range in the duration of a given stadium. At the end of the second stadium, the number of drupes in the foraging cage was increased to 18. The first time a nymph was observed in the foraging cage was considered the start of nymphal departure from the nest. Nymphal hatch was synchronized within a clutch. However, even under the minimally constrained conditions of this field experiment (no predation and guaranteed access to food), the average number of offspring ± S.D. decreased steadily from hatch ($n = 10$, 68.8 ± 27.7) to first sign of independence ($n = 10$, 45.6 ± 28.6) to emergence of first adults ($n = 8$, 27.9 ± 24.1) for a final average decrease of over 60%. The duration of first, second, third, fourth, and fifth nymphal stadia, determined by >50% of individuals molting to a given stage, was 2, 2, 4, 4, and 14 days, respectively (Nomakuchi et al. 2005). Nymphs remained in the nest feeding on provisioned drupes for several days after hatch and began to walk around the outside of, but remained near, the nest apparently when the drupe level became insufficient. Eventually they left the nest and made their way over to the foraging cage. Once in the foraging cage, they apparently did not return to the nest, though some apparently returned to the nest cage, and aggregated in the corner. Those in the foraging cage aggregated on drupes and fed.

Most cohorts began departing from the nest cage and relocating to the foraging cage at the fourth nymphal stadium (Fig. 4.16; Nomakuchi et al. 2005). However, in a cage where the mother had died during the late second/early third stadium, the nymphs left the nest during the third stadium. On the other hand, in another cage where the mother had died early, nymphs remained until the adult stage, probably because the mother had already provisioned many drupes. All females in this experiment had equal access to drupes, so clearly there is intra individual variation in the provisioning capacity that has nothing to do with resource availability. Once independence began, the average percentage ± S.E. of nymphs that had relocated to the foraging cage was 12.8 ± 9.4% on the first day and increased to 36.6 ± 27.9% on the second day after initiation of independence. As reported in Filippi et al. (2000), nymphs had relocated to the provisioning area, and, in fact, provisioning always continued until the death of the female (Nomakuchi et al. 2005); however, there was

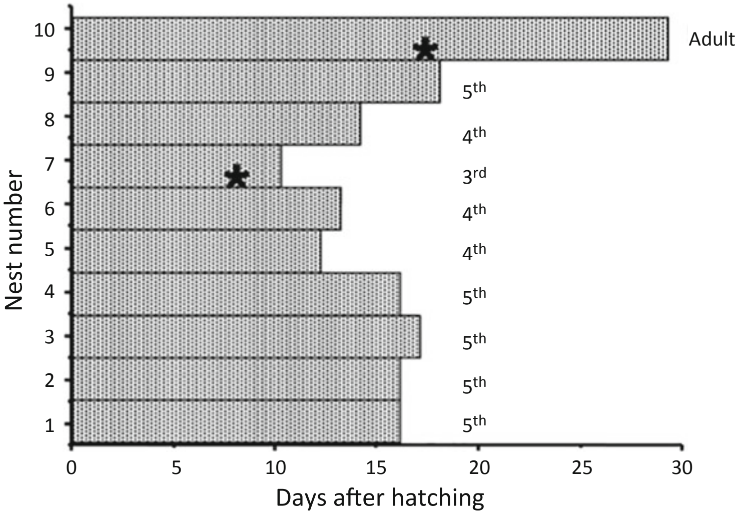


Fig. 4.16 Duration of nymphal nesting period. Stadium at the start of relocation from the nest to the foraging area is indicated on the right of each bar. Asterisks indicate death of the females that died before the start of nymphal independence. All other females died after nymphal independence. Adapted from Nomakuchi et al. (2005)

no significant difference between the duration of the nymphal nesting period and the duration of female provisioning (Wilcoxon test, $T = 9$, $n = 9$, $P > 0.05$).

To test our prediction that a progressively increasing number of drupes per day would be required to sustain nymphal development and that once the amount provisioned failed to be sufficient, the nymphs would depart from the nest an index of average provisioning rate (IAPR) was calculated. IAPR is a sequential daily calculation of the total number of drupes provided per cumulative number of nymphs in the nest until the day the first nymphs departed the nest for the foraging cage. The index values were subjected to a randomization test for evaluation. The IAPR value was positively correlated with the duration of the nymphal nesting period (Fig. 4.17; Pearson correlation coefficient, $r = 0.89$, $n = 9$, $p < 0.01$). However, it is possible that there was a spurious correlation between duration of the nymphal nesting period and IAPR because IAPR values tended to increase over days as a result of increasing provisioning frequency and decreasing number of nymphs. Therefore, an exact randomization test was performed to confirm the validity of the correlation. We were able to verify that departure from the nest cages began significantly earlier in nests that had fewer drupes provisioned ($p = 0.0421$; Nomakuchi et al. 2005).

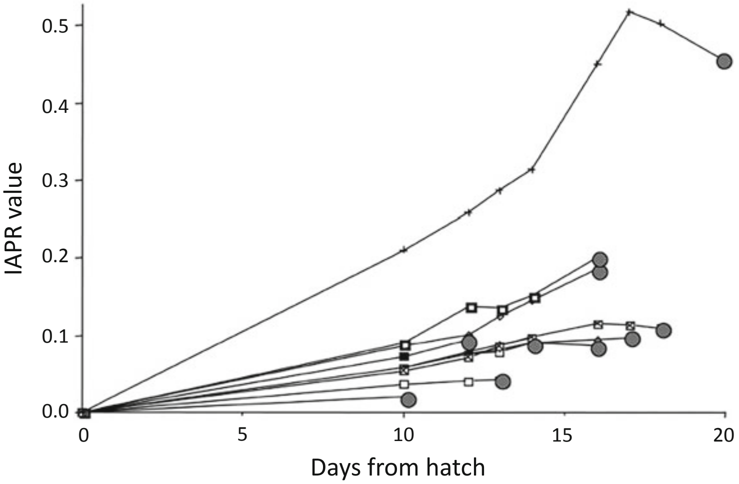


Fig. 4.17 Impact of maternal provisioning of drupes on duration of nymphal nesting stage. Each line represents the pattern of a different nest. Terminal point of line indicates beginning of nymphal independence. See text for explanation of IAPR value. Pearson correlation coefficient done for terminal points only. $N = 9$, $r = 0.89$, $p < 0.01$. Adapted from Nomakuchi et al. (2005)

4.15.2 Experiment 2: Impact of Provisioning Capacity on Timing of Nymphal Independence

To confirm the hypothesis that female provisioning capacity is positively correlated with the duration of the period prior to nymphal departure from the nest, we put females with an egg mass into a nest cage as in Experiment 1. We removed the mothers at the second stadium to avoid the possibility that another aspect of female care was affecting the duration of the nesting period prior to independence and provided the nymphs with poor or rich resource abundance in the nest. The number of drupes placed in the nests daily was regulated according to the number of nymphs in the nest each day using the IAPR values calculated in the previous Experiment. The number of drupes in the rich group was maintained at about four times the number in the poor resource group. The average number of days until departure from the nest in the poor resource group ± S.D. was 12.2 ± 2.7 days ($n = 6$), while the corresponding number of days in the rich resource group was 18.0 ± 3.1 days, $n = 5$. The duration of the nymphal stage prior to departure from the nest was significantly longer in the rich resource group (Fig. 4.18; Mann-Whitney U test, $p = 0.026$; Nomakuchi et al. 2005). Clearly, nymphs in nests with females that provision more drupes will depart for the foraging site later than those that are provisioned with fewer drupes.

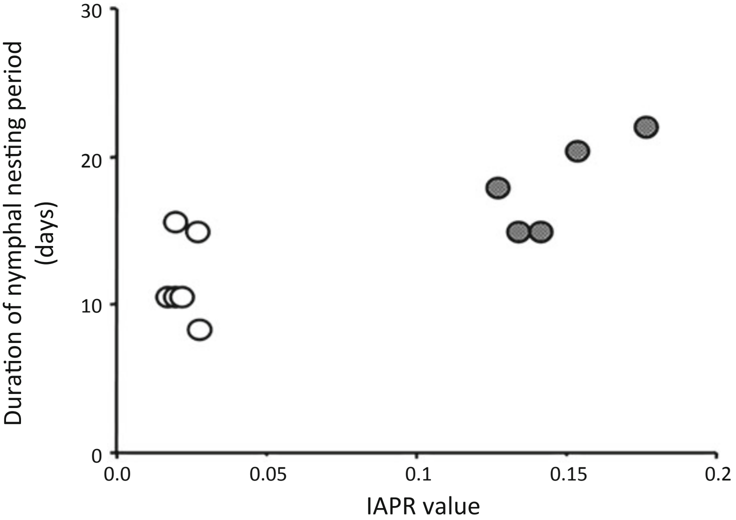


Fig. 4.18 Impact of provisioning level on duration of nymphal nesting period. Nymphs received either poor (open circles) or rich (shaded circles) provisioning. Females were removed at the second stadium for both conditions. Adapted from Nomakuchi et al. (2005)

4.15.3 Experiment 3: Distance and Success Rate of Nymphal Instars Reaching the Foraging Area

Finally, to determine how nymphal stadium and distance from the foraging area impact success of nymphs accessing the foraging area, in 2000 we reared nymphs with drupes in the laboratory at 25 °C from the egg stage to the second, third, and fourth nymphal stadia. The young nymphs were too soft to mark, so to ensure that any nymphs we encountered in this study were from among the ones released, we established an artificial foraging area more than 10 m from any host tree or subpopulation of *P. japonensis*. We placed 50 drupes in a 1 m^2 plot and then released 40–50 second, third, and fourth stadium nymphs either 1 m or 5 m from the "foraging site." We checked the number of nymphs that had reached the "foraging area" daily and removed them to avoid double counting. We repeated the trials five times for the second stadium nymphs and ten times for third and fourth stadium nymphs. We continued each trial until no more nymphs entered the "foraging area." There was a significant increase in success rate of nymphs as the stadia progressed (Fig. 4.19; Two-way ANOVA, $p < 0.001$; Nomakuchi et al. 2005). The success rate was also lower for nymphs released from further away; however, the combined effects of age and distance were not significant. Younger nymphs clearly have a much lower likelihood of successfully reaching the foraging area, particularly because many nests are located more than 5 m from the foraging area.

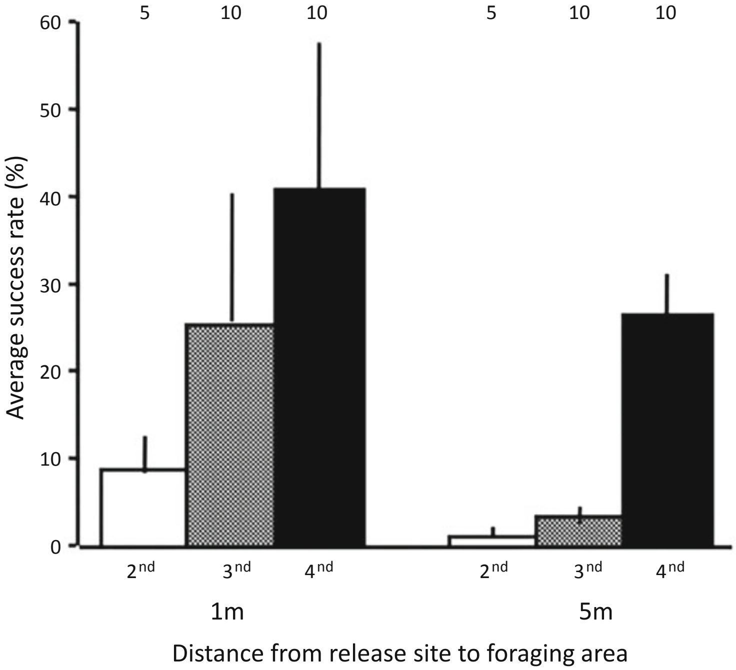


Fig. 4.19 Impact of distance from the foraging area on the success rate of different instars reaching it. Numbers above bars represent number of trials. Error bars represent S.D. Adapted from Nomakuchi et al. (2005)

4.16 Conclusions About the Impact of Provisioning on the Nymphal Nesting Period

The first series of experiments showed that, when excluding fifth instars who tend to aggregate on tree trunks, by far the greatest number of nymphs in the foraging area was in the fourth stadium (Figs. 4.13 and 4.16), suggesting that nymphs at that stage can succeed outside the nest much better than younger nymphs. On the other hand, the very low number of second and third instars in the foraging area suggests that when food becomes insufficient in the nest, they will be forced to leave. However, while only fifth instars seemed to be considerably less vulnerable to predation and fourth instars suffered equal predation rates to second and third instars (Fig. 4.14), the longer nymphs are exposed to predators during the vulnerable stages (second–fourth stadia), the more likely it is that they will succumb to predation before reaching the fifth stadium. Thus, nymphs leaving at the fourth stadium stand a far greater chance of reaching the fifth stadium and the adult stage than those leaving at the second or third stadium.

We have also demonstrated that the duration of the nesting period is positively correlated with the number of drupes that a female is able to provide (Figs. 4.17 and

4.18); however, because good drupes are scarce, the mother is not always able to provision enough to meet their needs so they will be forced to leave at a vulnerable stage. Moreover, the distance to the foraging area is negatively correlated with successful ability to reach it for all stadia, but the impact of distance is far greater for second and third instars than fourth instars (Fig. 4.19) who are much more mobile and better able to navigate the rough terrain as they make their way to the foraging area which can be up to 12 m away from the nest. It is a game of deadly trade-offs for nymphs who must balance the risk of starving in the nest, or being cannibalized by hungry siblings there, against the threat of predation and other physical hazards awaiting them outside the nest when they leave for the foraging area!

4.17 Provisioning of Nutrition *Before* Provisioning Drupes: Production of Trophic Eggs

We have mentioned the observation of newly hatched nymphs feeding on the egg cases (Filippi-Tsukamoto et al. 1995). Another observation that warranted explanation was that some eggs in *P. japonensis* egg masses invariably failed to turn pink, develop eyespots, and hatch. We decided to investigate whether females were in fact producing trophic eggs to feed their newly hatched offspring. Trophic eggs are eggs that are produced as a food for young, and young feeding on them is not considered oophagy or cannibalism because the mother has deliberately produced them for the express purpose of enhancing offspring development. Trophic egg production represents an extreme version of food caching that allows mothers to progressively provision young as a supplement to other food sources (Alexander 1974; Polis 1981, 1984; Mock and Parker 1997). Prior to our first study on trophic eggs in *P. japonensis*, production of trophic eggs had been documented in a variety of insect species but most commonly, by far, in the Hymenoptera (Wilson 1975; Polis 1981; Kukuk 1992; Choe and Crespi 1997; Iwanishi et al. 2003). Trophic eggs had also been reported in a few subsocial species, including a burrower cricket (*Anurogryllus muticus* DeGeer; West and Alexander 1963) and a cydnid (*Adomerus triguttulus* Motchulsky), a relative of *P. japonensis* with similar types of extensive parental care behaviors (Nakahira 1994). We now know that most of the parental cydnids do in fact also produce trophic eggs, and we will discuss those in Sect. 4.18. For foraging *P. japonensis* females, food availability is scarce, and provisioning efforts are not always successful. In fact, in some years the timing of drupe availability is totally out of synch with the timing of nymphal need (see Sect. 4.19). Cannibalism is also rampant in this species, and it would behoove *P. japonensis* mothers to evolve some mechanism to keep the young ones from eating each other while they are out trying to sequester a meal (Trivers 1985; Crespi 1992; Kukuk 1992; Mock and Parker 1997; Kim and Roland 2000)! The duration of the developmental period and the ultimate size of offspring can be greatly enhanced by trophic eggs (Baur 1992; Kam et al. 2000; Kim and Roland 2000; Kudo and Nakahira 2004). However, feeding on eggs does not necessarily mean that the female has produced trophic eggs; it could simply be cannibalism.

Perry and Roitberg (2006) proposed criteria that should be met prior to designating eggs that offspring feed on as trophic. In order to establish a cohesive and consistent definition of trophic eggs from an evolutionary standpoint, they proposed two hypotheses that could be tested to determine whether the putative trophic eggs had evolved. First, the eggs should differ phenotypically from fertile eggs, such that their production involves lower cost because females should invest less in them than in viable eggs destined to become offspring. Most often trophic eggs are smaller than viable eggs, rounder (Koedam et al. 2001) and have a softer shell, making them easier for young nymphs to penetrate. They might also have other structural differences, such as fewer or no micropyles, where the sperm penetrate the egg, as in the trophic eggs of *Adomerus triguttulus* (Kudo and Nakahira 2004). Second, the functional value of the trophic eggs should be determined. What is the adaptive value of the putative trophic eggs? We carried out a series of experiments to test these two hypotheses and to demonstrate that female *P. japonensis* produce trophic eggs to enhance offspring success.

4.17.1 Are Eggs Fed on by New Hatchlings Morphologically Different from Viable Eggs?

Female *P. japonensis* lay all their trophic eggs at the time of initial oviposition. Fertile and unfertilized eggs can be easily distinguished because the fertile eggs gradually turn from cream to yellow to pink and finally deep pink with red eyespots (Fig. 4.20). To eliminate the possibility that eggs being fed on by newly hatched

Fig. 4.20 Mother *P. japonensis* guarding her egg mass near hatch. Cream-colored eggs are unfertilized eggs; pink eggs are fertilized eggs; red dots are eyespots. Photo credit, Mantaro Hironaka

Table 4.4 Dimensions and weight of fertilized and unfertilized eggs

	Length (mm) Mean ± S.D.	Width (mm) Mean ± S.D	Weight (mg) Mean ± S.D
Fertilized eggs	1.51 ± 0.04	0.95 ± 0.03	$0.80 \pm 7.5 \times 10^{-2}$
Unfertilized eggs	1.47 ± 0.06	0.96 ± 0.04	$0.78 \pm 8.1 \times 10^{-2}$
P value (student's *t*-test	0.0001	0.22	0.0035

10 fertilized and 10 unfertilized eggs were measured from each of 5 egg masses for a total of 50 eggs of each type. Adapted from Hironaka et al. (2005)

nymphs were not simply eggs that had failed to get fertilized, we assessed whether fertilized and unfertilized eggs in an egg mass differ morphologically, other than color. We collected eight females with their egg masses from the field site at Mt. Hinokuma in Kanzaki Town, Saga, Japan, and brought them into the lab for incubation in individual clear plastic, lidded cups at 25 °C. Just prior to hatch, as evidenced by the clear presence of eyespots, mothers were removed. We then gently dissociated five egg masses and measured length and width of ten fertile and ten unfertilized eggs from each mass (total 50 eggs of each type). Individual egg weight was estimated by taking the average weight of the ten eggs that had been measured. While the average length of fertilized eggs (range 1.42–1.59 mm) was significantly longer than that of unfertilized eggs (range 1.33–1.6 mm), there was no difference in width between the two egg types, resulting in the fertilized eggs having an oval shape and the unfertilized eggs having a rounder shape (Table 4.4; Hironaka et al. 2005). Fertilized eggs were also significantly heavier than unfertilized eggs. Moreover, the unfertilized eggs were softer when pressure was applied with a tweezer (unpublished observation) and had fewer and flattened micropyles (S. Munshi and L. Filippi, unpublished data) compared to fertilized eggs. These phenotypic differences meet the first criterion proposed by Perry and Roitberg (2006) as necessary to conclude that females are producing trophic eggs.

4.17.2 Numbers/Proportions of Fertile and Unfertilized Eggs

In 2000, there was considerable variation in the average total number of eggs in an egg mass; among 51 dissociated egg masses, the average number of eggs was 130.5 ± 36.2. There was also considerable variation in the number of fertilized eggs in a mass (88.8 ± 34.2). There was less variation in the number of unfertilized eggs in a mass (41.7 ± 13.0). The average percentage of trophic eggs in an egg mass was 33.5 ± 11.9, showing the least amount of variation. The frequency distributions of these egg composition parameters are shown in Fig. 4.21.

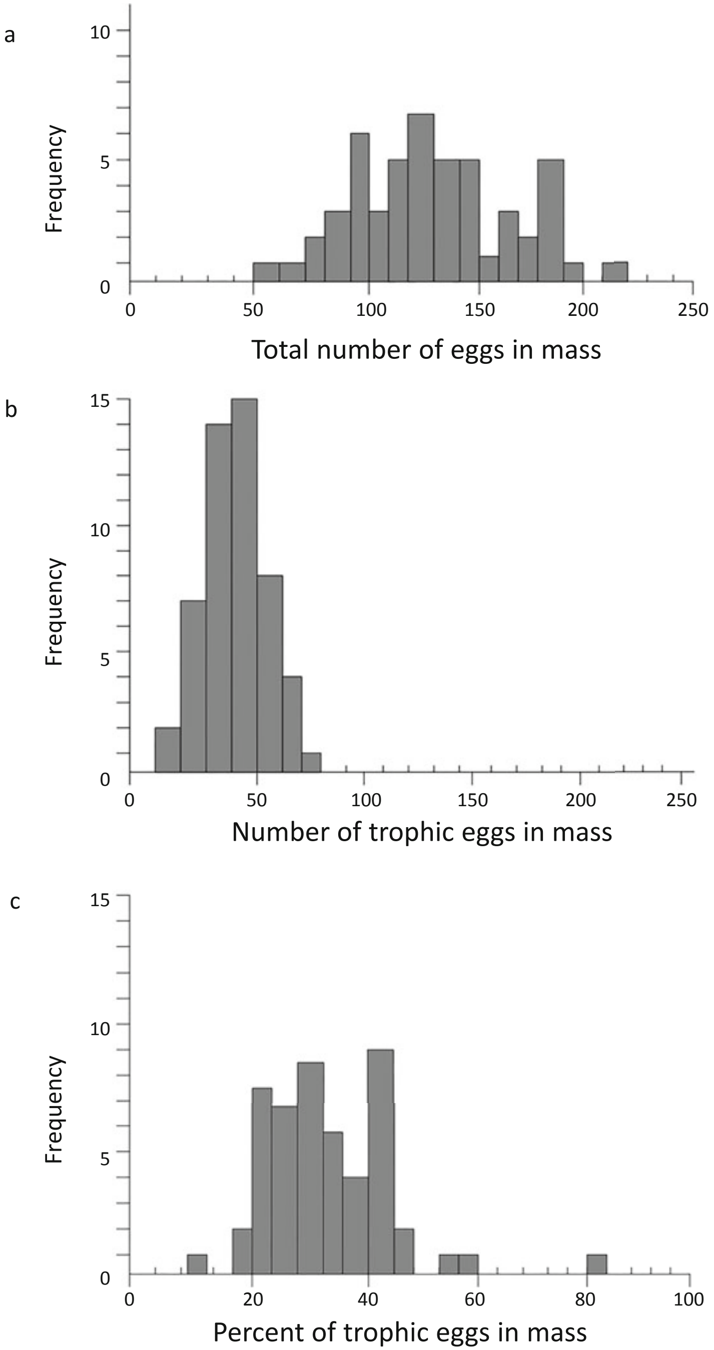


Fig. 4.21 Frequency distributions showing composition of egg masses. (**a**) Total number of eggs; (**b**) number of trophic eggs; (**c**) percent of trophic eggs in mass. $N = 51$ dissociated egg masses. Adapted from Hironaka et al. (2005)

4.17.3 Impact of Feeding on Unfertilized Eggs on Nymphal Weight at 24 h

Because newly hatched nymphs were seen probing egg cases immediately after hatch in the earlier studies (Filippi-Tsukamoto et al. 1995), we were interested in examining how that presumptive feeding impacted nymphal growth. A preliminary finding that 89.1% of trophic eggs in a mass are located on the outside of the mass (Hironaka et al. 2005) allowed us to perform a trophic egg removal experiment to assess this. We knew that by removing surface unfertilized eggs, most would be removed. For this study, we chose 36 females with egg masses that contained between 25 and 45% unfertilized eggs in their mass (see Hironaka et al. 2005 for details on how we calculated this). We separated the mother from all egg masses and, using a fine paintbrush (one of our favorite tools!), gently removed all trophic eggs from the surface of 18 of the egg masses (experimental group). We then allowed all females to retrieve their egg masses. The 18 egg masses that were left intact comprised the control group. When the eggs hatched, nymphs were clearly seen probing their egg masses. After 24 h we weighed the nymphs. Control nymphs that had access to the unfertilized eggs were significantly heavier by 16% at 24 h (range 0.94–1.7 mg) than the unfertilized egg removal nymphs (range 0.8–1.3 mg) (Fig. 4.22; Hironaka et al. 2005). Clearly, the control nymphs fed on those unfertilized eggs.

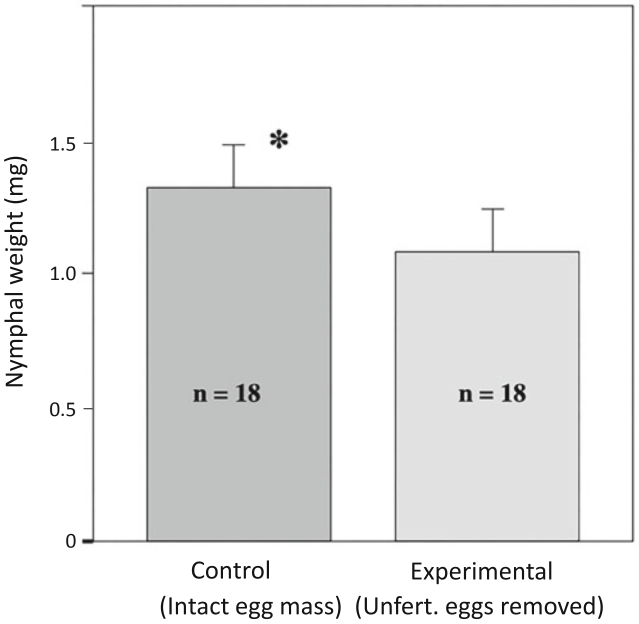


Fig. 4.22 Impact of removal of surface unfertilized eggs on average nymphal weight. *Mann-Whitney U test, $p < 0.002$. Adapted from Hironaka et al. (2005)

4.17.4 Impact of Access to Unfertilized Eggs on Nymphal Development Rate and Survivorship

There were other significant differences between nymphs with and without access to the unfertilized eggs. Twice as many first stadium nymphs had molted to the second stadium by 48 h (Mann-Whitney U test, $p < 0.005$; Fig. 4.23a; Hironaka et al. 2005), and survivorship at day 10 of nymphs with access to unfertilized eggs was over three times greater than that of nymphs deprived of access to unfertilized eggs (Mann-Whitney U test, $p < 0.003$; Fig. 4.23b; Hironaka et al. 2005). Nymphs clearly

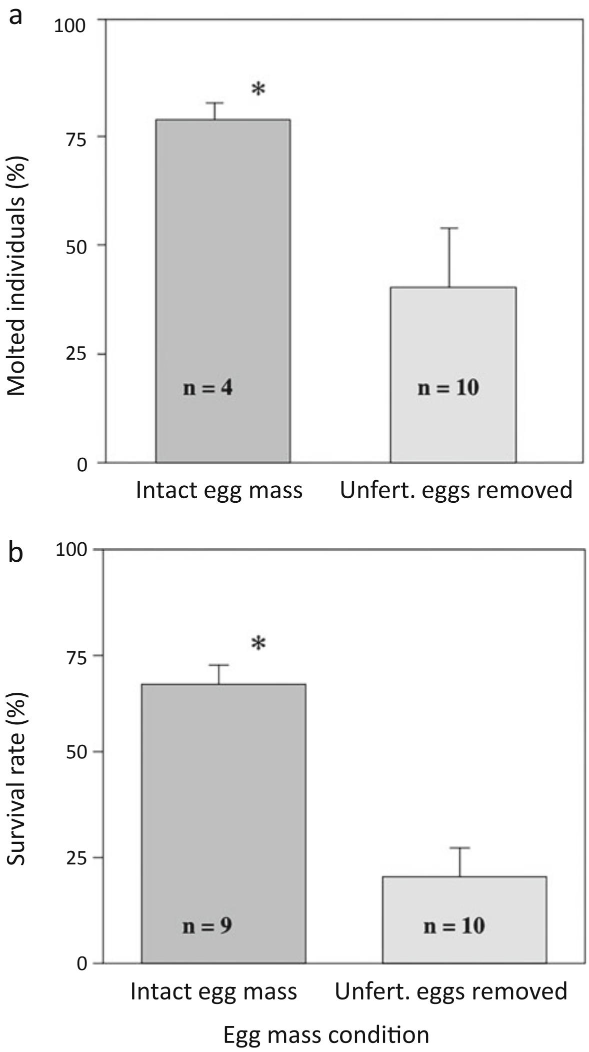


Fig. 4.23 Impact of access to unfertilized eggs on nymphal success. (**a**) Percent of nymphs that had molted to the second stadium by 48 h; Mann-Whitney U test, $p < 0.005$; (**b**) percent of nymphal survivorship at day 10; Mann-Whitney U test, $p < 0.003$. Adapted from Hironaka et al. (2005)

benefitted from access to unfertilized eggs because they were significantly heavier, molted to the second stadium significantly faster, and had significantly higher survivorship.

The findings of this study demonstrate that females are producing unfertilized eggs for nymphs to feed on; these eggs meet the criteria proposed by Perry and Roitberg (2006) for trophic eggs. They are phenotypically distinct from fertile eggs (Table 4.4) and, being smaller and more fragile, are undoubtedly less costly to make, and their adaptive value to offspring success has been demonstrated (Figs. 4.21, 4.22, and 4.23). Because of the scarcity and unpredictability of drupe availability, females often fail to find a drupe for a day or two after hatch. Trophic egg production as a way to ameliorate the poor resource condition (Kudo and Nakahira 2004) would fulfill the premise of the "ice-box" or "food-cache" hypothesis (Alexander 1974; Polis 1981, 1984). Trophic eggs can sustain young nymphs until drupes can be found. It is unlikely that nymphs feed on fertile eggs because of synchronized hatch (see Chap. 5), but hungry nymphs could feed on each other. Having access to trophic eggs should also mitigate a parent-offspring conflict by reducing cannibalism in the nest (Crespi 1992; Kukuk 1992; Mock and Parker 1997).

The pattern of deposition of trophic eggs is also adaptive. Because 89% of the trophic eggs are located on the surface of the egg mass (Hironaka et al. 2005), predation and physical damage will occur more on the less costly trophic eggs, and loss of fertile eggs is likely reduced (Nakahira 1994; Kudo and Nakahira 2004).

4.17.5 Risk-Sensitive Decision-Making as the Source of Variation in Trophic Egg Production

The large degree of individual variation in the number of trophic eggs produced and the percentage of the egg mass that is trophic eggs led us to the next study. We suspected that females were adjusting these numbers based on the resource availability and the condition of the female. Even within a given reproductive season, depending on whether a female oviposits early or late in the season, resource availability can fluctuate dramatically. Females that oviposit early have fewer females to compete with when their eggs hatch, and their young have no older nymphs to compete with for food once they depart for the foraging area. However, the female risks not having any drupes at all if they are not yet ripe when her eggs hatch. See Sect. 4.19 for more on this topic. On the other hand, laying ones' eggs later in the season might coincide with more mature drupes falling from the host tree for provisioning; however, there will also be more females to compete with for access to those drupes and, importantly, plenty of older nymphs for their offspring to compete with when they get to the foraging area. Phenotypic plasticity in deposition of fertile and trophic eggs would allow females to respond to the resource availability and their physical condition when developing and laying eggs (Bradshaw 1965; Fordyce 2006; Whitman and Agrawal 2009). In addition to assessing the food availability for provisioning, mothers should be able to gather information that would allow them to assess the level of resource constraint that offspring will encounter from competition with siblings and offspring from other cohorts when they become independent and relocate to the foraging area and adjust number, size, and types of eggs they produce accordingly (Mock and Parker 1997; Creighton 2005). We tested the hypothesis that timing of oviposition (early or late) and maternal phenotype (relative weight) were factors being used by females to make risk-sensitive decisions about the number, size, and types of eggs they produce. We predicted that females ovipositing later in the season would make more trophic eggs than those ovipositing earlier in response to the higher level of competition they and their offspring would encounter. We also predicted that relatively heavier females just prior to oviposition would produce more of both types of eggs as they would have more energetic resources to produce the eggs and to provision them (Filippi et al. 2012).

4.17.6 Temporal Variation in Female and Egg Parameters During the Nesting Season

During the nesting season of 2005, we mass marked pregnant females, easily distinguished by their distended abdomen, roaming about the nesting area searching for a nest with one of three different colors based on the phase of the nesting season (early, middle, late). The females had mated in the field and fed on the host trees there, presumably gathering information about resource availability and competition level while doing so. The first pregnant females were seen on May 29. Between May 29 and May 31, we marked 63 females and designated them the early group. Numbers of pregnant females dropped after that, and we marked only five females from June 1 to June 3. These females were designated as the middle group and were marked for purposes of exclusion from the early and late groups. We marked 46 females from June 4 to June 7 that were designated the late group. All females were released where we had found them as soon as they were marked and allowed to nest. After about a week, we began searching for the nests of the females we had labeled. To minimize confounding variables, we concentrated our search efforts on a 15 m^2 area that we recognized as being suitable for nesting and having consistent features throughout. We were able to recover 13 early females and 12 late females and their egg masses. We recorded the distance of each nest to the nearest appropriate-sized host tree and carefully transferred them with their nests to plastic cups containing leaf litter and soil and brought them back to the lab for incubation and then analysis. We incubated them at 25 °C, under a photoregime of 16L:8D (Filippi et al. 2012).

One day prior to the anticipated hatch date, based on the deep pink color of the fertile eggs and the dark red eyespots, the egg mass was separated from the female, and the female's weight and pronotum width were measured. We also weighed the egg mass and, after dissociating the egg mass with a fine paintbrush, counted the numbers of fertile and trophic eggs in each mass. In 2005, there were significant differences between early and late nesting females for all parameters measured except two (Table 4.5; Filippi et al. 2012). The nests of late nesting females were, on average, 2.5 m closer to the foraging site, but the range of distances was similar in both groups (2.5–12.1 m for early nesters and 3.1–12.7 m for late nesters). Nesting closer to the foraging area is considered a riskier behavior because of the lack of protective leaf litter and the greater density of foraging females and, later in the season, foraging nymphs; females would be expected to nest there when drupes or nests are particularly scarce (Filippi et al. 2002, 2005). The best nesting sites were probably saturated by the time late nesters were ovipositing.

The body weight of late nesting females was 6% heavier than that of early nesters (Table 4.5; Filippi et al. 2012). Because there was no difference in body size as measured by pronotum width, this difference in weight indicates that late nesting females had more fat body reserves after producing their eggs which could be used for provisioning. It is unclear whether these females started out with more fat body reserves after breaking reproductive diapause or if they actively fed on the host tree longer, accruing enough energy to produce eggs and build up their own reserves. The data from our earlier study on mating behavior indicate that fat body reserves after breaking diapause decrease for a time during the mating season before they build up again during feeding on the host tree (Fig. 3.5), which would support the latter scenario. Nesting further from the host tree would require more work getting to and from the foraging site and transporting drupes back to the nest. The extra body weight would support this additional effort.

The egg masses of late nesting females were nearly 20% heavier than those of early nesters. This extra weight was clearly relegated to trophic eggs, because although late nesters produced 10% more total eggs, they produced 16% fewer fertile eggs and 40% more trophic eggs. On average, a late nester's egg mass was comprised of 44% more trophic eggs than an early nester's (Table 4.5). Late nesting females were apparently responding to the likely shortage of drupes that would occur later in the season because provisioning females had been removing drupes from the foraging area for days before late nesters' eggs hatched and their third and fourth stadium offspring would enter a foraging stage overrun with clusters of older nymphs feeding on drupes.

4.17.7 Impact of Body Size on the Number of Eggs Produced

There was no correlation between body size (PNW) and the number of eggs produced for either group (not shown); however, there was a significant positive correlation between body weight and the total number of eggs produced for both early and late nesters (Fig. 4.24a; Filippi et al. 2012). Interestingly, the body weight of only the late nesters showed a significant positive correlation with the number of fertile eggs produced (Fig. 4.24b; Filippi et al. 2012). It is difficult to draw conclusions here because the outlier late female in the figure clearly impacted the significance, and when that female was removed from the analysis, the finding was no longer significant.

Table 4.5 Female and egg parameters of early and late nesting females

Timing of oviposition	Distance from host tree (m)	Female PNW (mm)	Female Bwt (g)	Egg mass wt (g)	Individ. egg wt (g)	Total no. eggs	No. fert. Eggs	No. trophic eggs	% trophic eggs
Early $n = 13$	8.70 ± 2.87	7.43 ± 0.19	0.18 ± 0.02	0.09 ± 0.01	$8.4 \times 10^{-4} \pm 5.59 \times 10^{-5}$	113.62 ± 12.37	74.54 ± 12.14	39.08 ± 13.38	34.08 ± 10.43
Late $n = 12$	6.35 ± 2.73	7.36 ± 0.29	0.19 ± 0.02	0.11 ± 0.02	$8.8 \times 10^{-4} \pm 9.75 \pm 10^{-5}$	126.92 ± 16.51	62.75 ± 13.09	64.17 ± 8.75	50.79 ± 5.89
$P =$	0.04	0.45	0.04	0.02	0.22	0.03	0.03	**0.0001**	**0.0001**

Values are means ± S.D. PNW = pronotum width. Female BWT = body weight just prior to hatch. Adapted from Filippi et al. (2012)

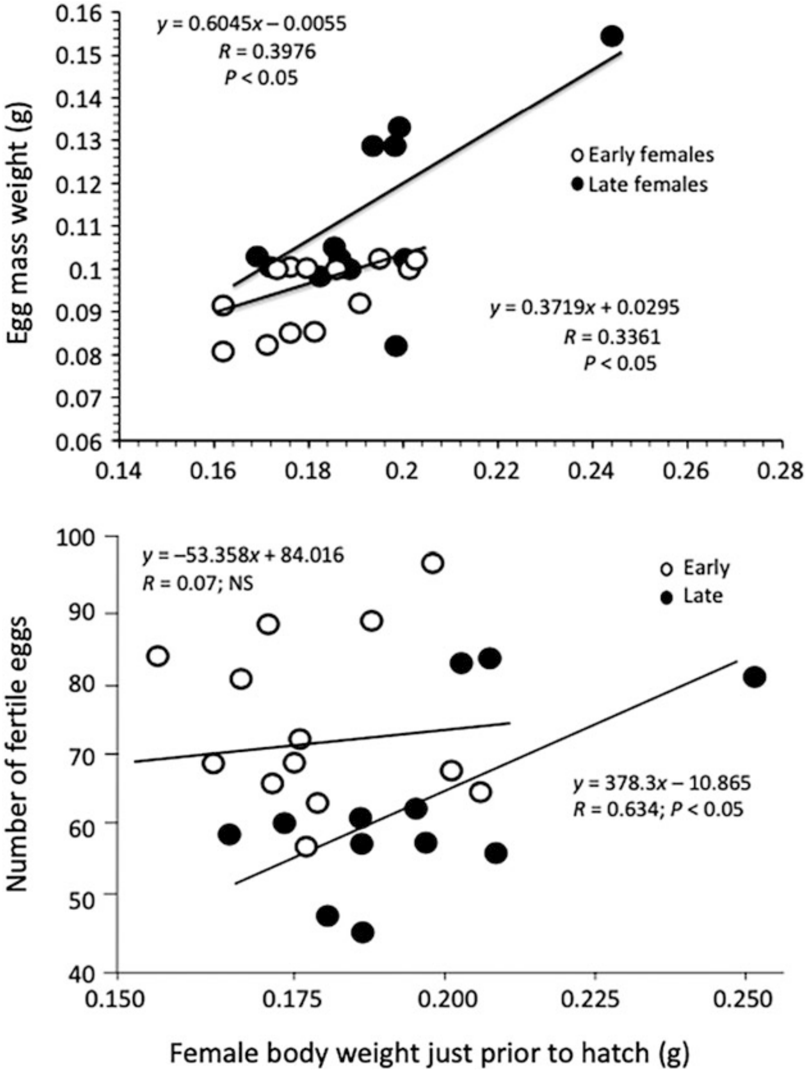


Fig. 4.24 Relationship between female body weight just prior to hatch and egg abundance. (**a**) Total egg mass; (**b**) fertile eggs. Adapted from Filippi et al. (2012)

The degree of variation in a data set is always an intriguing source of more questions when the variation is real, and not a source of error. The tendency for the earlier females to have a much greater degree of variation in the number of trophic eggs produced than late females (33% of the average for early females and 10% of the average for late females; Table 4.5) was curious, particularly because that difference in variation was not present for fertile eggs (~16% and ~21% of the average for early and late females, respectively), but the difference was not significant. LF recalls being

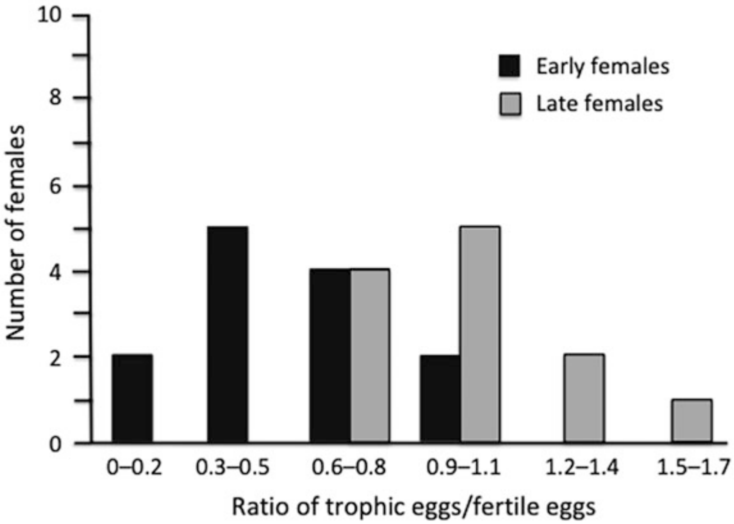


Fig. 4.25 Frequency distribution of the rate of trophic eggs produced per fertile egg. Adapted from Filippi et al. (2012)

frustrated when analyzing the data, because although we detected something intriguing was afoot, nothing stood out until we arranged the data as number of trophic eggs per fertile egg in each group. And there it was! Most late females produced at least one trophic egg per fertile egg (1.06 ± 0.27, mean ± S.D.), while very few early females did so and more than half of the early females produced 0.5 or less trophic eggs per fertile egg (0.55 ± 0.24, mean ± S.D.; Fig. 4.25).

There is a physiological limitation to the amount of provisioning a female can manage. Females should be designed to produce an appropriate number of eggs based on their ability to provision those nymphs (Lack 1947; Monaghan and Nager 1997). The data suggest that the number of fertile eggs that early females produced might be the maximum number that a female could provision and any extra body fat reserves that early females possessed were relegated to trophic eggs. On the other hand, while both groups showed a positive correlation between body weight and total egg number, only late females showed that same correlation between body weight and the number of fertile eggs. The data suggest that to mitigate an anticipated scarcity of drupes, late females obligatively produce eggs such that the number of fertile eggs matches trophic and, when resources allow, facultatively produce additional fertile eggs.

We carried out a binomial generalized linear model (GLM) analysis to determine the factors that had the greatest impact on the percentage of trophic eggs that a female incorporated into her egg mass. Timing of oviposition and distance of the nest from the foraging area had the greatest impact (Table 4.6). Late females produced a higher percentage of trophic eggs, as did females nesting closer to the foraging area. This suggests that late females, who likely nested closer to the

Table 4.6 Coefficient table of binomial GLM for analysis of percentage of trophic eggs

Coefficients	Estimate	SE	*X* value	*P*-value
Intercept	−0.37288	0.38439	−0.970	0.332
Early/late oviposition	0.57169	0.08702	6.570	0.0000
Distance from host tree	−0.05775	0.01403	−4.116	0.0000
Female BWT Just prior to hatch	−1.95321	1.95897	−0.997	0.319

GLM generalized linear model. Adopted from Filippi et al. (2012)

foraging site because more distant, protected nest sites were saturated with early nesting females, were responding to the disturbance to the nest and competition that their nymphs would experience from the many provisioning females and foraging older nymphs.

