論文の内容の要旨

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Studies on the reproductive investment in the Adzuki bean borer, *Ostrinia scapulalis*
（アズキノメイガの繁殖投資に関する研究）

Reproductive success is one of the important measurements of the fitness of insects. The study of reproductive success is required for understanding of evolutionary biology. Several studies focused on reproductive investment in females because investments by females are more important for reproductive fitness. Like females, the study of male reproductive investment is required for understanding of evolutionary biology. Male reproductive success depends on the increasing number of females that he can inseminate. Nevertheless, reproduction can be costly for males when they provide nutritious ejaculates or nuptial gifts to the females during copulation. As a result, males should use a wide variety of strategies to allocate their resource carefully to reduce their reproductive cost.

Lepidopteran species are particularly interesting for exploring variation in male pre- and post-copulatory reproductive strategies. Males use various form of courtship displays (e.g., visual, acoustic and olfactory stimuli, etc.) during courtship to attract females. Moreover, males of many Lepidopteran species provide a nuptial gift in the form of capsule, called "spermatophore" that contains sperm and accessory gland nutrients (e.g., carbohydrate, protein, water, etc.) to females during copulation. The production of spermatophore is known to be physiological costly for males because of the time required to recover before producing another spermatophore. Evolutionary theory predicts that it may function as "*paternal investment"* (i.e., increasing female reproductive output with male donated nutrients) and/or "*male mating effort"* (i.e., delaying female re-mating). Males transfer two types of sperm, nucleated, fertile "*eupyrene*"

sperm and anucleated, non-fertile "*apyrene*" sperm. The production of sperm can be limited for males because the number of sperm transferred by male declined with successive matings. Several studies reported that male donated nutrients are needed for egg production and somatic maintenance of females. Among nutrients, protein can be a limiting resource for males because most of the proteins come from larval feeding stage and males can obtain small amount of protein as adult stage, especially for nectar feeding species in Lepidoptera. Thus, the protein contained in a spermatophore is also likely to be an important component of male resource allocation strategy.

The adzuki bean borer, *Ostrinia scapulalis* (Lepidoptera: Crambidae), is a monandrous species as almost all females mate only once throughout her life whereas males are polygynous. Like other *Ostrinia* species, males find conspecific females by following a female-derived sex pheromone and show a courtship display after landing close to the female. During copulation, males transfer a nuptial gift called "spermatophore" containing sperm and nutrients to females. *O. scapulalis* is a multivoltine (i.e.one generation in spring, two or three generation in autumn), a facultative diapause mediated by short day length and low temperature and enters diapause as a mature fifth-instar larva. The mature larvae stop feeding and enter diapause in late summer (July – August) and emerge in spring (May – June) and resume their development. In this thesis, I investigated variation in reproductive investment of *O. scapulalis.*

Age-related male reproductive investment in courtship display and nuptial gift

Due to a trade-off between current and future reproduction, costly reproductive investments should be increased towards the end of a lifespan when the probability of reproduction becomes low (terminal investment hypothesis). I investigated age-related changes in male reproductive investment towards courtship display and the spermatophore in three age classes (young, middle-aged and old) of *O. scapulalis*. As predicted, old males had higher mating success than young and middle-aged males in no- choice tests. Moreover, two-choice tests revealed that middle-aged males had a higher success rate than young males because of their higher courtship frequency rather than any female preference for them. It was found that old males produced a larger spermatophore than young and middle-aged males, suggesting greater reproductive effort. The protein content of spermatophores also tended to increase with male age. Despite the age-related variation in spermatophore size and protein content, age did

not affect female fecundity or longevity. A decrease in the number of sperm in the older males might counteract the nutritional benefit of larger spermatophores. Alternatively, fitness components other than longevity and fecundity may be influenced by male age. The male strategy for allocation of resources across successive matings (mating history) remained unclear in this study because only virgin males were used.

Differential allocation of nuptial gift by virgin and mated males

Re-mating by males has profound effect on male resource allocation and female fitness consequences in several lepidopteran species. Here, I investigated the effect of male mating history on male spermatophore investments and female fitness in *O. scapulalis*. My results showed that spermatophore size and protein content decreased from first mating (virgin males) to second mating (mated males) within the same males. Males are expected to allocate most of their resources only in their first matings and saving large amount of resources for expected future matings may be disadvantage because the numbers of male life-time matings should be low and operation sex ratio (OSR) is always male-biased as females are no longer to re-mate after first mating. Spermatophore size and nutrient contents can vary depending on the number of days between matings. In the present study, one day interval between first and second mating was not sufficient to be able to produce the second spermatophore as large as the first one. For males of the same age, transferred spermatophore size and protein content was higher in virgin males (3-d-old) and in mated males (3-d-old), indicating that mating history per se rather than male age resulted in decreased in spermatophore investment. However, female fecundity and longevity were not affected by male mating history, that is, whether she mated with virgin or mated males, within the same male or males of the same age. Although spermatophore size and protein content declined after first mating, males might be able to produce more or constant amount of other components of spermatophore (e.g. sperm and other nutrients), which are also important for female reproduction, in second mating. These results suggest that there is a cost of mating to males although males can increase their reproductive fitness by re-mating.

Female condition-dependent allocation of nuptial gift by males

The males of many insect species transfer a spermatophore, i.e. a proteinaceous capsule containing sperm, to females during copulation, and this may also function as a nuptial gift. If

production of the spermatophore is costly and variations in the quality of females are large, males may strategically allocate their investment based on the quality of the mate in order to maximize their own reproductive success. I examined the size and protein content of spermatophore transferred to females of different ages and body sizes, and also to water-deprived and water-replete females in *O.scapulalis*. Males transferred a spermatophore of a smaller size or with less protein to older females, smaller females, and water-deprived females. These results indicated that *O. scapulalis* males manipulated their reproductive investment based on the conditions of the mate. I also demonstrated that older males varied their resource allocation to a greater extent in response to female conditions than younger males. Thus, resource allocation by the males of this species was modulated by both female conditions and the age of the males.

Effect of diapause on post-diapause reproductive investment in male and female

Diapause is a strategy for