The hypothesis that the resource exposure as a function of timing of oviposition was determinant in the number of trophic eggs produced was supported (Fig. 4.25; Tables 4.5 and 4.6). Moreover, the hypothesis that maternal phenotype (body weight) was also a determinant was also supported (Fig. 4.24; Table 4.5). The data clearly indicated that females have the capacity to adjust the number and types of eggs they produce to best respond to the availability of the resource, which is based on numbers of drupes produced by the host tree and competition with foraging females and older nymphs at the time their nymphs will be foraging. The findings that late females produce on average about one trophic egg for each of the hatchlings to feed on while early females produce about one half of a trophic egg per nymph (Fig. 4.26) suggests that, because of less resource availability to later nymphs, trophic eggs are much more important to the success of late offspring than early ones. It will be of great interest to explore whether there is a genetic predisposition to when and how females oviposit, or whether it is simply a plastic response to the resources each female was able to accrue prior to diapause. Also, clarifying the cues females use to assess resource level would be a fascinating undertaking. Do they "count" the number of females they are exposed to while they are moving about the host tree feeding to produce eggs? There are no drupes on the ground when females descend the host tree to oviposit, so they cannot be assessing resource level by the number of drupes that they encounter on the ground while looking for a nest site. It could also be that they are "counting" the absolute number of drupes on the host tree that they encounter versus the number that have been fed on by other females. All intriguing questions, so much left to discover!

4.18 Timing and Manner of Trophic Egg Deposition in the Cydnid Relatives of *P. japonensis*

We have stated that 89% of trophic eggs produced by *P. japonensis* females are deposited on the outside of the egg mass, that they are all deposited at the time of initial oviposition, and that survivorship was critical to having access to trophic eggs

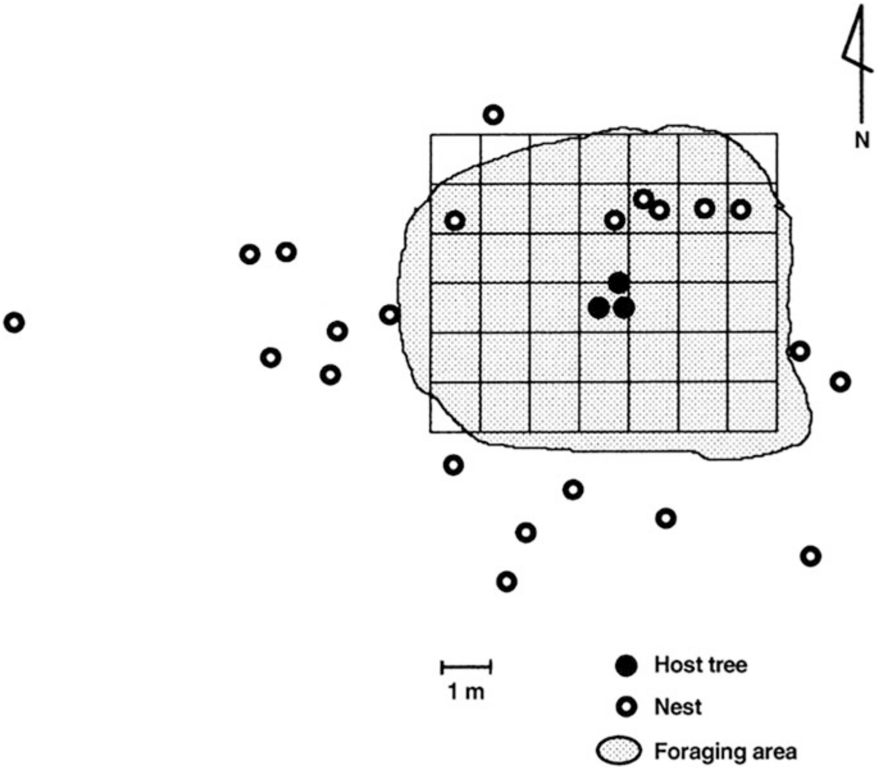


Fig. 4.26 Natural nesting and foraging areas at field site in 1995. Adapted from Filippi et al. (2002)

(Hironaka et al. 2005). The related Japanese cydnid provisioners do things quite differently. *Adomerus triguttulus* lays most trophic eggs in the original egg mass but continues to add trophic eggs during the egg guarding phase (Nakahira 1994). The timing and pattern of deposition of trophic eggs by *Adomerus variegatus* mothers are very similar to what is seen in *P. japonensis* (Mukai et al. 2010). *Canthophorus niveimarginatus* mothers, on the other hand, produce two types of trophic eggs based on timing of deposition. A small number of trophic eggs are deposited on the outside of the initial egg mass, and mothers also deliver trophic eggs to nymphs upon hatch. Thus, females produce both pre- and post-hatch trophic eggs (Filippi et al. 2009). As the mother is releasing the post-hatch trophic eggs, nymphs climb up to her abdomen and gather on the eggs to feed. Nymphs cannot survive the first stadium without access to trophic eggs because fragile first instars cannot penetrate the seed to access the endosperm (Baba et al. 2011). Mothers of *Adomerus rotundus* also produce these two types of trophic eggs, though the number of pre-hatch trophic eggs is apparently much smaller and not all females produce them (Inadomi et al. 2014). Sadly, mothers of *Sehirus cinctus*, the only provisioning cydnid in North America, do not produce trophic eggs (personal observation, L.F.).

One might speculate that the reason for pre- and post-hatch eggs allows for some flexibility in these iteroparous species. If the first egg batch should fail, then another one can quickly be produced. However, if the first batch is successful, the mother releases the less costly eggs upon hatch as food. The ecological significance of the different patterns of trophic egg production, and of no trophic egg production in *S. cinctus*, warrants further investigation.

4.19 Verifying Risk-Sensitive Decision-Making by Nesting Mothers

We discovered answers to some of the ultimate "whys" regarding the adaptiveness of maternal guarding of eggs and nymphs, progressive provisioning, and production of trophic eggs. We know that guarding protects against predation (Filippi-Tsukamoto et al. 1995, Filippi et al. 2000; Nomakuchi et al. 2001) and that in the absence of a provisioning female, even when predators are eliminated, the altricial offspring cannot gain enough access to good drupes to sustain growth and development, so careful choosing of good drupes by mothers is another adaptive function of progressive (Filippi et al. 2000; Nomakuchi et al. 2005). We found that survivorship of nymphs is positively correlated with duration of the nesting period, which is positively correlated with provisioning capacity of the female (Filippi et al. 2000; Nomakuchi et al. 2005). We discovered that trophic egg production by females is essential for success of offspring because often mothers cannot find good drupes for a few days after hatch (Hironaka et al. 2005). We also discovered that females manipulate the composition of their egg mass, producing more or fewer fertile and trophic eggs, based on resource availability, competition, and the mother's level of energetic stores (Filippi et al. 2012). However, a true understanding of the ecological constraints that lead to the evolution of behaviors, and the consequences of those behaviors on reproductive success, requires examination of the ecological parameters, such as resource availability, competition level, predation level, and weather effects, and their effect on patterns of behavior and reproductive success over several years. The natural behaviors observed in each of the previous field studies only provided a snapshot of the behaviors that occur in response to the specific conditions that prevailed during the year of the study. Particularly in environments where conditions are not stable and the resource is ephemeral, unpredictable, and scarce, evolution should favor parents that can use risk-sensitive decision-making to respond to the prevailing conditions in a way that would minimize costs and optimize their average fitness gains (Caraco 1980; Bulmer 1994). Because foraging and defense against predation are both essential but mutually exclusive behaviors, evolution should favor individuals that can assess and respond to the intensity of predation risk and balance their allocation of resources to these behaviors optimally (Lima and Dill 1990; Lima and Bednekoff 1999). In the case of nesting *P. japonensis*, the biggest defenses against predation are prolonging the duration of the nesting period and guarding of eggs and nymphs by the mother, and duration of the nesting period is positively correlated with provisioning success of the female. Clearly, a careful balance between provisioning and guarding is required for the female to both be away from the nest often enough to provide sufficient drupes and be present in the nest often enough to ward off would-be predators. Provisioning capacity is determined by the abundance of drupes available to provisioning females, the intensity of competition, and the distance of the nest from the foraging site. Females invest considerable energy traveling over great distances to and from the foraging area, laden with heavy drupes. Moreover, as the provisioning season coincides with the rainy season, females regularly have the added constraint on provisioning capacity of navigating the rough terrain in a flooded environment. Are females adapting their behaviors to best mitigate all these constraints?

Although trophic egg production is a major component of parental care that *P. japonensis* females use to enhance offspring success, and we have determined that females can adjust egg numbers and proportions using adaptive risk-sensitive decisions, we did not have long-term data on trophic egg production in the field. Therefore, this long-term study was to assess only the progressive provisioning component of parental care. To verify that females were using risk-sensitive decision-making to enhance provisioning success, we analyzed data on the prevailing ecological conditions (abundance of good drupes, intraspecific competition, and weather) and the behaviors and physiological responses that were under female control (timing of oviposition and distance of the nest from the foraging area, both of which affect access to the resource and intensity of competition, and the duration of provisioning activity during the provisioning seasons of 4 years <1995, 1997, 1998, 1999>). We correlated the variation in these parameters with provisioning success of females (number of drupes provisioned). We tested four hypotheses: (1) the availability of drupes would be the greatest determinant of female risk-sensitive behavior. We predicted that the intensity of both intraspecific competition and inclement weather would be negatively correlated with provisioning capacity. (2) The greater exposure to predation and disturbance by other foraging females of nests within the foraging area because of sparse leaf litter and vegetation (Tsukamoto and Tojo 1992) combined with the heavy rains that prevail during the provisioning season should make nesting in the foraging area a risky behavior. Females were predicted to opt for the risky nest site only under severe conditions (extreme scarcity of drupes, extreme weather that would make the repeated and lengthy trips of progressive provisioning particularly costly). (3) Successful provisioners would adapt physiologically by adjusting the timing of oviposition so that provisioning coincides with the period when good drupes are available in the foraging area. (4) Finally, we hypothesized that females nesting according to the parameters in the first three hypotheses would have the greatest provisioning success.

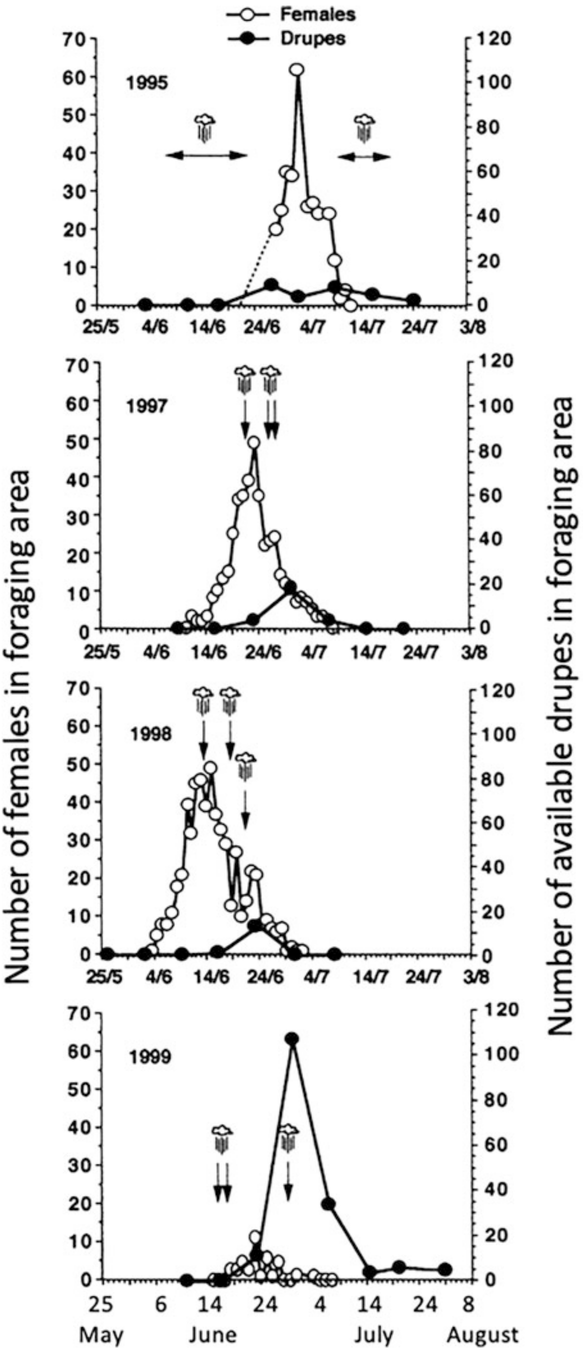
The site that was used for the 4 years surveyed contained three host trees in a triangular pattern about 30 cm away from each other with overlapping crowns at the center of the foraging area. The ground under the crowns was cleared somewhat to facilitate observations, and a 6 × 7 m quadrat was established. Each year we individually marked with liquid paint markers all females entering the foraging area during the first 10 days or so of the provisioning season and labeled as many nests as we could find (Fig. 4.26). To assess the relative number of good drupes available to foraging females, we set two seed traps under the collective crowns of the host trees as described in Sect. 1.11. The traps were set on opposite sides of the trees, about 80 cm from the trunks. As an estimation of relative drupe number and quality, drupes that fell into the traps were collected weekly, counted, and bisected to determine the endosperm content. Drupes containing more than 40% endosperm were considered good (Nomakuchi et al. 1998).

4.19.1 Variation in Duration of Provisioning Season and Individual Provisioning Activity

The duration of the provisioning period over the 4 years ranged from 16 to 30 days and averaged 24.25 days. The start of the provisioning season varied over the 4 years by about 2 weeks, beginning on about June 18th in 1995, June 11th in 1997, June 4th in 1998, and June 19th in 1999 (Fig. 4.27; Filippi et al. 2002). The provisioning period lasted for 21, 27, 30, and 16 days in 1995, 1997, 1998, and 1999, respectively. To assess the intensity of intraspecific competition, hourly counts of all females, marked and unmarked, in the foraging area were made daily during the most active provisioning period of the day, from early afternoon until evening, and the peak value for each day was used as the relative number of females for the day. The numbers of foraging females rose sharply over several days, peaked for a few days, and then dropped sharply. There were dramatic fluctuations in the peak numbers of foraging females over the 4 years, ranging from 13 to 65. In 1997 we also observed and found that provisioning occurred throughout the night, but the numbers of females foraging at night were half those observed in the daytime.

Marked individuals were recorded during each count each day to determine how long individuals continued to provision. The number of days individual females provisioned ranged from 1 to 25 over the 4 years but varied widely from year to year (range; average ± SE, 1–10 days; 3.5 ± 0.21 days; $n = 114$ in 1995; 1–19 days; 6.72 ± 0.39; $n = 156$ in 1997; and 1–25 days; 6.05 ± 0.34; $n = 137$ in 1998). There were significant differences between individual provisioning durations of 1995 and 1997 and between 1995 and 1998 (Kruskal-Wallis, post hoc for multiple comparisons $p < 0.001$; Filippi et al. 2002). Because only one census per day was done in 1999, that year was not included in this calculation. However, among the 3 years, the shortest duration of the overall provisioning period was in 1995, and, perhaps not surprisingly, that year also had the shortest duration of individual provisioning. This suggests that individual provisioning of females in 1999, when the overall duration of the provisioning period was 5 days shorter than that of 1995, was probably even shorter than that of 1995.

Fig. 4.27 Variation in seasonal changes in numbers of female *P. japonensis* and available good drupes in the foraging area during four different years. Symbols above arrows represent days of torrential rain. Dotted line in female count for 1995 is estimation. Adapted from Filippi et al. (2002)



4.19.2 Variation in S. jasminodora *Drupe Availability and Weather During the Provisioning Season*

The data for the 4 years show interesting and varied patterns (Fig. 4.27; Filippi et al. 2002). The numbers of total and good drupes collected in the seed traps over the course of the provisioning season for each of the 4 years are presented in Fig. 1.10. The total numbers of drupes collected varied from 120 to 270 over the 4 years, but the percentage of good drupes varied from 5 to 95%, and the year that 270 drupes were collected (1998) had the lowest percentage of good drupes (5%). 1995 and 1999 had similar numbers of drupes (~180), but in 1995, only 19.4% were good, while in 1999, over 95% of the drupes were good! (Fig. 4.27; Filippi et al. 2002). Of course, females did not have provisioning access to drupes that were still on the host tree and could only provision drupes that had fallen to the ground; the torrential rains that preceded the peaks in the drupe numbers likely brought the drupes down and made them available for provisioning. An interesting pattern that would have been a serious constraint to provisioning females is that in most years the numbers of provisioning females were very high relative to the numbers of drupes and the peak number of females searching for drupes to provision occurred *before* the peak number of good drupe availability on the ground (Fig. 4.27; Filippi et al. 2002). Of course, we only collected drupes from a small portion of the entire crown, so there were surely more drupes for females to provision than the very small numbers presented in the graph; however, clearly the availability of good drupes is poor, at best, most years when females are provisioning. It was particularly poor in 1998 when females were provisioning for nearly 3 weeks before good drupes were collected in the seed traps. This would severely constrain provisioning capacity and offspring success. Good drupes were more abundant when nymphs were out foraging, but unless mothers can provision the nest to keep the young safe until the mid-third instar, offspring success will be severely limited. Notably, in 1999, the pattern was reversed. There were very few provisioning females, probably because of frequent nest failure the previous year, and a superabundance of good drupes. It is likely the abundance of drupes in 1999 while females were provisioning meant that females could find good drupes easily, had little competition for them, and were able to find and stockpile many drupes in a much shorter period of time. This would also explain the shorter duration of the provisioning period that year (Fig. 4.27). Interestingly, early females comprised between 60 and 66% of all provisioning females each year, and for each of the 3 years, the early provisioners always provisioned significantly longer than late females: (mean ± S.E.) 3.99 ± 0.27 days ($n = 73$) and 2.29 ± 0.25 days ($n = 41$) for early and late females, respectively, in 1995 (Mann-Whitney U test, $p < 0.001$); 8.5 ± 0.58 days ($n = 93$) and 4.0 ± 0.4 days ($n = 63$) for early and late females, respectively, in 1997 ($p < 0.001$); and 6.75 ± 0.48 days ($n = 91$) and 4.65 ± 0.37 days ($n = 46$) for early and late females, respectively ($p < 0.05$) (Filippi et al. 2002). It is likely that the paucity of good drupes earlier in the season meant that it took longer for females to find and provision their nests with enough drupes to sustain the young to a safer stage.

4.19.3 Intensity of Competition for the Resource, Weather, and Provisioning Capacity of Females

As a measure of the intensity of intraspecific resource competition for provisioning females in each of the 4 years, we divided the total number of good drupes collected over each provisioning season by the highest number of females counted among all the censuses in the foraging area for that season (female density index, FDI). A larger FDI indicates a lower intensity of intraspecific competition. FDI values ranged from 0.28 (1998) to 13.92 (1999) (Table 4.7). The findings indicate that there is enormous variation in intraspecific competition from year to year. Remarkably, intraspecific competition was 25 times greater in 1995 and 1997 and 50 times greater in 1998 than it was in 1999. These differences apparently drive population fluctuations from year to year. Interestingly, although the FDI values for 1995 and 1997 were very similar, the average provisioning capacity of females in 1995 was about 1/3 of that in 1997. In fact, the average provisioning capacity of females in 1995 was very similar to that of females in 1998, when the FDI was only 0.28, which was less than half that in 1995. We attribute the poor provisioning capacity in 1995 to the torrential rain that persisted for 2 weeks in early June, at least 1 week of which was during the time females would have been provisioning, and then again for about 5 days late in the provisioning season (Fig. 4.27). As mentioned, the entire nesting season from the egg stage through provisioning coincides with the rainy season. Daily rain is normal and expected, but persistent torrential rains are less common and certainly would disrupt provisioning behavior and even lead more directly to nest destruction because of flooding.

In fact, it is surprising that the population did not crash the following year because of the number of consecutive days with torrential rain in 1995. The first week of torrential rain occurred when females were sheltered in their nests under the leaf litter guarding their eggs. It seems not too many nests got washed away. Provisioning females during that second week in early June would not have fared as well. It is also of interest that despite the very short provisioning season in 1999 (16 days), females were able to provision nests with so many drupes. Undoubtedly, the very large FDI that year meant that females had a very easy time finding good drupes, making many

Table 4.7 Drupe availability, female density and provisioning capacity for four provisioning seasons

	Number of good drupes[a]	Number of females[b]	FDI	No. of drupes provided (mean ± SE) (n)
1995	36	62	0.58	10.9 ± 1.9 (11) a
1997	26	48	0.54	31.4 ± 4.6 (22) b
1998	14	50	0.28	11.2 ± 2.5 (34) a
1999	167	12	13.92	36.6 ± 9.2 (8) b

FDI female density index (no. good drupes/female). Numbers followed by different letters were significantly different (Kruskal-Wallis, $p < 0.001$). Adapted from Filippi et al. (2002)

[a] Total number of good drupes collected in seed traps (see text)

[b] Number of females at peak density (see text)

Fig. 4.28 *S. jasminodora* seedlings emerging in 2000 from drupes in a nest from the 1999 nesting season. Nest was 4.56 m outside the range of fallen drupes

round trips to the foraging area and bringing many to the nest in a very short period of time. At this field site, we have seen such a high proportion of good drupes available to provisioning females only twice, about once every 10 years. Anecdotally, and of considerable interest, is what we observed the following year (2000). We found several tiny *S. jasminodora* seedlings emerging from drupes in one of the 1999 nests that was 4.56 m away from the foraging area (Fig. 4.28)! Clearly, those drupes were not fed on and had been spared. Females keep provisioning until they literally drop dead, and with the great number of good drupes in 1999, they apparently provisioned more than was needed before dying of exhaustion and ending the season quite early. Now for the interesting question. Were there so many good drupes simply because there were so few females feeding on the host tree, thus leaving many more drupes undamaged and able to ripen fully before falling to the ground? Or, more intriguing, is the host tree deliberately manipulating the production of good drupes so that the *P. japonensis* population will crash every 10 years or so, allowing some of the over abundantly provisioned drupes that have been dragged far from the host tree to germinate? Coevolution? This is a tough question to answer as the life span of the tree is long and it is not amenable to cultivating outside its natural habitat (alas, we have tried!). It would be a brilliant strategy for the tree, because seedlings cannot emerge under the crown, likely because of allelochemicals produced by the mother tree, and we have never seen any other potential seed disperser, such as a bird or mammal, feed on the nasty tasting drupes.

We counted the number of drupes in marked nests at the end of the nesting season. The range and degree of variation among females in provisioning capacity varied considerably within a year and over the 4 years (Fig. 4.29; adapted from

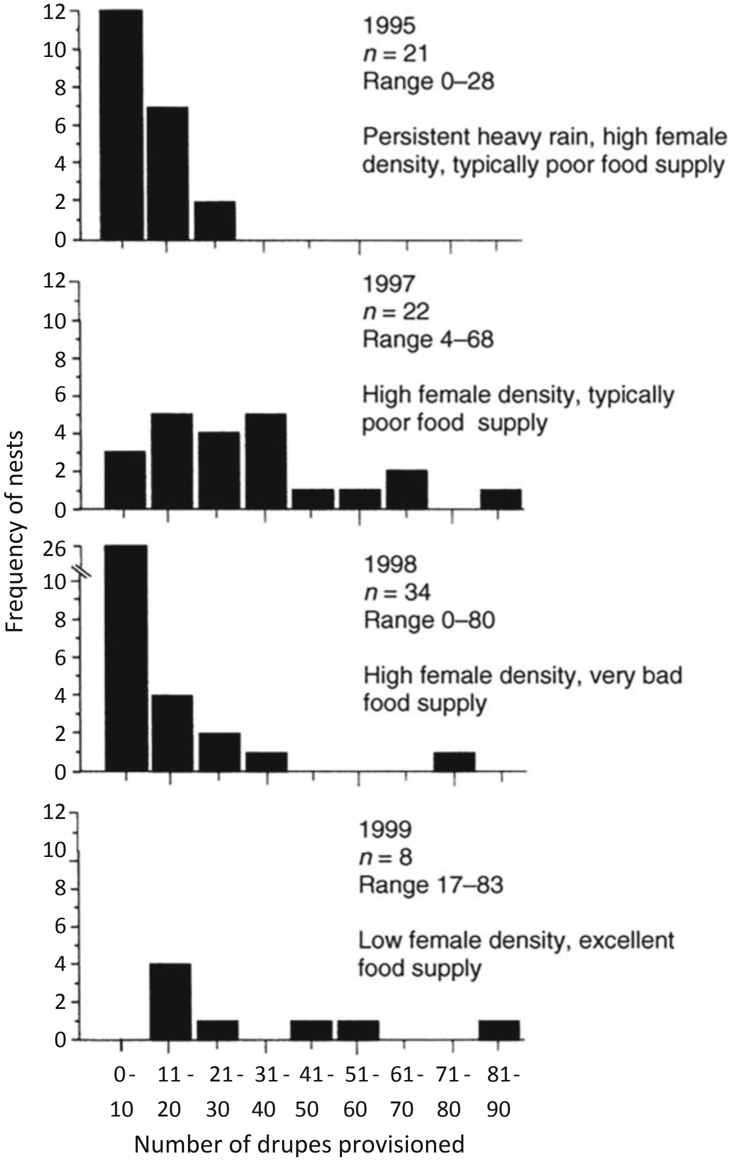


Fig. 4.29 Frequency distribution of drupe provisioning success of females at the end of four provisioning seasons. Adapted from Filippi et al. (2002)

Filippi et al. 2002). In 1995 and 1998, 57% and 76%, respectively, of females provisioned less than or equal to ten drupes. Moreover, three females in 1995 and two females in 1998 were unable to provision any drupes at all. Except for one super successful female in 1998, the range and pattern of variation in provisioning capacity were similar in both years. No females in 1995 and only two females in 1998 managed to provision more than 30 drupes. These 2 years had severe, identifiable constraints (weather in 1995 and very intense competition because of very poor resource level in 1998) that were likely responsible for the poor provisioning.

The range of provisioning capacity in both 1997 and 1999 was much broader and more evenly distributed (Fig. 4.29). In 1997, a typically poor year with no extreme weather issues, 86% of the females provisioned greater than or equal to 11 drupes, and nearly half of the females provisioned more than 30 drupes. Remarkably, in 1999, 100% of the females provisioned more than 17 drupes, and nearly half provisioned more than 40 drupes.

4.19.4 Variation of Nest Distribution over the Four Nesting Seasons

As predicted, females had an overwhelming preference for nesting outside the foraging area (Fig. 4.26; Table 4.8), particularly when there were no severe stressors. Preference in some years was also apparently dependent on the stage in the provisioning season (early or late) that the female was active. A similar variation in nest site preference was seen in the trophic egg study (Filippi et al. 2012). As predicted, most females in all years nested outside the foraging area, and females only nested inside the foraging area in the two particularly stressful years (1995 and 1998). There were significantly different nesting patterns (inside/outside for early and late nesters) for each of those 2 years (Fisher's exact test, $p < 0.01$ for both years).

Table 4.8 Frequency of early and late nests inside and outside the foraging area

	Stage in provisioning season		
	Nest site	Early	Late
1995[a]	Inside	6	0
	Outside	5	10
1997	Inside	0	0
	Outside	14	8
1998[a]	Inside	0	6
	Outside	20	8
1999	Inside	0	0
	Outside	6	2

[a] Fisher's Exact test, $p < 0.01$. Adapted from Filippi et al. (2002)

4.19.5 Variation in Provisioning Capacity Based on Nest Location and Active Timing

As predicted, opting for the riskier behavior led to greater provisioning capacity under stressful conditions (Fig. 4.30; Filippi et al. 2002). In terms of timing of provisioning activity during the provisioning season, later should be riskier because the provisioning female would have more intraspecific competition and her young would be competing with older nymphs when they got to the foraging area, so foraging later in the season should be the preferred option under severely constrained conditions. The rain in 1995 was an unpredictable event that a female would not have had information about before she laid her eggs. However, in 1998, females would have been able to assess food availability and intraspecific competition while feeding on the host tree to make their eggs. In 1998, good drupes were not available until late in the season, so females nesting later had an advantage while provisioning, and they had a strong tendency to provision more drupes; however, the difference was not significant (Fig. 4.30; range 0–22 and 1–80 for early and late females, respectively). Interestingly, in 1999 all females nested just outside the foraging area. They apparently did not perceive a problematic degree of intraspecific competition and disturbance to their nests because of the low density of females, and there was a

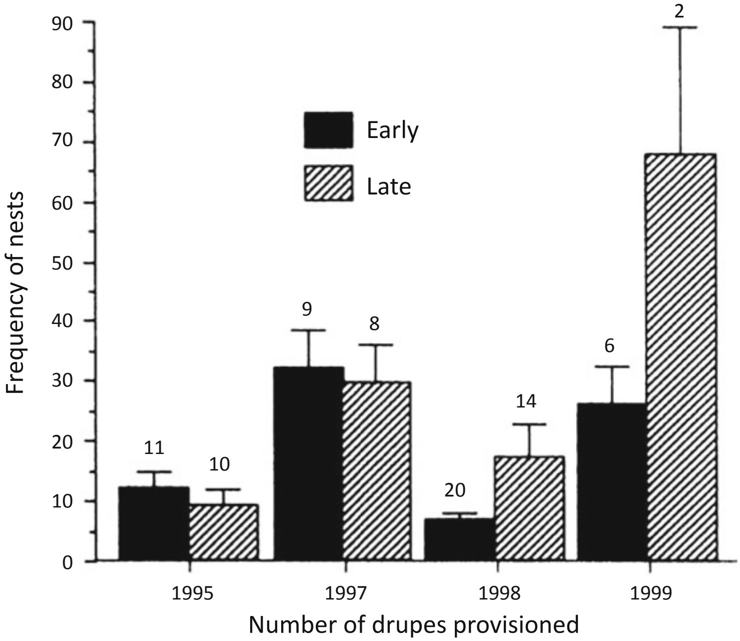


Fig. 4.30 Impact of active stage in provisioning season (early or late) and provisioning success of females in four provisioning seasons. Numbers above bars indicate sample size. Error bars represent S.E. Adapted from Filippi et al. (2002)

Table 4.9 Mean no. of drupes provisioned per early and late nest inside and outside the foraging area

		Average number of drupes provisioned per nest			
		Early	n	Late	n
1995	Inside	14.9 ± 4.0	6	–	–
	Outside	9.0 ± 3.2	5	9.4 ± 2.7	10
1998	Inside	–	–	6.8 ± 1.9 a	6
	Outside	6.8 ± 1.2 a	20	25.4 ± 8.9 b	8

Means ± SE. Numbers within group followed by different letters are significantly different (Kruskal-Wallis with *post hoc* Bonferroni adjustment $p < 0.01$. Adapted from Filippi et al. (2002)

huge surge in drupes later in the season (Fig. 4.27), so provisioning later was the best strategy that year. Late females provisioned more than twice as many drupes on average than early nesting females, but because of the small sample size, statistical analysis was not possible (Fig. 4.27). There was no difference in the number of drupes provisioned by early and late females when there was no severe resource constraint (1995 and 1997) (range, 0–27 and 0–28 for 1995 early and late females, respectively; 2–68 and 4–57 for 1997 early and late females, respectively). Apparently, risk-sensitive nesting in terms of timing in the provisioning season is only realized under extreme resource conditions.

There were only 2 years to compare where nests had been found both inside and outside the foraging area (1995 and 1998). We compared the provisioning success of early and late females both inside and outside the foraging area for those years (Table 4.9). We predicted that females nesting and provisioning according to the risk-sensitive constraints proposed would provision more drupes. While there was no significant difference in 1995 between early and late females (Fig. 4.30; Table 4.9), physiological constraints that we did not assess (fat body content, etc.) could have made the strategy used optimal. In 1998, there was no difference in provisioning capacity between early/outside and late/inside nests. However, there was a significant difference between late inside and outside nests. In 1998, there were very few good drupes available early in the season. It appears that provisioning late was optimal that year and females nesting inside the foraging area might have suffered from thieving females. We will cover this in detail in Chap. 6; however, it bears mentioning here that females were observed entering other females' nests and removing drupes that the resident female had provisioned. We expect that females nesting within the foraging area should experience thievery more often because of the high density of females foraging there and the lesser amount of leaf litter and ground cover to conceal the nests.

4.19.6 *Conclusions Regarding Risk-Sensitive Decision-Making*

We conclude that *P. japonensis* females show flexibility in their behavioral and physiological patterns and apparently use risk-prone behavior under *extreme* ecological conditions. In other words, hypothesis 1 was supported under the constraints of severe ecological conditions of severe weather (persistent torrential rain) and extreme resource constraint, assessed by both absolute abundance and intraspecific competition (Figs. 4.28 and 4.29; Tables 4.7, 4.8, and 4.9), but also under extreme resource abundance. Females opted for risk-prone responses and provisioned more when exposed to these conditions. The flexibility was still present under moderate conditions; however, it resulted in no difference in provisioning capacity. Adaptive variation among individuals can be maintained in a population, particularly in species with as little dispersal as *P. japonensis*. Risk-sensitive behavior could become prevalent over evolutionary time and be maintained when the benefits allow individuals to respond favorably to unpredictable and stressful conditions (Clutton-Brock 1991; Budaev 1999).

Hypothesis 2 was also supported: the riskier nest site (inside the foraging area) was only chosen when conditions were constrained and led to good provisioning success (Tables 4.8 and 4.9). In support of the third hypothesis that females would make physiological adjustments such that they were provisioning when drupes were most abundant, females that nested later when the resource was not available until late in the provisioning season provisioned more drupes than early females (Fig. 4.30 and Table 4.9). Support for the first three hypotheses results in support for the fourth hypothesis that females adjusting the timing and location of their provisioning activities according to the constraints outlined provision the most drupes (Figs. 4.27, 4.28, and 4.29 and Tables 4.7, 4.8, and 4.9).

The extreme behavior of progressive provisioning is essential to *P. japonensis* offspring success (Filippi et al. 2000), and females seem to have an “on” switch to begin provisioning triggered by the hatching of their eggs but no “off” switch. They provision until they die of exhaustion, even when their offspring have already left the nest. In this semelparous species, which literally puts all its eggs in one basket, evolution should favor any physiologically feasible adaptation that would enhance the provisioning capacity of females because of the overwhelming impact it has on offspring success. A major evolutionary innovation would be the ability to respond to the unpredictable and highly variable conditions that provisioning females encounter, in other words, risk-sensitive decision-making. We have presented drupe data for only 4 years here (Figs. 1.10 and 4.27) but collected drupe data for 8 years. The resource is not only ephemeral, but good drupe availability fluctuates from very poor to abysmal and is only occasionally (every 10 years) very good. It is under the backdrop of these poor resource and weather conditions that the physiology and behaviors of *P. japonensis* have evolved. We have described in Chap. 2 other ways that *P. japonensis* optimizes use of the resource, which is both rich in content when it is “good” and poor in terms of abundance. Only inseminated females feed, the metabolic rate is dramatically suppressed, in females, especially, and males don't ever feed as adults but use their remaining reserves to mate with numerous females before they perish when those reserves are depleted. In the face of these extreme traits, it seems a matter of course that risk-sensitive decision-making, the ability to respond adaptively to the conditions one is faced with, should also evolve in this species.

In response to the great variation and unpredictability of the ecological conditions *P. japonensis* females have faced during the nesting season over their evolutionary history, it appears that natural selection has favored risk-sensitive decision-making in nesting behaviors as one additional mechanism to maximize fitness (Luttbeg and Warner 1999).

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Lisa Filippi initiated the work on *Parastrachia japonensis* at Mt. Hinokuma Park in Kanzaki Town, Japan, where she earned her M.S. and doctoral degrees elucidating many of the amazing physiological and behavioral traits of this fascinating insect through field and laboratory studies. She is currently a Professor of Biology at Hofstra University in New York.

Mantaro Hironaka in fact applied to Saga University in order to eventually join the *P. japonensis* research team. He proved to be a brilliant, enthusiastic, energetic, and creative member of the team who contributed significantly to a variety of behavioral studies. Mantaro went on to get his doctoral degree in Dr. Tojo's laboratory from the United Graduate School of Agriculture, Kagoshima University, focusing on the neurological processes involved in the complex navigation that *P. japonensis* uses when provisioning. He is currently an Associate Professor at the Department of Applied Entomology, Faculty of Bioproduction Science, Ishikawa Prefectural University.

Shintaro Nomakuchi an ecologist focusing on insect behavior, came to Saga University in 1993 as an Associate Professor, and was keen to share his very valuable expertise and join the *P. japonensis* research team, which he eventually headed. The work on clarifying the quality of the habitat, the techniques for monitoring the subpopulations and for carrying out a variety of ecological and behavioral studies, as well as the methods for performing robust data analyses, all got a hefty boost, and we were off and running. He is a Professor Emeritus of the Faculty of Agriculture at Saga University.

Chapter 5
Parent-Offspring Communication

Hiromi Mukai and Shintaro Nomakuchi

Abstract Parent-offspring communication is an indispensable capability for many social insect species that allows them to make full use of elaborate and complex parental care activities. *Parastrachia japonensis* Scott (Hemiptera: Parastrachiidae) is one such species. This chapter explains two astonishing phenomena, brilliant examples of parent-offspring communication via vibration that were recently discovered in this species. The first half of the chapter delves into parent-embryo communication that regulates the timing of egg hatching. Parent-embryo communication ensures synchronized hatch and mitigates sibling cannibalism. The second half of the chapter explores parent-nymph communication that involves a mother calling nymphs to gather on the drupes that she brings back to the nest. Parent-nymph communication ensures that nymphs gain equal access to food and likely keeps them hidden from predators until the mother returns to the nest. These communication behaviors are discussed from the viewpoint of fitness of both parents and offspring.

Keywords Hatching · Provisioning · Prenatal and postnatal parent-offspring communication · Substrate-borne vibration · Synchronization

5.1 Introduction

Parent-offspring communication involves transmission and processing of information about physiological and/or ecological conditions or requirements from parent to offspring or from offspring to parent. It appears that in subsocial insects, including *Parastrachia japonensis* Scott (Hemiptera: Parastrachiidae), parent-offspring

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communication often plays an important role throughout the process of all parental care behaviors in the family (Cocroft and Hamel 2010; Costa 2006). For example, nymphs of a subsocial treehopper clustered on a tree branch call their mother via substrate vibration for defense against an approaching enemy; the mother then approaches the enemy and threatens it, even if there is risk to her own survival, leading not only to increased fitness for the nymphs but also for the mother (Cocroft 1999). Substrate vibration from nymphs induces defensive behavior by the mother in this case. Moreover, larvae of subsocial burying beetles physically touch the parents' head with their legs on a carcass ball in a nest chamber when the larvae want food. In response to this larval begging, parents feed the larvae by regurgitation (Rauter and Moore 1999; Smiseth and Moore 2004). In this study, physical contact from the larvae induced feeding behavior by parents. These examples demonstrate that parent-offspring communication is likely to be essential for all interactions between parents and offspring in the process of parental care activities to ensure that all care behaviors are carried out successfully, e.g., defense against predators and feeding.

The studies discussed above exemplify the case where offspring are the signalers and parents are the receivers; however, examples where the parents or other siblings are the signalers and offspring are the receivers are also known. For example, it has been reported that hatchlings of the burying beetle direct their movement toward the sound that a parent makes in the nest (Costa 2006) and that, in order to maintain group cohesiveness, family members of treehoppers (Cocroft 2005) and sawflies (Fletcher 2007) recruit other family members to a new feeding site using substrate-borne vibration. However, to the best of our knowledge, there is no report demonstrating that a parent of another subsocial insect repeatedly uses vibration signals to communicate with offspring each time it progressively provisions the nest.

Can we identify any specific conditions that might shed light on why parent-offspring communication has evolved in subsocial insects, particularly communication that serves as an interaction tool for care behaviors between parent and offspring and not in insects whose parents do not engage in post-ovipositional care of offspring? There are probably at least two conditional factors that could have promoted the evolution of parent-offspring communication in subsocial insects.

The first factor relates to a basic condition of the physical parent-offspring relationship. One of the most obvious features that the emergence of post-ovipositional parental care has brought to the insect is the very state that parents and offspring remain together at a specific site, e.g., a nest, for a period of time. For example, in many insect species with guarding behavior of eggs and/or post-hatched offspring by parents, the parents and offspring remain together continuously even after oviposition has completed. This inevitability of cohabitation could have led to the occurrence of "family" in subsocial insects. Because family members would have interactions among themselves, approaching or contacting each other, especially during various types of intra-familial actions operating through parental care or sibling competition, they would have been able to use various kinds of stimuli or displays functionally working through short distances as signals to inform other family members about their own condition or requirements or to determine the same type of information from them. Even low-cost, weak stimuli that can only be transmitted in an isolated and small nest would have been enough to use as functional signals in a family. The lower cost of the transmission of information would have favored the evolution of intra-familial communication.

The second factor stems from theories of the evolution of animal communication. Communication is the system that involves transmission of any information between signalers and receivers. Because signals are actions or structures that signalers display to manipulate the receivers, they are considered to co-evolve with the response of the receivers: the signals evolve toward improved effectiveness in manipulation of the receivers, and the response of the receiver evolves against the improved manipulation by the signalers. In this way the signals continue to evolve to be ever more effective against antagonistic responses by receivers, etc., instead of evolving for simple improvement in the effectiveness of information transmission (Davies et al. 2012). Therefore, signals must include honest information on average because dishonest signals are costly to the receivers. Receivers would abandon utilization of dishonest signals, and, ultimately, the signal-receiving system would be eliminated. Behavioral ecologists have discussed several necessary conditions that must be met for signals to be kept honest, including index, handicap, and common interest (Davies et al. 2012). In the evolution of parent-offspring communication in subsocial insects like *P. japonensis*, what condition might have been most important for signals to remain honest? It could be the common interest between parent and offspring through their kinship. Because family members have a kinship with each other, selfish signals of one member in the family would be accepted by the other members in the family as long as direct or indirect fitness of the receivers increases.

In this chapter, we will first explain how *P. japonensis* mothers communicate with embryos to synchronize hatch (Sect. 5.2) and then how they communicate with post-hatched offspring during provisioning behavior to synchronize nymphal feeding (Sect. 5.3). We will also discuss how these types of communication enhance offspring success.

5.2 Parent-Embryo Communication

5.2.1 Maternal Substrate Vibrations as Cues of Exquisite Synchronous Hatching

Embryos of most animals develop while being protected by a capsule such as an eggshell. In contrast to more developed stages, generally embryos and newly hatched young are vulnerable to dangerous and changeable environments (Warkentin 2011a). Particularly during hatching, when embryos emerge from their egg capsules, simultaneous changes in the physical environment and their development status, as well as their biotic interactions, present the highest risks for feeble embryos. Therefore, hatching at a suitable timing is a vital survival strategy.

Animal parents of several species use a particular type of care during the hatching moment, i.e., “hatching care,” to increase their offspring’s chances of survival. For instance, some avian parents actively assist their offspring in emerging from a hard shell at hatching. Parents gently peck the shell with their bills immediately before hatching to facilitate the emergence of the chick (Tremaine 1974; Birmelin and Wolter 1986; Newton 1986; Bond et al. 1988). Just before hatching, chicks chirp from inside their eggs, and this sound causes parental hatching assistance behavior. It is strongly predicted that such a close parent-embryo relationship while hatching has led to the evolution of special signals and communication systems (called “parent-embryo communication”); however, studies on static embryos have scarcely been advanced.

This section introduces the interaction and communication between the parent and embryo during hatching in the subsocial shield bug, *Parastrachia japonensis* (Heteroptera: Parastrachiidae), discovered by our research group (Mukai et al. 2014). As described in Chap. 4, *P. japonensis* mothers show complex maternal cares that aid nymphs after hatching, such as production of trophic eggs, protection of nymphs, and progressive provisioning (Tachikawa and Schaefer 1985; Tsukamoto and Tojo 1992; Filippi et al. 2001; Hironaka et al. 2005). There have been many reports on the maternal care of *P. japonensis* for the “active” nymphs after hatching; however, there have been few reports on care of “static” embryos. To explore this topic, we undertook a study to answer the following questions:

Do female *P. japonensis* care for embryos before or at the moment of hatching?

If so, what kind of parental care for hatching does the female show?

Exquisite Synchronous Hatching

The female *P. japonensis* weaves its proboscis through the interstices of the egg mass and attaches the proboscis to the eggs with saliva. Thus, the egg mass is protected by suspending it between the long legs of the female, such that the eggs are kept elevated without sticking to the ground. This is thought to have the effect of preventing predators such as ants from approaching the egg mass from the soil surface and protecting from insecticidal pathogens (Fig. 5.1).

The eggs have a whitish cream color immediately after being laid (Fig. 5.2a), but they gradually become pale pink over 10 days. Hatching begins soon after the eye spots become visible and nearly all eggs have turned red (Fig. 5.2b).

The hatching of the *P. japonensis* egg mass occurs synchronously. Hosokawa et al. (2012) reported that mothers of *P. japonensis* start to excrete a copious amount of symbiont-containing white mucus from the anus onto the egg mass at around 40 min before egg hatching (median 39 min; range 30–50 min; $n = 10$). After the excretion behavior, the mothers lifted the egg mass using the proboscis and resumed the egg-guarding posture (Fig. 5.3a). Then, after a while, the nymphs broke the eggshells all at once and began to hatch. By 10 min after the start of hatching, 90.3 ± 7.3% (mean ± SD) of the embryos had emerged from their shells (Fig. 5.3b). Newly hatched nymphs remained motionless for a few minutes and then gradually

Fig. 5.1 Egg-guarding female *P. japonensis*. Females hold the egg mass in the nest, suspended on the stylets, lifted off the ground. Photo credit, Mantaro Hironaka

Fig. 5.2 Female *P. japonensis* guarding egg mass. The eggs are (**a**) whitish cream color immediately after being laid, and (**b**) fertile eggs turn pink with visible eyespots just before hatching

began to move (Fig. 5.3c). They started to walk around actively and aggregated on the egg mass within 5 min (Fig. 5.3d). The newly hatched nymphs immediately took up the mucus secretion and the trophic eggs with their respective proboscises (Fig. 5.3e). Interestingly, it was found that similar batch hatching was not observed when the egg mass that had been smeared with symbiotic bacteria was separated from the female parent (Fig. 5.3f-j). In such egg masses, hatching occurred bit by bit after the first individual started to hatch. After 30 min from the end of excretion, about half of the individuals in the egg mass remained in the shell.

This suggested that the symbiont-containing mucus secretion did not affect the timing of hatching and that female involvement after mucus secretion was most likely occurring to induce synchronous hatching.

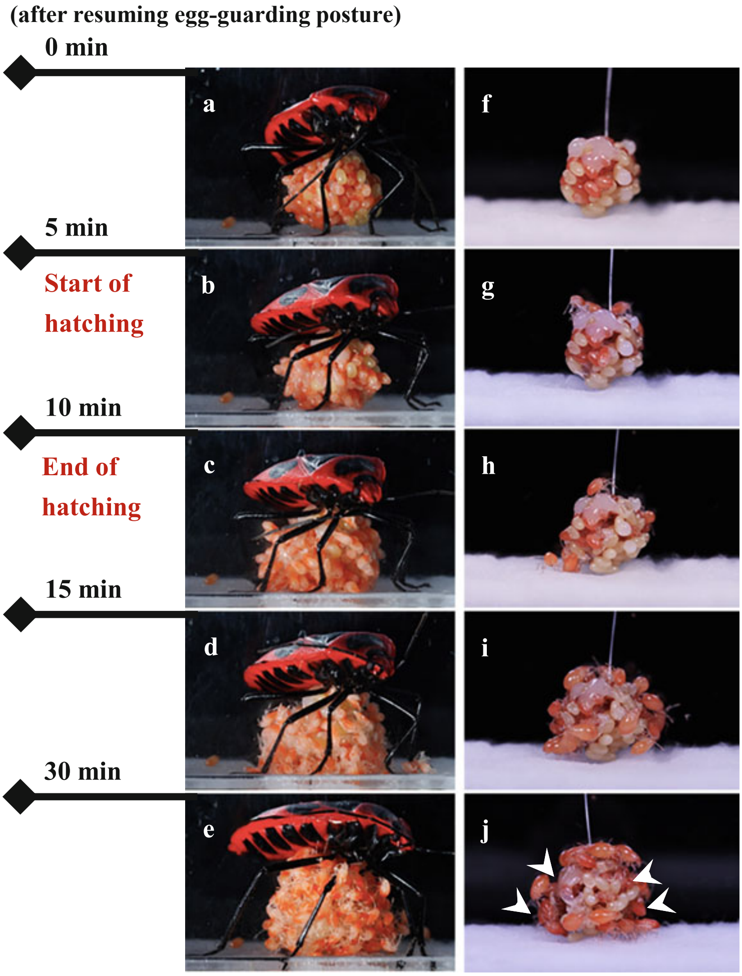

Fig. 5.3 The hatching processes of control (**a**–**e**) and mother-removed egg mass (**f**–**j**). Arrowheads indicate nymphs that are still hatching or which remain in the eggshell (**j**). Whitish eggs are trophic eggs. Adapted from Mukai et al. (2014)

Vibrating Behavior by Mothers

We hypothesized that the synchronous hatching in *P. japonensis* was related to the female's behavior at the moment of hatching. To monitor maternal behavior, we observed seven mothers holding a mature egg mass, which turned pink and which developed conspicuous eye spots immediately before hatching. And it was then that we discovered the unique maternal "vibrating behavior."

The video monitoring observation revealed that all mothers showed a physical vibration at the time of nymphal hatching. Mothers vibrated on their suspended egg

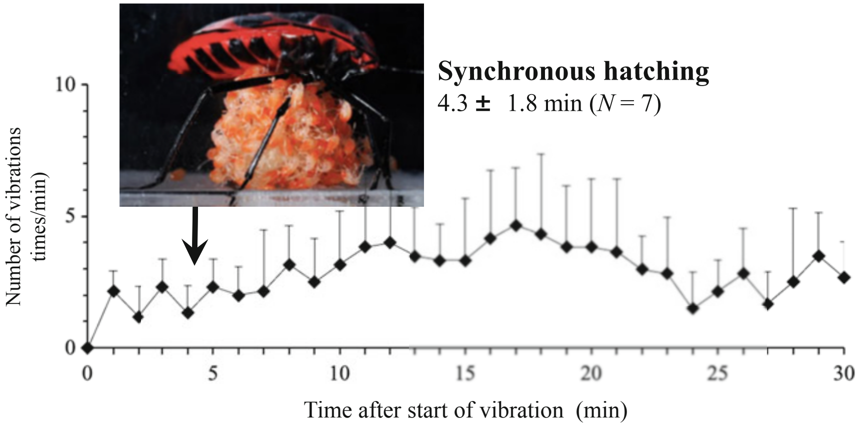

Fig. 5.4 Maternal vibration at the moment of hatching. Black arrow indicates the initiation of synchronous hatching. $n = 7$. Error bars represent SD. Adapted from Mukai et al. (2014)

mass while maintaining the egg-guarding posture. Mothers showed intermittent faint vibrating behavior, i.e., they did not move for several tens of seconds after the prior vibration; then they vibrated their body only once. Figure 5.4 shows the occurrence pattern of maternal vibration. Means + SD are shown for numbers of vibration/min from the start of vibration generated by seven mothers, and at the time indicated by a black arrow, the synchronous hatching began suddenly.

Observation using high-speed photography revealed that maternal vibration comprised only a faint single vertical motion, moving the abdomen slightly, just 2–3 mm vertically up and down. The single vertical motion took approximately 0.3 s to move the mother's abdomen. To quantify the temporal pattern of the maternal vibration, we counted the abdominal movements per minute for 30 min from the beginning of the vibrating behavior (Fig. 5.4). Mothers vibrated, on average, a total of 87.5 ± 1.8 times (mean ± SD), constituting 2.9 ± 1.8 times per minute of vibration during this period. Although no distinct peak of the maternal vibration or remarkable change of the mother's behavior was observed, highly synchronous hatching began at 4.3 ± 1.8 min (Fig. 5.4) after the start of vibration. Some mothers continued the vibrating behavior for more than 6 h.

5.2.2 *Effect of Maternal Vibration on Hatching Pattern*

Does the maternal vibration affect hatching pattern?

To verify whether the maternal vibration served to synchronize hatching, we monitored the temporal pattern of hatching of eggs assigned to one of four groups: control (with mother) group, mother-removed group, and two artificial vibration groups.

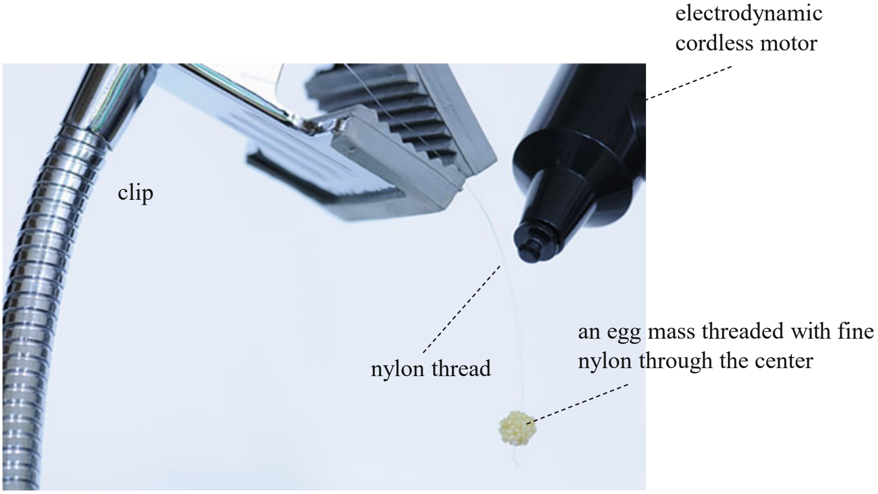

Fig. 5.5 Experimental design of artificial vibration

The vibration of the female parent was reproduced using a cordless motor (Fig. 5.5). In the mother-removed group and two artificial vibration groups, we isolated mature egg masses from mothers immediately after they finished the excretion behavior. Then we weaved a fine nylon thread through the midline of each egg mass and artificially suspended it by holding it with a clip. For the artificial vibration, we used two different vibration patterns: intermittent artificial vibration and continuous artificial vibration. For the intermittent vibration group, we provided artificial vibration using a cordless motor during 15 min from the end of mucus excretion. We switched the motor on for an instant every 30 s with the cordless motor attached to the end of the nylon thread. For the continuous vibration group, we provided continuous artificial vibration for 15 min from the end of excretion.

The results clearly revealed that when we exposed the egg masses to intermittent artificial vibration, the embryos began to hatch rapidly one after another during approximately 5 min after the start of vibration. The average percentage of hatched eggs ± SD was 77.6 ± 11.8% within the first 30 min, which was a similar level to the control group (90.3 ± 7.3%). However, when the mothers were removed from the egg mass, the pattern of hatching changed drastically. In the mother-removed group, about half of the embryos failed to hatch, and the other half hatched asynchronously. Embryos took a long time to hatch, with only 5.0 ± 4.5% of the embryos hatching within the first 30 min (Fig. 5.6). Interestingly, when we exposed egg masses to continuous artificial vibration, the embryos also took a long time to hatch, with only 14.9 ± 13.4% of the embryos hatching within the first 30 min (Fig. 5.6). The embryos that had not hatched during the observation never hatched thereafter; all blackened and died. These results suggest that the embryos sense whether the female parent is vibrating or not and respond by synchronizing the timing of their hatching.

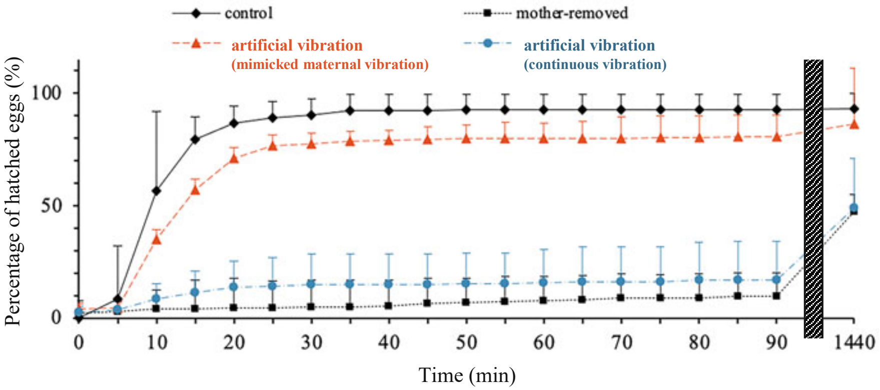

Fig. 5.6 Hatching pattern of embryos exposed to the different treatments. Means + SD are shown for the percentage of hatched eggs at 5 min intervals for the first 90 min and for 24 h from the start of hatching. Adapted from Mukai et al. (2014)

Embryos enclosed in the eggshells have a sophisticated mechanism for distinguishing maternal vibration.

5.2.3 Maternal Care as a Necessary Cue for Embryo Hatching

Mukai et al. (2012) described the functions of hatching care as classifiable into two groups: “hatching assistance” and “hatching regulation.” Hatching assistance, a form of parental care performed by parents to provide some assistance for their young, enhances hatching success, as shown by parents of most avian species: they break into the hard eggshell to assist their young (Tremaine 1974; Birmelin and Wolter 1986; Newton 1986; Bond et al. 1988). Hatching regulation is a form of parental care that is performed by parents to provide some physical or chemical stimulation for embryos to regulate the hatching pattern, as seen in subsocial spiders: mothers adjust the egg-hatching time in response to the threat of predation (Li 2002). The hatching care of *P. japonensis* is evidently different from other examples of hatching assistance in avian, crocodilian, and arachnid species.

In *P. japonensis*, mothers give only a faint vibration to the egg mass. In fact, even when we provided continuous artificial vibration to the isolated egg mass that did not match the temporal pattern of maternal vibration, the hatching tended to fail and to occur asynchronously (Fig. 5.6). This observation revealed that it was not the actual physical vibration that induced shell rupture. However, we also found that when we provided continuous artificial vibration, embryo hatching was not only asynchronous but also some embryos failed to emerge from their shells. This suggests that the female *P. japonensis* vibrations in a particular pattern serve as a necessary cue for hatching.

Warkentin (2011a) classified hatching systems related to hatching cues into two groups: “spontaneous hatching (SH)” and “environmentally cued hatching (ECH).” “Fixed hatching” or “normal hatching” is synonymous with spontaneous hatching used in hatching studies of amphibians (Warkentin 2011b) and reptilians and avians (Ewert 1991). SH is defined as a hatching system that operates without an external cue (Warkentin 2011a; Gomez-Mestre et al. 2008; Saifur et al. 2010). SH occurs at a consistent stage in development or, potentially, after a consistent embryonic period, irrespective of environmental conditions. In contrast, ECH is defined as a “decision” based on information. It might depend on a specific behavioral or physiological process that is cued environmentally (Warkentin 2011a). For an animal species that depends entirely on an SH system, or on both SH and ECH, embryos hatch with no stimulation. In a species depending solely on ECH, hatching will not occur without the environmental cue. Embryos die when the energy reserves become exhausted. For example, in some solitary zooplankton *Daphnia* species, embryos never hatch without moisture stimulation (Arnott and Yan 2002). It is likely that the hatching system of *P. japonensis* is a type of ECH only. Therefore, interception of the maternal vibration or providing continuous artificial vibration might affect the hatching success.

In Mukai et al. (2014), the form of hatching care was redefined as below: “Hatching assistance” is a form of parental care by which parents assist hatching by reducing the physical burden of embryos. “Hatching regulation” is a form of parental care by which the parents regulate the temporal pattern of hatching without reducing the burden. We have classified the function of hatching care in accordance with ascertaining whether parental care examined in previous studies served (1) to improve the hatching success and (2) to influence the hatching pattern. Therefore, in addition to these indexes, we must incorporate consideration of a new index: (3) how parental care is related to embryo hatching. In this paper, we proposed and assessed a more appropriate definition and verification methods of functions related to hatching care than those reported in the literature (Mukai et al. 2012). Our proposals are expected to clarify previous and future studies of parental hatching care. Future studies are expected to extend the classification of hatching care and to elucidate the evolutionary background of hatching care and parent-embryo interactions.

5.2.4 Maternal Vibration and Synchronous Hatching in Closely Related Species

Closely related species to *P. japonensis* also have been found to display maternal vibration and synchronous hatching. Burrower bugs, *Adomerus rotundus* (Heteroptera: Cydnidae), exhibit maternal care including defense against predation and provision of food. We previously found that female *A. rotundus* also displayed

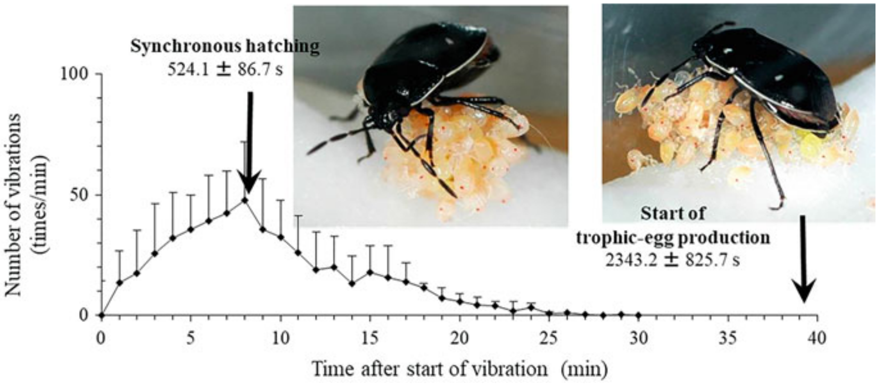

Fig. 5.7 Maternal hatching vibration in *A. rotundus*. Black arrows indicate each event of maternal care. Adapted from Mukai et al. (2012)

physical vibration (shaking the body rhythmically) while maintaining the egg-guarding posture, i.e., holding the egg mass under the thorax between the forelegs and midlegs, and promote synchronous hatching (Mukai et al. 2012). However, although both the hatching care of *P. japonensis* and *A. rotundus* includes vibration of their egg masses, some important differences are apparent in the features of the vibrating behavior.

One notable difference is the occurrence pattern of the vibrating behavior. In *A. rotundus*, the maternal vibration increased initially over time, reaching a peak at approximately 9 min after the start of vibration, when most of the eggs hatched (Fig. 5.7). This vibrating behavior lasted about 23 min. However, in *P. japonensis*, the maternal vibration did not have a distinct peak. Rather, it gradually increased and then plateaued. Vibrating behavior of *P. japonensis* lasted interminably over 6 h from the beginning of vibration, even after almost all embryos' hatching had already finished (Fig. 5.4). Detailed temporal patterns of vibration were also different in these two species. In *A. rotundus*, maternal vibration apparently took place intermittently with alternate occurrence of two conspicuous periods: a vibration period and a pause period. The vibration period included several pulses, which comprised the single vertical motion of the vibrating mother's body. The vibration of the female *A. rotundus* was composed of intermittent pulse groups in the low-frequency region below 100 Hz. On the other hand, *P. japonensis* mothers provided a faint vibration to the suspended egg mass, i.e., mothers vibrated their bodies only once per several tens of seconds.

Among closely related species of some crabs, the mothers of which generate abdominal pumping at hatching, some comparisons have been made of the frequency and the occurrence pattern of maternal pumping behavior (De Vries et al. 1991; Christy 2011; De Vries and Forward Jr. 1991). The differences between the hatching care are presumably attributable to the physiological constraints of eggs, such as their respective sizes (De Vries and Forward Jr. 1991). Considering that, differences between *P. japonensis* and *A. rotundus* might be derived from

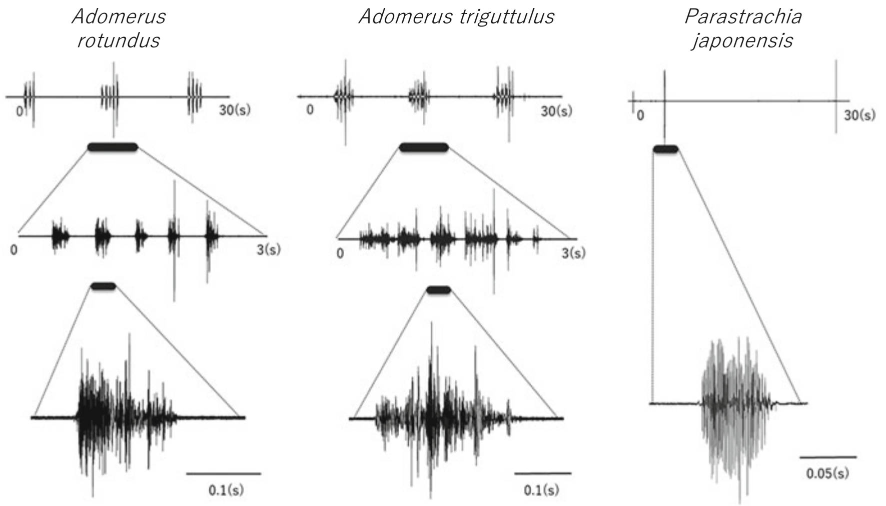

Fig. 5.8 Time-based waveform of maternal hatching vibration in three species, *Adomerus rotundus*, *A. triguttulus*, and *Parastrachia japonensis*. Adapted in part from Mukai (2019)

physiological constraints. Some species of burrower bugs (Cydnidae: Sehirinae) also show maternal care, including egg guarding, production of trophic eggs, protection of nymphs, and progressive provisioning (*Sehirus cinctus*, Sites and McPherson 1982; *Adomerus triguttulus*, Nakahira 1994; *Canthophorus niveimarginatus*, Filippi et al. 2009; *Adomerus variegatus*, Mukai et al. 2010; *Adomerus rotundus*, Inadomi et al. 2014). Moreover, in some species of burrower bugs, maternal hatching vibration has also been observed. Measuring the vibration with a non-contact-type Laser Doppler Vibrometer revealed that *Adomerus triguttulus*, which has a similar life history to *A. rotundus*, shows a similar vibrational pattern to that of *A. rotundus* (Fig. 5.8). To elucidate the diversity of the stimulus in hatching care, future studies should examine more details of the physiological and ecological constraints of these species and other subsocial bugs practicing complex maternal care resembling that of *A. rotundus* and *P. japonensis* provided by mothers.

5.2.5 Adaptive Function of Maternal Care at the Moment of Hatching

Recent studies have also provided results that explain the adaptive significance of simultaneous hatching. Mukai et al. (2018) tested the hypothesis that synchronous hatching induced by maternal vibration in *A. rotundus* prevents sibling cannibalism. Mothers and their mature egg masses were allocated to three groups: synchronous hatching by maternal vibration (SHmv), synchronous hatching by artificial vibration

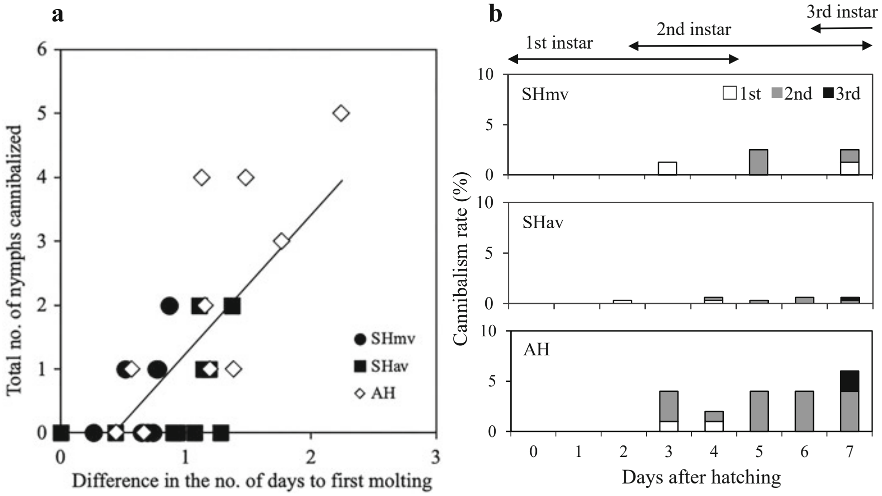

Fig. 5.9 (**a**) Correlation between number of days to first molting and total number of *A. rotundus* nymphs cannibalized and (**b**) timing of sibling cannibalism within clutch. *SHmv* synchronous hatching by maternal vibration; *SHav* synchronous hatching by artificial vibration; *AH* asynchronous hatching. Adapted from Mukai et al. (2018)

(SHav), and asynchronous hatching (AH). Then they investigated the influence of each hatching strategy on the occurrence of sibling cannibalism of eggs and early instar nymphs in the laboratory. No difference in the proportion of eggs cannibalized was observed among the three groups. However, the proportion of nymphs cannibalized was higher in the AH group than in the SHmv group (Fig. 5.9, Mukai et al. 2018). The number of days to first molting within clutch was significantly higher in the AH group than in the SHmv group (Fig. 5.9a). After examining the timing of cannibalism, it was clear that cannibalism occurred with high frequency during the period of molting from the first instar to the second instar, and also from the second instar to the third instar (Fig. 5.9b). We sometimes observed early instar nymphs being eaten by late instar nymphs. However, we often observed that immediately after molting, late instar nymphs were at a high risk of being eaten by early instar nymphs (Fig. 5.10). Molting individuals have a thin integument and cannot escape or protect themselves from conspecific or interspecific predation (Dick et al. 1990; Soluk 1990), so they are considered to be an affordable and familiar food source for starving nymphs. Such within-clutch interactions caused by AH may have been an evolutionary driving force for the unique maternal hatching regulatory system and synchronous hatching in *A. rotundus*. Endo and Numata (2020) also revealed evidence supporting that synchronized hatching observed in the brown marmorated stink bug stimulated by vibratory cues (Endo et al. 2019) helps eggs to avoid being cannibalized by their earlier-hatched siblings. They also show that other stink bugs with no synchronized hatching have little or no risk of sibling egg cannibalism (Endo and Numata 2020). Sibling cannibalism and synchronized hatching are likely to be closely related.

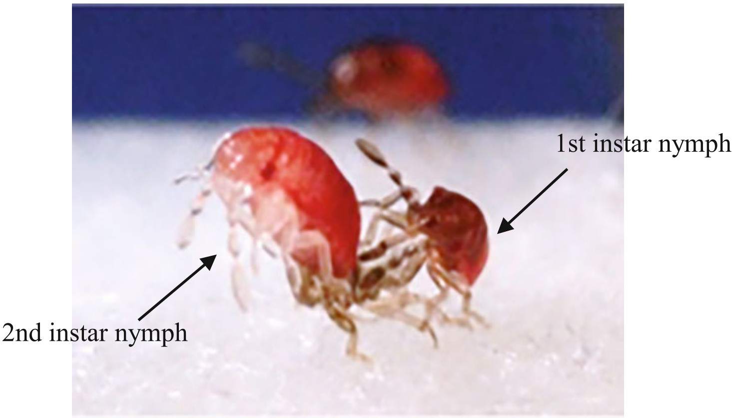

Fig. 5.10 Sibling cannibalism of newly molted second instar by first instar nymph in *Adomerus rotundus*. Adapted from Mukai et al. (2018)

Synchronous hatching also could help the newly hatched nymphs form large aggregations more easily. Such aggregations might improve hatchling survival rates by effectively reducing the predation rate through cooperative defense (Henry 1972; Godfray et al. 1991), maintaining favorable conditions (Lauber and Darvas 2009), and/or obtaining food resources by group foraging (Way and Cammell 1970; Ralph 1976; Inoue 1986). In addition, synchronous hatching might function to keep the developmental stages synchronous, thereby enabling parents to provide care that is appropriate to the developmental stage of all or most nymphs at any given time. In several precocial avian species, some newly hatched chicks leave the nest after a few hours and start self-feeding under parental care. If the other eggs in the nest have not yet hatched at this point, then parents face the problem of whether to choose egg-guarding or offspring-protecting, which are two mutually exclusive behaviors. To avoid this dilemma, parents might synchronize hatching of chicks strategically, thereby eliminating the need for decision-making that would result in neglecting some offspring (Stoleson and Beissinger 1995). In any of these scenarios, some offspring would necessarily be neglected for part of the time. To give all offspring optimal care with optimal timing and to economize the duration of care, mothers might prompt eggs to hatch synchronously. It is also possible that synchronous hatching could increase the reproductive rate of the parents by shortening the parental care period.

In subsocial stink bugs, the nymphs often spend 10–15 days (third instar or fourth instar) under the protection of the female bugs. During this period, the nymphs live in dense aggregations of siblings in the nests and depend on trophic eggs and seeds of host plants supplied by the female parent for their food. In *P. japonensis*, synchronized hatching might also have other functions for hatched nymphs such as activating the taking up of the mucus secretion, which might be involved in uric acid recycling during the diapause period (Kashima et al. 2006; Hosokawa et al. 2010). Given these unique ecological peculiarities, in addition to the cannibalism avoidance described above, the influence of the parental care behavior of the female parent may be the background for the evolution of synchronous hatching. The acquisition of parent-embryo communication through vibration is an important means to achieve that synchronous hatching.

5.2.6 Conclusions About Parent-Embryo Communication in Subsocial Stinkbug

Shield bugs and stink bugs specialize in vibration communication, and it is highly possible that they have acquired a communication system at the time of hatching by fully adapting the already existing vibration reception system (Cocroft and Rodriguez 2005; Čokl 2008). It is known that *P. japonensis* also has the vibrational receptors called "Femoral Chordotonal Organs (FCOs)" in all six legs (Nishino et al. 2016). In addition, it has been confirmed that just prior to hatching the embryos actively move inside the eggs. From these facts, it is considered that in *P. japonensis*, embryos immediately before hatching actively receive vibrational signals and construct bidirectional communication between parents and embryos. In future studies, we would like to clarify the process by which the evolution of the unique hatching communication system by the subsocial shield bug embryos occurred in the exposed biological environment.

5.3 Parent-Nymph Communication

Mothers of *P. japonensis* communicate with offspring at different stages of development. Although we have already explained about the communication between mothers and eggs, we have discovered that females also communicate with post-hatched nymphs when feeding them in the nest. Females emit a vibrational call after returning to the nest with a drupe; the nymphs then gather on the drupe. We designated this vibrational call "provisioning call." We will explain here how mothers emit the provisioning call to post-hatch nymphs and how nymphs respond to the call. Most of what we present in this section is based on the results about female "provisioning call" that were reported by Nomakuchi et al. (2012).

In this section, we first introduce a story about the discovery of the provisioning call, in addition to the behavioral conditions of a parent and nymphs when calling, and then we describe the vibrational characteristics of the provisioning call itself and, further, its functions. We consider that the provisioning call is one of the signals in the communication system between a signaling mother and receiving nymphs.

5.3.1 Provisioning Call

Discovery of the Provisioning Call

One of our research bases for this species, *P. japonensis*, was the Laboratory of Animal Behavioral Ecology, Department of Applied Biology, Faculty of Agriculture at Saga University, where a variety of intensive research in terms of parental care behavior, intra-familial conflict, and cooperation in this species has been carried out for a couple of decades. Although in the early stages of our research, most of our investigations were carried out in the field, we gradually moved to laboratory experiments to enable more controlled testing of various hypotheses about parental behavior. In most laboratory experiments, females (50–100 individuals) guarding their egg mass under leaf litter were collected together with their egg mass from our field site at Mt. Hinokuma, Kanzaki City, Saga, Japan, and were introduced into the laboratory in late May. Collected females and their egg masses were individually placed into transparent plastic cups (about 10 cm in diameter and about 5 cm in depth) filled with forest bed debris collected from around the actual nests in the field and kept in incubators under conditions of 25 °C and 14-h light/10-h dark. Using these females, several experiments have been carried out to observe parental behavior under laboratory conditions. Mantaro Hironaka, who had recently completed his doctoral dissertation on this bug, was the first to notice a faint buzzing sound when females returned to their nests laden with drupes. It was fortunate that he recognized the sound by chance while engaged in another procedure during a series of experiments unrelated to communication. This was just the moment when the "provisioning call" was discovered. The call was so low that it would never have been recognized in the field because of the background noise. It is considered that there are three fortunate conditions of the study that have contributed to discovery of the provisioning call. First, although the provisioning call could be a vibrational signal probably adapted to the physiological receiving capabilities of conspecifics, it also partially clearly involved audible airborne sounds that could be heard by a human. If it were only vibrational, we might never have recognized it. Second, our experimental observations were performed in the convenient environment of the laboratory under which sufficiently quiet conditions were possible instead of in the noisy field. Additionally, we could simply move our ears close to the cups containing a nest to hear the call. Third, the cups housing the nests were transparent so that observers could monitor the behaviors of females and nymphs from every direction when females were emitting the call.

5.3.2 When Do Females Emit the Call?

In our investigation of the provisioning call, we first sought to determine exactly when females emitted the call, especially questioning whether the call was limited to

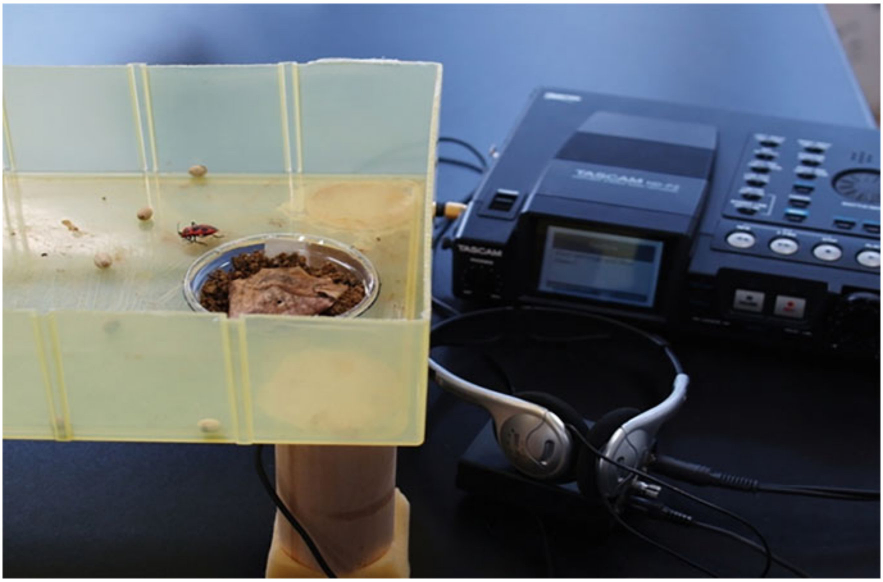

Fig. 5.11 Observation table with a nest cup set into the bottom of the container/table and the digital recorder. Note the female foraging for drupes on the bottom of the container after leaving her nest

a particular time that coincided with any specific actions or behaviors by females and/or nymphs during parental care. The observation was carried out in the laboratory using a rectangular plastic container that was configured into a table (40 cm in length, 30 cm in width, 15 cm in height, with four legs, Fig. 5.11). A hole was cut into the bottom of the table where the nest cup could be set, such that the upper edge of the cup was flush with the bottom surface of the table where drupes that the females could collect were scattered, and the rest of the transparent nest cup protruded below the base of the container/table. In this way, the females could easily leave and return to the nest over the cup edge when foraging for a drupe, and activities inside the nest cup could also be easily monitored. Females that returned to a nest were continuously observed as long as possible. In other words, unless the female burrowed into the debris around the nest, we could observe her behaviors; simultaneously, all vibrations and/or sounds observed were also measured. All vibrational signals emitted through the cup wall were collected using a contact microphone (HOGA original contact microphone, TWA-3S, Kyoto City, Kyoto, Japan: http://www.hoga-kyoto.com/) attached to the outside of the bottom of the nest cup and were recorded on a digital sound recorder (TASCAM, HD-P2) with a 44.1 kHz sampling frequency (Fig. 5.11). Additionally, a condenser microphone (Audio-Technica, AT9842, Machida City, Tokyo, Japan) was set 2 cm above the nests to collect the airborne sound.

More than 100 females caring for nymphs were monitored according to the above procedure, and the sequence of behaviors that follows for one female was consistent for all females observed. Initially the call began to sound just when the female entered the nest cup, and it continued to be clearly heard, emitting from the direction of the female as she moved about in the nest. Observation of the female's body posture when the call was emitted revealed that the female was shaking her body while maintaining a narrow gap between the dorsal abdomen and wings, similar to the posture of calling crickets. These observations demonstrate that the call was definitely being emitted from the female. Further, we came to realize that that the call from the female was coordinated with a series of female behaviors during the process of provisioning the nymphs in the nest. The female began to call as soon as she entered the nest dragging the drupe that she had collected and continued to call intermittently in bouts inside of the nest while plowing her way through the nest debris, which included plant roots, organic soil, and rotten leaves. Upon encountering an aggregation of nymphs, the female placed the drupe next to the nymphs, but still kept it attached to her proboscis. The nymphs then began to approach and cling to the drupe that the female still covered with her body. When many nymphs had gathered and clung to the drupe, fully covering its surface, the female finally released the drupe from her proboscis and stopped calling. The female then moved about 2 cm away from the feeding nymphs and remained there for just a few minutes before departing the nest to collect the next drupe. Observations were terminated when the female left the nest, or at 10 min after the female dropped the drupe, even if she remained in the nest. This behavioral sequence was nearly identical in all cases that we observed ($n = 40$). Therefore, the provisioning call could be a necessary component of a programmed behavioral sequence comprising all the provisioning actions. In fact, we assumed that the call would serve as a special signal that females use to call the nymphs in the nest so they could easily and quickly encounter the food brought into the nest.

Further, we discovered that the call collected by the contact microphone on each observation of a female consisted of multiple sound bouts with various sound lengths. The length of all sound bouts and their gaps were recorded from actual hearing of the call with a headphone. The duration of each call was measured from start to finish with a stopwatch for individual females. Table 5.1 shows the results of four collected variables: the total number of sound bouts, the longest bout length, the percentages of short sound bouts (<2 s), and the total length of all sound bouts through an individual call for two nymphal stadia, the first and the third (Nomakuchi et al. 2012). The number of sound bouts was significantly larger, and the total length

Table 5.1 Pattern of sound bouts in a provisioning call through nymphal growth (the first and the third stadia). Adapted from Nomakuchi et al. (2012)

N	No. bouts	Longest bout length (min)	% Short bouts	Total length of bouts (min)
1st 17	55.8 ± 4.1	1.12 ± 0.33	56.5 ± 3.5	36.16 ± 3.53
3rd 13	26.2 ± 4.6	2.17 ± 0.46	55.7 ± 4.6	12.90 ± 2.17
U	353.0	226.0	265.5	356.0
P	0.0002	0.1215	0.9499	0.0001

of all bouts was significantly longer, during the first instar than the third instar. There was no significant difference in the length of the longest sound bout between the first and the third instars. Furthermore, there was also no significant difference in the percentage of short sound bouts between the first and the third instars. The fact that the number of sound bouts and the total length of all sound bouts decreased possibly resulted in a shortening of the entire provisioning call with the progression of nymphal development. If the provisioning call functions to gather nymphs to the drupe that the females were trying to provide them, the present results are reasonable because older nymphs with better maneuverability should be able to approach the drupe more easily and quickly on their own.

5.3.3 Vibrational Characteristics of the Provisioning Call

We further tried to investigate vibrational characteristics of the provisioning call using the sound data that we had recorded. We had collected and recorded the call through the wall of the plastic nest cup to examine how females emit the provisioning call and how the call is associated with behavior of females and/or nymphs through observations in the laboratory. However, we realized that the wave quality of the call might have been transformed into a biased one when passing through the plastic wall. Therefore, we felt strongly that we should measure the call again by a more appropriate method, one that involved less wave transformation, so that we would be able to analyze its vibrational properties more appropriately. Thus, we decided to collect the call again from wild females more directly, under natural field conditions and without the plastic cup.

On a fine day with almost no wind at our field site at Mt. Hinokuma, three nests with third instars were chosen to collect the calls for analysis of their vibrational properties. Once we located a nest in the field, we gently inserted the short arm of an L-shaped iron clamp into the soil just under the nest, and the other arm of the clamp, sticking out, from the ground was attached to the contact microphone. We considered that the metal clamp would work as an extended probe for the contact microphone. We collected all vibrational pulses emitted from the nest when females returned with a drupe. Furthermore, to identify all background noises, we also collected the noise when females were absent from their nest. All vibrational pulses were recorded on a digital sound recorder, and data were input into a personal computer and analyzed with fast Fourier transform (FFT) analysis using the bio-acoustic analyzing software, Avisoft SASLab Pro (Berlin, Germany).

Although the provisioning call sounded just like a weak buzzing or fluttering sound, composed of a set of alternately repeating intermittent sound parts (sound bouts) and gaps (silent intervals) to the human ear, the vibrational properties of a segment of a provisioning call collected by the contact microphone in the field are shown in Fig. 5.12. Figure 5.12a indicates a waveform of the segment of the provisioning call by a female that was calling in her nest. The call clearly consists of repetitive clusters (bouts) of waves with varying range and amplitude. Some bouts

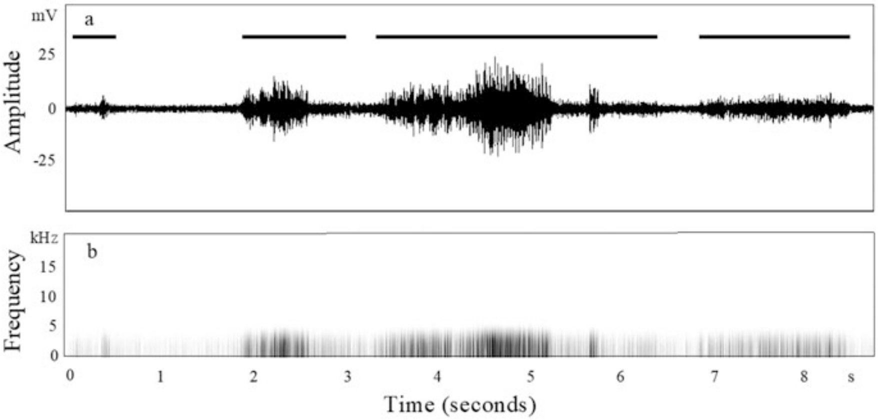

Fig. 5.12 The vibrational properties of the provisioning call. An oscillogram (**a**) of a segment of a provisioning call is shown. The provisioning call consists of a repetition of clusters of waves with varying range and amplitude. Substantial vibration parts with higher amplitude and other parts with lower amplitude or background noise repeatedly appeared, matching the intermittent sound occurrence (sound bouts: marked with bold horizontal bars) heard by the observer. A spectrogram (**b**) of the same segment of the provisioning call is shown below. Adapted from Nomakuchi et al. (2012)

maintain higher amplitude for 2–130 s, probably indicating audible parts, while the gap parts may represent the less audible segments with lower amplitude. Figure 5.12b shows the spectrogram from the FFT analysis of the same segment, indicating that, consistent with the waveform, the power spectral density was apparently concentrated on a relatively lower level of frequency, varying somewhat in power and frequency.

On the other hand, we compare the power spectra of the same segment of the collected call as above with another segment which was collected when the female was absent from the same nest (as background noise) to identify the actual components of the spectra of the call (Fig. 5.13a, b). The component of power spectrum of the provisioning call can be clearly discriminated from that of the background noise with an occurrence in the range of 0–1 kHz. This result suggests that the provisioning call is basically composed of substrate-borne vibration, although partially including airborne sound, probably as a by-product. This may be supported by the fact that this insect has no auditory senses that can capture airborne sound; it can only receive vibrational senses for substrate-borne vibration (Takanashi et al. 2019).

Observations in the laboratory indicate that the provisioning call apparently comes from females. How do females emit the provisioning call? Calling females were shaking their bodies, maintaining a narrow gap between the dorsal abdomen and wings, suggesting that the females may emit the call from this body vibration. However, it is still unknown how females produce the call. All we know at present, besides the body vibration, is that the body part likely related to production of the call is a complex "membrane" structure on the abdomen. They have an exposed membrane between the thorax and abdomen on the dorsal side under their wings

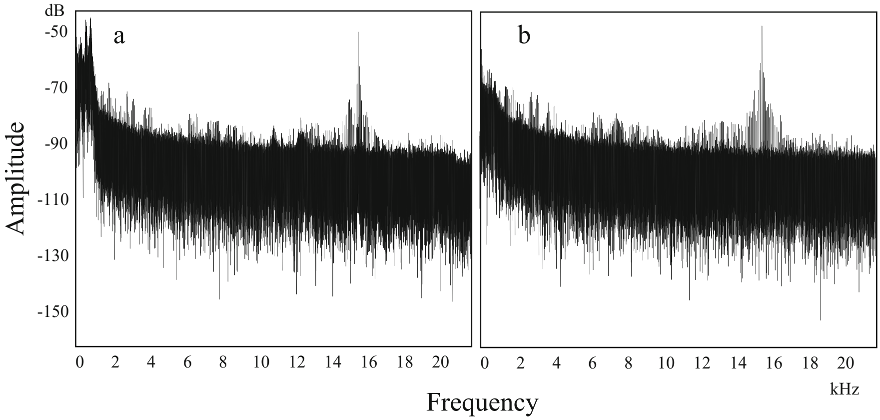

Fig. 5.13 The power spectra of a segment of the provisioning call when a female was calling in her nest (**a**) and of background noise when the female was absent from the same nest (**b**). The substantial component of the provisioning call that could be clearly discriminated from the background noise is recognized in the lower range of 0–1 kHz. Adapted from Nomakuchi et al. (2012)

(Fig. 5.14). Further, inside their body near the dorsal abdomen, multiple coeloms divided by another internal membrane spread from the small cavity under the exposed membrane between the first and second dorsal abdominal tergite. When we slit the exposed membrane experimentally, the amplitude of the call was

Fig. 5.14 Dorsal abdomen of female specimen with the four wings removed. Dorsal abdomen has a characteristic membrane between the first and the second tergites. This exposed membrane seems to play a role in amplifying the call emitted by females because the volume of the call was extremely reduced when it was slit or hardened by super glue

extremely reduced, although the call could still be heard. Thus, it seems that the exposed membrane is the organ that functions to amplify the call, not to produce the call, as the call producing "timbal" in some hemipteran insects like cicadas. Although the precise role this membrane structure plays in the provisioning call remains unknown, we expect that it has an important function for the emittance of the call.

It has already been reported that every *P. japonensis* individual produces an "alarm call" when stimulated by touching and blowing on a hibernating group of bugs. The alarm call could be a high pitch (about 10 kHz) stridulatory sound made by scraping the edge of the scutellum and forewing (Filippi-Tsukamoto et al. 1995b). Even if a few individuals of a group are stimulated by attacks of vertebrate predators, their responsive body movements involved with the stridulatory sound gradually expand throughout the group, often leading to large responses by the whole group. As the alarm call eventually emerges simultaneously from every individual in the group, it could become a loud sound, loud enough even for human observers to be able to hear clearly from about 1 m away. By the way, since it is known that *P. japonensis* has no auditory senses for airborne sound (Takanashi et al. 2019), then who hears this alarm call? It could be that the call is meant to startle vertebrate predators, e.g., birds and mammals, instead of alarming conspecific group members. Because *P. japonensis* has triglycerides that are presumed to be unpalatable or poisonous to vertebrate predators (Tojo 2008), this alarm call could be considered to function as a kind of aposematism like the sounds of rattle snakes.

The provisioning call is entirely different from this alarm call. Although the provisioning call was slightly audible to human observers, it may have a functional role mainly as a substrate-borne vibration because most of the sound power was distributed in the lower frequency (less than 1 kHz) range. Further, nymphs might be able to receive a substrate-borne vibration sound more easily than an air-borne sound, because they are usually hiding in the complex inner substrate of the nest, in crevices of debris, fragments of broken and half decayed leaves or tree twigs, in the nest. They may sense substrate-borne vibration better through their legs while standing on a piece of debris in the nest. It is also known that individuals of *P. japonensis* actually have special organs to sense vibration, the chordotonal organ, in their legs instead of senses for recognition of audible sounds (Takanashi et al. 2019). In fact, Gogala et al. (1974), in a leg removal experiment, reported that males in a closely related family, Cydnidae, in which *P. japonensis* was included until Sweet and Schaefer (2002) elevated the genus *Parastrachia* to a new family, Parastrachiidae, need their legs to receive a rival's vibrational call.

It is generally known that individuals in Cydnidae have two mechanisms to produce sound or vibration in communication, a tymbal mechanism and stridulation (Gogala et al. 1974). Tymbal vibration typically has relatively lower frequency signals, which would be effective in communicating through substrate-borne vibration. On the other hand, stridulatory sound has higher frequency signals, which would be effective in communicating through airborne sound (Gogala 1985). We have not yet established which part of the body of female *P. japonensis* is responsible for this vibration production. However, several findings in the present study suggest that the tymbal mechanism could be responsible for the provisioning call for the following reasons. First, the provisioning call basically ranges in the lower region of frequencies that are not usually included in the stridulatory sound in Cydnidae (Čokl et al. 2006). Second, *P. japonensis* has a characteristic membrane structure in the dorsal part of the abdomen, including not only the tergal membrane between the first and second tergum of the abdomen but also an internal membrane structure separating a number of vacant chambers within the abdomen. These membranes could contribute to the vibration production and/or amplification, as Gogala (1985) mentioned about a tergal tymbal in Cydnidae. Third, we also observed that females of *P. japonensis* appeared to vibrate their abdomen while maintaining a small gap between the abdomen and wings, when emitting the provisioning call (unpublished). Therefore, we suspect that the provisioning call could be produced by the membrane structure as the tymbal system.

5.3.4 Function of the Provisioning Call

Why do females emit the provisioning call? From the fact that females begin to call only when they return and enter their nest with a drupe and end the call when they give the drupe to nymphs in the nest, we formulated a hypothesis that the provisioning call functions to call nymphs hiding deep in the crevices in the nest to gather on a drupe. We carried out an experiment using a playback call to test this hypothesis.

As the playback call for the experiment, we employed a vibration sequence (5 min) made by repeatedly combining a specific segment of an actual provisioning call recorded in the Hinokuma field (about 30 s) that contained minimal background noise. We played this constructed playback call to all families in the experiment, assuming that the specificity of the provisioning call for individual families, if present, may be weak because of how remarkably irregular the pattern of actually recorded provisioning calls was even for a given female. To carry out the playback experiment, we used wild families that were collected from the Hinokuma field site and maintained individually in nest cups in incubators as described above. We set the nest cup on the observation table. We placed a drupe at the center of the nest after removing the leaf covering on the nest and connected one end of a steel probe to the drupe and the other end to a contact speaker (Ceramics speaker unit “CEMI,” SANEI HOUSE Co. Ltd., Sagamihara, Kanagawa, Japan) in order to transmit the playback call in the nest through the drupe and any nest debris.

We prepared 29 families with 27.3 ± 6.2 (mean ± SD) individuals of third instars whose mothers were removed from the nests. We exposed each family to two conditions, a drupe with no call (control) and a drupe with the playback call (treatment), sequentially with an interval of 24 h between the two conditions. We changed the order of the conditions to eliminate any order effects: the first 15 families were given the treatment condition first and then the control condition second, and the remaining 14 families were given the control condition first and then the treatment condition second. The response of nymphs to these conditions was

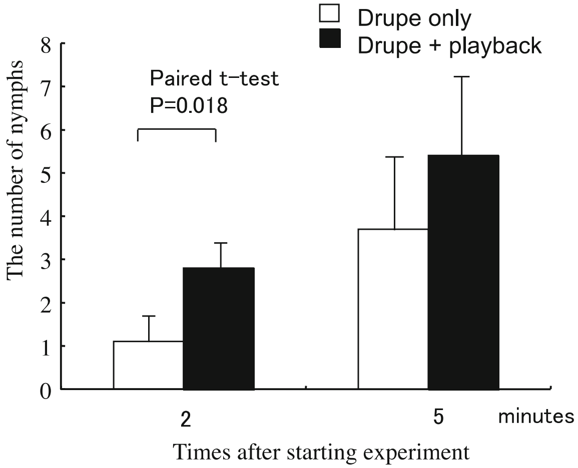

Fig. 5.15 The effect of the provisioning call to nymphs. The numbers of nymphs that gathered on and clung to a drupe placed in a nest under the conditions with a playback of the provisioning call (shaded columns) and with no call (open columns) at 2 and 5 min after the start of experiment are shown. A significantly greater number of nymphs had gathered on the drupe with the call than that with no call at 2 min after start of the experiment. Error bars represent standard error. Adapted from Nomakuchi et al. (2012)

assessed by counting the number of nymphs that had gathered on the drupe at 2 and 5 min after the start of the trial (setting a drupe and probe in the nest for the control condition and starting the playback call additionally for the treatment condition), respectively.

We obtained relatively clear results from this experiment. Significantly greater numbers of nymphs under the treatment condition (about 10% of total nymphs in the family) in which the nymphs were exposed to the drupe and the playback call at the same time gathered on the drupe, compared with those under the control condition (about 4% of total nymphs in the family) (only a drupe) at 2 min after the start of the trial (Fig. 5.15; paired *t*-test: $t = 2.505$, df $= 28$, $P = 0.018$). On the other hand, at 5 min after the start of the trial, the difference in the number of nymphs that gathered on the drupe between the treatment and control conditions was not significant, although the average number of nymphs under the treatment condition tended to be greater than that under the control condition (paired *t*-test; $t = 1.106$, df $= 28$, $P = 0.278$). The statistical significance for the result at 2 min after the start of the trial was consistent even when the Bonferroni method for multiple comparisons was employed. This suggests that at least in the earlier period from the start of the provisioning call, the number of nymphs gathering on a drupe is enhanced by the call.

The hypothesis that the provisioning call functions to call nymphs hiding deep in the nest to gather on a drupe was supported. However, the role of the provisioning call was not definitively established because when the nymphs in the nest were exposed to only the playback call without a drupe, there was a lesser tendency for them to gather on or near the probe (Nomakuchi, unpublished). This means that the call alone does not attract nymphs to a drupe. It seems that nymphs may need the odor of drupes as a cue to recognize the existence of the drupe and/or to locate where it is. What actually is the provisioning call doing to enhance the attractiveness of a drupe to nymphs when it is accompanied by the call in a nest? Our second hypothesis to address this question was that the provisioning call may function to provide nymphs with information about the mother's return to prevent nymphs from dispersing or continuing to hide inappropriately, misidentifying the female as an enemy when she enters the nest with a drupe. In the field, predators such as carabid beetles sometimes intrude in the nest, in which case the nymphs should disperse from their nest or remain hidden inside the crevices of debris (Filippi-Tsukamoto et al. 1995; Nomakuchi et al. 2005). Therefore, it would be adaptive for nymphs to receive a signal that would allow them to recognize when their mother enters the nest, such as the provisioning call, so as to avoid inappropriate dispersal or hiding when they could be feeding.

It is likely that the provisioning call may also have the effect of not only stopping useless evasive behavior of nymphs when the female returns to their nest but also synchronizing feeding on the drupe in the early period. This could lead to several adaptive results, such as enhancing feeding efficiency of nymphs. To understand why, it will be helpful to explain how nymphs actually feed on a drupe. Nymphs feed on the endosperm enclosed by the hard shell in a drupe (Nomakuchi, unpublished). Similar to other heteropterans, *P. japonensis* nymphs insert the proboscis, inject saliva containing digestive enzymes, and suck up the digested liquid endosperm (Shuh and Slater 1995). If the drupe is too hard for a single or a few nymphs to manage, or saliva from a single or a few nymphs is not enough to digest the endosperm, then the feeding efficiency for individuals should increase by group feeding (Kalin and Knerer 1977; Tsubaki and Shiotsu 1982; Lawrence 1990). In fact, the more nymphs that fed on a drupe together, the more liquid was obtained per nymph on average (Nomakuchi, unpublished). Therefore, the effect of the provisioning call may include a factor of enhancing nymphal feeding efficiency by inducing more nymphs to begin feeding on the drupe simultaneously.

The provisioning call may also reduce variance in feeding opportunity and feeding amount among nymphs. Our observations of the nymphal response when given a drupe in the nest without the provisioning call revealed that nymphs gradually gathered to the drupe, resulting in an asynchrony in the timing of nymphal approach to the drupe, probably according to individual differences in boldness of nymphs, their original location in the nest, and their mobility within the nest debris. This may inevitably cause differences among nymphs in the amount of food acquired from a given drupe, likely having a negative impact on female fitness under sibling competition for food (Mock and Parker 1997; Godfray and Parker 1992). Thus, the provisioning call might also serve as a countermeasure by the mother against excessive sibling competition for food among nymphs.

Another fascinating possibility to explore regarding communication between parent and offspring in this system is that of begging by nymphs. When the female enters the nest, nymphs regularly climb on her body, probing it with their proboscis. In response, the mother typically rocks her body from side to side and shakes them off. However, we have also observed on many occasions groups of nymphs gathered and feeding on a female carcass, much as they would feed on a drupe. We assume these are females that had recently died or become too weak to shake the nymphs off. In fact, a preliminary study in which an anesthetized female was presented to a group of nymphs, the nymphs quickly gathered on the female and probed her. After about an hour, the weight of the female had decreased indicating that the nymphs had fed on her. This raises an important question that needs to be explored: is the behavior of nymphs climbing on the female's body, making physical contact with her, and even attempting to probe her with their proboscis, in fact, a form of begging behavior where the nymphs are signaling their hunger to the female? We hope to answer this question in future studies.

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Hiromi Mukai another student in Dr. Nomakuchi's laboratory and a clever and enthusiastic researcher with fine observational skills also completed a doctoral degree under Dr. Nomakuchi at the United Graduate School of Agriculture, Kagoshima University, with her excellent work that clarified the process by which egg-guarding females synchronize the timing of egg hatching. She is currently a researcher at the Department of Forest Entomology, Forestry and Forest Products Research Institute.

Shintaro Nomakuchi an ecologist focusing on insect behavior, came to Saga University in 1993 as an Associate Professor, and was keen to share his very valuable expertise and join the *P. japonensis* research team, which he eventually headed. The work on clarifying the quality of the habitat, the techniques for monitoring the subpopulations and for carrying out a variety of ecological and behavioral studies, as well as the methods for performing robust data analyses, all got a hefty boost, and we were off and running. He is a Professor Emeritus of the Faculty of Agriculture at Saga University.

Chapter 6
Foraging Strategies

Mantaro Hironaka, Shintaro Nomakuchi, and Lisa Filippi

Abstract *Parastrachia japonensis* Scott (Heteroptera: Cydnidae) females progressively provision nests with drupes of the host tree, *Schoepfia jasminodora*, a scarce, ephemeral, and unpredictable resource. To optimize use of the resource, they have evolved several extraordinary behaviors to increase the success of the only egg mass the female will produce in its lifetime. These behaviors include guarding of eggs and nymphs, trophic egg production, and progressive provisioning. To accomplish provisioning, they have evolved a complex navigation system that allows them to forage repeatedly over great distances and time and successfully return to the nest during both day and night. During inspection of the fickle drupes, females employ a sequence of behaviors that ensure they transport only the best ones to their nest. Females steal from each other while foraging and even raid conspecific nests. In response, defensive behaviors against thievery have evolved. This chapter explores the mechanisms and evolutionary implications of these foraging behaviors that provisioning females use to enhance their fitness.

Keywords Alternative tactic · Chemical cue · Foraging · Kleptoparasitism · Navigation · Orientation · Resource competition · Resource quality · Selective foraging · Visual compass

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6.1 Introduction

Foraging to feed oneself requires the ability to locate and recognize an appropriate food resource, the ability to defend it from conspecific and interspecific competitors, and the ability to process it. Progressive provisioning is a particular type of foraging behavior that requires the provisioning parent to not only be successful in those endeavors but also be able to make repeated trips between the nest and the food source. It occurs commonly in birds and mammals, but, outside the Social Hymenoptera, is rare among insects. In addition to the cluster of cydnids alluded to in Chap. 4, a handful of solitary wasps in disparate genera progressively provision paralyzed insects to the nest to feed larvae (Field 2005), and burying beetles progressively provision larvae (Eggert and Sakaluk 1995; Trumbo 1996, 2012). Earwig females also progressively provision young (Kölliker 2007), as do male assassin bugs (Reduviidae) (Ralston 1977; Machado and Trumbo 2018). As discussed in previous chapters, appropriate food, that is, drupes that are of high enough quality to be worthy of transportation to the nest, are, in most years, very few and far between. The search for an acceptable drupe can take hours and requires traversing a tortuous path over rough terrain and, often in miserable, rainy weather (Filippi-Tsukamoto et al. 1995; Filippi et al. 2002). In this chapter, we first describe the fascinating behaviors that make it possible for *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) females to navigate the complex provisioning trip. We then explain the exhaustive process that provisioning females use to determine the acceptability of a drupe.

There are trade-offs and risk-sensitive decisions to be made while foraging (Davies et al. 2012). Provisioning females must compete with the many other provisioning females they encounter in the foraging area, each female with the single goal of securing as many of the precious drupes for its young as it can. We describe the trade-offs involved in nest site preference in terms of provisioning efficiency and the risk of losing drupes to females who employ an alternative tactic of stealing (kleptoparasitism) over self-foraging, as well as how females defend themselves against theft of drupes from the nest. Finally, we explore whether kleptoparasitism arises as a purely opportunistic behavior or whether it has evolved as an alternative tactic in the larger provisioning strategy.

6.2 Visual and Chemical Orientation in the Provisioning Excursion

6.2.1 Navigating the Provisioning Trip

As described in previous chapters, during the provisioning season in June, females of the shield bug, *P. japonensis*, nest under fallen leaves and excavate a small hole as a nest under the leaf litter where they lay their eggs, and then guard the egg mass.

Unless there is an accident, the bugs do not leave the nest during the 2 weeks of egg protection (Filippi-Tsukamoto et al. 1995). Nests are built at a depth of at least 5 cm below the ground surface, and some nests are built under a single fallen leaf. Perhaps because of this, the nests do not have a clear tunnel structure, and the entrances to the nests that they enter and exit are not made of compacted soil, but rather look like gaps in fallen leaves (Fig. 6.1).

The nests are usually built a few meters, or in some cases more than 20 meters, away from the host tree, *Schoepfia jasminodora* (Santalales: Schoepfiaceae) (Filippi-Tsukamoto et al. 1995). Because of the spatial distribution of the nests and host trees, females must first move several meters or more to reach a host tree under which the drupes for the nymphs to feed on have fallen. How do they find this foraging area and the drupe? Next, after finding the drupes of the host tree, the females must return without error to their own nest. In the forest, it is almost impossible for us to find the nest entrance of *P. japonensis* which has no identifiable cues. The nest entrance is a small opening with a diameter of about 1 cm between overlapping fallen leaves on the ground surface and appears to have no identifiable visual characteristics. How do the females reach their nest after searching for food? The navigation task of finding a goal such as a nest or a drupe on such a two-dimensional surface is called x, y-orientation (Dyer 1998). In this section, we present what is known about the mysteries of the navigational task during provisioning excursions in *P. japonensis*.

In late June, when the nymphs hatch, the female walks out of the nest, finds the drupes under the host tree, and brings them back to the nest at our field site, Mt. Hinokuma Park (Tsukamoto and Tojo 1992). For example, during the provisioning season of 2000, provisioning behavior was observed at a 15 × 15 m study site at noon; females searching for drupes were first observed on June 19 and peaked on June 26, and the last provisioning female was observed on July 9 (Hironaka et al. 2003b). During this observation period, 10 days were sunny, 8 days were cloudy, and 3 days were rainy, but provisioning behavior was observed regardless of the weather conditions. We also counted females searching for or transporting drupes at the study site for 24 h at 2-h intervals during the peak of the provisioning season.

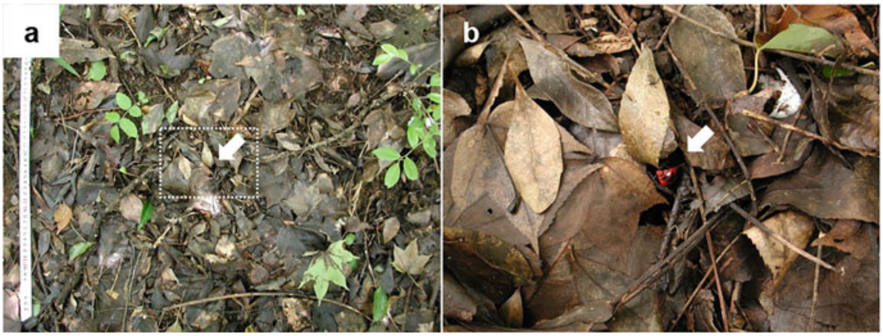


Fig. 6.1 A typical nest entrance (**a**) and the female peeking out of it (**b**). The area of the dotted line is enlarged and shown as (**b**). The nest entrance is typically about 1 cm in diameter, which allows the female to pass through. Arrows indicate an entrance on the ground

Many females were observed regardless of the time of day, indicating that they were provisioning not only during the day but also at night.

Field observations of the paths of the provisioning excursion of females who had experienced drupe transporting many times revealed that they achieved a very interesting path from leaving the nest to returning to the nest (Hironaka et al. 2007a, c). After leaving the nest entrance, the female first started walking toward the direction of the host tree. It appeared that all females had a predetermined direction for foraging when they left the nest. However, their walking paths were quite tortuous. If no drupes were found where they were headed, they walked in a complex path over a large area. When the female finally found a drupe with sufficient endosperm to merit being transported to the nest (Nomakuchi et al. 1998; also see next Sect. 6.3), it always oriented toward its own nest in a straight line close to the shortest path (Fig. 6.2; Hironaka et al. 2007a). When the female reached the vicinity of its own nest, it started to search around the nest entrance. We call the stage from the departure from the nest to the discovery of a drupe the foraging phase and the stage from the start of drupe transport to returning to the nest the homing phase.

Apparent differences between the orientation behavior of the foraging and homing phases were observed. In the foraging phase, the bug left for the previously foraged point, frequently changing direction. Sometimes it stopped walking and raised and waved its antennae. On the other hand, in the homing phase, the drupe-

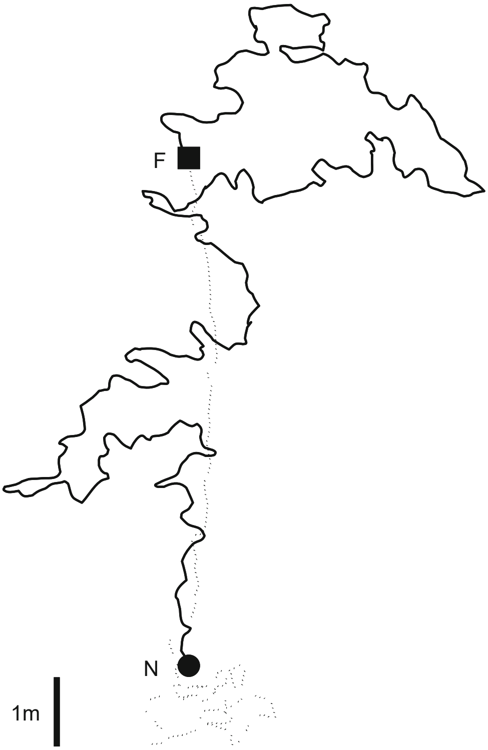


Fig. 6.2 A typical example of a diurnal provisioning excursion of *P. japonensis*. The continuous and dotted lines indicate the foraging and homing paths, respectively. The bug searched arduously until it encountered a drupe. After accepting a provided drupe, it took a direct path back to the nest over coarse terrain (dotted line). N (black circle): nest; F (black square): location where the bug discovered the drupe. Adapted from Hironaka et al. (2007a)

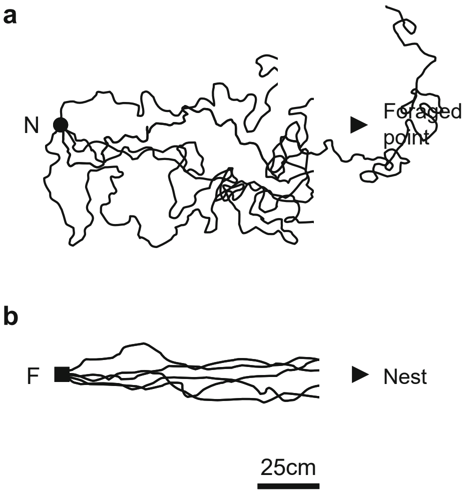


Fig. 6.3 The walking paths in the foraging and homing phases. We plotted the foraging (**a**) and homing (**b**) paths of five individuals to a distance of 1 m. N (black circle): nest; F (black square): location where the bug discovered the drupe. Arrows indicate the direction of the goals of each phase

laden bug walked rapidly, straight to its nest. Thus, the walking paths in the foraging phase clearly differed from those in the homing phase. The foraging bug showed a winding path, and, as a result, its path covered a wider area compared with that of the homing bug (Fig. 6.3; Hironaka, unpublished data). We measured the walking speed and the degree of curvature of the paths of 23 individuals who were repeatedly provisioning in the field (Hironaka, unpublished data). The walking speeds in the foraging and homing phases were 71.7 ± 19.8 (mean ± SD) cm/min and 86.4 ± 23.6 cm/min, respectively. The foraging speed was significantly slower than the homing speed (Wilcoxon signed-rank test: $P = 0.0007$). The sinuosity indexes of the paths taken in the foraging and homing trips were 1.6 ± 0.3 (mean ± SD) and 1.1 ± 0.1, respectively. The sinuosity of the foraging trip was significantly higher than that of the homing trip (Wilcoxon signed-rank test: $P < 0.0001$).

6.2.2 *Outbound Trip*

As described in Chap. 3, female *P. japonensis* develop their ovaries by feeding on the endosperm of drupes on host trees for 1 week to 10 days. When the ovaries are fully developed, the gravid female descends from the tree and searches for a nesting site while walking away from the host tree. However, exactly how this behavior of departing from the tree is carried out has not been sufficiently observed. The female roams through the fallen leaves to find a suitable place to build a nest with a thick layer of leaf litter; it burrows into the leaf litter to create a small chamber in which it can barely change its posture and lays a single round egg mass. The eggs hatch approximately 2 weeks later. The female usually leaves the nest within 48 h after hatching to perform the first provisioning behavior.

In order to find drupes to feed its nymphs, the bug must orient itself and reach an area under the host tree that serves as the foraging area of provisioning female *P. japonensis*. During the provisioning period of 2003, many host drupes were scattered over the ground, and 13 nests were discovered in the study site (Fig. 6.4; Hironaka, unpublished data). The total number of drupes around the four host trees was 307. Of these 307 drupes, 116 (37.8%) had an endosperm that was at least 50% developed and were considered to be good drupes. Some drupes fell from the tree and rolled out of the crown area (the area of ground directly under the crowns of the four host trees). The drupes were distributed over the uneven ground in a pattern of loose patches (foraging area), and no notable differences in the distribution of good and bad drupes were observed. Nests were observed along the periphery of the foraging area. In 2006, we observed the drupes and nests and recognized the same tendencies regarding the distribution and quality of drupes.

If a female memorizes some kind of positional relationship between the host tree and its own nest when it searches for a nesting site, it will be able to orient toward the direction of the relevant foraging area without losing its way after leaving the nest, even on its first provisioning bout. However, based on several observations of first-time provisioning behavior, we expect that the female will not remember the direction and distance of the host tree on which it was feeding 2 weeks ago. The first time a female left the nest, it went out in a random direction relative to the

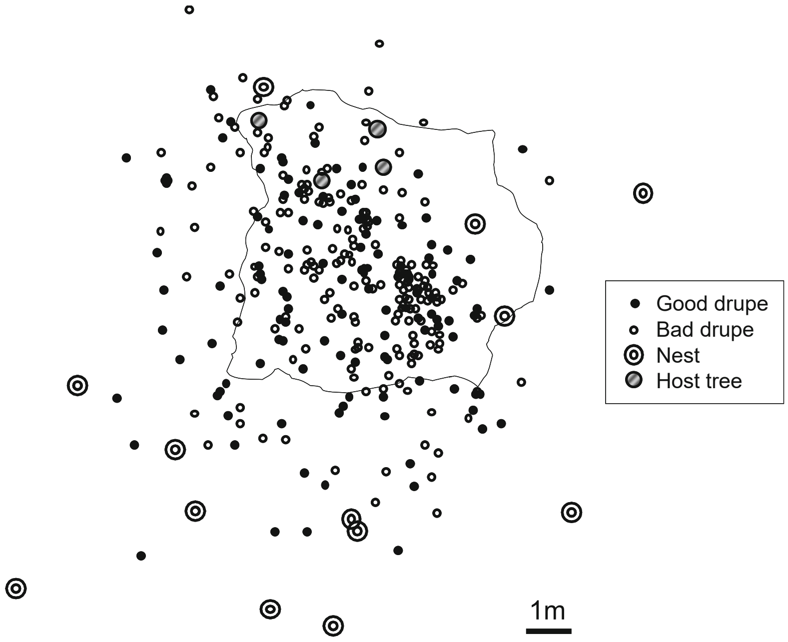


Fig. 6.4 A map of the fallen drupes and nests around the host tree at the busiest provisioning period in 2003. The solid line outlines the area of ground directly under the overlapping crowns of the host trees

direction of the nearest host tree (Hironaka et al. 2007c). As a result, the female repeatedly searched around the nest and returned to it empty-handed without ever reaching the foraging area. We expected that the female would gradually expand its search direction and range and eventually reach the foraging area. Once a female arrives at the foraging area under the host tree and discovers a drupe, it is able to orient toward the direction of the host tree during the next foraging phase (Hironaka et al. 2007c). However, we were able to set a "foraging area" in the absence of the host tree and train the female, whose entire nest, along with the female, we had transported to the constructed foraging site. This led us to believe that bugs do not aim in the direction of the host tree. Therefore, we conducted an experiment to distinguish whether the bug was heading toward the host tree or returning to the place where it had found the previous drupe before (Hironaka, unpublished data). To clarify which of the two directions a bug orients toward, we experimentally established the condition whereby the direction to the host tree does not correspond with the direction to the previously foraged point (Fig. 6.5a). We chose several nests and laid twine between each nest and the nearest host tree. The direction to be foraged (foraged direction) was determined as an angle of 45° counterclockwise to the twine of the nearest host tree, and we laid the twine on that path. Under natural conditions, we looked for a bug which had successfully conveyed a drupe and chose only those females that found the drupe within an angle of 40°, centering on the host tree direction. Next, we tracked the second foraging trip of the selected females and

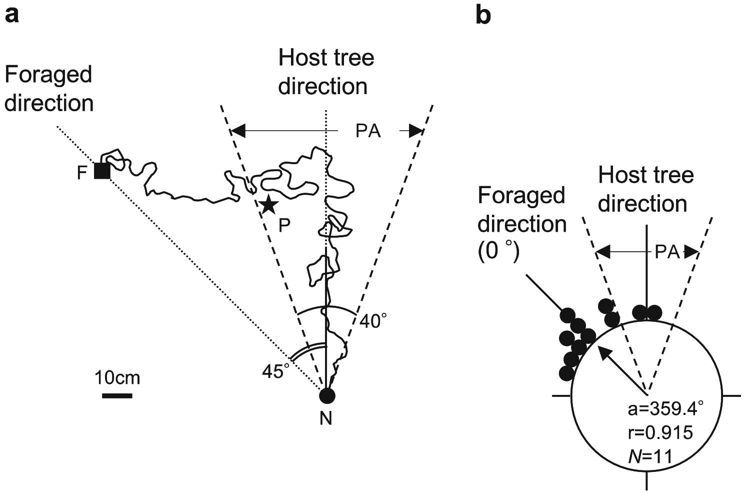


Fig. 6.5 A schematic drawing of the experiment (**a**) and the distribution of foraged directions (**b**). The locations of the nest and of the drupe found in the first run are denoted by N (black circle) and P (black star), respectively. On the first trip, the bug discovered a drupe in the area within an angle of 40° centering on the host tree direction, as shown by PA. The solid line shows an example of an actual foraging path, and F (black square) shows the location foraged on the second foraging trip. Solid circles in b indicate orientation angle for each bug. The arrow indicates mean vector. *a* mean angle of orientation; *r* length of the mean resultant vector; *N* number of animals tested

waited until each bug crossed the twine representing the previously foraged direction. When the foraging female crossed the twine, we gave it a drupe, and it started to home to the nest. Using this experimental procedure, the angular differences between the foraged points of the first and second trips were never less than 25°. When the female started the third foraging trip, the direction of departure from the nest at a distance of 0.5 m was measured. A bug that found a drupe at a location in the direction of the host tree on the first foraging trip homed along the line between the host tree and its nest. On the second trip, the bug roughly oriented toward the previously foraged point (P in Fig. 6.5a). A typical example of a foraging path taken on the second foraging trip is shown by the solid line in Fig. 6.5a. We waited until the bug crossed the line of the determined previously foraged direction and gave it a ripe drupe (F in Fig. 6.5a). Figure 6.5b shows the orientational direction individual females took on the third trip (filled circles). The figure reveals significant clustering in the previously foraged direction (V test: $P < 0.0001$). This result shows that *P. japonensis* females did not leave for the host tree, but that they, instead, oriented toward the direction where they had obtained a drupe on the most recent foraging trip.

In the final stage of the foraging phase, females use the odor of the ripe pulp as a cue to find good drupes, as shown by Y-tube experiments (Nomakuchi et al. 1998). This will be explained in detail in the next Sect. 6.3. Drupes filled with endosperm release volatile chemicals such as ethyl acetate, acetic acid, and ethanol when the pulp ripens (Hironaka, unpublished data). It is inferred that the female eventually reaches the host drupe after being attracted by these chemicals.

6.2.3 Inbound Trip

Upon reaching the drupe, the females assess it for tens to hundreds of seconds (Nomakuchi et al. 1998). When a female determines that the drupe is good enough and should be provisioned, it immediately begins to return to its nest. At this time, the bug looked around once at the location where the drupe was found and then walked out with its longitudinal body axis set roughly in the direction of the nest. The female did not lose sight of the nest's direction and returned to it along a straight path, even though she walked over and around various obstacles on the ground surface along the way. As a rule, once females reached the periphery of their nests, they made a sharp turn and then switched to a searching behavior in which they frequently changed direction (Fig. 6.6; Hironaka et al. 2003a, c). In other words, the homing behavior of *P. japonensis* consists of two phases: a "straight run phase" to reach the periphery of the nest and a "wandering phase" to search for the nest in the immediate vicinity of the entrance.

We measured whether homing behavior changed between provisioning periods in the field (Hironaka et al. 2003a). Two parameters that indicate the accuracy of orientation, "homing direction" and "starting position of wandering behavior," were examined in early females within a few days of the start of provisioning and in later females 10 days after the start. The homing direction was defined as the

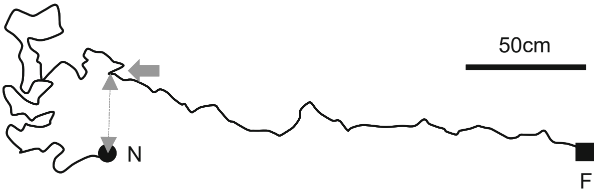


Fig. 6.6 A typical homing path of *P. japonensis*. N (black circle): nest; F (black square): location where the bug discovered the drupe. After direct homing for ca. 2 m, the bug usually performs a sharp turn (see arrow) and switches its behavior to search. Dotted line indicates the length between the 'wandering point' and its nest entrance. Adapted from Hironaka et al. (2003a)

direction of the body axis of the females 1 min after they began walking. The starting position of the wandering behavior was defined as the straight distance from the nest entrance to the wandering point (Fig. 6.6). At the same time, we measured the "wandering time," which is the time from the start of the searching behavior to the final arrival at the nest entrance. The results showed that there were no differences in the homing direction (*V* test: earlier homing, $P < 0.0001$; later homing, $P < 0.0001$) and the starting position of wandering behavior (Mann-Whitney *U* test: $P = 0.209$) between earlier and later homing and that both females that carried a drupe for the first time and females that carried them repeatedly returned along a straight path toward their own nest (Fig. 6.7a–c). On the other hand, a significant difference was

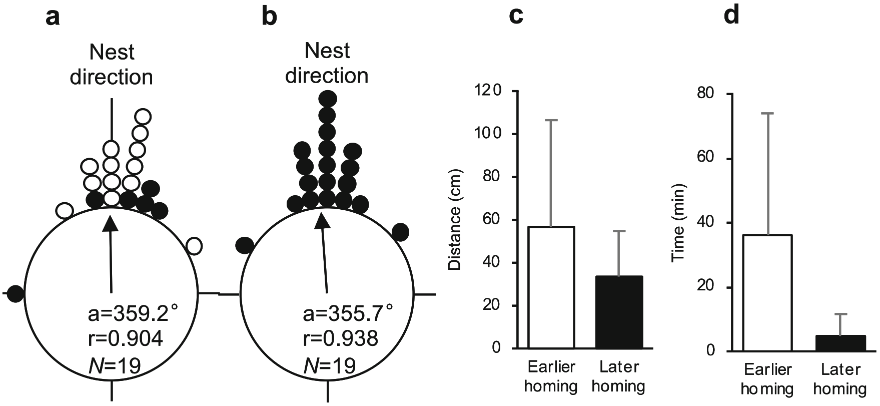


Fig. 6.7 Behavioral elements during the homing phase in the earlier and later provisioning periods. Circular graphs showing the distribution of "homing direction" of earlier (**a**) and later (**b**) homing bugs. Small circles around the circular graph indicate the 'homing direction' of individual bugs. White circle in the earlier homing graph (**a**) shows the homing direction of initial homing bug. Arrows indicate the mean resultant vectors (*a* mean direction of the resultant vectors, *r* the radius of the circle corresponds to vector length = 1, and *N*; sample size). The distances between the "wandering point" and the bug's nest (**c**) and the "wandering time" (**d**) were also measured. Mean values with SD (vertical bars) are represented in the histogram. Adapted from Hironaka et al. (2003a)

observed in the wandering time to finally arrive at the nest entrance (Fig. 6.7d). The wandering time for the nest was dramatically reduced from an average of 36.1 min in the earlier homing to 4.7 min in the later homing (Mann-Whitney U test: $P < 0.0001$).

6.2.4 Chemical Cue Used in Nest Searching

What cues do *P. japonensis* use to finally reach their own nest during the wandering phase? We conducted a field experiment to determine whether odor is an important cue in the final stage of the inbound trip as it is in the outbound trip. In the wandering phase, we blocked the compound eyes and/or antennae of homing females with paint and released them near the nests to determine whether they could enter their own nests (Hironaka et al. 2007a). Females whose eyes were covered with silver paste found the nest entrance and entered through it. On the other hand, the females whose antennae were covered with watercolor paint did not notice the nest entrance even when they passed close by it. The nest contains the odor of the nymphs, the provisioned drupes, and the female's own odor. We conducted an experiment to determine which odor was used as a cue for the female to return to its own nest (Hironaka et al. 2007a). We placed a gravid female in an individual lidded, clear plastic cup filled with substrate (leaf litter), where it deposited an egg mass and cared for its nymphs upon hatch (Fig. 6.8). After the start of provisioning behavior, we created four other artificial cups by placing nymphs and substrate in the new cups while the female was out of the nest. The four new nests consisted of the female's own nymphs and own substrate, nymphs of another female and the original female's substrate, the female's own nymphs and alien substrate, and alien nymphs and alien substrate. When these were presented to the female in the wandering phase, it returned not only to the nest of its own nymphs and substrate but also to the nest of its own substrate, although the nymphs had been replaced by those of other individuals. This strongly suggests that the substrate that comprises the nest and its surroundings contains cues used by the female to orient toward its own nest.

So, what kind of substrate cues are there? We suspected that the nest substrate might have some kind of odor emitted from the female itself. When rearing *P. japonensis* in artificial nests using Kimwipes® as a substrate, the white Kimwipes® turn into a brownish color and have a distinctive sweet smell that becomes stronger as the days pass. When we attached a centrifuge tube to the anus of the female, we found that it excreted yellowish-brown excrement at intervals of several days during the egg protection and provisioning periods (Fig. 6.9). We hypothesize that this excrement serves as an individual identification pheromone to distinguish its own nest from other nests. This hypothesis that the female gradually marks the nest with her own excrement nicely explains the shorter wandering time in the later stages of the provisioning period. These facts indicate that chemical cues derived from the nest substrate play an important role at least in

Fig. 6.8 A photograph of the artificial nest used in the experiment. Red granular objects are second instar nymphs aggregating on a black ripe drupe. Each egg-guarding female was reared in a clear plastic cup lined with substrate. The cup was covered with a perforated plastic lid to prevent escape. After eggs hatched, we continued to rear them together with their mother and ripe drupes, until the experiment

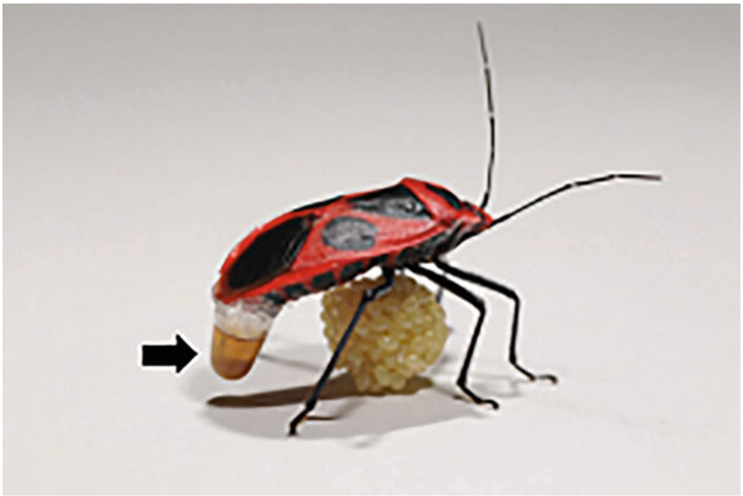


Fig. 6.9 An egg-guarding female with excreta being collected. A centrifuge tube was glued over the anus and left there until the end of provisioning behavior. The arrows indicate the excrement accumulated in the container

the final stage of homing. Can the use of chemical cues also explain the homing behavior of the straight run phase of the inbound trip?

6.2.5 Path Integration System

R. Wehner, one of the leading authorities on insect orientation and navigation, suggested that insects performing provisioning navigation use three different navigation systems to return to their starting point, the nest (Wehner and Wehner 1990). One is a path following system, such as a pheromone trail, which follows the outbound path directly, another is a path integration system, which accumulates the direction and distance of its own movement from the starting point, and the third is a map-based system, which stores surrounding landmarks and determines the starting point based on the memories of the landmarks. The easiest way to find out which system a given navigator uses is to conduct a displacement experiment. We artificially displaced a female before homing to another location and observed the path of its subsequent return (Hironaka et al. 2007a, c). We gently picked up a female that was searching for drupes, artificially displaced it to another location, and immediately gave it a drupe. It then initiated homing behavior. If the displaced bug loses its homing direction and wanders around, a path following system might be used. If the bug directly orients to the position where the nest should be, we can say that it is a path integration system. Or, if the bug can precisely relocate to the nest, it is likely to be using a map-based system. What kind of homing path did the displaced bug follow? After being displaced to a location on the opposite side of its nest, the bug did not head for the true nest, but headed directly in the direction of the fictive nest, that is, where the nest should have been if it had been displaced along with the female. When the bug had walked almost the same distance as that between the capture location and its true nest, it made a sharp turn and showed typical wandering behavior (Fig. 6.10; Hironaka et al. 2007c). Day and night, the bugs could not recognize any change in the position of the nest when they were artificially displaced to any location and oriented precisely toward the location where the nest had been before the displacement. The results of this experiment indicate that *P. japonensis* females depend on a path integration system in which the direction and distance of outbound movement are constantly monitored, and the vector accumulated from the outbound path is reversed at the time of homing to form the home vector, in the straight run phase (Hironaka et al. 2007a, c). To the best of our knowledge, this is the first report in the heteropterans of a navigational strategy using path integration system.

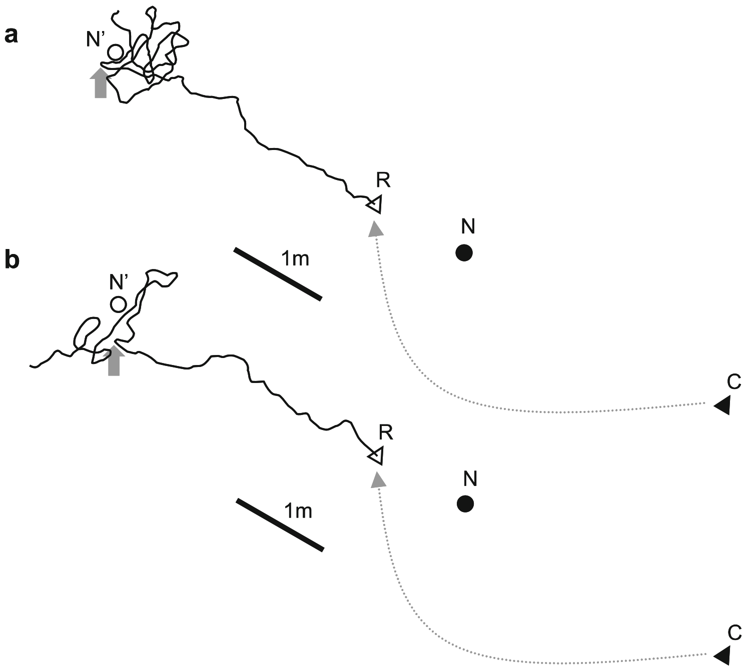


Fig. 6.10 Homing paths in a diurnal (**a**) and nocturnal (**b**) displacement test. An example of the homing path for 10 min. N (black circle): nest; C (black square): point of capture; R (white up-pointing triangle): point of release; N′ (white circle): location of fictive nest (approx. 180° from true nest site). The bug was displaced from C to R (curved arrow). The gray arrow indicates the point where the bug initiated wandering behavior. Adapted from Hironaka et al. (2007c)

6.2.6 Visual Compass Mechanism

Animals that navigate using a path integration system measure the route of their outbound trip from the starting point, such as a nest to the current location using some kind of compass and distance meter, and accumulate direction and distance information (Collett and Collett 2000). In addition, when they return to the starting point according to the homing vector obtained by path integration, navigators check whether the orientation direction has deviated from the homing vector and how much of the distance in the homing vector it has consumed. In other words, navigators always need directional and distance information in the outbound and inbound trip. Here is what we know from field and laboratory experiments about the cues from which *P. japonensis* obtain directional information.

It is known that insects obtain directional information from various environmental cues such as the sun, polarized light in the sky, geomagnetism, wind, and idiothetic cues (Wolf 2011). Which of these mechanisms does *P. japonensis* use? We first conducted a field experiment to determine whether vision of *P. japonensis* plays an important role in the reception of direction or distance information. We applied a light-blocking silver paste to the compound eyes of a female just before it started its homing trip and examined whether it was able to orient toward its nest. We drew a circle with a radius of 50 cm centered on the foraged point, the starting point for returning to the nest (homing), and investigated which point on this circumference the bug would reach (Fig. 6.11a; Hironaka et al. 2003b). The orientation angle of the bugs whose eyes were coated with transparent enamel was distributed around the direction of the nest, indicating that all the bugs headed for the nest accurately (Fig. 6.11b; Hironaka et al. 2003b). On the other hand, bugs with their eyes blocked with silver paste oriented toward various directions, and some of them headed for the completely opposite direction to the nest (Fig. 6.11c; Hironaka et al. 2003b). *P. japonensis* identifies the homing direction by looking at something on the forest floor during the daytime.

In an additional field experiment in which the dorsal or ventral half of the compound eyes of homing bugs was blocked, we found that the directional cues were perceived by the dorsal side (Hironaka, unpublished data). To investigate the possibility that *P. japonensis* females use the sun compass, which is used by many insects (Wehner 1984), we had bugs perform provisioning behavior in a laboratory arena with a single bulb-shaped fluorescent lamp. In the arena, the bug repeated its characteristic foraging and homing behavior, which was the same as in the field. When the position of the light source was shifted by 180° horizontally during the

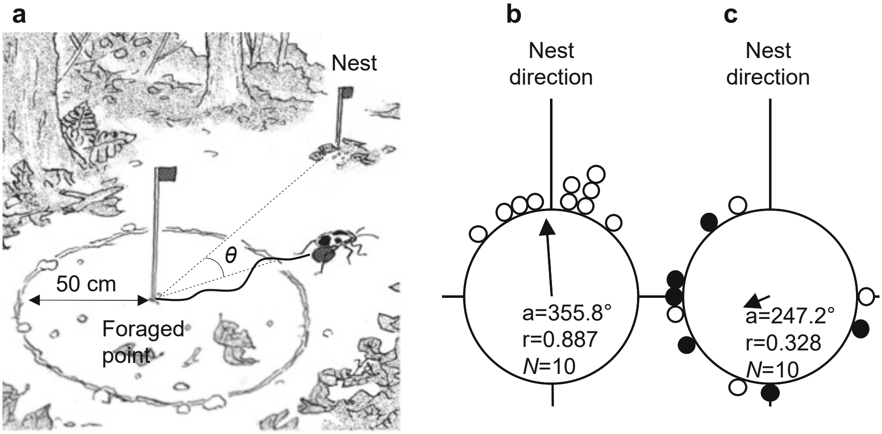


Fig. 6.11 Schematic representation of direction measurement and homing direction during the day. We marked the point arrived at by the homing female along the circumference of a circle with a radius of 50 cm drawn with the foraged point at its center and calculated the orientation angle (**a**). The orientation angle is the angle (θ) between the direction of the arrival point and the direction of the nest seen from the foraged point. The distribution of homing directions of females with their compound eyes and ocelli painted with clear enamel (**b**) or silver paste (**c**) are shown on sunny (white circles) and rainy (black circles) days. Arrows indicate mean vector. *a* mean angle of orientation; *r* length of the mean resultant vector; *N* number of animals tested. Adapted from Hironaka et al. (2003b) and Hironaka (2014)

homing phase, the bugs suddenly changed their orientation direction and walked toward the starting point of the homing phase as if they were returning to the path they had walked. From a series of behavioral experiments, it became clear that *P. japonensis* females can use light sources as compass markers in the daytime (Hironaka et al. 2007b).

However, as mentioned earlier, *P. japonensis* can return to its nest with a straight path even at night, when the sun compass is not available, using a path integration system (Hironaka et al. 2007c). Therefore, we conducted a similar eye-blocking experiment to determine whether the visual compass was also used at night. Females that had their eyes blocked with silver paste lost the homing direction regardless of the nighttime weather (Hironaka et al. 2003b).

6.2.7 Canopy Cue in Nocturnal Homing

What do provisioning *P. japonensis* females look at in the forest at night to obtain directional information? The first possibility to consider is celestial cues, such as the moon and stars (Wehner 1984), and the pattern of linear polarization in the night sky due to moonlight (Dacke et al. 2003). These celestial cues are often used to navigate by flying insects and insects that live in open environments such as desert and sandy beaches. However, in the forests where *P. japonensis* lives, the celestial cues do not provide a stable compass reference because they are surrounded by dense woody vegetation. In fact, bugs accomplish accurate orientations in the foraging and homing phases even on rainy nights when these celestial cues are not available (Hironaka et al. 2003b).

Another overhead visual cue available in the forest is the canopy, the layer of upper branches and leaves of the trees that covers the navigator. In 1980, B. Hölldobler showed experimentally that forest-dwelling ants determine their orientation from images of the surrounding canopy when returning to the nest. He named this behavioral ability canopy orientation as a novel system of animal navigation and reported it in *Science* (Hölldobler 1980). However, to date, little is known about how animals use canopy images, such as whether they look at the entire canopy or just part of it. Furthermore, whether some animals use the canopy as an orientation cue at night has not yet been revealed.

To gain some understanding about this, we went into the forest at night and turned off the outdoor headlights. In the darkness of the forest, we couldn't see anything for a while, but as our eyes gradually became adapted to it, images of the canopy that surrounded us appeared. Through gaps in the canopy, we could see the night sky, which was only slightly brighter than the canopy. The contrast between the night sky and the canopy created a complex canopy image (Fig. 6.12; Hironaka 2014). Do *P. japonensis* females look at the canopy in the forest at night, and if so, how do they get directional information from the canopy?

If the bug uses the canopy image as an orientation cue, this means that it will be using some part of the canopy as a compass reference. Therefore, we hypothesized

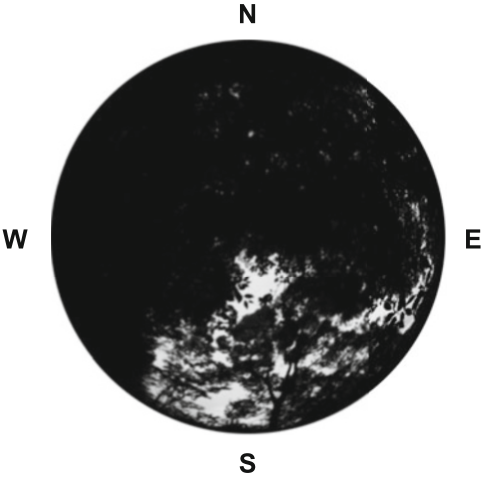


Fig. 6.12 A hemispherical photograph taken above a nest of *P. japonensis*. Canopy gaps of various sizes are scattered over bugs. At this location, relatively large gaps (white areas) were observed from the zenith to the south. Adapted from Hironaka (2014)

that it uses a canopy gap as a compass reference to determine the direction and tested this hypothesis in a field experiment. A box-shaped experimental arena was set up outdoors in a location with little artificial light, and a female was allowed to nest and provision inside the arena (Fig. 6.13a). The sides and ceiling of the arena were covered with dark blue plastic boards, and one circular hole was placed on the south side of the ceiling. The hole and dark blue plastic board simulated the situation where there is one gap opening in the canopy cover. In this arena, we confirmed that a *P. japonensis* female was able to reach the "foraging area" in the center of the arena, assess a good drupe, and then carry it back to the nest. After observing this, the next time the female reached the "foraging area," we rotated the ceiling board by 180° such that the position of the artificial gap was changed from south to north. The female then started to walk in the complete opposite direction to its nest (Fig. 6.13b). In other words, *P. japonensis* used the position of the canopy gap as a compass reference for path integration in the nocturnal homing (Hironaka et al. 2008a, b).

6.2.8 Conclusion: Navigation Ability in Subsocial Bugs

What kind of cues and behavioral mechanisms do insect navigators use to accomplish their navigation? This question has been studied intensively in the hymenopterans, which include bees and ants. Hymenopteran insects build complex nests in which they congregate and live highly social lives. As a result, they exhibit a behavior called central place foraging, in which they go from a central point, such as a nest, to feeding sites, obtain food, and then return to the central point again. To accomplish this provisioning navigation, it is thought that the hymenopterans have acquired precise orientation ability and various navigation systems. However, provisioning navigation is not the exclusive property of bees and ants. Provisioning

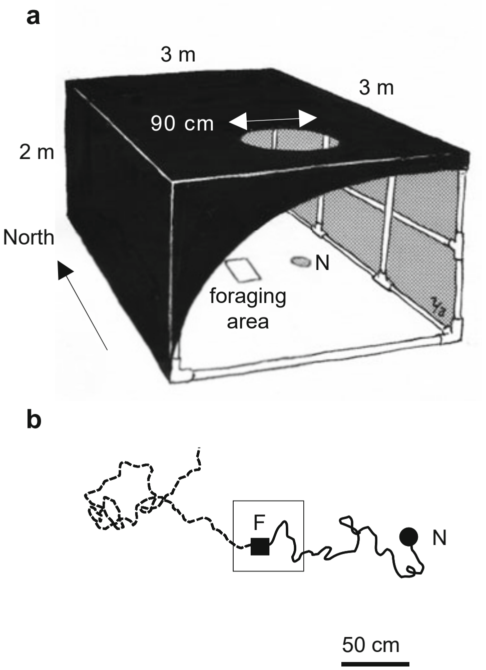


Fig. 6.13 Schematic representation of the experimental arena (**a**) and a typical provisioning path of a bug (**b**). The walls and ceiling were made of dark blue plastic board. A round hole simulating a canopy gap was opened in the ceiling; initially it was positioned south from the center of the arena. A square 'foraging area' containing several drupes was located in the center of the arena. The experimental nests were set on the ground 1 m from the center of the 'foraging area'. The solid and dashed lines indicate the foraging and the homing paths, respectively. N (black circle): nest; F (black square): location where the bug discovered the drupe. Adapted from Hironaka et al. (2008a, b)

navigation has been observed in several species of subsocial bugs, including *P. japonensis*. In addition, several other taxa of subsocial insects, such as burrow-building crickets (Beugnon and Campan 1989) and earwigs (Radl and Linsenmair 1991), also perform provisioning navigation. Among subsocial bugs belonging to Cydnidae and Parastrachiidae (or subfamilies Parastrachiinae), provisioning behavior has been observed in six species: *Sehirus cinctus*, *Adomerus triguttulus*, *A. variegatus*, *A. rotundus*, *Canthophorus niveimarginatus*, and *P. japonensis* (Mukai et al. 2010; Inadomi et al. 2014). In all of these species, the provisioning path from departure to return has been observed. Of these, five species, *A. triguttulus A. variegatus*, *A. rotundus*, *C. niveimarginatus*, and *P. japonensis*, have been observed to return to the nest using a direct homing path (Fig. 6.14). And among these, preliminary experiments have confirmed that *S. niveimarginatus*, *A. triguttulus*, and *A. rotundus* use a visual compass-based path integration system for provisioning navigation, in addition to *P. japonensis* (Hironaka, unpublished

Fig. 6.14 Seed-provisioning subsocial bugs, *Adomerus triguttulus* (**a**), *A. variegatus* (**b**), *A. rotundus* (**c**) and *Canthophorus niveimarginatus* (**d**), oriented toward their nest after attaching a seed of their host plants to the tip of their proboscis

data). This strongly suggests that the path integration system using a compass and a distance meter is not the exclusive property of hymenopteran insects, but has evolved in a wide range of insect taxa. Even more interestingly, the remaining species, *S. cinctus*, appears to use a trail pheromone for provisioning navigation. Kight (1995) observed that *S. cinctus* females traced back along the outbound path with remarkable precision, even when parts of the return route involved movement away from the nest. However, there is no experimental evidence for the trail pheromone.

There are still many mysteries in the orientation and navigation abilities of *P. japonensis*, even at the behavioral level. For example, it is not at all clear about the distance meter that is required in addition to the compass in the path integration system. They may be using "odometers" in their legs like *Cataglyphis* ants (Wittlinger et al. 2006) or optic flow like honeybees (Esch and Burns 1996). The canopy compass is also a mystery. The forest canopies encountered by provisioning females of *P. japonensis* are complex and diverse. Instead of the simple structure artificially shown in our field experiment, the gaps in the canopy are fine and numerous. How does a provisioning female choose a gap as a reference point for its compass? The shape and size of the canopy gap will change significantly over long-distance provisioning trips and in some cases will disappear. The visible gap may be temporarily obscured by a tree trunk or other object during provisioning excursion and by wind. Canopy gaps are unstable compass markers, unlike celestial cues. How do bugs solve these problems? The unique orientation and navigation of *P. japonensis* in the forest still hold many fascinating secrets that await better understanding of the behavioral abilities in animal navigation.

6.3 Process Used by Females to Identify a "Good" Drupe

One of the most enjoyable studies carried out in the field took place in 1994 when we sought to understand how females were deciding which drupes to transport back to the nest. This study was truly fun!

As we have described, we regularly saw hundreds of drupes on the ground; when females were provisioning, they most often passed most drupes by without a glance. We wanted to clarify the cues that females were using to select the perfect drupe worthy of transport back to the nest to feed their hungry nymphs. As we have described in Sect. 4.3.1, on average, the drupes that females dragged back to their nests weighed from 1.7 to more than 5 times their body weight. They transported drupes at a formidable speed of over an average distance of 254 cm (range 160–430 cm) (Tsukamoto and Tojo 1992). We have not specifically assessed the cost of dragging one drupe back to the nest, but one can assume it is considerable both in terms of the energetics and the time involved. Nymphs will leave the nest prematurely if the female fails to provide sufficient food, so it behooves mothers to make the most of their provisioning efforts and only transport those drupes that are most nutritious back to their nest. To that end, natural selection should favor mothers who are able to carefully screen drupes and distinguish between those that are good and those that are of poor quality. During the provisioning season of 1994, we carried out a series of five field experiments to identify the cues that females were using to select "good" drupes (Nomakuchi et al. 1998).

First, we observed females provisioning in the field to see precisely what they were doing as they roamed around the foraging area and searched for a "good" drupe. In 1994, nests were distributed in a range of 5–15 m from the foraging area. Females began leaving their nests to provision from the third week of June, and provisioning behavior continued until early July. Hundreds of drupes were on the ground under the host trees. Females walked with their antennae in front of them waving about. They ignored most of the drupes around them, but occasionally a female stopped at a drupe, palpated it with the antennae, and grasped it with the front four legs. Then she rolled it around with those front four legs for a few seconds, keeping it under the abdomen. Next, the female inserted the proboscis into the drupe. The female repeatedly inserted and withdrew the proboscis from the drupe. This "tasting" behavior continued for about 10 s to several minutes, until the female either rejected the drupe by dropping it and walking away to inspect another drupe or accepted it by piercing it even further and hastily dragging it back to its nest (Nomakuchi et al. 1998).

Females were also seen directly competing over drupes. The competitive bouts proceeded as follows. After one female found a drupe, inspected it, determined it was “good” and was hastily returning to its nest, she would be intercepted by several other provisioning females, each trying to aggressively pull the drupe away from the others. On one such occasion, we saw eight females engaged in such a tug-of-war for 2 h! Eventually, a few females gave up and went off to forage in the traditional manner, but several clung to the drupe even as the victorious female was dragging it away! These competitive bouts were especially common when two drupes were connected to the same bit of stem and could be dragged off together. It seemed like the different movement of the female once a “good” drupe had been secured, switching from the stop and start movement and repeatedly changing direction to a beeline straight run in a very fast, continuous pace, clued other females to the fact that one of them had secured a “good” drupe and initiated the tugs-of-war. More on this curious behavior in Sect. 6.4!

6.3.1 Relationship Between Drupe Features and Acceptability to Provisioning Females

Drupes on the ground had an outer skin that varied in color, based on maturity of the drupe. Most were green (least ripe) or a bright cherry red, indicating that most drupes fall from the tree before they are ripe. As drupes matured, they became dark red. Some very ripe drupes were purple or black, and there were also drupes that had ripened to the point that their skin and the fleshy part of the drupe got washed away altogether. Those drupes were tan or whitish and much smaller. At the natural field site, we observed the rate at which provisioning females encountering each of the different colored drupes accepted or rejected them. Females accepted only about 35% of green drupes, but they accepted 71% of red drupes and 100% of dark red, purple, and skinless drupes (Fig. 6.15; Nomakuchi et al. 1998). They were clearly able to distinguish which drupes were most ripe.

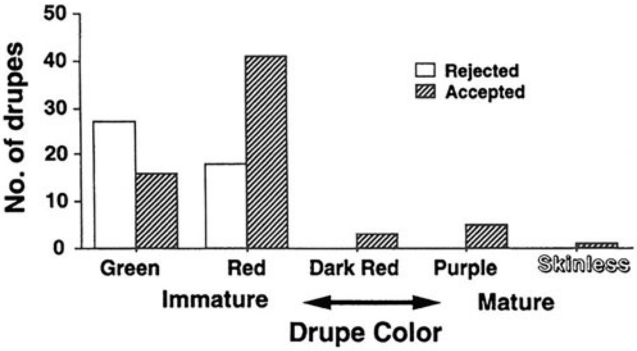


Fig. 6.15 Relationship between drupe color and acceptability to provisioning female *P. japonensis* in the field. Total drupes, $n = 97$. Adapted from Nomakuchi et al. (1998)

The drupes that females accepted were slightly, but significantly, larger than those that were rejected, probably because the outer skin thickens as the drupe matures until it falls off (Fig. 6.16a; Student's t test, $p < 0.04$; Nomakuchi et al. 1998). They were also significantly heavier (68%), likely reflecting a more developed dense endosperm (Fig. 6.16b; Student's t-test, $p < 0.002$).

Drupes are composed of an outer skin, an inner shell, a fibrous layer, and endosperm. As drupes mature, the fibrous layer is gradually converted to endosperm (Fig. 6.17; Nomakuchi et al. 1998). Apparently to develop their eggs inseminated females sequentially feed on many yellow and green drupes on the host tree that only have between 5 and <20% endosperm. After weighing and measuring the drupes, we split them open to estimate the endosperm content. The average endosperm content of accepted drupes was over four times greater (78%) than rejected drupes (Fig. 6.18; Nomakuchi et al. 1998), indicating that females can assess the amount of

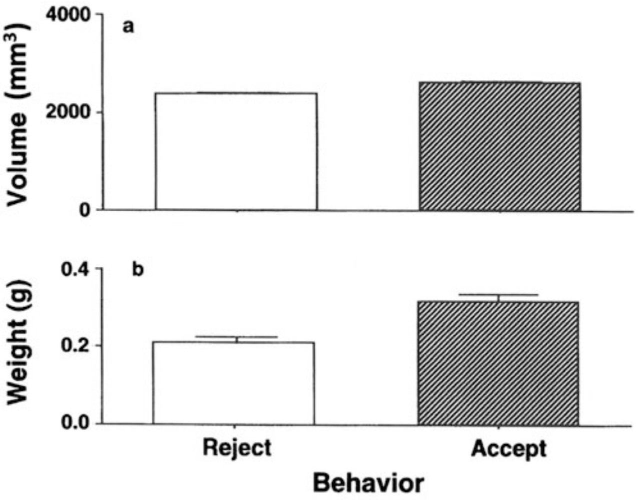
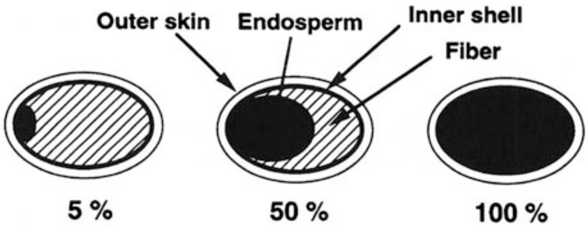


Fig. 6.16 Relationship between drupe volume and weight and acceptability to provisioning female *P. japonensis* in the field. Measurements done on 44 drupes that were rejected and 52 drupes that were accepted. Values represent means ± SE. (**a**) volume, Student's t-test, $p < 0.04$; (**b**) weight, $p < 0.002$. Adapted from Nomakuchi et al. (1998)

Fig. 6.17 Internal composition of drupes of different maturity. Fiber is gradually replaced with endosperm as the drupe matures. Adapted from Nomakuchi et al. (1998)



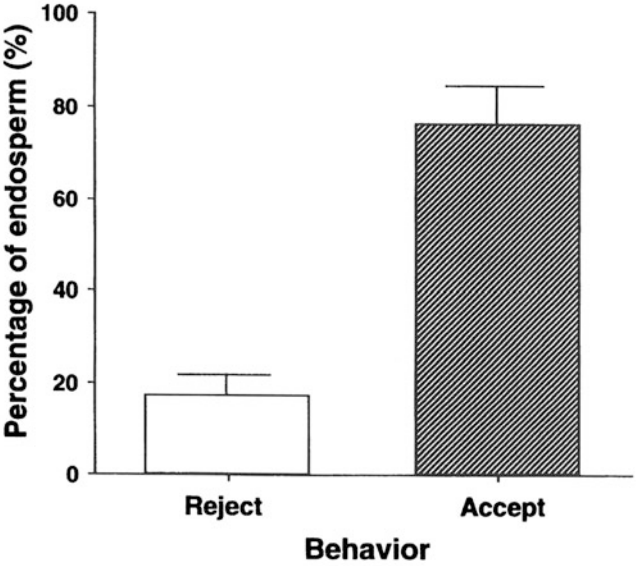


Fig. 6.18 Relationship between amount of endosperm in a drupe and acceptability to provisioning *P. japonensis* females in the field. Values represent mean ± SE. Mann-Whitney U test, $p < 0.0002$. Adapted from Nomakuchi et al. (1998)

endosperm inside a drupe and were primarily bringing "good" drupes back to the nest to feed their young. Now that we had verified that females were actually choosing drupes that were more mature and had higher endosperm content, we set out to identify the cues they were using to identify "good" drupes.

6.3.2 Initial Cues Used by Provisioning Females that Led to Approaching a Drupe

We carried out a choice test and a non-choice test in the field to determine whether females were using volatile cues or color when they decided to approach a drupe. We placed a Y tube in the field and put a green drupe in one short end and a red drupe in the other short end. The female was allowed to enter the long end and choose either of the drupes. Of ten trials, nine females chose the arm with the mature drupe. Moreover, when 15 females were exposed to a green drupe that had its skin cut, all of them approached the drupe, although before it had been cut, they ignored it completely (Nomakuchi et al. 1998)! These findings indicated that females use volatile cues to initially approach a drupe, not color; the volatiles in green drupes are probably in very low concentration and remained unrecognizable by females until the skin was broken.

6.3.3 *Weight as a Possible Cue to Accept a Drupe*

Although females approached the green drupes that had been cut, after probing them and grasping them in their four front legs, rolling them a bit, every female rejected them. So, what other cues were the females assessing to verify the quality of the drupe? We thought weight might be a factor. To test this, we peeled the skin off green drupes to release more volatiles that would attract the female and injected water into the drupes to make them heavier. We presented these weighted drupes to ten females. The females approached them, probed them with the proboscis, and rolled them around in their legs, but, alas, they did not accept them. So, something else must be the main cue.

6.3.4 *Nonvolatile Juices in the Outer Skin as a Possible Cue to Accept a Drupe*

We tested whether the taste of juices in the outer skin acted as a cue for acceptance of a drupe. We peeled the juicy skin off very ripe drupes and smeared the juice all over immature green drupes (13 trials) and wooden dummies that were about the size and shape of a drupe carved from untreated wooden branches from the forest floor (11 trials). Interestingly, all females approached, inspected, and dragged off the flavored substrates of both types (Nomakuchi et al. 1998). This indicates that females are tasting the drupes when they probe them, and the taste is determinant in whether they accept the drupe.

6.3.5 *Consistency of Drupe as a Possible Cue for Its Acceptability*

To clarify whether provisioning females recognized that the hard inner endosperm was the target, and not just the odorous and flavorful skin, in a choice test 17 provisioning females were presented with just the juicy skin of a mature drupe and a wooden dummy drupe encased in the skin of a mature drupe. All females approached both bait items, but only dragged off the fragrant skin-encased wooden dummy (Nomakuchi et al. 1998). This demonstrated that females require cues from the volatile chemicals, the taste, and the solid core.

6.3.6 Can Females Recognize Just the Endosperm with No Chemical Cues from the Outer Skin?

In a final choice test to determine whether females were able to recognize the final goal of their foraging efforts, the endosperm, in the absence of the attractive chemical cues, 34 provisioning females were presented with the fragrant juicy skin and inner shell of a mature drupe and a bare, mature endosperm whose outer skin was carefully removed such that no juices contacted it. Four females approached the endosperm, inspected it, and dragged it off. Two of 30 females that approached the skin and inner shell dragged the drupe off. Nine of the 28 females that rejected the skin accidentally came upon the endosperm, inspected it by probing and rolling it, and dragged it off. In other words, they did not extend and flagellate their antennae in the direction of the endosperm as if they were attracted to it, as they did with the fragrant skin. The remaining 19 females that had initially inspected the skin just wandered off and never came upon the endosperm (Nomakuchi et al. 1998). Clearly, it is difficult for provisioning females to find endosperm without the juices of the outer skin, stressing the importance of these chemical cues as an attractant to initially finding a good drupe. In the field, when a drupe matures to the point where the skin falls off, the inner shell remains and there would usually be dried juices on the outside of the endosperm that emit the fragrant, attractive volatile chemical cues, so there is no need for selection to have favored females that could recognize endosperm with no juice fragrance.

Finally, we compared the time it took from approach to acceptance of the bait for transport back to the nest of 11 females that had accepted a wooden dummy drupe encased in the skin of a mature drupe and 10 females that had accepted an endosperm. Females took significantly less time to inspect and accept the endosperm than the skin-encased wooden dummy drupe (Fig. 6.19; Mann-Whitney U test, $p < 0.01$; Nomakuchi et al. 1998). Females accepted the endosperm in about 1/6 the amount of time required for females to accept and transport to the nest a fragrant dummy drupe. The females apparently recognized that something was off but decided to risk the time and energy cost should it turn out not to be a good drupe and transported it to the nest.

6.3.7 Conclusions About Drupe Selection Process

We summarize the drupe selection process in Fig. 6.20 (Filippi et al. 2001). Females were most directly attracted to approach the bait when it had the fragrant odor of a mature drupe, but only accepted it if it also had the texture of a hard endosperm. They could recognize just the endosperm if they touched it by accident, but never approached it in the absence of the appropriate chemical cues.

The data demonstrate that females use reliable cues to distinguish good drupes from bad. They choose the most mature, heaviest, and largest drupes with high

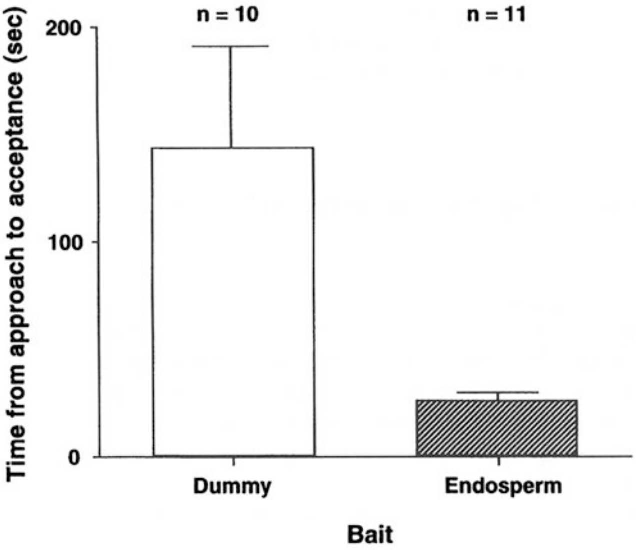


Fig. 6.19 Inspection time of provisioning *P. japonensis* females in the field when exposed to fragrant skin-encased wooden dummy and endosperm. Mann-Whitney U test, $p < 0.01$. Adapted from Nomakuchi et al. (1998)

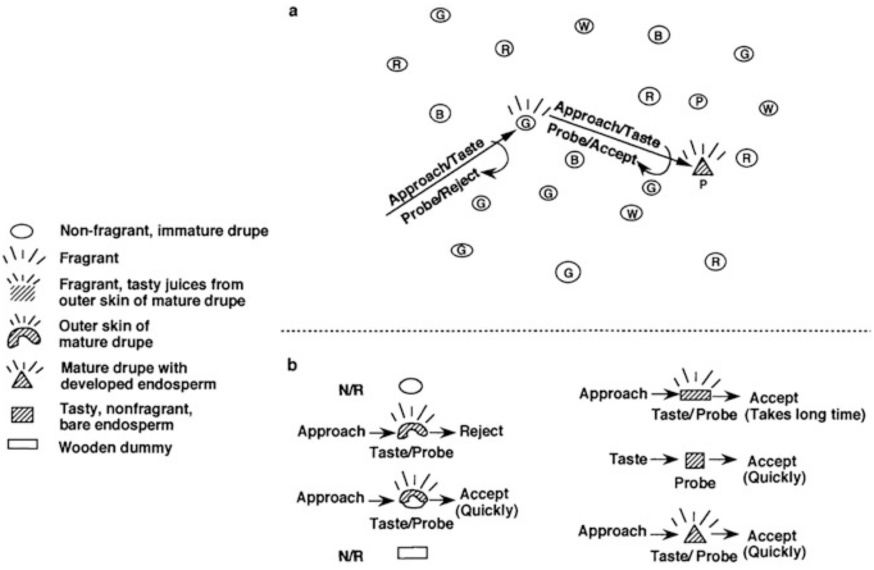


Fig. 6.20 Behaviors of provisioning female *P. japonensis* prior to accepting or rejecting a drupe. (**a**) Behaviors in response to natural drupes in the field; (**b**) Composition of manipulated and artificial drupes and female response. Adapted from Filippi et al. (2001)

percentages of endosperm (Figs. 6.15, 6.16, 6.17, and 6.18). They are attracted to and approach drupes in response to volatile and tactile chemical cues and accept them for transport to the nest if they also have a dense core that is assessed by weight and probably also by probing with the proboscis. Under natural conditions, these criteria are sufficient to ensure that females are selecting only good quality drupes to provision their nests.

6.4 Alternative Foraging Behavior: Kleptoparasitism

As alluded to in Sect. 4.19.5, females were sometimes seen removing drupes from the nests of other females, essentially usurping a resource that the resident female had invested considerable time and energy to procure. Depending on how commonly it occurs, this behavior, known as kleptoparasitism (Rothschild and Clay 1952; Brockmann and Barnard 1979; Iyengar 2008), could be quite costly to the resident female. A variety of selective pressures that can fluctuate widely promote the evolution of inter- and intraspecific stealing from another individual's diligently provisioned cache. The availability of the resource in both quantity and quality (Nilsson and Brönmark 1999), density of intraspecific or interspecific competitors (Scott 1994), and the tendency for host nests with stored food to be aggregated (Elgar 1988) can drive the evolution of kleptoparasitism. Increased density of foragers combined with increased handling time of the resource leads to increased rates of kleptoparasitism (Brockmann and Barnard 1979). However, while the motivation of kleptoparasites to steal from a particular individual will be affected by these selective pressures, the age (Tso and Severinghaus 1998), condition, and response of the host (Elgar 1988; Cangialosi 1991) will play a big role in determining the success of a kleptoparasitic event for the parasite and the cost to the host. Because of the likely fluctuation in all these parameters, risk-sensitive behaviors should be adopted by both the parasite, to enhance the likelihood of success, and the host, to minimize the cost.

Risk-sensitive behavior, primarily in response to food availability, was shown to occur with regard to trophic egg production (Filippi et al. 2012) and nest site preference (Filippi et al. 2002) in *P. japonensis*. When one considers the severe constraints imposed by the unpredictable and poor resource (Nomakuchi et al. 1998; Filippi et al. 2002) that have favored the evolution of such extensive and unusual parental care behaviors, including progressive provisioning, and extreme physiological adaptations (Tojo et al. 2005; Kashima et al. 2006; Hosokawa et al. 2012, 2013) and add on the further limitation of obligative semelparity, it is not at all remarkable that stealing to procure the precious resource should also occur in this eclectic species. In fact, provisioning females would be expected to assess the likelihood of finding a good drupe and use risk-sensitive decision-making to choose a foraging strategy that is most likely to meet with success. If search time is extraordinarily long, and a provisioning female comes upon either another female who has secured a good drupe and is returning to its nest or a nest that has been provisioned with many good drupes, stealing should be the optimal behavior. In other words, when the payoff from stealing exceeds that from self-foraging, provisioning females should opt for kleptoparasitism (Brockmann and Barnard 1979; Broom and Ruxton 1998; Flower et al. 2013). We set out to determine how common kleptoparasitism is in *P. japonensis* and to test the hypothesis that the prevalence for nest sites that are outside the foraging area is associated with reduced risk of kleptoparasitism. We predicted that females nesting within the foraging area would suffer a higher rate of kleptoparasitism and lower success overall up to the time of nest abandonment because their nests would be physically more exposed to predators and to kleptoparasitism because of sparse leaf litter and protective vegetation (Tsukamoto and Tojo 1992); the higher density of foraging females inside the foraging area should also lead to more opportunistic thievery there than outside the foraging area (Filippi et al. 2002).

We carried out a manipulated field study during the provisioning season of 2000 (June and July) at our field site at Mt. Hinokuma Park in Saga, Japan. Three clustered large host trees, the crowns of which largely overlap, serve as the resource for the subpopulation at this site. The foraging area encompasses the area under the overlapping crowns and slightly downhill of the trees where the fallen drupes roll. The radius from the center of the three trees to the edge of the foraging area is about 3.5 m and is relatively bare of ground cover. On the other hand, just outside this area, where most females tend to nest, the ground is covered with abundant leaf litter and vegetation. We searched for nesting females inside and outside the foraging area, but at this site in 2000, nesting females were only found outside the foraging area. We collected 32 females and re-established their nests, preparing 16 nests inside and 16 nests outside the foraging area. We set each egg mass-bearing female into a small depression in the soil and covered the nest with leaf litter. To prevent predator disruption to the nests prior to hatch, we enclosed each nest in a polypropylene cylinder (10 cm diameter, 15 cm height) sealed with a plastic bag and sprayed water on the nest daily. We carefully inspected the nests daily, and upon hatch, the cylinders were removed so the females could forage freely. The inside nests were about 1.5 m from the edge of the foraging area, and the outside nests were about 1.5 m outside the edge of the foraging area (Fig. 6.21; Filippi et al. 2005). Because the amount of energetic reserves a female entered the provisioning season with would affect her provisioning capacity, we measured an initial female energy index (body weight <g>/pronotum width <mm> of all females). There was no significant difference between females in the two groups. The parameters assessed throughout the provisioning season included incidence of kleptoparasitism (fewer drupes at inspection than at the previous inspection), number of drupes provisioned, female vigilance (presence of female in the nest when inspected), development rate and survivorship of young, persistence of nest, and resource pressure (good drupes/ female, described in detail in Sects. 4.19.2 and 4.19.3).

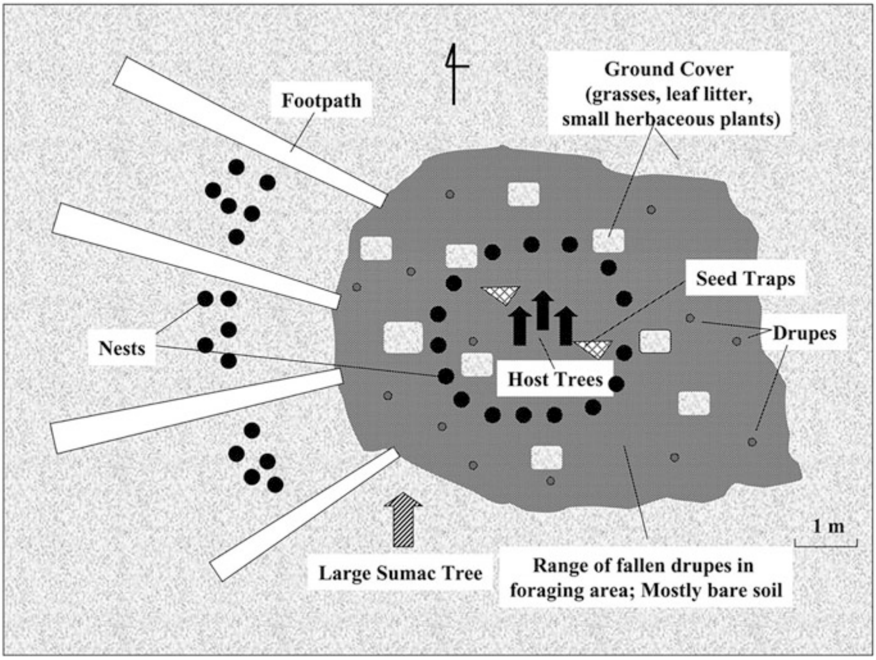


Fig. 6.21 The manipulated field site at Mt. Hinokuma in Kanzaki Town, Saga, Japan, in 2000. Nests were established inside (darkly shaded area around the host trees) and outside the foraging area. Lightly shaded areas represent ground cover (grass, leaf litter, herbaceous plants, etc.), which was sparse inside the foraging area. Adapted from Filippi et al. (2005)

6.4.1 Factors Related to Rate of Kleptoparasitism

Interestingly, both inside and outside nests suffered approximately equal rates of kleptoparasitism; however, though the difference was not quite significant and consistent with our prediction, inside nests had nearly twice as many drupes removed from them as outside nests (Table 6.1). The values in the table for stolen drupes are also most likely very conservative, because, to minimize disturbance, we only checked the nests every other day. It is possible, and even likely, that many

Table 6.1 Conditions within nests inside and outside the foraging area

	Inside ($n = 16$)	Outside ($n = 16$)	Mann-Whitney U test: $P =$
No. kleptoparasitized nests	14/16	13/16	NS
Mean no. drupes stolen (± SE)	4.0 ± 0.72	2.2 ± 0.56	0.06
Mean final no. drupes provided (± SE)	13.9 ± 2.5	6.5 ± 2.0	0.025
Female presence/absence in nest	50/78	42/92	NS

Adapted from Filippi et al. (2005)

more drupes during unobserved times had been provisioned and then stolen. Females tended to remember a good site and return to it repeatedly.

The resource pressure in 2000, calculated using the method in Table 4.7, revealed that the FDI was extremely low (i.e., high resource pressure) in 2000 (0.29) and very similar to that in 1998 (0.28), as was the provisioning capacity. Interestingly, no females were found nesting inside the foraging area early in the season that year either.

Pattern of Drupe Availability

Despite the greater number of drupes stolen from inside nests, provisioning by inside females increased steadily throughout the critical third stadium. On the other hand, provisioning by outside females increased at a much slower rate than inside females and, in fact, plateaued during the middle of the still very vulnerable second stadium (Fig. 6.22; Filippi et al. 2005). We waited until all nests had been abandoned before counting the final number of drupes available to nymphs. Surprisingly, despite the higher rate of kleptoparasitism, in the end, inside females still provisioned significantly more drupes than outside females (Table 6.1)! It is noteworthy that even

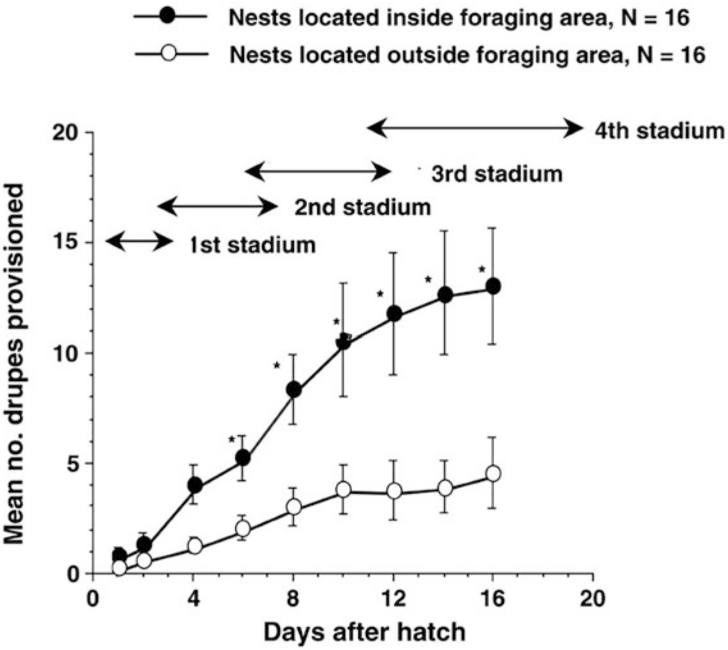


Fig. 6.22 Relationship between nest location and provisioning capacity of females throughout the provisioning season of 2000. Error bars represent S.E. *Mann-Whitney U test, $p < 0.05$. Adapted from Filippi et al. (2005)

though inside females ultimately provided more than twice as many drupes as outside females, the average number was still among the lowest we have seen over the years (Filippi et al. 2002), reflecting the very poor resource availability in 2000.

We expected that those females able to find drupes more easily would spend more time guarding nymphs in the nest. However, while females were present in the nest at 54% of the observations in the inside nests and 46% of the time in the outside nests, at this sample size the difference was not significant (Table 6.1). The extremely low number of good drupes in 2000 probably required that all females spend as much time as possible foraging.

6.4.2 Effect of Energetic Reserves on Provisioning Capacity

We found no significant relationship between energetic reserves and the final number of drupes provisioned by inside females; however, this was not the case with outside females. There was a significant positive correlation between energetic reserves and the final number of drupes provisioned by outside females (Bartlett $\chi 2$, $p = 0.027$). Recall that no females had prepared nest sites within the foraging area in 2000; we randomly *assigned* the females we collected from their chosen nest sites outside the foraging area to nest sites inside and outside of the foraging area. Because none had actually chosen either of those sites, it is possible this finding is an artifact of the experimental design. Also, we suspect that the inside nests prepared by us had better litter cover than would normally occur within the foraging area, and that could have made it safer for inside females and their nests than if the nests were completely natural. This finding bears further scrutiny under more natural nesting preference conditions.

6.4.3 Development and Survival Rates of Nymphs Inside and Outside the Foraging Area

Nymphs in inside nests developed significantly faster than those in outside nests (Fig. 6.23; Filippi et al. 2005). The stage of development was significantly advanced for nymphs in inside nests by day 4, and the numbers of nymphs in outside nests dropped off dramatically by day 6 (Fig. 6.23). Survivorship was also dramatically lower for outside nests (Fig. 6.24; Filippi et al. 2005). In fact, outside nests had lower survivorship than inside nests from the first day of observation and showed a sharp decrease in survivorship of 37% between days 4 and 6, while inside nests showed a much more gradual decline and leveled off at 40% by day 10. Survivorship of outside nymphs had dropped to about 10% by day 10. These findings of lower success in outside nests surprised us as over the years females have shown a clear preference for nesting outside the foraging area (Filippi et al. 2002). Why would

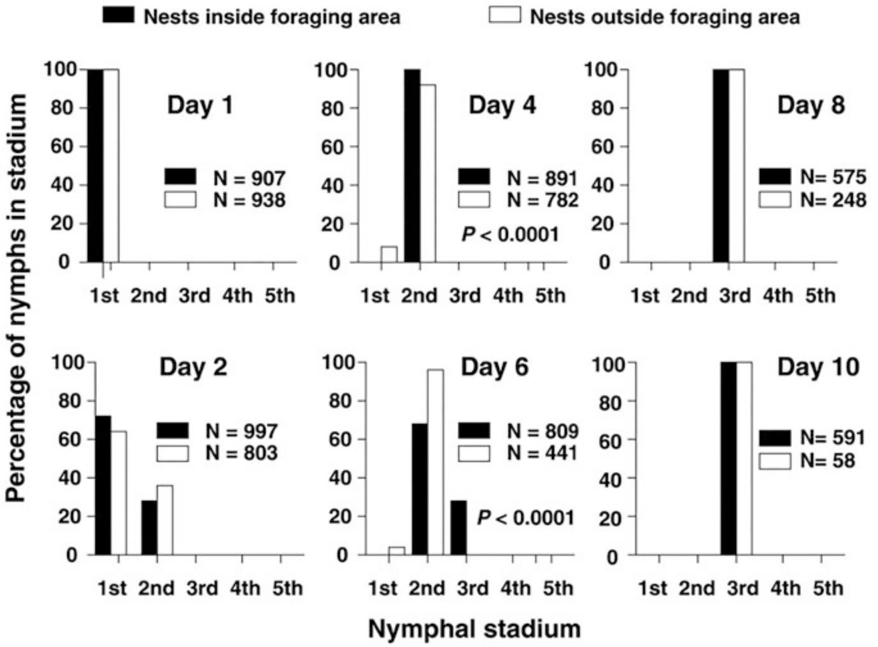
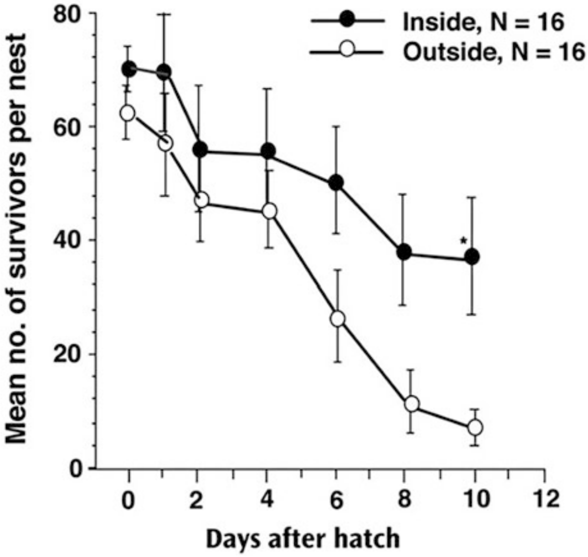


Fig. 6.23 Effect of nest location on development rate of nymphs. Adapted from Filippi et al. (2005)

Fig. 6.24 Relationship between nest location and survivorship of *P. japonensis* nymphs. Survival curves plotted by Cutler-Ederer method. Error bars represent S.E. Mann-Whitney U test, $p = 0.02$. Adapted from Filippi et al. (2005)



there be such a clear preference for nesting outside the foraging area if the inside nesters do better? We consider this in Sect. 6.4.5.

6.4.4 Persistence of Nests Inside and Outside the Foraging Area

Initially, and as predicted (finally!), the number of intact nests was slightly higher than that of inside nests. However, by day 6 the pattern was reversed, and, contrary to our prediction once again, by day 8 the difference was significant with twice as many inside nests as outside nests remaining intact (Fig. 6.25; Filippi et al. 2005). It is highly likely the very poor provisioning capacity of the outside females leads to early departure of the nymphs from the nest.

6.4.5 Conclusions of This Study

So what did we learn through this study? Well, we certainly discovered that kleptoparasitism is not a rare behavior at all in this species but is in fact quite widespread (Table 6.1), at the very least under the resource conditions of 2000 at this particular site. We don't know if it is as common every year as it was in 2000,

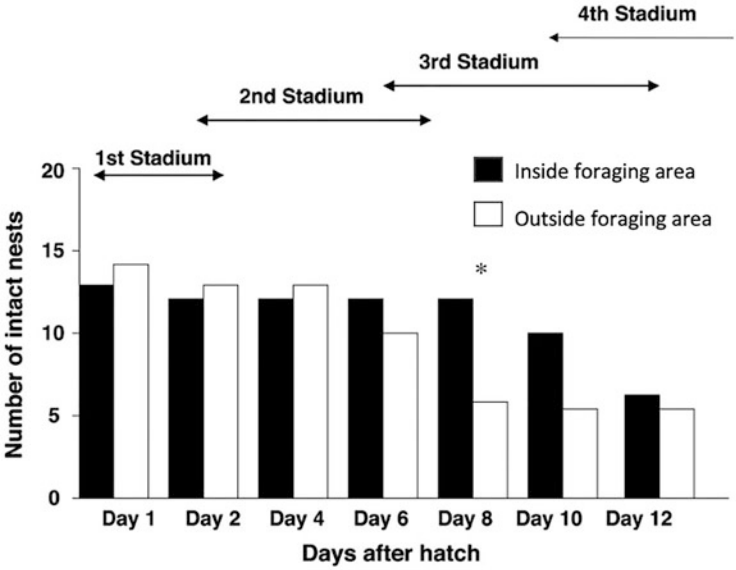


Fig. 6.25 Persistence of nests inside and outside the foraging area during the nesting stage. Adapted from Filippi et al. (2005)

but with the extremely poor resource conditions that year, almost all nests had experienced some kleptoparasitism. We were also humbled by the number of our predictions that turned out to be wrong. However, never to be discouraged, we attribute several of our miscalculations to our keen desire to make sure the inside nests did not disappear so early in the study that we would have been unable to collect any data at all. In hindsight, the inside nests were far better protected with leaf litter than they would have been under natural circumstances, which is likely why no females were nesting there to begin with! Moreover, the biggest factor that every field ecologist must always contend with when interpreting results of field studies is, of course, the fact that there are invariably multiple ecological conditions that are beyond our control and that very likely affect the results of our studies in different ways in different years. For example, we could not have predicted the very poor resource this year, which surely affected the outcome of our study. But what we can do is incorporate as many of the relevant factors that we encountered into our interpretation of the results. And we can only EVER say that the results of our study apply to the conditions at this particular site in this particular year. Even within our small Mt. Hinokuma, there were at least 59 subpopulations at the time of this study, and the ecological conditions, including resource abundance, varied considerably from site to site.

So, it seems that when the resource pressure is as severe as it was at this site in 2000, females nesting outside the foraging area (where we found them nesting initially) have very poor success (Figs. 6.23, 6.24, and 6.25). We concluded that the combination of travel time to and from the foraging area and the extreme scarcity of good drupes (increased handling time) was devastating to the success of outside females that season. Such conditions would increase the benefit of the stealing strategy (Hockey and Steele 1990). However, there are trade-offs involved and costs and benefits associated with each tactic, and at this point in our research we were unclear whether stealing was limited to opportunistic encounters or whether it had evolved as an alternative foraging tactic. At any rate, a decision to steal or self-forage will depend on a variety of factors, and the range of options extends from self-foraging only to stealing only; there will come a point where the costs of sticking to one tactic outweigh the benefits and a switch will occur (Broom and Ruxton 1998). Risk-sensitive decision-making would mitigate the economic costs involved such that each option would be chosen when the payoff associated with it outweighs the costs (Brockmann and Barnard 1979; Broom and Ruxton 1998; Koops and Giraldeau 1996; Flower et al. 2013). Having the plasticity to respond to the ecological conditions encountered is the key to success.

For the host, the costs of stealing are obvious, but what might be the cost to the kleptoparasite? Mantaro Hironaka observed behaviors during this study that suggested stealing from a nest was not without cost, at least when the host female is present. Host females were seen aggressively engaging with the would-be kleptoparasite, and so began our quest to answer another series of questions regarding the evolution of strategies by the host to counter the costs of kleptoparasitism.

6.5 Guarding Behavior Against Intraspecific Kleptoparasites

Kleptoparasitism of food that a parent has invested energy into procuring can occur outside the nest, along the route back to the nest with the food item, or inside the nest after the provision has been deposited (Field 1989a, b, 1992; Villalobos and Shelly 1996). Similar to what has been reported for solitary wasps (Field 1989a, b, 1992; Villalobos and Shelly 1996; Kurczewski and Spofford 1998), provisioning *P. japonensis* females who have succeeded in finding a drupe are often accosted by other foraging females on their way back to the nest, and lengthy tugs-of-war and grappling over the prized drupe may ensue (Filippi-Tsukamoto et al. 1995; Filippi et al. 2002). Because the loss of provisions that were acquired at great cost could severely impact a female's fitness, natural selection should favor the evolution of strategies that would mitigate the risk of kleptoparasitism. Reports of defensive behavior against kleptoparasitism are scarce, but brooding females of the dung beetle *Copris lunaris* aggressively defend dung balls against potential thieves (Klemperer 1982; Costa 2006). We undertook a study to investigate the effectiveness of guarding and defensive strategies against kleptoparasitism in *P. japonensis*.

6.5.1 Guarding Behaviors Displayed

During the provisioning season (June and July) of 2000, we set out to identify whether females could effectively mitigate the threat of intraspecific kleptoparasitism. We hypothesized that there would be specific and effective defensive behaviors against intraspecific kleptoparasites. To verify the resident female in a nest, we marked the elytra of 67 egg-guarding females and labeled the nests with our regular trusted chopstick flags. Once the provisioning season got underway, when we located a marked female foraging for a drupe, we set a "good" drupe in front of her. The female took up the drupe and dragged it back to her nest. We gently removed the leaf litter to observe what was happening in the nest, specifically the female's behavior, the stage of nymphal development, and the nymphs' behavior. After depositing the drupe in the nest, the female remained holding the drupe for a time as the nymphs gathered on it. We called this the holding phase (Fig. 6.26a). The duration of the holding phase decreased significantly with progression of the nymphal stadia (Fig. 6.27; two-way ANOVA, $p < 0.001$). The holding phase could clearly be distinguished from feeding because the proboscis remained still rather than moving up and down as is seen during feeding. After the holding phase, the female shifted off the drupe and remained beside it and the feeding nymphs. We called this behavior the attending phase (Fig. 6.26b). Once a female initiated the attending phase for a newly deposited drupe, she never reverted to the holding phase.

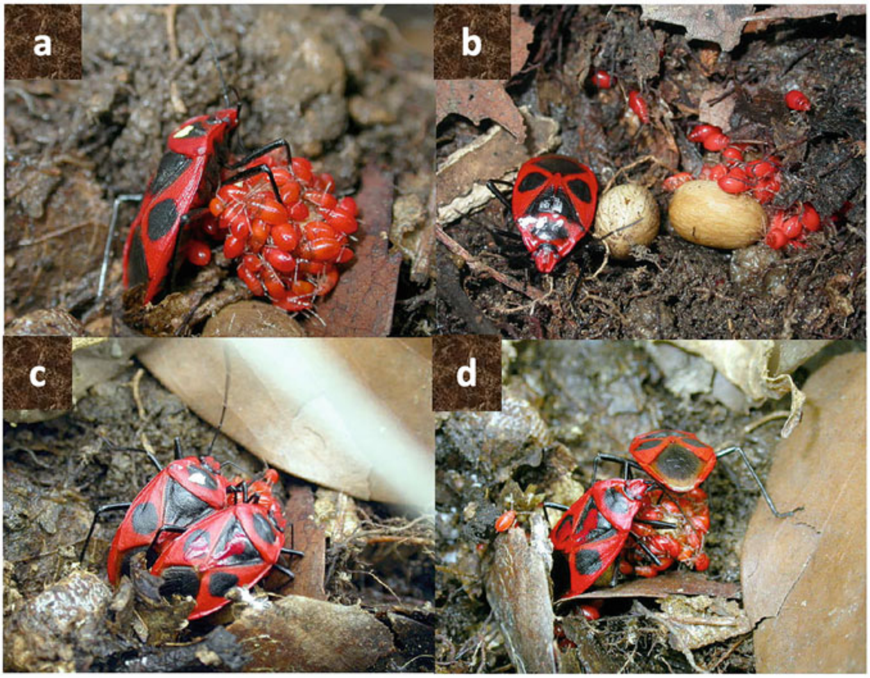
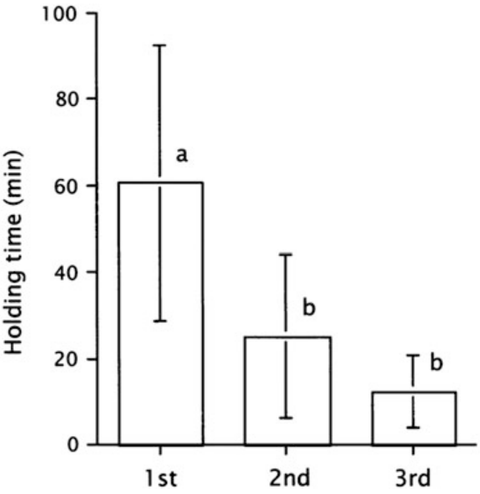


Fig. 6.26 Guarding behaviors displayed by resident *P. japonensis* females in the nest. (**a**) Typical holding behavior. The female that just returned to the nest with a drupe is holding the drupe, which is covered by a cluster of nymphs; (**b**) typical attending behavior; (**c**) resident female blocking intruder from gaining access to drupe; (**d**) resident female kicking intruder with hind legs. Adapted from Hironaka et al. (2008b)

Fig. 6.27 Female holding time for each nymphal stage. $N = 20$ for each stage. Error bars indicate S.E. Different letters above bars indicate significant difference (Scheffe's F test at $p < 0.05$). Significant decrease as developmental stage progressed (two-way ANOVA, $p < 0.0001$). Adapted from Hironaka et al. (2008b)



6.5.2 *Effectiveness of Guarding Behavior Against Intruders*

To observe the effectiveness of these behaviors against intraspecific kleptoparasites, 5 min after initiation of the holding phase, we carefully placed another foraging female right next to the entrance of the resident female's nest. The intruding female immediately entered the nest and attempted to remove the drupe. We observed the response of the 20 resident females to an intruder during the holding phase. Females displayed either no response or one or both of two different guarding behaviors in response to different levels of threat from an intruding female (Fig. 6.28). Upon contact with the antennae of the intruding female, the resident female, still holding the drupe, waved its antennae rapidly and emitted a soft chattering sound. The intruder attempted to insert its proboscis in the drupe and steal it, but the resident female (still in the holding phase) guarded the drupe by presenting its dorsum to the intruder, much like a shield, and attempted to push the intruder away (Fig. 6.26c). The resident female also sometimes kicked at the intruder (Fig. 6.26d). The altercation continued for up to 10 min and ended when the intruder either successfully

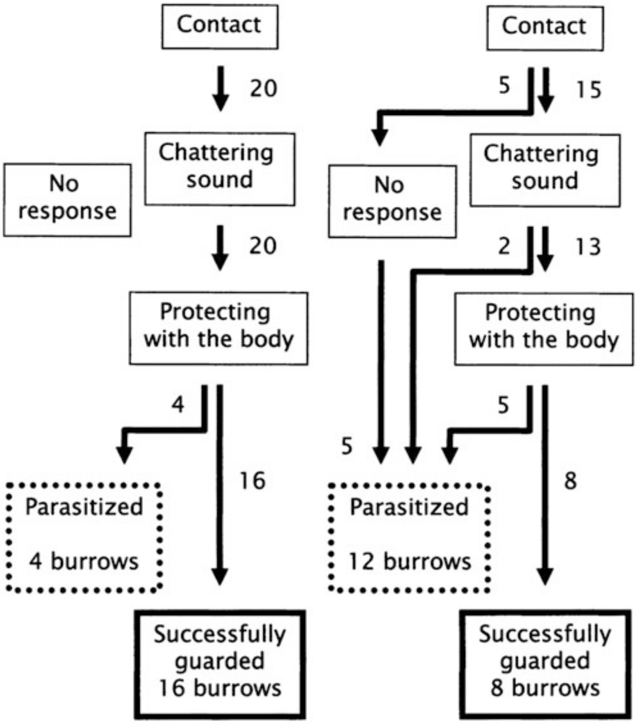


Fig. 6.28 Kinematic diagrams of guarding behaviors displayed against an intraspecific kleptoparasite. (**a**) behaviors displayed during the holding phase; (**b**) behaviors displayed during the attending phase. Numbers represent number of times behavior was observed. 20 burrows were observed in each phase. Adapted from Hironaka et al. (2008b)

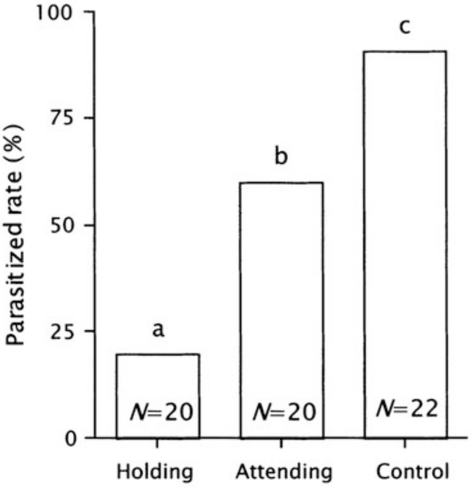


Fig. 6.29 Effectiveness of guarding phases as defense against intraspecific kleptoparasitism. Fisher's exact test, $p = 0.0128$. Control nests had female removed after nests were provisioned. Adapted from Hironaka et al. (2008b)

snatched the drupe and scampered off or relinquished its attempt to steal the drupe and left in defeat.

On the other hand, when we set a foraging female at the entrance to the nest 5 min into the attending phase, the response of the resident female was very subtle, with five attending females showing no observable response at all (Fig. 6.28). When the female was prevented from returning to her well-provisioned nest, ~90% of the nests were parasitized and nearly all the provisioned drupes were stolen (control group, $n = 22$; Fig. 6.29). Nevertheless, females in both the holding and attending phases demonstrated some success at defending drupes from thievery compared to the control group. However, behaviors displayed during the holding phase were significantly more effective at mitigating kleptoparasitism. Only 20% of nests were successfully parasitized during the holding phase ($n = 20$), while fully 60% of nests in the attending phase were parasitized (n = 20).

6.5.3 Summary of Guarding Behavior

The hypothesis that *P. japonensis* females display guarding behaviors to effectively reduce kleptoparasitism was supported (Figs. 6.26, 6.27, 6.28, and 6.29). We have demonstrated that kleptoparasitism occurs as an alternative strategy for foraging females to provision their nests (Filippi and Nomakuchi 2016; this work, Sect. 6.4). Nests of the solitary wasps discussed above and those of *P. japonensis* are spatially clumped, a condition that favors the evolution of kleptoparasitism (Eickwort 1975). It is, thus, reasonable that defensive behaviors to thwart the thievery should also evolve. When a female of one of these species that has finally procured food is accosted on its way back to the nest, the tugs-of-war and grappling behaviors ensue. If accosted just outside the nest, the female rushes into the nest with the food (Filippi-

Tsukamoto et al. 1995; Villalobos and Shelly 1996; Kurczewski and Spofford 1998; Filippi et al. 2002). The entrances to spatially clumped nests tend to be inconspicuous, and such is the case with *P. japonensis.* The female slips under a leaf that looks like any other leaf but is in fact the entrance to the nest (Tsukamoto and Tojo 1992). Hiding the entrance to the nest each time the female leaves is a strategy used by some wasps (Villalobos and Shelly 1996; Kurczewski and Spofford 1998). To minimize the risk of kleptoparasitism, female *P. japonensis* prefer to nest outside the foraging area, which has less protective leaf litter (Filippi-Tsukamoto et al. 1995).

Because the constraints on the different species vary, so will the particulars of the defensive behaviors. Being constrained to semelparity, *P. japonensis* has more to lose than the iteroparous solitary wasps who tend multiple nests at a given time. The wasps return to a nest, drop off the food, and leave to get more. Once sufficient food is provisioned, the mother does not return to the nest again (Villalobos and Shelly 1996). On the other hand, until they die from exhaustion, *P. japonensis* females divide their time between guarding the nest and provisioning. Searching time for drupes can take more than 2 h, so females often return to the nest intermittently even if they have not found a drupe and spend some time guarding the nest against predation and kleptoparasitism (Filippi et al. 2005; Hironaka et al. 2007a, b, c). Thus, females are faced with a precarious balancing act that involves splitting their time between the arduous task of searching for the essential food and mitigating theft of that precious resource by females intruding into an undefended nest.

While both phases of guarding behavior by *P. japonensis* females were significantly more effective at reducing theft of drupes than no behavior, the behaviors during the holding phase were much more effective than those during the attending phase (Fig. 6.26; Hironaka et al. 2007a, b, c). Why is this phase such an effective deterrent to kleptoparasitism? This seems to be related to the time-consuming series of behaviors that females engage in when searching for a drupe (Nomakuchi et al. 1998). The intruding female will first approach the drupe in response to the volatile chemicals it emits. Then she must pierce the skin and probe the drupe repeatedly to assess that it has a solid endosperm and that it tastes right. Then she must grasp the drupe with her fore- and mid-legs and roll it around to assess the weight. The posture and behaviors exhibited by the resident female during the holding phase essentially make the drupe inaccessible to inspection by the intruding female, who most often eventually gives up and leaves.

If the holding phase is so effective, why then do females release the drupe and move off to the side for the attending phase? Behavior of the voracious nymphs could shed some light on this, and it can also help us to understand why the duration of the holding phase goes from an average of 60 min during the first nymphal stadium to just over 10 min during the third stadium (Fig. 6.27). Nymphs gather on the drupe that the female has brought into the nest, but some also gather on the female and attempt to insert their proboscis through her exoskeleton to feed on her! We observed nymphs feeding on their mother inside the nest on four occasions; in three cases the diners were third instars, and in the remaining case they were fourth instars. It likely happens much more than that, and the older the nymphs, the more likely they are to attempt to feed on the mother. Clearly, the mother has another trade-off to deal with: the decision about how long to remain in the very effective holding phase and possibly become the victim of premature cannibalism, which would, of course, result in no more provisioning, and when to let go of the drupe, spending a few minutes attending and living to provision another day!

The nymphs are feeding while the female is holding the drupe, and the longer she holds it, preventing an intruding female from stealing it, the more they can feed. It is also very possible that the reason the female is holding the drupe is because she is secreting an enzyme to aid in digestion of the endosperm by the nymphs. This hypothesis requires further exploration; however, it would be compatible with providing time for the young to feed on the drupe before an intruder can steal it. It is also possible that a drupe that has been fed on by nymphs for the duration of the holding time might be unacceptable to intruding females.

6.6 Kleptoparasitism: An Opportunistic Behavior or an Evolved Alternative Tactic?

Intraspecific kleptoparasitism is widespread across taxa and among vertebrates has been reported in mammals, birds, reptiles, and amphibians. Among invertebrates, it has been documented in insects and spiders (reviewed in Iyengar 2008). Because the victim loses the resource, along with the energy invested to procure it, and the thief stands to gain considerably because the cost of searching is eliminated, the consequences of kleptoparasitism can have profound evolutionary implications (Brockmann and Barnard 1979; Iyengar 2008). The majority of examples of kleptoparasitism represent facultative and even opportunistic behavior. An individual happens upon another individual transporting the desired food item and, because the interloper is looking for food and has found it (the fact that another individual found it first is irrelevant), it tries to claim it. Representing the other end of the spectrum, cases of obligatory kleptoparasitism have also been identified (Brockmann and Barnard 1979; Field 1989a, b, 1992). In between these two extremes lies the condition whereby kleptoparasitism has evolved as an alternative tactic within a larger foraging strategy. The success of a kleptoparasitic tactic could have important consequences on the fitness of both the victim and the thief.

Parastrachia japonensis is severely constrained by an unpredictable and scarce resource, drupes of the host tree *Schoepfia jasminodora* (Filippi-Tsukamoto et al. 1995; Filippi et al. 2002). Their numbers are sporadic, and the quality of the drupes is very inconsistent and most often unacceptable to provisioning females. Because of these conditions, the search and handling time for the precious resource is extremely lengthy. Females can search for up to 2 h before finding a drupe worthy of inspection and then invest several minutes exposed to conspecific foragers and to predators while they inspect each drupe to make sure it is of high quality (Filippi-Tsukamoto et al. 1995; Nomakuchi et al. 1998). Lengthy handling times leave the foraging female vulnerable to exploitation by kleptoparasites (Sivinski et al. 1999). Foraging females steal from females in the process of inspection or soon after the drupe has been accepted and the female is heading off with it in haste (Filippi-Tsukamoto et al. 1995l Filippi et al. 2005). We suspect the hasty return to the nest with the drupe in tow, a very different movement pattern from the many other foraging females in the area engaged in a stop and start tentative inspection of drupes, serves as a cue to foraging females that a high-quality drupe has been procured. We once observed eight females engaged in a tug-of-war over a drupe that lasted for at least 2 h (Fig. 6.30; Filippi and Nomakuchi 2016). We consider this type of stealing to be opportunistic, although the ability to recognize the cue that a good drupe had been found must have evolved.

It is of interest to clarify the proximal mechanisms that could facilitate the evolution of kleptoparasitic behaviors. We considered that the behavior of entering a conspecific female's nest to steal a drupe seemed to be more deliberate than the opportunistic behavior of stealing from a foraging female. Thus, we tested the hypothesis that stealing from a nest was, indeed, evolved an alternative tactic to self-foraging that provisioning females use to provide high-quality drupes to offspring. In support of this hypothesis, we predicted that females would (1) be actively attracted to the nests of conspecific females and (2) that they would deliberately enter the nests to steal high-quality drupes.

Fig. 6.30 Eight females engaged in a tug-of-war over a prized "good" drupe. This went on for over 2 h. Arrows indicate other unacceptable drupes in the image. Adapted from Filippi and Nomakuchi (2016)

From June 3 to 10, 2008, we collected egg-guarding females with their original nest substrate of leaf litter and soil from the field site described previously and maintained them individually in the laboratory in lidded, clear plastic cups (8 cm d, 4 cm h) at 25 °C and a photoregime of 16L:8D. Within 24 h of hatch, the nest cups were transferred to an experimental arena designed as follows (Fig. 6.31; Filippi and Nomakuchi 2016). A clear plexiglass box was constructed (35 × 18 × 15 cm, L × W × H) with a plexiglass/mesh lid. The bottom of the box was layered with 5 cm of soil and sparse leaf litter. A hole large enough to accommodate a nest cup was dug 1 cm from either end of the box. Thus, when the nest cups were set into the soil in the box, the tops of the cups were flush with the soil and leaf litter so that the females could enter and leave their nests freely. The test female's nest was set at one end of the box, and an experimental nest was set at the other end. Unless otherwise stated, none of the test females had experienced provisioning prior to the trial. To avoid confounding effects from female interactions, the experimental nest did not have a female.

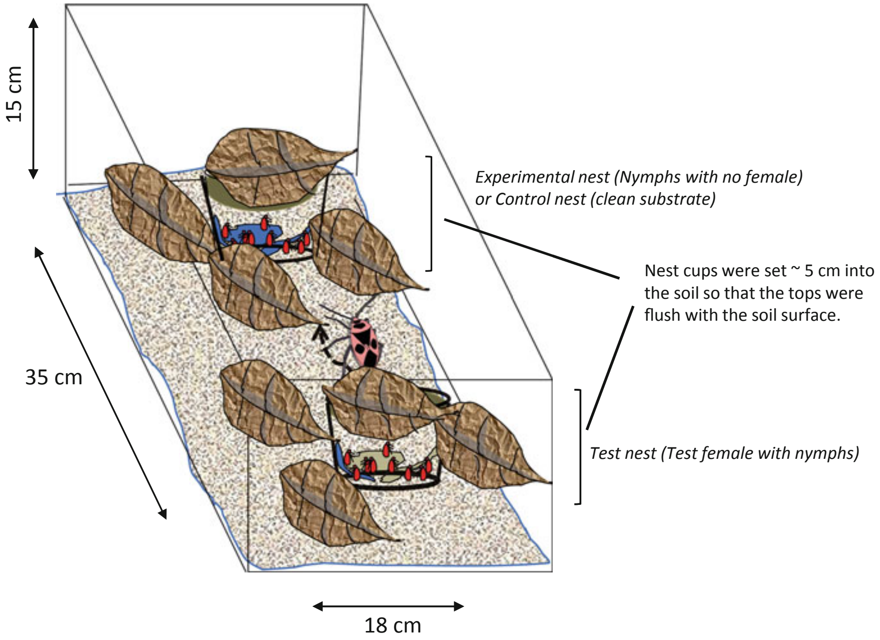


Fig. 6.31 Experimental arena. Test female with nymphs and original nest substrate (soil and leaf litter) in clear plastic cup in foreground; experimental nest (nymphs with nest substrate but no female) or control nest (clean substrate) in clear plastic cup at opposite end of plexiglass container. The bottom of the container was covered with 5 cm of soil and sparse leaf litter. Nest cups were set into the soil. The female could enter and leave nest freely. Adapted from Filippi and Nomakuchi (2016)

6.6.1 Are Females Attracted to Other Female's Nests?

To determine whether females were actively attracted to conspecific nests, the "other" nest was either an experimental nest containing the nymphs and substrate of a conspecific nest ($n = 12$) or a nest cup containing control substrate (clean substrate with no nymphs; $n = 12$). After removing the lid of the test nest, the test female could leave her nest to forage. We counted the number of females that entered the experimental and the control nest. Eight times as many females entered the experimental nest containing nymphs and substrate of a conspecific nest, compared to the cup with the clean substrate (Fig. 6.32a; Fisher's Exact Test, $p < 0.005$; Filippi and Nomakuchi 2016). Females were significantly more attracted to conspecific nests compared to control nests.

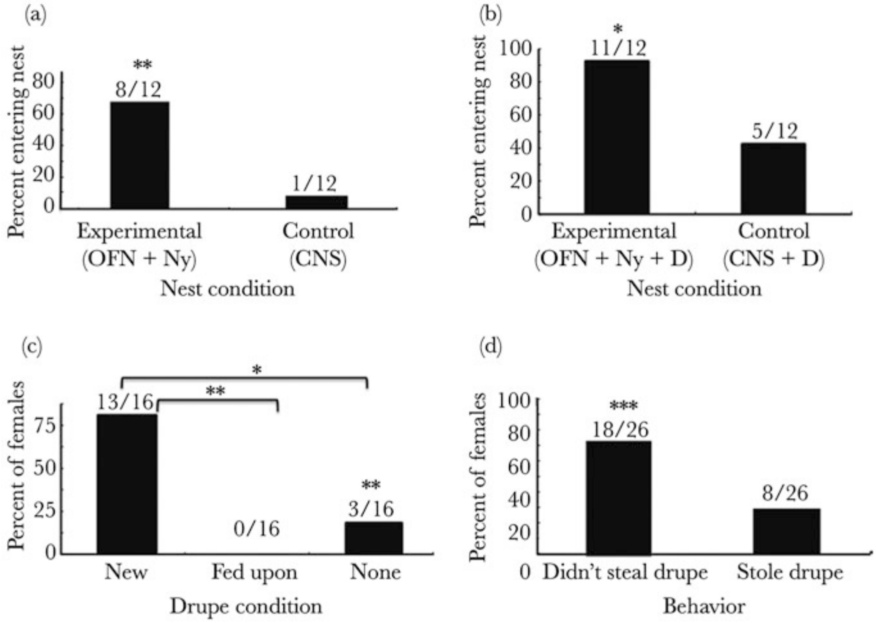


Fig. 6.32 Rate of intrusion into "other" female's nest and stealing behaviors of provisioning females. (**a** and **b**) *OFN + Ny* "other" female's nest with nymphs; *CNS* clean nest substrate (soil, leaf litter from field site); *D* drupe. *Fisher's Exact test, $p < 0.05$; **$p < 0.005$. (**c**) Females entered and then left intruded nest with a new drupe, a fed on drupe, or no drupe (none). *$\chi 2$ Goodness of Fit test, $P < 0.05$. **$p < 0.001$. (**d**) test female's nest was switched with OFN; provisioning female entered her nest (in the OFN location) and subsequently left the nest with or without a drupe. ***$\chi 2$ Goodness of Fit test, $p < 0.0001$. Test females in (**a**), (**b**), and (**c**) had not provisioned before the trial; test females in d had stolen one drupe prior to the trial. Adapted from Filippi and Nomakuchi (2016)

6.6.2 Testing the Attractiveness of a Good Drupe vs a Conspecific

To test whether the attractive force of a conspecific nest with a drupe was stronger than that of a drupe alone, the trial was repeated, but this time the experimental nest was either a conspecific nest containing a good drupe ($>80\%$ endosperm; $n = 12$) or a control nest containing clean substrate and a good drupe ($n = 12$), and the number of test females that entered each nest was counted. More than twice as many females entered the conspecific nest with the drupe compared to the control nest with the drupe (Fig. 6.32b; *, Fisher's Exact test, $p < 0.05$; Filippi and Nomakuchi 2016). Females were significantly more attracted to conspecific nests with a drupe than the control nests with a drupe, supporting the prediction that females are actively attracted to conspecific nests.

6.6.3 Could Females Be Entering a Conspecific Nest for Some Other Reason?

It is possible that females entered the nest to disturb the nest (interference competition) or that they mistook it for their own nest. To determine whether females were actually entering a conspecific nest to steal a drupe for the purpose of providing high-quality food for their nymphs, we placed one good drupe and one drupe that had been fed on extensively by nymphs in the experimental conspecific nest. We removed the lid on the test nest and monitored the female's behavior. For those that entered the experimental nest, we observed the female until it left the experimental nest to determine if a drupe was stolen, and if so, which one ($n = 16$). All 16 females entered the experimental nest. No females stole the fed-upon drupe and over four times as many females emerged with the good drupe, compared to those who emerged with no drupe (Fig. 6.32c; $*\chi2$ Goodness of Fit test, $P < 0.05$. $**p < 0.001$; Filippi and Nomakuchi 2016), supporting the prediction that females were entering conspecific nests to steal a good drupe.

6.6.4 Does the Female Find Her Own Nest and Other Nests by Location or by Odor?

To determine whether the sequence of behaviors by nest kleptoparasites happens via odor or location cues and whether females return to their own nest using odor or location cues, we allowed a female to leave her nest and steal a drupe from the experimental nest. After the test female returned to her nest with the stolen drupe and then set out to forage again, we quickly switched the location of the two nests and placed a fresh drupe in the putative conspecific nest ($n = 26$). We monitored the female to determine whether she would enter the putative experimental nest and whether she would leave with the drupe. All females entered the putative conspecific nest; however, less than half of the females emerged from the nest with a drupe (Fig. 6.32d; ***$\chi 2$ Goodness of Fit test, $p < 0.0001$; Filippi and Nomakuchi 2016), suggesting that females approached the previously visited experimental nest by location cues and then, recognizing that the nest was their own nest using chemical cues, did not remove a drupe.

6.6.5 Conclusions

The findings support the hypothesis that nest kleptoparasitism is not merely an opportunistic behavior but is an evolved tactic within the larger foraging strategy during progressive provisioning. Outside of the Hymenoptera, to the best of our knowledge, this is the first report of conspecific kleptoparasitism that involves approaching a victim's nest when it does not contain a resource reward. Females are actively attracted to the nests of conspecifics (Fig. 6.30a); they preferred to enter nests of conspecifics that contained a good drupe rather than clean substrate with a good drupe (Fig. 6.30b), entered nests to steal good drupes (Fig. 6.30c), and could distinguish their own nest using volatile cues even when it was switched with the conspecific nest and so did not remove drupes from it (Fig. 6.30d). Thus, in addition to progressive provisioning (Tsukamoto and Tojo 1992; Filippi-Tsukamoto et al. 1995), feeding limited to nymphs and inseminated females (Tsukamoto et al. 1994), suppression of metabolic rate to sustain the adult stage for up to 2 years in the absence of feeding (Tojo et al. 2005), selective foraging behavior (Nomakuchi et al. 1998), complex navigation behaviors during provisioning (Hironaka et al. 2003a, b, 2007a), production of trophic eggs (Hironaka et al. 2005; Filippi et al. 2012), and behaviors to defend against kleptoparasitism (Hironaka et al. 2008b), nest kleptoparasitism as an alternative tactic during provisioning is yet another evolutionary innovation that has enabled *P. japonensis* to optimize use of the severe resource constraints that it has experienced over its evolutionary history.

Considering that females have effective defense strategies against nest intruders (Hironaka et al. 2008a, b), it is curious that females preferred to enter conspecific nests with a drupe over clean substrate nests with a drupe. However, an earlier study demonstrated that females were absent from the nest at nearly 70% of the inspections, likely out foraging (Filippi et al. 2005); thus, there is a good chance that the intruding female will not meet with resistance. And should the resident female be present in the nest, it is also possible that she will be weakened from provisioning over many days and would not be a formidable defender. Thus, the cost of stealing might not be significant.

Another curious aspect of the host tree also suggests why attraction to a stockpile of drupes might be favored. The host tree grows in such a way that certain twigs produce and drop good drupes, while others do not, leaving hotspots of drupes on the ground. If a hotspot is encountered, the female will remember it and, using its keen navigation behaviors, return to it repeatedly. A well-provisioned nest would resemble a hotspot, and, with a bit of tweaking, co-opting the sensory and behavioral mechanisms used during provisioning to target conspecific nests would be a very efficient way to forage, indeed (Breed et al. 2012). Moreover, females clearly recognize the odor of other nests and avoid them when returning to their nest with a drupe because they never mistakenly provision conspecific nests (Filippi-Tsukamoto et al. 1995; Filippi et al. 2000; Hironaka et al. 2007a). To adopt the alternative tactic of nest thievery, they would then be attracted to the conspecific nest.

Other conditions that favor the evolution of nest thievery are the high density of nests with stockpiles of drupes in the foraging area and the fact that the stockpiles of drupes have already been vetted by females and so would require less handling time. Compared to self-foraging, the shorter amount of time spent exposed to predation and aggressive encounters with other provisioning females while foraging would be attractive (Broom and Ruxton 1998). These factors should also weigh heavily in favor of adopting the alternative tactic of nest kleptoparasitism. Females with the ability to be attracted to both drupes and conspecific nests should enjoy considerable success. If that ability is genetic, the trait and nest kleptoparasitism as an alternative foraging tactic should be favored by natural selection for provisioning females.

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Mantaro Hironaka in fact applied to Saga University in order to eventually join the *P. japonensis* research team. He proved to be a brilliant, enthusiastic, energetic, and creative member of the team who contributed significantly to a variety of behavioral studies. Mantaro went on to get his doctoral degree in Dr. Tojo's laboratory from the United Graduate School of Agriculture, Kagoshima University, focusing on the neurological processes involved in the complex navigation that *P. japonensis* uses when provisioning. He is currently an Associate Professor at the Department of Applied Entomology, Faculty of Bioproduction Science, Ishikawa Prefectural University.

Shintaro Nomakuchi an ecologist focusing on insect behavior, came to Saga University in 1993 as an Associate Professor, and was keen to share his very valuable expertise and join the *P. japonensis* research team, which he eventually headed. The work on clarifying the quality of the habitat, the techniques for monitoring the subpopulations and for carrying out a variety of ecological and behavioral studies, as well as the methods for performing robust data analyses, all got a hefty boost, and we were off and running. He is a Professor Emeritus of the Faculty of Agriculture at Saga University.

Lisa Filippi initiated the work on *Parastrachia japonensis* at Mt. Hinokuma Park in Kanzaki Town, Japan, where she earned her M.S. and doctoral degrees elucidating many of the amazing physiological and behavioral traits of this fascinating insect through field and laboratory studies. She is currently a Professor of Biology at Hofstra University in New York.

Chapter 7
Nematodes Exploiting *P. japonensis*

Toyoshi Yoshiga, Ryusei Tanaka, and Etsuko Okamura

Abstract Nematodes are ubiquitous in soil, and their ability to adapt to different environments has enabled them to be an evolutionarily successful group of organisms. Two types of nematodes have been isolated from *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae), parasitic and phoretic/necromenic. At least two species of mermithid nematodes parasitize the bug. Because *P. japonensis* is a sedentary insect that is soil dwelling and remains in very limited areas where *Schoepfia jasminodora* drupes, their sole food, are available, the bug would be an attractive host for these parasites. Synchronized emergence of mermithids from the host during its late last nymphal stage indicates that the mermithids infect early nymphal stages feeding on drupes on the ground and leave their host in the area where nymphs develop. This suggests that the mermithids have synchronized their life cycle to adapt to the host's life cycle. The other nematode, the phoretic/necromenic *Caenorhabditis japonica,* forms a species-specific association with the bug. The nematode leaves the female bug in the bug's nest and propagates on damaged eggs, nymphal cadavers, and drupes, and dauer larvae, the non-feeding and host-finding stage of the nematode, are reproduced. The nematode uses nymphs for transport and as a food source if they die. The association is biased to female bugs in adulthood, and dauer larvae on the bug enter quiescence, which enables the nematode to reach the bug's nest in the next reproductive season. Characteristic active host-seeking behavior of *C. japonica* dauer larvae, such as nictation, waving, and jumping, and the desiccation tolerance of dauer larvae on the bug appear to have

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evolved to associate with their carrier. The unique life cycle of *P. japonensis* has created special niches, and different types of nematodes have been adapting to them. Conversely, these associated nematodes might have been influencing the behavior, ecology, and life history of *P. japonensis*.

Keywords Commensalism · Necromeny · Parasite · Parasitism · Phoresy

7.1 Introduction

Nematodes are vermiform, unsegmented, bilaterally symmetrical pseudocoelomates. Like insects, they belong to the Superphylum Ecdysozoa, comprised of protostome animals that grow by molting (Aguinaldo et al. 1997; Telford et al. 2008). Most nematodes are microscopic because they live in the water film between soil particles. They are ubiquitous in soil and are the planet's most abundant metazoan (Bongers and Ferris 1999). They are an evolutionarily successful group of organisms, as are insects. At present, about 28,000 species have been described (Luc et al. 2010), but the total number of species is estimated to be a million or more (Lambshead 2004). According to molecular phylogenetic analysis based on the small subunit of ribosomal RNA gene, three major lineages exist within the phylum: the subclasses Chromadoria, Enoplia, and Dorylaimia (De Ley 2006). Nematode species are further divided into 5 clades (Blaxter 2011) or 12 clades (Bird et al. 2015).

Some nematodes are microbivores, feeding on organisms such as bacteria, fungi, and small eukaryotes, and others are plant feeders, carnivores, or omnivores. Free-living is the basic life cycle for nematodes, but some have acquired parasitic stages in their life cycle. The group of nematodes that has associations with insects is called entomophilic nematodes. These nematodes are further subdivided into phoretic (transfer), necromenic (propagation on decaying host), pathogenic, and parasitic relationships.

While *Parastrachia japonensis* Scott (Heteroptera: Parastrachiidae) aggregates on leaves of evergreen trees for several months of the year, this species is basically a soil-dwelling insect that spends its reproductive period, winter hibernation, and summer estivation burrowed in soil or on the soil surface. During its soil-dwelling phases, the shield bug has opportunities to encounter and contact other soil-dwelling organisms including nematodes. In the course of research of the shield bug, two types of nematodes have been isolated, parasitic and phoretic/necromenic. In this chapter we describe the biology of the two types of nematodes that exploit *P. japonensis* and infer the interaction between *P. japonensis* and nematodes.

7.2 Parasitic Nematode: Mermithid Nematodes

Mermithid nematodes are obligate parasites of invertebrates that belong to Dorylaimia (De Ley 2006). They are widely distributed in both aquatic and terrestrial habitats. Most of their hosts are insects, but spiders, crustaceans, earthworms, leeches, and mollusks also serve as hosts (Poinar Jr. 1979). A second stage infective juvenile emerges from an egg and actively seeks out a potential host. Once contact is made, the nematode begins penetrating through the integument of the insect with the aid of a stylet and enzymatic secretions. The nematode initiates development in the host's hemocoel. After completing its period of growth and development as a parasitic juvenile, the mermithid leaves the host, generally by boring through the integument. Upon entering the environment, the postparasitic juveniles molt to the adult stage and then mate, and the females deposit their eggs (Poinar Jr. 1979). Mermithids have relatively long bodies among the nematodes ranging from approximately 0.6 to 46 cm depending on the species, and the body size varies even within a given species between sexes and depending on the developmental condition (Poinar Jr. 1979). Because a mermithid's exit from its host results in the death of the host, some mermithids have been studied as biological control agents for insect pests such as mosquitoes, grasshoppers, and brown plant hoppers (Poinar Jr. 1979; Kaiser 1991). However, many mermithids apparently take a year to complete their life cycle, and most mermithids have never been reared completely through their life cycle in the laboratory; thus, limited information including species description is available.

7.2.1 Prevalence of Mermithids in P. japonensis

Mermithid nematodes typically parasitize heteropteran insects (shield bugs) in both the nymphal and adult stages (Riberiro and Castiglioni 2008; Tarla et al. 2011; Kamminga et al. 2012; Stubbins et al. 2016). However, emergence of mermithids from *P. japonensis* primarily occurs during the final nymphal stage, and they rarely emerge from adults (Iryu et al. 2020). Infection rates in *P. japonensis* populations range from 0 to 16.1% (mean, 6.2%). Fresh weight (1.2–55.3 mg, mean 21.8 mg) and length (8.8–26.1 cm, mean 18.9) of emerged mermithids also vary, but width is less variable (range 0.11–0.17 mm). A single parasitism is the typical case, but hyperparasitism is also found (Fig. 7.1).

All mermithid nematodes emerging from *P. japonensis* are postparasitic juveniles, and they have not been identified to species because of the difficulty of identification using postparasitic juveniles, which have fewer distinctive morphological characteristics, and the difficulty of obtaining the adults necessary for morphological identification. Molecular phylogenetic analysis using 18S ribosomal RNA genes suggests that at least two species of mermithids parasitize *P. japonensis* (Fig. 7.2; Iryu et al. 2020). One type is closely related to a mermithid, *Ovomermis sinensis,* detected from *Helicoverpa armigera* in China. The other nematode type is closely related to a mermithid, *Amphimermis* sp., detected from a wide range of insects in China. Because very limited information is available on mermithid species in Japan, it is difficult to infer the host specificity of the nematodes.

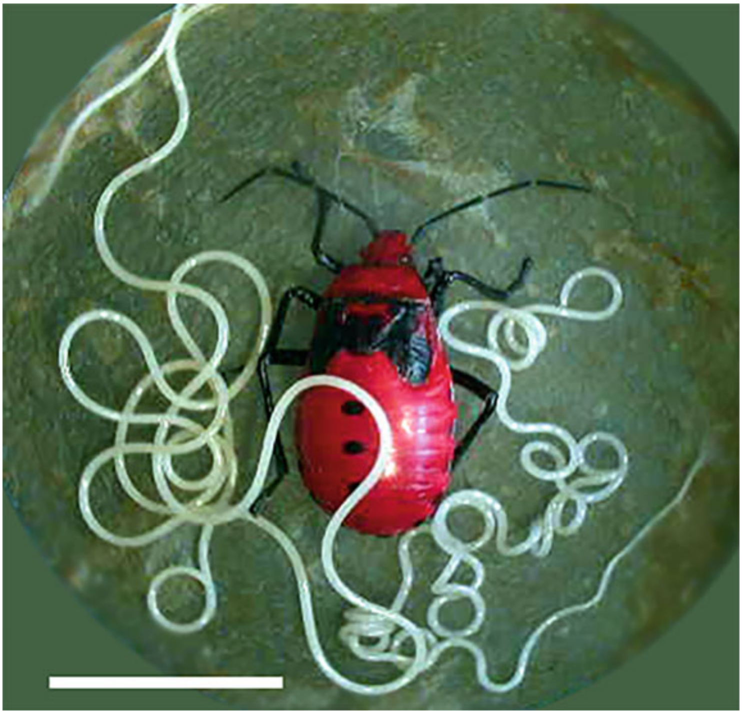


Fig. 7.1 Last instar nymph of *P. japonensis* and two emerged mermithid nematodes. White bar indicates 1 cm. Adapted from Iryu et al. (2020)

7.2.2 Synchronization of Life Cycle

Interestingly, emergence of mermithids is predominantly limited to the last nymphal stage of the host and coincides with the time just prior to eclosion of adult of *P. japonensis*. Because *P. japonensis* adults spend much of their life history aggregating on leaves of broadleaf evergreen trees and plants, there are very limited opportunities for soil-dwelling nematodes to contact the host insect. One such opportunity arises during development of *P. japonensis* nymphs while feeding on drupes of *Schoepfia jasminodora* scattered on the ground. Apparently mermithids infect the bug during the early nymphal stages, and thus their emergence seems to be synchronized with that of the nymphs. According to the life cycles of known mermithid species and *P. japonensis*, the life cycle of mermithids associating with *P. japonensis* in Saga is as follows. Postparasitic juveniles emerge from *P. japonensis* nymphs in late July to early August. They overwinter in the soil and mature and mate in the spring. Mated females lay eggs and infective second stage juveniles hatch in June. The infective juveniles then seek hosts and encounter *P. japonensis* nymphs, the most abundant insect that infective juveniles will face

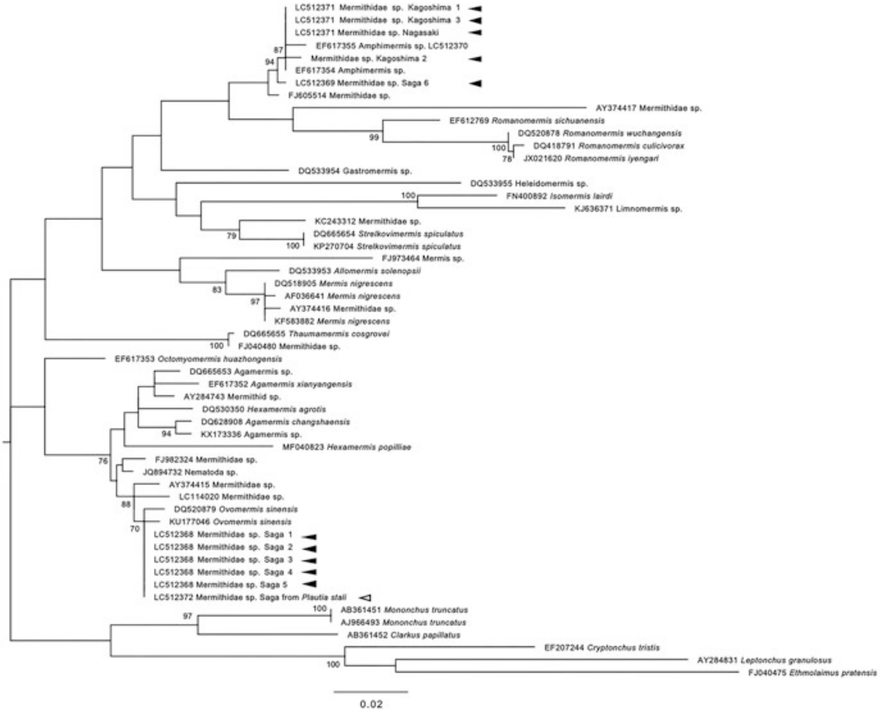


Fig. 7.2 Maximum-likelihood tree based on partial sequences of the 18SrRNA. The bootstrap support values are indicated at the node. Black arrowheads indicate mermithids from *P. japonensis*. Adapted from Iryu et al. (2020)

in the area at the time. The juveniles infect and grow in the nymphs for several months, and well-fed juveniles emerge from nymphs.

Mermithids usually have relatively broad host ranges and can infect different types of insects. However, once mermithids start utilizing *P. japonensis* as a host, because it is a sedentary insect that reproduces only over the same limited area where the drupes are available every year, their life cycle could be synchronized with that of *P. japonensis*. We still are not sure of the specificity of the mermithids with *P. japonensis*, but the specificity of the mermithids to *P. japonensis* will increase with repeated infection cycles, ultimately resulting in speciation of the mermithids. The host-parasite interaction will also drive physiological and behavioral adaptations of both *P. japonensis* and the mermithids.

7.2.3 Impact of Mermithid Parasitism on P. japonensis

Insect parasitism by mermithids often causes deformation, sterilization, and behavioral changes of host insects (Kaiser 1991; Vance 1996; Choo et al. 1995). However, in the case of *P. japonensis*, no apparent morphological or behavioral change is observed in the nymphs before mermithids emerge from their host. In fact, infected nymphs cannot be distinguished from noninfected nymphs before mermithids emerge from their host. However, immediately after emergence, the host nymph dies. It has been suspected that the cause of host death by mermithid emergence is loss of hemolymph, but in the case of *P. japonensis*, there is no apparent loss of hemolymph after nematode emergence, and the death is caused by other factors.

Because mermithid emergence from a host results in the death of the host insect, infection by mermithids is lethal for the host and one of the main factors in host mortality. High infection rate may result in a decrease of the host population. As mentioned, *P. japonensis* reproduces in a limited area where drupes of its host tree are available every year and nymphs feed on drupes on the ground, which provides an opportunity for mermithids to infect the host bug. If adults were to remain on the ground until the following reproductive season, they would be a good target for the nematodes. Thus, behavioral and/or physiological adaptations to avoid infection from mermithids would be expected to evolve. Aggregation of *P. japonensis* on the leaves of evergreen trees and changes in the aggregation sites during the course of the year are two of the mysterious behaviors seen in *P. japonensis*. Movement of the aggregations from the soil to the leaves of trees and changing the aggregation sites could represent behavioral adaptations of *P. japonensis* to avoid predators and infection from microbes and mermithids.

7.3 Phoretic/Necromenic Nematode: *Caenorhabditis japonica*

Nematodes move a very limited distance by themselves. However, some nematodes move from one place to another by coopting involuntary aid of larger invertebrates such as insects as a carrier. Such a relationship is called phoresis (Sudhaus 2008). A special type of phoresy in which the nematodes stay on their carrier waiting for the death of hosts and propagate on decaying hosts is called necromeny (Sudhaus and Schulte 1989; Sudhaus 2008). A bacteria-feeding and phoretic/necromenic nematode was isolated from *P. japonensis* and named *Caenorhabditis japonica* (Kiontke et al. 2002). Since the discovery of *C. japonica*, research has revealed an intimate association between this nematode and *P. japonensis*. *C. japonica* uses *P. japonensis* as a carrier, and the nematode propagates using the bug’s cadaver, which suggests phoretic and necromenic association with *P. japonensis*. Here, we describe the life history of *C. japonica* in association with *P. japonensis* along with behavioral and physiological characteristics found in *C. japonica* dauer larvae.

7.3.1 *The Genus* Caenorhabditis *and Phylogenetic Relationship*

Caenorhabditis is the nematode genus in the order Rhabditida in a chromadorian lineage to which the famous model organism *Caenorhabditis elegans* belongs. *C. elegans* was the first multicellular organism whose whole genome sequences were reported in 1998. It is the only multicellular animal whose cell lineage and neural network have been clarified. Since Sydney Brenner started using *C. elegans* as a model organism for developmental biology in 1974, researchers using *C. elegans* as a model organism have been gradually increasing, and *C. elegans* is studied in over a thousand laboratories worldwide. In spite of its status as an advanced experimental model system, the natural life history of *C. elegans* has not been well elucidated because of the difficulty of finding the nematode in the field. Extensive surveys of *C. elegans* and *Caenorhabditis* species have been conducted, leading to the discovery of new *C. elegans* strains and other *Caenorhabditis* species. Currently 72 *Caenorhabditis* species have been identified, and dozens of new species will be added over the next few years. There are several groups in the genus *Caenorhabditis*. The "Japonica group" to which *C. japonica* belongs forms a sister clade with the "Elegans group," and together they form the "Elegans supergroup" (Kiontke et al. 2011).

Caenorhabditis species are sometimes called "Fruit worm," likened to fruit flies, *Drosophila*, which are often found in rotten fruits in the fields, because *Caenorhabditis* species are often found in decaying fruits and plant materials. Interestingly, many of the *Caenorhabditis* nematodes associate with invertebrates (Kiontke and Sudhaus 2006). For example, *C. elegans* has a phoretic association with isopods, snails, and some other invertebrates (Frézal and Félix 2015). Usually, phoretic nematodes are generalists who use different invertebrates as a carrier with low host specificity. Specialists are confined to a few species such as *C. japonica* associated with *P. japonensis* and *C. drosophilae* that use *Drosophila* flies in the Arizona desert (Kiontke 1997).

Because of its microscopic size and the difficulty in re-isolation, research on the natural life history and ecology of nematode species including *Caenorhabditis* species is often challenging. Taking advantage of the specific association of *C. japonica* with *P. japonensis*, study on the natural history of *C. japonica* was undertaken and, as a result, *C. japonica* has become one of the few *Caenorhabditis* nematodes whose natural habitat, distribution, and life history have been well studied.

7.3.2 *Distribution*

P. japonensis is distributed from Kyushu, Japan, to China, and the origin of *P. japonensis* is considered to be Yunnan Province, China (Zhu et al. 2013).

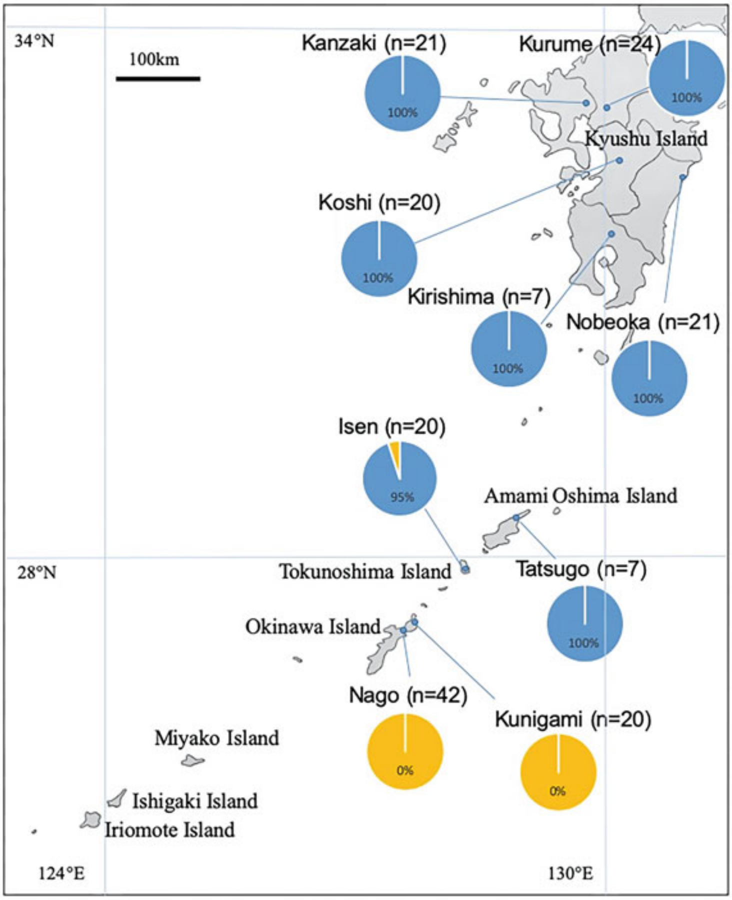


Fig. 7.3 Distribution of *C. japonica*. The percentage of hosts carrying *C. japonica* in each *P. japonensis* population is indicated. n, number of insects examined. No *P. japonensis* was detected from islands located in west of Okinawa Island. Adapted from Yoshiga (2018)

C. japonica has been found on adult females and nymphs of *P. japonensis* from all of the *P. japonensis* populations in Kyushu, Shikoku, Amami, and Tokunoshima Islands examined thus far. Surprisingly, *C. japonica* has not been detected in *P. japonensis* populations in Okinawa Island (Fig. 7.3; Yoshiga 2018). Although the distribution of *P. japonensis* is reported in mainland China, there is currently no information available on *C. japonica* in those areas.

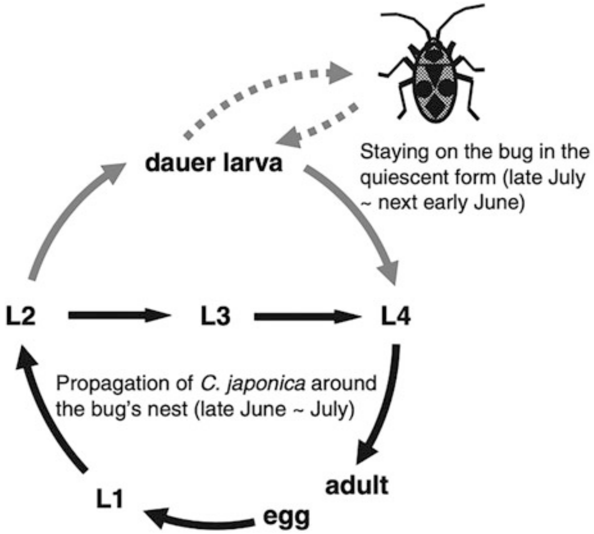
The genetic diversity among populations of *C. japonica* is less than one third that seen in other outbreeding *Caenorhabditis* species. Its specialist host association is considered to contribute to the lower genetic variation (Li et al. 2014). In addition, its limited distribution may also contribute to its low nucleotide variation. There is no information on the origin of *C. japonica*, but the low genetic variation reflects its limited distribution and suggests its origin is in the region between Tokunoshima and Kyushu islands in Japan (Yoshiga 2018).

7.3.3 Life Cycle

Nematodes develop from embryo to adults through four larval stages (L1–L4). Like most other nematode species, *C. japonica* has two sexual forms, male and female, and they mate and reproduce amphimictically (Fig. 7.4). *C. japonica* can be cultured on an agar plate seeded with *Escherichia coli* as other bacterial-feeding nematodes. It can grow in temperatures as low as 3 °C and as high as 30 °C. Generation time of nematodes varies depending on the species and conditions. In the case of *C. japonica*, it takes 3–4 days at 25 °C for one generation, which is similar to other *Caenorhabditis* species. According to the morphology of the mouth and the pharyngeal parts, *C. japonica* appears to be a microbe feeder. We still do not know what *C. japonica* actually feeds on in the wild, but it might feed on microbes such as bacteria and monocellular eukaryotes, along with insect cadavers and organic materials digested by microbes where they propagate.

C. japonica is a colonizer that has a boom-and-bust lifestyle: When food is available, they propagate rapidly. As nematode density increases with food source depletion, young larvae develop to the alternative third larval stage called dauer larva, a developmentally arrested stage that is similar to the larval diapause of insects. Dauer larvae are the non-feeding stage for survival under unfavorable conditions and, thus, the mouth and anus are closed, the intestinal cavity shrinks, and a dauer-specific cuticle that is resistant to chemicals and desiccation is formed.

Fig. 7.4 Life cycle of *C. japonica*. Adapted from Tanaka et al. (2012)



In addition, before entering the dauer stage, nematodes store nutrients such as lipids and glycogen mainly in the intestine and epidermis and use them as a nutrient source during the dauer stage, allowing them to survive without feeding (Wolkow and Hall 2013). When food sources are available under favorable conditions, dauer larvae molt to fourth stage larvae and then become adults. Although dauer larva is the diapause stage of nematodes, it is also the migratory/foraging stage that is absolutely essential for the association with *P. japonensis* and thus they move about actively.

7.3.4 Synchronized Life History with **P. japonensis**

P. japonensis has synchronized its life history to that of the host tree *S. jasminodora*. Likewise, *C. japonica* appears to have synchronized its reproduction period to that of *P. japonensis* (Fig. 7.5). When *P. japonensis* is in adult reproductive diapause on the leaves of evergreen trees, *C. japonica* found on the body surface under the wing (usually between body segments) are in the dauer larval stage (Fig. 7.6). Nematodes are essentially aquatic organisms that live in a film of water in the soil; they stop moving when they are subjected to <100% humidity and gradually die. Because the bug aggregates on the leaves and nematodes on the bug are subjected to light

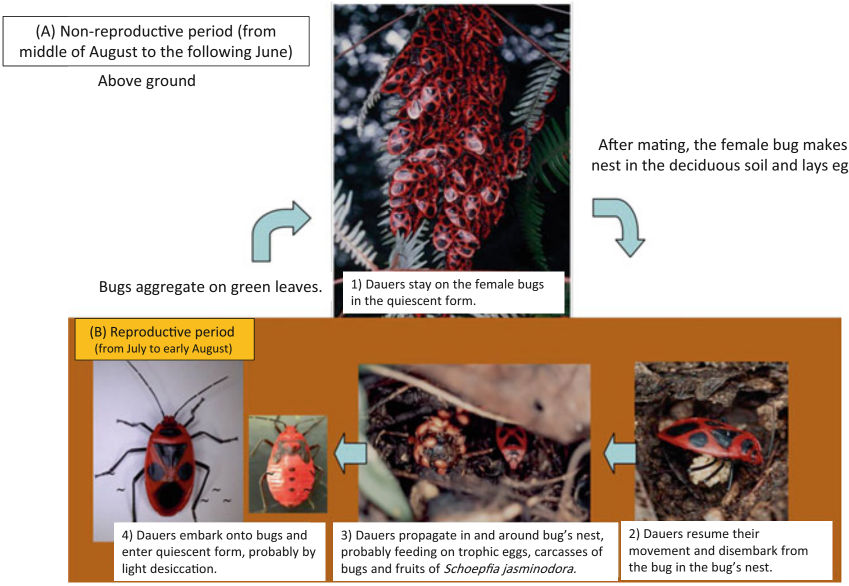


Fig. 7.5 Synchronized life history of *C. japonica* with *P. japonensis*. (**a**) *C. japonica* spends its non-reproductive period at the dauer larval stage on *P. japonensis* adults aggregating on the evergreen leaves. (**b**) *C. japonica* enters reproduction period after mother bugs start laying eggs in the soil. Figure created from data presented in Yoshiga et al. (2013), Okumura et al. (2013a), Okumura and Yoshiga (2014)

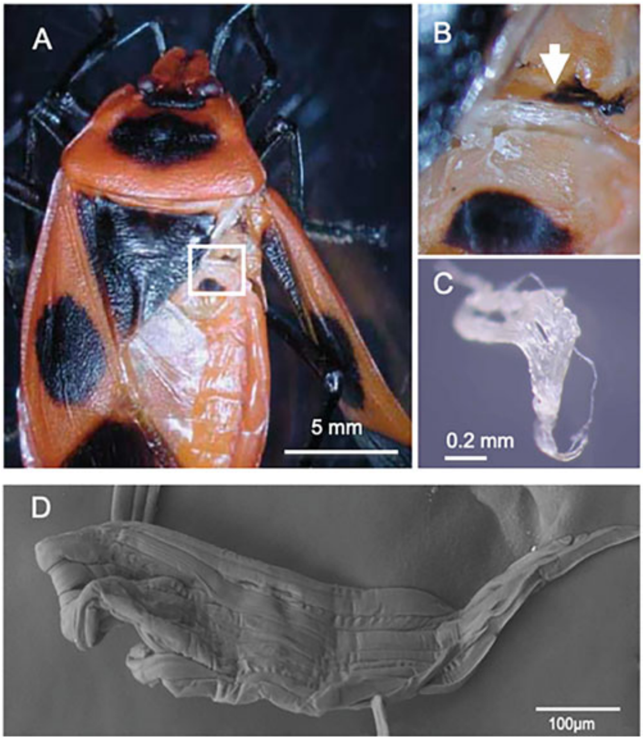


Fig. 7.6 (**a**) The site where dauer larvae were detected on the back of *P. japonensis*. (**b**) An enlargement of boxed area in A. Arrow indicates a clump of quiescent dauer larvae. (**c**) A clump of dauer larvae picked up from the back of a bug. (**d**) Cryo-scanning electron micrograph of a clump of dauer larvae. (Adapted from Yoshiga et al. 2013; Tanaka et al. 2010)

desiccation, the mass of nematodes on the bug is partly desiccated and looks like a small piece of abiotic fibers (Fig. 7.6c, d).

Resuming mobility is possible only under conditions of 100% humidity, which is achieved only when the host bug goes into the leaf litter or soil to make a nest where it lays its eggs during the rainy season from late May through June. The high humidity in the nest animates *C. japonica* dauer larvae on the bug, and they resume their mobility. Around the time when *P. japonensis* nymphs have hatched, dauer larvae leave the mother bug and propagate in the bug's nest. Because the number of nematodes on the mother bug decreases suddenly, it is likely that departing *C. japonica* utilize a cue that is related to nymphal hatching, such as odors from the hatched nymphs, fluid in the eggs, and/or symbiotic bacteria of *P. japonensis*.

After hatching, nymphs feed on trophic eggs (nutritive eggs) in their egg mass. They also feed on nymphs that failed to hatch and those that delayed hatching. The nutrients in the trophic eggs and nymphal cadavers in the nest could be the initial food source for *C. japonica* after leaving the mother bug. After the eggs hatch, mother bugs bring drupes of *S. jasminodora* to their nymphs in the nest; nymphs feed on the endosperm of the drupes. *C. japonica* can be found on drupes in the nest, which indicates that *C. japonica* propagates by feeding on the microbes and juice of drupes in the nest. Because nymphs and nematodes utilize endosperm and fruit, respectively, there seems to be no competition for food source between them. After the mother provisions the nest for about 10 days, or after she dies, nymphs scatter and feed independently on drupes on the ground. During this period, *C. japonica* can be found on the fruit of *S. jasminodora* laying on the ground outside of the nest. *C. japonica* is not detected on fruit where *P. japonensis* nymphs are absent. Since active *C. japonica* dauer larvae can usually be found on the nymphs, and the nematodes are found on drupes where *P. japonensis* nymphs are present, *C. japonica* appears to use the nymphs for transfer to the fruits, and newly reproduced dauer larvae on the fruits board the nymphs.

Well-fed *P. japonensis* nymphs aggregate on the leaves or trunks of nearby trees, and a nymph just before adult eclosion disperses and prepares for eclosion on a leaf or trunk in isolation. Immediately after the nymphal cuticle begins to shed, dauer larvae hiding under the wing pad of a fifth instar nymph come out and attempt to migrate toward a newly emerging adult. The dauer larvae that successfully board a teneral adult crawl under the wing, but the unsuccessful ones remain on the shed cuticle. This boarding behavior of *C. japonica* can be observed on both male and female *P. japonensis*. After completion of eclosion and hardening of the exoskeleton, newly emerged bugs aggregate once again on the trunks and leaves of nearby trees. Dauer larvae on the bugs are subjected to light desiccation even when they are under the host's wing because the humidity at the aggregation site is considerably lower than the 100% humidity required; therefore, they become quiescent. Both bugs and nematodes wait for the next reproductive season.

C. japonica can be found on *P. japonensis* throughout the year but cannot be isolated from soil or drupes after the reproductive period of the bug even at sites where nests of *P. japonensis* and drupes of *S. jasminodora* had been present. In the soil, a *C. japonica* dauer larva has limited longevity because it is physiologically adapted to associate with *P. japonensis* (described below). Thus, *C. japonica* cannot survive in soil and can only survive on *P. japonensis*.

7.3.5 Species Specificity and Adult Female **P. japonensis**-*Biased Association of* **C. japonica**

Several other invertebrates, such as coleopteran and other hemipteran insects and isopods, occur at sites inhabited by *P. japonensis*. However, *C. japonica* has never been detected on any invertebrates other than *P. japonensis* (Yoshiga et al. 2013). *C. japonica* can be found on the drupes of *S. jasminodora* where *P. japonensis* nymphs are present, but they are not found on drupes where *P. japonensis* does not occur. In addition, *C. japonica* can be found in every *P. japonensis* population in Kyushu, Shikoku, and Amami Islands, and almost 100% of adult females are

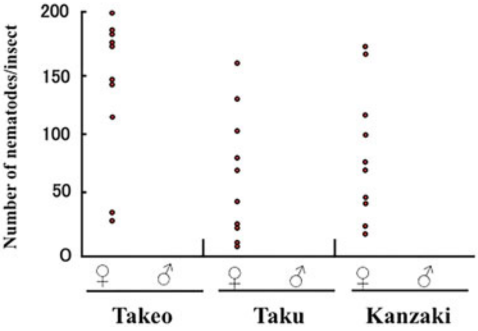


Fig. 7.7 Female-biased association of *C. japonica* at three different *P. japonensis* populations in April. *C. japonica* were found on the *P. japonensis* females but not on the males. Each dot indicates the number of dauer larvae per insect ($n = 10$). Adapted from Yoshiga et al. (2013)

associated with *C. japonica*, but they are very rarely found on adult males. These data suggest that *C. japonica* associates with *P. japonensis* in a species-specific manner and the association is *P. japonensis* female-biased (Fig. 7.7).

Female host-biased or female host-specific association is not commonly found in nematodes but has been reported in some species, for example, *Fergusobia* nematode and *Fergusonia* fly mutualism (Currie 1937; Davies et al. 2001) and *Sphaerularia* nematode/*Bombus* bee or *Vespa* hornet parasitism (for reviews, see Bedding 1984; Sayama et al. 2007). The mechanisms involved in the specific or biased association in these associations are not yet well understood. In addition, these nematodes have different life histories and are phylogenetically quite different from *C. japonica*. In the genus *Caenorhabditis*, such biased association has not been reported. Thus, the female-biased association in *C. japonica* appears to have evolved independently during speciation and association with *P. japonensis*.

Monitoring of the field fluctuation of nematode populations on males and females of *P. japonensis* has been conducted throughout the year in Saga (Fig. 7.8). In spring when *P. japonensis* enters the reproductive period, *C. japonica* dauer larvae are detected almost exclusively on females and rarely on males that enter reproduction. After the bugs mate, nematodes are still detected on gravid and egg-guarding female bugs. However, nematodes leave mother bugs after the eggs hatch. Nematodes are then detected on nymphs. Soon after adult eclosion in late July, usually less than ten dauer larvae individuals are observed on both male and female bugs. However, there are up to several hundred more dauer larvae on female bugs than on male bugs, and the number on male bugs decreases to zero by the following spring in the field. Nematodes are always found on females that remain in the aggregations in a state of reproductive diapause during the reproductive period and throughout the year but are rarely found on males. *C. japonica* can be found on every *P. japonensis* nymph, and there seems to be no preference by *C. japonica* between the sexes of the carrier bug until adult eclosion, but the association is biased to female bugs thereafter.

It is ecologically reasonable for *C. japonica* to associate only with females because nematodes on females can reach the food source after they mate and lay their eggs in the nest under the leaf litter or in the soil, while those on males cannot as male bugs die soon after mating and are not near drupes. We still do not know the mechanism involved in the changes of nematode number on the sexes of the bug.

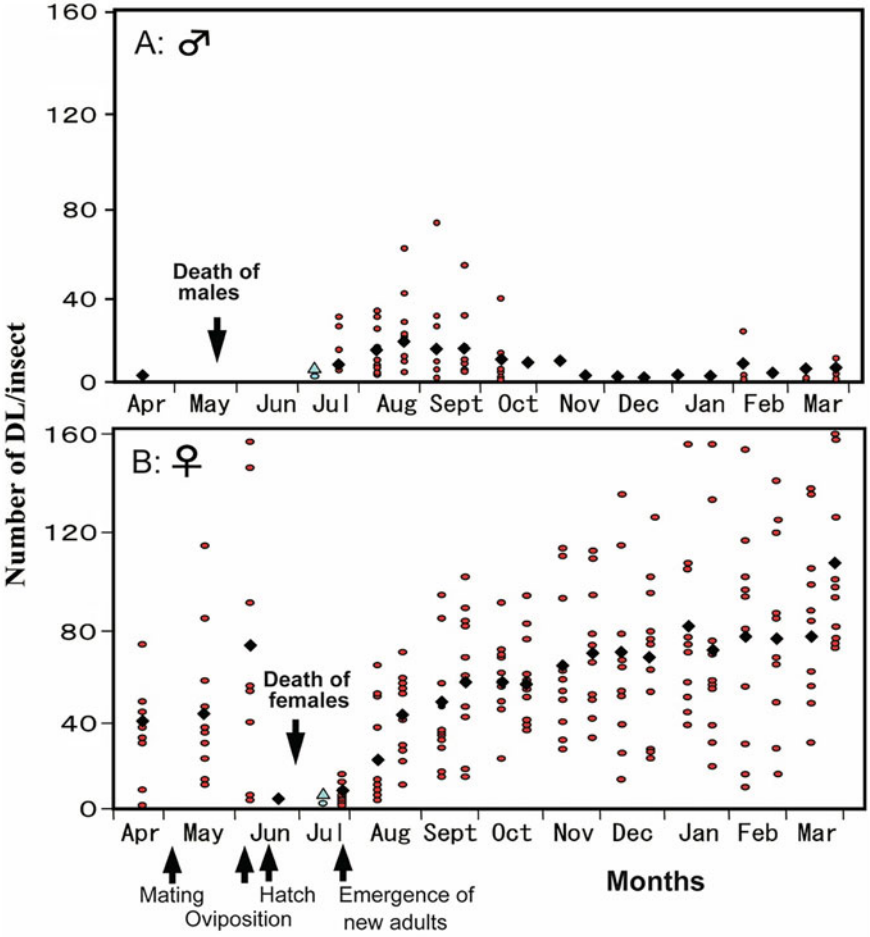


Fig. 7.8 Seasonal fluctuation of dauer larvae on *P. japonensis*. Each dot indicates the number of dauer larvae on a bug. Each rhombus indicates the mean number of nematodes on the insects on each sampling date ($n = 10$). Blue triangles indicate the mean nematode numbers on a fifth instar nymph ($n = 10$). (Adapted from Yoshiga et al. 2013)

Movement of nematodes from male bugs to female bugs appears to be impossible while in the aggregations on trees because of their extreme vulnerability to desiccation, and thus they are immobile. It is impossible for nematodes to propagate on the bug in the leaves since there are no food sources available. One possibility is that *C. japonica* may propagate during the bug's summer estivation and winter hibernation in the soil. In soil, where 100% humidity can be achieved, nematodes can resume their activity. It is probable that nematodes are animated and propagate on dead host insects, and the newly produced dauer larvae board live adults during their estivation/hibernation in the soil. Attraction of *C. japonica* dauer larvae to male and female adults of *P. japonensis* is the same in laboratory experiments (described below in details), suggesting that the female-biased association is not caused by a difference in attractiveness, but that it could be related to behavioral and/or physical differences between the sexes. Males are smaller than females and can thus be subjected to more severe desiccation; this would lead to greater death of the nematodes on males compared to those on the larger females which provide nematodes with higher humidity. Another possibility is the grooming behavior that is frequently seen in *P. japonensis* males, which fly about the aggregations during mating, while females remain sedentary. Males frequently groom in between flights, opening their wings and rubbing their body parts such as antennae, legs, and dorsal body with their hind legs. This grooming might remove the associated nematodes.

7.3.6 Characteristic Behavior of **C. japonica** *Dauer Larva*

As described above, association with *P. japonensis* is necessary for *C. japonica* to continue their life history. *C. japonica* appears to have developed characteristic behaviors of seeking and boarding their carrier and the ability to efficiently recognize their carrier insect to ensure the association. During the study of *C. japonica*, several interesting behaviors have been observed in the dauer larvae of *C. japonica*.

Nictation, Waving, and Jumping

One of the interesting behaviors observed in *C. japonica* dauer larvae is nictation, waving, and jumping. Nictation is a sit-and-wait behavior and is associated with host-finding. It is often observed in the dauer stages of phoretic nematodes as well as parasitic stages of entomopathogenic nematodes and vertebrate-parasitic nematodes. Nematodes lift most of the body in the air and are supported by only the tip of their tail (Reed and Wallace 1965). By reducing the surface tension forces holding the nematode to the substrate, nictation can increase the nematodes' ability to attach to passing insects. Nictating nematodes sometimes wave their body (waving). Waving nematodes are considered to be sensing the odor from potential hosts, and these behaviors facilitate encountering their host or carrier. In *C. japonica*, newly emerged dauer larvae crawl up substrates, nictate, and wave (Fig. 7.9). These behaviors will help them find and attach to *P. japonensis* passing by. In entomopathogenic nematodes, only those species that forage on the soil surface are likely to evolve the use of nictation behavior. In phoretic nematodes, on the other hand, nictation is commonly observed. This indicates that nictation is one of the important behaviors conserved in phoretic nematodes and these phoretic nematodes including *C. japonica* forage on the soil surface.

In *C. japonica*, nictation continues seconds to minutes or longer. During nictation, *C. japonica* dauer larvae often jump into the air. The jumping suddenly occurs during nictation and waving, and the nematodes disappear out of sight in the blink of an eye. This jumping behavior in nematodes has only been seen in the

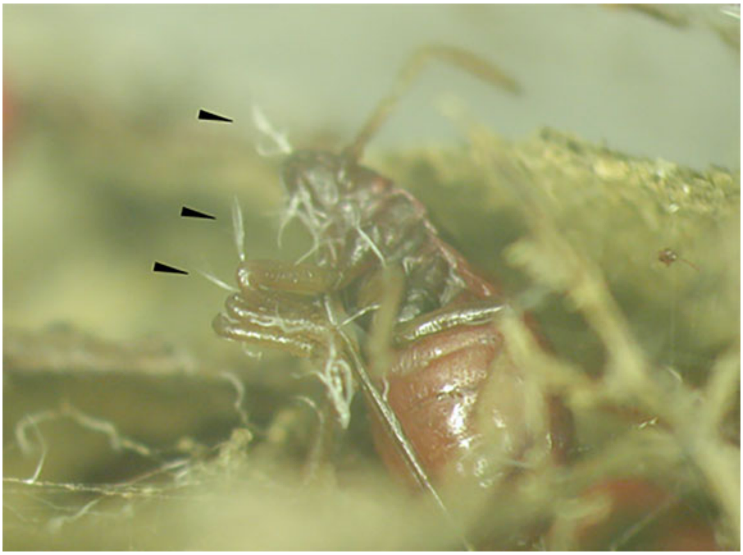


Fig. 7.9 Nictation of *C. japonica* dauer larvae on the cadaver of a *P. japonensis* nymph. Arrowhead indicates nictating nematodes

infective or phoretic stages of species associated with insects (Campbell and Kaya 1999). It is considered to facilitate attachment to passing insects on the ground. In the entomopathogenic nematode *Steinernema carpocapsae*, CO_2 and odors emitted by insects stimulate jumping (Hallem et al. 2011). *S. carpocapsae* infective juveniles are able to change the direction of jumping in response to information from the environment and tend to jump toward the source of the cues (Campbell and Kaya 1999). There is no detailed information on the condition that evokes and stimulates *C. japonica* jumping, but it is probable that jumping of *C. japonica* dauer larvae is also stimulated by the presence of *P. japonensis*.

Negative Gravitaxis

Another characteristic behavior demonstrated in *C. japonica* dauer larvae is negative gravitaxis (Okumura et al. 2013b) (Fig. 7.10). *C. japonica* dauer larvae need to contact *P. japonensis*, which move over the surface of leaf litter, and newly reproduced *C. japonica* dauer larvae have a tendency to move upward, which may help them to contact their carrier bug. Laboratory experiments demonstrate that *C. japonica* dauer larvae show negative gravitaxis behavior. When nictating dauer larvae are inoculated at the center of an agar plate and the plate is placed horizontally, the inoculated nematodes move upward, while non-nictating dauer larvae do not. The upward movement is observed in the absence of a host insect. The percentage showing this upward movement changes with the age of the dauer

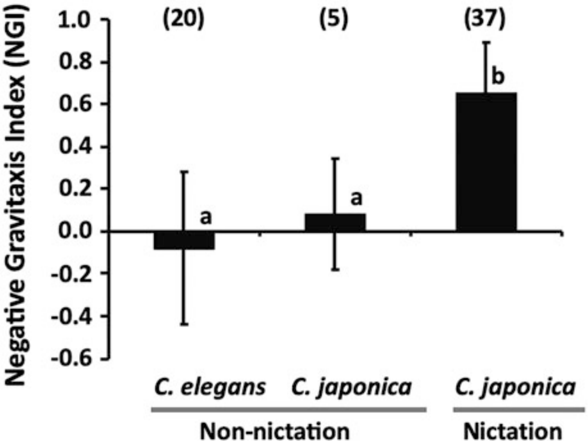


Fig. 7.10 Comparison of negative gravitaxis between *C. japonica* and *C. elegans* dauer larvae. Approximately 20 dauer larvae were inoculated onto a 9 cm NGM plate, the plate was set vertically, and the number of nematodes that moved upward and downward from the inoculation point were counted 1 h after inoculation. Error bars indicate ± S.D. Different letters above the columns indicate a statistically significant difference detected by ANOVA with the Bonferroni/Dunn test ($P < 0.0001$). Adapted from Okumura et al. (2013b)

larva: a higher percentage of nictating dauer larvae collected from young nematode cultures show negative gravitaxis compared to those from older cultures. Thus, it might be that only newly reproduced dauer larvae with enough energy are able to perform such behavior. The negative gravitaxis may facilitate reaching the surface of the leaf litter and soil particles after dauer larvae are reproduced, followed by nictation and waving. The mechanism involved in the negative gravitaxis is unknown, but the fact that only the dauer larvae showing nictation significantly move upward indicates that the behavior is associated with nictation. In other words, the negative gravitactic nematodes may show nictation. It is possible that neurons that regulate nictation may also regulate negative gravitaxis. There is no information about where and how gravity is perceived in nematodes. Mechanoreceptors play an important role in detection and response to gravity in many animals including *Drosophila* (Barbercheck and Duncan 2004).

Gravity is a constant stimulus on Earth, and many organisms are able to perceive and respond to it. In nematodes, positive and negative gravitactic behavior has been reported in many species, but further experiments concluded the behavior to be either unbiased (random) or passively biased by dragging a heavy tail (Croll 1970). The discovery of negative gravitaxis in *C. japonica* represents the first time it has been clearly demonstrated in nematodes. Because *C. japonica* has negative gravitaxis and some other nematodes apparently show positive and negative gravitactic behavior, it is probable that other nematodes also sense gravity and use it in their life history.

Odor Recognition and Host Orientation

The first important step for phoresy is host orientation. To associate with *P. japonensis* efficiently, *C. japonica* has to sense the presence of *P. japonensis*, move toward it, and recognize it. In addition, there are many other invertebrates, such as arthropods, including insects, spiders, centipedes, millipedes, and isopods, and mollusks including slugs and snails in the same habitat utilized by *C. japonica*. *C. japonica* dauer larvae have to distinguish *P. japonensis* from other animals. Although some nematodes have photoreceptors that perceive light and respond to it, nematode eyes or ocelli are too primitive to see as we humans do. *C. japonica* does not even have apparent eyes. In the case of *C. japonica*, the most probable medium for seeking a host is chemical cues because nematodes have a sensitive chemoreception system.

Several steps are required for *C. japonica* dauer larvae to associate with *P. japonensis*: perception of the presence of *P. japonensis*; orientation to, contact with, and recognition of host; boarding the host; and staying on the host. Before *C. japonica* can orient toward *P. japonensis*, it first needs to recognize its presence and then decide the direction to move. Volatiles travel in the air very quickly and thus are useful as a signal from a distant place. Because *C. japonica* dauer larvae seek *P. japonensis* that move on the surface of the leaf litter, the nematode apparently uses volatiles for their orientation to *P. japonensis*. A Y-tube olfactometer in which volatiles pass through one arm of the Y-tube and clean air passes through the other and test animals are allowed to choose the preferred odors is commonly used to assess insect olfactory response. A modified Y-tube olfactory assay demonstrates that *C. japonica* dauer larvae are attracted to the direction of odors from *P. japonensis* but not to that from other insects (Okumura and Yoshiga 2014) (Fig. 7.11). These findings indicate that *C. japonica* dauer larvae use volatile chemical cues for host orientation and can distinguish the odors from their carrier insect from others.

The next step is recognition of the host insect after contact. In laboratory experiments, when *C. japonica* dauer larvae and different species of shield bugs or an isopod are co-incubated in a 6 cm Petri dish, *C. japonica* boards *P. japonensis* but not other invertebrates (Okumura et al. 2013c). However, there was no significant difference between the number of nematodes that boarded male and female *P. japonensis*. These results suggest that, while *C. japonica* dauer larvae are able to distinguish *P. japonensis* from other insects, they cannot distinguish the sex of *P. japonensis*.

C. japonica dauer larvae on *P. japonensis* are usually found under the wing pad of fifth instar nymphs. In addition, the dauer larvae that move onto *P. japonensis* during eclosion usually move under the wing. These observations suggest that dauer larvae are attracted to and board *P. japonensis* and then move to the dorsal thorax. Hexane extracts of *P. japonensis* attract *C. japonica* dauer larvae, and the larvae remain in the chemical after attraction (Okumura et al. 2013c). These studies suggest that *C. japonica* dauer larvae can recognize the odor from *P. japonensis* and orient

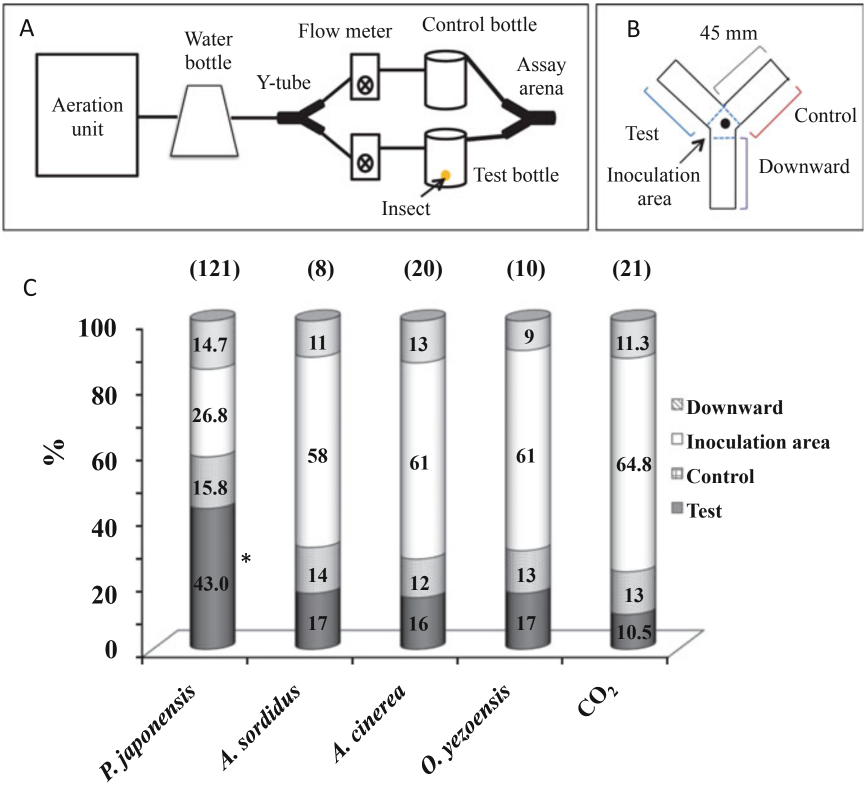


Fig. 7.11 Olfactory assay using *Caenorhabditis japonica* dauer larvae. (**a**) Olfactometer. The olfactometer consists of an air pump, a water bottle containing deionized water, a Y-shaped glass tube (inner diameter, 12 mm), two flow meters, two bottles, and an assay arena (Y-shaped glass tube). Air flow rate was adjusted to 0.4 L/min by using a flow meter. Approximately 30–100 dauer larvae were inoculated at the center of the inoculation area, and the assay arena was set vertically (**b**). (**b**) Enlargement of the assay arena. Approximately 10 min after the start of the experiment, the number of nematodes in the test, control, inoculation, and downward areas was determined. The small circle in the inoculation area indicates the nematode inoculation point. (**c**) Olfactory response of *C. japonica* dauer larvae to four insect species and CO_2. A significantly higher number of nematodes moved toward volatiles emitted by *P. japonensis*. In contrast, more than half of the inoculated dauer larvae stayed within the inoculation area when other insects or CO_2 was used, and there was no attraction to other insects or CO_2. Data were analyzed using the generalized linear model (GLM). *$P < 0.001$. Adapted from Okumura and Yoshiga (2014)

toward their carrier. Once the contact with their carrier is made, they crawl up and remain on the carrier using chemicals from the insect. The chemicals that attract *C. japonica* have not been identified, as yet. However, the information indicates the presence of a species-specific kairomone that is directly released by the host insect, and *C. japonica* dauer larvae appear to use it for host orientation, recognition, and association. Allelochemicals elicit a physiological or behavioral response between different species (Huettel 1986; Riga 2004), and kairomones are allelochemicals that elicit a positive response from the receiving organism. The specialized trophic ecology of *P. japonensis* association with *S. jasminodora* may have helped to develop a specific kairomone for *C. japonica*. The identification of a species-specific kairomone or a specific blend of chemicals in *P. japonensis* may be a key factor for clarifying the association between *C. japonica* and *P. japonensis*.

Host seeking is one of the most critical steps for parasitic and phoretic nematodes. Nematodes perceive and respond to a wide variety of stimuli including chemical and mechanical stimuli, temperature, and light. They also respond to magnetic fields and electric currents (Lee 2002). Some nematodes such as adult females of a mermithid species, *Mermis nigrescens*, that parasitize locusts have reddish pigments and show apparent positive phototaxis. The positive phototaxis is suspected to allow them to climb vegetation to lay their eggs after rain showers to then be fed on by locusts. However, most nematodes that inhabit soil have not developed organs that receive light signals, and so they cannot use visual information. Skin-penetrating infective larvae of some vertebrate-parasitic nematodes of endothermic hosts are positively thermotactic. This will improve the probability that the nematodes will make contact with and enter a suitable host (Lee 2002). However, nematodes that parasitize or form phoretic associations with insects, which are ectotherms, cannot use thermotaxis for host seeking. A potential host-finding stimulus that might be used instead by *C. japonica*, in addition to the chemical cues already discussed, could be vibration, if *P. japonensis* produces vibration. *S. carpocapsae* is reported to utilize vibration of its host to orientate (Torr et al. 2004).

Behavioral Changes After Association with *P. japonensis*

For parasitic nematodes such as entomopathogenic nematodes, the host is the destination for the journey of infective juveniles, after which they start growing and propagate in the host. However, boarding *P. japonensis* is not the end of the journey for *C. japonica* dauer larvae because it is a temporary place to stay and the propagation site is essentially different. Dauer larvae on the nymphs have to leave the nymphs at an appropriate place with appropriate conditions in order to propagate and enter their growth stages, or they have to move to newly ecdysed nymphs or adults during molting or eclosion. Dauer larvae on adults have to remain on adult females until they lay eggs in a nest under the leaf litter, but they also have to leave their carrier at the appropriate time. The process appears to be very complex, but dauer larvae ultimately have to make the right decisions at the different stages and conditions in order to successfully continue their life cycle using a variety of cues depending on the conditions.

To find carrier bugs and board them, behaviors such as negative gravitaxis, nictating, waving, and jumping are useful, but these behaviors do not help them leave their carrier. *C. japonica* dauer larvae have to do the opposite behaviors to get off their carrier. In addition, chemicals including volatiles that are used for host seeking and recognition can be obstacles when it is time to leave the carrier to propagate. They have to now show the opposite response to the attractant. The cues necessary for the behavioral changes are not well understood, but physiological changes of the nematode after boarding *P. japonensis* will be involved in this along with environmental conditions such as chemical cues and humidity.

A *C. elegans* adult hermaphrodite consists of only approximately 1000 cells, which include 302 neural cells (Corsi et al. 2015). Dauer larva is the alternative third stage and appears to have fewer cells. *C. japonica* is a relatively close species to *C. elegans*, and the cell numbers and organization of the two species appear to be similar. It is amazing that even this kind of small and simple animal with just a small number of neurons can recognize and respond to a complex set of conditions and make the appropriate decision at each stage in order to complete their life history successfully.

7.3.7 Characteristic Physiology of C. japonica *Dauer Larvae*

Nematodes often face unfavorable environmental conditions such as a lack of food and desiccation. Unlike insects that can fly to a favorable place to ensure survival, nematodes are less mobile and cannot relocate by themselves. To survive such unfavorable conditions, nematodes have developed several strategies depending on the group of nematodes and species. Dauer larva is the stage for surviving under unfavorable conditions in rhabditid nematodes, and they can usually survive for several months without feeding. The dauer stage is homologous to the infective third juvenile stage of entomopathogenic nematodes such as *Steinernema* and *Heterorhabditis* and animal parasitic nematodes such as *Strongyloides*. During this study on *C. japonica*, some interesting physiological characters of dauer larvae have been clarified.

7.3.8 Longevity of Dauer Larvae

Dauer larva is the surviving stage nematodes pass through until favorable conditions for propagation appear. For example, *C. elegans* dauer larvae can survive for several months without feeding by lowering their metabolism and using stored nutrients such as lipids and glycogen in the intestine and epidermis. Similarly, dauer larvae of other phoretic nematodes and infective juveniles of entomopathogenic nematodes and parasitic nematodes also survive for a long period without feeding. However, the longevity of *C. japonica* dauer larvae that do not associate with *P. japonensis* is surprisingly short compared to *C. elegans* dauer larvae and these infective juveniles (Tanaka et al. 2012). When *C. japonica* and *C. elegans* dauer larvae are placed in buffer solution or soil at 25 °C, survival of *C. japonica* dauer larvae decreases dramatically after a week, and all of them die by day 20, while that of *C. elegans* remains high even after 10 days (Fig. 7.12). Survival of *C. japonica* dauer larvae in soil is also low. Live nematodes cannot be recovered from soil 10 days after addition

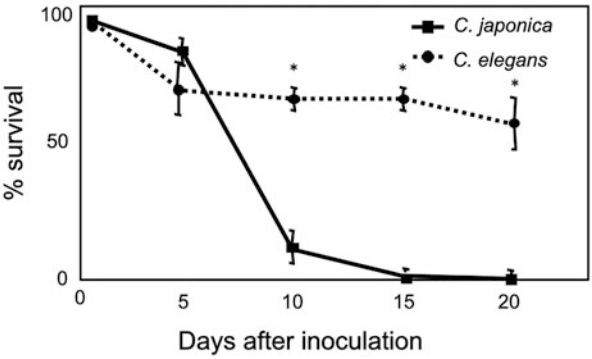


Fig. 7.12 Changes of survival in buffer. *C. japonica* or *C. elegans* dauer larvae were placed in 10 mL M9 buffer in a 9 cm Petri dish at a density of 100 dauer larvae/mL, and survival rates were examined at 25 °C. *Significance of $P < 0.05$, determined by the Kaplan-Meier test. Adapted from Tanaka et al. (2012)

of *C. japonica* to the soil. These results coincide with the findings that *C. japonica* is rarely detected from the soil where *P. japonensis* propagates in the field and are indicative of the short longevity of *C. japonica* dauer larvae that do not associate with *P. japonensis*.

Why is the longevity of *C. japonica* dauer larvae that are not associated with *P. japonensis* so short compared to other dauer larvae? Dauer larvae of *C. japonica* are very active as they migrate to *P. japonensis* and show behaviors such as negative gravitaxis, nictating, waving, and jumping. These aggressive, carrier-seeking behaviors are usually accompanied with consumption of energy and oxygen. One possibility is that the active host-seeking behavior depletes their energy reservoir, which results in their short longevity. However, triacylglycerol, which is the main energy reservoir for dauer larvae, is still present when the dauer larvae die (Tanaka et al. 2012). The other possibility is that their active movement results in the production of excess amounts of reactive oxygen species (ROS) which damage nematode cells. When the oxidizing stress tolerance of dauer larvae is compared between *C. japonica* and *C. elegans* exposed to paraquat and hydrogen peroxide solution, survival rate of *C. japonica* is significantly lower than that of *C. elegans* (Tanaka et al. 2012). Protein carbonyl accumulation that is related to aging can serve as an indicator of oxidative damage of *C. elegans*. The carbonyl concentration is significantly higher in *C. japonica* than in *C. elegans* initially, and it increases significantly with each passing day. These data indicate that the low oxidizing stress tolerance of *C. japonica* dauer larvae compared to *C. elegans* and the increase in production of reactive oxygen species during the active carrier-seeking behavior seem to be the main causes of the reduced survivorship during the dauer stage rather than the shortage of energy (Tanaka et al. 2012).

We still do not know the reason why the stress tolerance of *C. japonica* is low. Dauer larvae in other species that inhabit soil usually do not enter the quiescence that *C. japonica* dauers do and have to survive until food is available under favorable conditions. Nematodes with less mobility, low energy consumption, and long longevity might have been selected for, and oxidative stress tolerance, which is important for long survival, might have increased. On the other hand, the *C. japonica* dauer larvae that show active movement might have more opportunities to board

P. japonensis and survive. After boarding *P. japonensis*, *C. japonica* dauer larvae enter a quiescent state by being exposed to light desiccation on adult bugs, and then their animation is suspended (Fig. 7.6). The ametabolic state does not produce ROS, and animated dauer larvae molt to the fourth propagative stage soon after recovering from the dauer stage. Thus, the system to remove ROS in dauer larvae might not have evolved well in *C. japonica*, or it might have been lost during speciation because it was not needed. This appears to be an interesting physiological adaptation of *C. japonica* resulting from the association with *P. japonensis*.

Desiccation Tolerance

Desiccation is one of the major environmental stressors for terrestrial soil nematodes that cannot move by themselves over long distances; they cannot escape from dry conditions. Some soil nematodes have acquired desiccation tolerance, but the tolerance varies depending on the species. Anhydrobiosis is a metabolic state induced by desiccation in which measurable metabolic processes stop. Nematodes that have entered anhydrobiosis can survive under extreme desiccation for long periods. After boarding a *P. japonensis*, *C. japonica* dauer larvae on the bug are usually subjected to desiccation, and they become quiescent. Because the nematodes on *P. japonensis* are usually partly desiccated and survive for months, desiccation tolerance of *C. japonica* dauer larvae seems to be high, and they appear anhydrobiotic. However, *C. japonica* does not, in fact, enter anhydrobiosis. When newly produced and active *C. japonica* dauer larvae are exposed to relatively light desiccation conditions (85% relative humidity), they gradually stop their movement, their survival gradually decreases, and all are dead by day 15 (Tanaka et al. 2010). Although the desiccation tolerance of *C. japonica* dauer larvae is apparently higher than that of *C. elegans* which dies within a few days under the same conditions, the survival is lower than those entering anhydrobiosis. Interestingly, if *C. japonica* dauer larvae are exposed to the same desiccation conditions on *P. japonensis*, survival of the nematodes on the bug remains high for at least several months. *C. japonica* dauer larvae themselves have some desiccation tolerance but not enough to survive desiccation on their own. The survival of the nematode can only be enhanced on *P. japonensis*. In addition, when only nematodes are exposed to desiccation conditions, they often get contaminated with fungi. On the other hand, those with *P. japonensis* do not. This observation suggests the possibility that *C. japonica* is protected not only from desiccation but also from microbe infection while the unanimated dauer larvae are on the bug.

Influence of *C. japonica* on *P. japonensis*

There are several benefits that *C. japonica* derives from associating with *P. japonensis*: *C. japonica* uses *P. japonensis* as a transfer mechanism to the propagation site as well as a shelter from desiccation and microbial infection. On the other hand, we still do not know if there is an advantage or even a neutral effect for *P. japonensis* to be associated with the nematode. At the moment, the advantage is limited to the nematodes, and the relationship between *C. japonica* and *P. japonensis* seems to be commensalism.

Because all the *P. japonensis* populations in Kyushu Island where *P. japonensis* is populated associate with *C. japonica* and there is no *P. japonensis* population without the nematode on the island, there is a possibility that an advantage for *P. japonensis* has been established. A possible advantage for *P. japonensis* could be suppression of microbe infestation caused by nematode scavenging of the *P. japonensis* nest. There are egg shells after hatching and dead nymphs during hatching or ecdysis in the nest. Those could be a source for microbe infestation which may affect *P. japonensis* survival. *C. japonica* is a colonizer that feeds on rapidly growing bacteria and unicellular eukaryotes as well as other nutrients they can ingest. Feeding on the decaying carcasses by nematodes before microbe infestation may suppress microbe infestation in the bug's nest, which could prevent microbe infection in nymphs and help increase nymphal survival.

One of the most well-studied phoretic relationships between a nematode and an insect is the association between the pine wood nematode *Bursaphelenchus xylophilus* that causes pine wilt disease and its vector insects, sawyer beetles (*Monochamus* spp.) (Linit et al. 1983; Kobayashi et al. 1984). In this association, nematodes are transferred to pine woods by their carrier beetles, and transferred nematodes infect from the site bitten by the beetles. Nematodes propagate in the pine wood, which results in pine wilt. Wilted pines can be a favorable host tree for oviposition and growth of the sawyer larvae. Thus, it is essentially a phoretic relationship (commensalism), but there is a mutualistic benefit between them. There is an interesting report that nematode pheromones called ascarosides produced by *B. xylophilus* induce ecdysone production in the beetle and promote beetle pupation. On the other hand, once the beetle develops into the adult stage, it secretes ascarosides that attract the dispersal fourth-stage nematodes of *B. xylophilus* and facilitate transport to the next pine tree (Zhao et al. 2016). Because *C. japonica* and *P. japonensis* have formed an intimate species-specific association, there is a possibility that chemicals such as ascarosides produced by *C. japonica* and/or *P. japonensis* may influence the growth and survival of their partners.

7.4 Conclusion

In this chapter, two types of nematodes that associate with *P. japonensis*, parasitic mermithid nematodes and phoretic/necromenic *C. japonica*, have been described. Mermithid nematodes appear to be one of the important causes of death of *P. japonensis* during the nymphal period. We do not know the host range of the mermithids currently found on *P. japonensis*, but they could be a specialist as they have synchronized their life cycle to that of *P. japonensis* over the years. The other nematode, *C. japonica*, has formed an obligate phoretic/necromenic association with

P. japonensis. The carrier bug functions as a shelter to protect the nematodes from desiccation and microbial infection as well as transport of nematodes to the food resource. This relationship between the nematode and the bug seems to be commensalism thus far, but there is a possibility that the intimate association may have led to benefits for the bug.

Parastrachia currently consists of two species: *P. japonensis* and *P. nagaensis*. *P. japonensis* shows a continuous distribution from the Hengduan region of China to southern Japan, while *P. nagaensis* exhibited sporadic distribution between eastern India and southwestern China (Schaefer and Kikuhara 2007; Zhu et al. 2013). The ecological research on *P. japonensis* has been conducted exclusively in Saga, Japan, and information is limited to that area. No ecological and physiological information on *P. nagaensis* is currently available. In addition, there is no information on the nematodes associating with these bugs in the Eurasian Continent. It will be of interest to investigate the ecology of *P. japonensis* and *P. nagaensis* in the continental populations along with the nematode association with these bugs. Ecological differences along with geographical distribution of *P. japonensis* populations and between the species of *P. japonensis* and *P. nagaensis* may influence the association with nematodes. In contrast, the difference in the associating nematodes may influence the ecology of *P. japonensis* if nematodes have any influences on the bug. Further, it will clarify the origin of *C. japonica* as well as mermithids found in Japan.

P. japonensis has synchronized its life cycle to the host tree *S. jasminodora*. The unique life cycle of *P. japonensis* adapted to *S. jasminodora* has created a special niche which can be used for smaller animals such as nematodes. Different types of organisms have been influenced by the bug, and at least two types of nematodes have adapted to the life cycle of *P. japonensis*. *P. japonensis* has contributed an important role for creating a niche followed by evolution of specialistic nematodes. To adapt to the life history of *P. japonensis*, *C. japonica* dauer larvae have developed special abilities. Conversely, there is a possibility that these nematodes associating with *P. japonensis* might also have influenced the ecology and life history of *P. japonensis*. Specialization of organisms could be a driving force for speciation and coevolution of associating organisms.

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Ryusei Tanaka another energetic and talented student, earned his doctoral degree working in Dr. Yoshiga's lab investigating the complex relationships that nematodes have with *P. japonensis*, particularly the survivorship mechanisms of *C. japonica*. Dr. Tanaka is currently an Assistant Professor at the Faculty of Medicine at Miyazaki University.

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Chapter 8
Future Directions: Where Do We Go from Here?

Lisa Filippi and Shintaro Nomakuchi

Abstract In recent years, six species within the Superfamily Sehirinae that progressively provision seeds/drupes/nutlets to their young have been identified, one cydnid in North America and four cydnids and *Parastrachia japonensis* (Parastrachiidae) in Japan. This chapter begins with a reflection on some of the similarities and differences among the members of this parental cluster. It then considers the various directions that future research on *P. japonensis* should take, such as comparison of the life history with that of *P. nagaensis*, clarification of the ecological factors that regulate the proportion of individuals that refrain from mating until the second spring, and the mechanism of symbiont transfer that makes the 2-year lifespan without feeding possible. We refer to the questions that remain about mating behavior, regulation of trophic egg production, and parent-offspring communication, as well as the need for further exploration on navigation behaviors, particularly the mechanisms used at night. Finally, we stress the need for studies to confirm unpublished findings presented in this chapter regarding suppression of sibling cannibalism while the female is away from the nest.

Keywords Cannibalism · Cydnidae · Parent-offspring conflict · Mating behavior · Navigation · Parastrachiidae · Provisioning insects · Sibling rivalry · Symbionts · Trophic eggs

8.1 Introduction

Our first public announcement about the remarkable progressive provisioning behavior of *P. japonensis* took place over 30 years ago at the 35th Annual Conference of the Japanese Society of Applied Entomology and Zoology. We were quite

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excited to be presenting our findings and were certain the news about the first evidence of a phytophagous terrestrial heteropteran that progressively provisioned young would be the hottest topic at the conference! Well, that was until we discovered through the conference buzz that Taiichi Nakahira from Hokkaido University, who was equally excited about presenting what he thought was going to be a first-ever discovery, was planning to present very similar findings about his research organism, a cydnid, *Adomerus triguttulus* (Nakahira 1991). It is fair to say that we were both just a tad deflated when we found out about each other's discovery, but nevertheless rose to the occasion, and our respective stories were well-received. Since that time, and to our delight, research in the Nomakuchi Animal Behavioral Ecology laboratory at Saga University has revealed similar parental care behaviors in three additional Japanese cydnid species, *Adomerus rotundus* (Inadomi et al. 2014)*, A. variegatus* (Mukai et al. 2010), and *Canthophorus niveimarginatus* (Filippi et al. 2009).

8.2 Similarities and Differences Among Members of the Parental Cluster

The three cydnid species investigated by the Nomakuchi laboratory display similar parental care behaviors to *A. triguttulus*, *P. japonensis*, and the single North American representative of this cluster, *Sehirus cinctus* (Sites and McPherson 1982; Kight 1997). They all have egg-guarding and progressive provisioning; however, there are differences in the patterns of when provisioning begins. Mothers of some species repeatedly leave the nest during the guarding phase to feed, while others never feed during this phase (Table 8.1). One species begins provisioning at the second nymphal stadium, while others begin soon after hatch. A non-parental cydnid, *Adrisa magna*, drags drupes to a sheltered site and feeds in solitary and in aggregations in the laboratory (Takeuchi and Tamura 2000), and LF has observed other unidentified Japanese and American cydnids transporting seeds to feed under leaves in the field. This behavior of transporting drupes to a sheltered site would minimize the risks of conspecific competition and predation while feeding and could be a preadaptation for progressive provisioning.

There is also variation in parity among the species. *Parastrachia japonensis* is unique among this club of provisioning cydnids in its obligate semelparity (Table 8.1; Filippi et al. 2002), which is likely a response to the severe constraint presented by the fickle food resource. Obligate semelparity indicates that the selective pressure for effective provisioning is much greater in *P. japonensis* than it is in the provisioning cydnids which have multiple opportunities to reproduce.

Additionally, the five cydnids provision very tiny seeds, certainly tiny relative to their body size (Fig. 8.1). The winged seed that *A. variegatus* provisions is somewhat more cumbersome than the other mint seeds; however, the female drags it along behind its body so that it does not interfere with walking (Fig. 8.1b), and the overall

Table 8.1 Comparison of parameters related to trophic egg production and timing of provisioning in parental bugs

Species	Parity	Mother leaves eggs to feed	Pre-hatch trophic eggs	Post-hatch trophic eggs	Resource constraint	Predation risk	Reference
Parastrachia japonensis	Semelparous	No	Yes	No	Yes, severe	Yes	Hironaka et al. (2005)
Adomerus triguttulus	Iteroparous	Yes	Yes	No	No	Yes	Kudo and Nakahira (2004)
Adomerus rotundus	Iteroparous	No	Yes, but very few	Yes	No	Yes	Inadomi et al. (2014)
Adomerus variegates	Iteroparous	No	Yes, but very few	No	Yes, moderate-severe	Yes	Mukai et al. (2010)
Canthophorus niveimarginatus	Iteroparous	No	Yes	Yes	Yes, moderate	Yes	Filippi et al. (2009)
Sehirus cinctus	Iteroparous	Yes, in lab	No	No	No	Yes	LF, pers. observations in lab

Fig. 8.1 Seed provisioning subsocial bugs. (**a**) *Adomerus triguttulus* transporting deadnettle (*Lamium* spp.) seed*;* (**b**) *A. variegatus* transporting winged seed of Japanese elm (*Ulmus davidiana*)*;* (**c**) *P. japonensis* transporting (*Schoepfia jasminodora*) drupe*;* (**d**) *A. rotundus* transporting *(Lamium amplexicaule*) seed; (**e**) *Canthophorus niveimarginatus* transporting kanabiki-sou (*Thesium chinense*) seed*;* (**f**) *Sehirus cinctus albonotatus* transporting purple deadnettle (*Lamium purpureum*) seed. (**a**, **b**, **d**, **e**) Photo credit, Mantaro Hironaka

weight difference pales in comparison to the unwieldy and heavy drupes that *P. japonensis* females provision (Fig. 8.1c). Agrawal et al. (2005) concluded that the cost of the provisioning component of parental care for *S. cinctus* is negligible compared to the egg-guarding component; however, in the laboratory, LF has observed female *S. cinctus* feeding in the nest. If female feeding in the nest also occurs in the field, it would decrease the cost of provisioning even further. Moreover, it is reasonable for species that provision seeds that are very small relative to the size of the female to incur minimal cost, but we suspect the cost is greater for *P. japonensis*. Particularly when several drupes are attached to a single stem and are all dragged back to the nest together, the energy required for *P. japonensis* females to transport such a load relative to body size over a distance as great as 15 m is likely to be considerable. Although constrained by semelparity, which precludes the cost to future reproductive success, there is likely a cost in terms of the number/size of fertile eggs produced.

Another important trait for which variation exists between members of this provisioning cluster distinguishes the Japanese members from their single North American peer. *Sehirus cinctus* is the only member of the provisioning club that does not also produce trophic eggs. LF has verified this with both *S. cinctus cinctus* and *S. c. albonotatus*. Trophic egg production is essential to the success of *P. japonensis* and *A. rotundus* offspring. The very poor quality of the available drupes experienced by *P. japonensis* means that mothers often cannot find acceptable drupes in a timely fashion to provision the nest, and trophic eggs would mitigate the risk of starvation and cannibalism until acceptable drupes are provided (Filippi et al. 2002; Hironaka et al. 2005). In the laboratory, first instar *C. niveimarginatus* could not penetrate intact *Thesium chinense* seeds, but they could successfully feed on the endosperm when the tough outer drupe coat was removed. Post-hatch trophic eggs sustain nymphs until they reach the second stadium and can penetrate and feed on the seeds (Baba et al. 2011). Both *A. triguttulus* and *P. japonensis* have been shown to increase the ratio of trophic eggs produced per viable egg under resource-constrained conditions (Kudo and Nakahira 2005; Filippi et al. 2012). Moreover, resource availability affected the impact of trophic egg feeding on *A. triguttulus*; nymphs developed faster when allowed to feed on trophic eggs in the presence of abundant resource, but survivorship was not affected. However, when deprived of trophic eggs under resource-limited conditions, survivorship was severely reduced. In fact, nymphs failed to molt to the second stadium (Kudo and Nakahira 2004). Interestingly, the rate of cannibalism (nymphal feeding on viable eggs) did not appear to be impacted by access to trophic eggs. Moreover, it is curious that cannibalism has not been observed in *S. cinctus*, the only species in this group that does not produce trophic eggs. Thus, resource constraints in terms of abundance and ability of the young to access the nutritional component of the resource and mitigation of cannibalism appear to have been the main factors promoting the evolution of trophic egg production in the other species.

8.3 Future Directions

8.3.1 Describing the Sister Species

Clearly there is much need for comparative studies that manipulate the ecological conditions of the members of this parental club, but with regard to *Parastrachia* research, where do we go from here? Detailed studies on the behavior of the sister species alluded to in Chap. 1 are certainly called for, but unfortunately, the species remains elusive. SN searched in likely areas of Laos and Vietnam, two countries within the reported range of *P. nagaensis;* however, both trips were unsuccessful. We continue to hope that we will find it one day and be able to clarify the resource, ecological constraints, and behaviors of this shy sister.

8.3.2 Ecological Cues, Suppression of Metabolic Rate, and Physiological Innovations Using Bacterial Symbionts

No work has been done to identify the ecological cues used by *P. japonensis* to regulate the number of individuals that become reproductively active each spring, while the remaining individuals continue their prolonged reproductive diapause without feeding until the following spring (Chap. 2). Is it the winter temperatures, which might also affect the productivity of the host tree? Or is it determined even prior to hibernation based on some condition experienced during development of the nymphs before they become adults? And, to be sure, there are more secrets that await discovery regarding the remarkable physiological adaptations that allow of *P. japonensis* adults to survive for 2 years without feeding, such as nitrogen recycling, which is dependent on a symbiotic bacterium. The curious timing of vertical transfer of the bacterial symbiont to eggs that is also seen in *Brachypelta atterima* (Cydnidae) requires clarification. Why do all the other parental cydnids secrete the symbiont together with the adhesive coating at the time of oviposition, yet *P. japonensis* (Hosokawa et al. 2013) and *B. atterima* (Schorr 1957) only do so just prior to hatch? Studies on the roles and functions of the bacterial symbionts continue to shed light on the intimate relationship *P. japonensis* has with its gut microbiota and their evolutionary history.

Of course, the fascinating gregarious nature of *P. japonensis* warrants further scrutiny and is the subject Tojo Sensei was investigating just prior to his death. What factors determine the size of the aggregations, which range from just a few individuals to thousands. Humidity did not appear to be a major factor, but this should be explored again.

8.3.3 Lingering Questions About Mating Behavior

Questions about mating behavior remain unresolved, as well (Chap. 3). What is the function of the male thrusting behavior? Time-lapse freezing of pairs in copula might clarify that and would allow visualization of the sperm on its path to the spermatheca. It would also be fascinating to verify whether any kind of sperm precedence pattern is at play here or even whether multiple males fertilize a female's brood.

8.3.4 Regulation of Trophic Egg Production

Future studies on trophic egg production (Chap. 4) to determine whether the timing of oviposition is genetically predisposed or facultative are needed. It would also be of great interest to determine the cues that females use to assess resource abundance. Are they assessing abundance while they feed on the host tree to produce eggs and simultaneously assessing competition level based on how many drupes had already been fed on by other females?

8.3.5 Parent-Offspring Conflict and Sibling Rivalry

In addition to assessing the relative and absolute costs of guarding, progressive provisioning, and trophic egg production, there is a need for further studies on parent/offspring communication and conflict. Moreover, in this semelparous species with no opportunity for future reproductive success, there is much potential for intra-brood sibling conflict. The earliest instars start off at very similar weights, largely due to synchronized hatching (Chap. 5). They then feed cooperatively, aggregating on the drupe, completely covering it, because injecting the digestive enzyme into the drupe together facilitates feeding. This cooperative feeding results in fairly similar weight gains among the first and second instar nymphs. However, as the nymphs mature, the feeding pattern gradually transitions to competitive, and weight disparity among nymphs becomes apparent. Sibling conflict from this perspective requires further investigation.

We also know that mothers communicate with their offspring using vibrational stimuli, but research on potential begging cues by offspring is lacking. While volatile chemicals might be effective begging cues when multiple broods are involved, we anticipate that more individual cues are used with intra-brood sibling conflict in semelparous species such as *P. japonensis.* Future investigations are needed to identify the cues used by *P. japonensis* nymphs in sibling rivalry.

8.3.6 Navigating at Night

Navigation has not been explored extensively in the cydnids, but it seems likely that it is much more developed in *P. japonensis*, which must navigate great distances and tortuous paths between the nest and the food source than the cydnid progressive provisioners which nest among the seeds that they provision. Much has been discovered about how *P. japonensis* navigates while provisioning (Chap. 6), but many questions remain. Future investigations could focus on the criteria females use to choose a canopy gap to navigate by. While celestial cues are stable and the changes through the night predictable, canopy gaps will vary with even a breeze. It is curious that females rely on this fickle cue to provision at night.

8.3.7 Mitigation of Sibling Cannibalism

While cannibalism by nymphs is regularly observed in the field, we rarely observe it in the nest. It is likely that females suppress cannibalism when they are in the nest, however the female is often absent from the nest. What then keeps the young from eating each other while their mother is out searching for dinner? We carried out a preliminary study in an attempt to sort this out (unpublished data; Filippi et al. 2009).

In the laboratory, we established five different nest conditions in lidded clear plastic rearing cups (~10 cm diameter, 4 cm height). Nests contained soil, leaf litter, and five nymphs, and five to seven cups were prepared for each of the five conditions. Because we were trying to ascertain how cannibalism is mitigated when the female is out foraging, the mother was removed from all nest cups. The conditions of the nests varied with regard to nymph relatedness and substrate familiarity (Fig. 8.2a). Every nest in the group ($n = 5$) where all nymphs were unrelated and the substrate was foreign (Group E) showed evidence of cannibalism by 24 h (Fig. 8.2b). On the other hand, just 1/7 (14.3%) nests in the group of siblings only in their original substrate showed evidence of cannibalism by 24 h. When substrate was foreign but the nymphs were all siblings (Group B), this percentage increased to 29%. However, in the group with the original substrate, but one stranger among the siblings (Group C), the percentage of cannibalized nests jumped to 85.7%! This was a higher percentage than the group with one stranger and unfamiliar substrate (Group D; 57.4%). Interestingly, we found that in the nests that were cannibalized, a sibling was just as likely to be the victim as a stranger (Fig. 8.2c). By 10 days without food or mother, cannibalism increased in all groups, but was more than twice as prevalent in the two groups with unrelated nymphs than in the group with all sibs and original substrate (Fig. 8.2d). The finding that fewer nests in the group with both one foreign individual and foreign substrate had lower cannibalism than the group with only one foreign individual but original substrate is puzzling. However, the results suggest that in the presence of chemical cues from siblings and the original nest substrate cannibalism is suppressed, but nymphs are released from suppression and are free to cannibalize once the chemical cues are unfamiliar. This is reasonable as the only time nymphs should experience unfamiliar chemical cues in nature is after they become independent and relocate to the foraging area with thousands of unrelated nymphs. This study needs to be repeated with a larger sample size and expanded to verify specific factors in the nest that might serve as chemical cues. A likely target might be the liquid waste excreted by the female that is apparently used in homing (Chap. 6).

We anticipate that further investigation and comparison of the ecological parameters that have led to the evolution of complex parental care behaviors in *P. japonensis* and the provisioning cydnids will continue to contribute valuable information to our understanding of the selective pressures that lead to the evolution of parental care and strengthen the value of this exclusive parental club as a model system in parental care research.

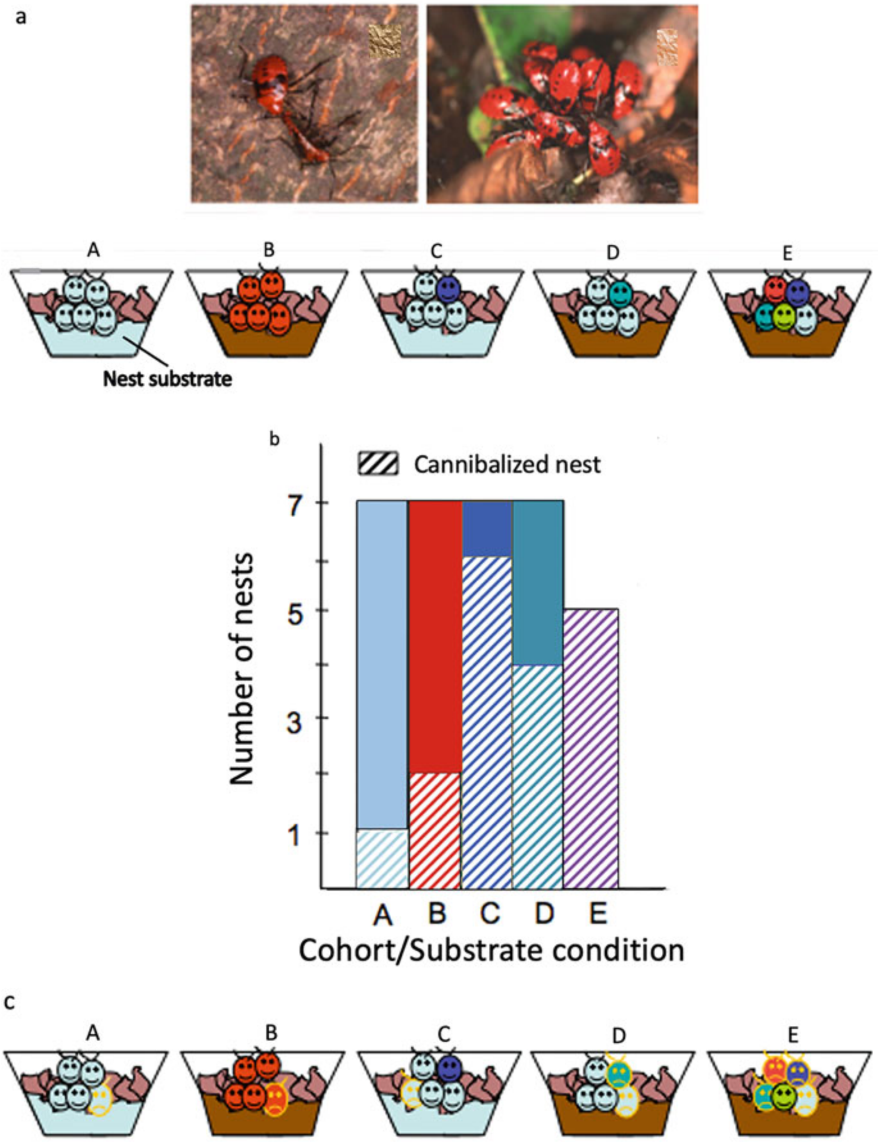

Fig. 8.2 The impact of chemical cues on sibling cannibalism. (**a**) Upper, fifth instars cannibalizing another fifth instar in isolation (left) and in aggregation (right). Lower, third instar nymphs were exposed to different nesting conditions, as indicated by the color of the nymphs and substrate: (A) all siblings in original nest substrate; (B) all siblings in "other nest" substrate; (C) four siblings and one stranger in original siblings' nest substrate; (D) four siblings and one stranger in stranger nest substrate; (E) five unrelated nymphs in strange nest substrate; different color nymph is the stranger. (**b**) Number of nests that had evidence of cannibalism at 24 h. *, Fisher's exact Test, 2 × 5 contingency, $p = 0.008$. (**c**) Target of cannibalism indicated by yellow outline on nymph. (**d**) Percent of nests in each group that had been cannibalized over 10 days in the absence of food and mother. (Unpublished data; conference abstract, Filippi and Nomakuchi 2009)

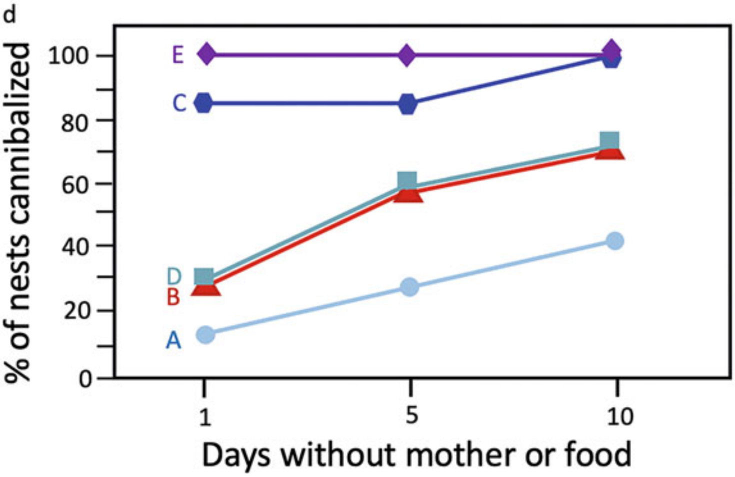

Fig. 8.2 (continued)

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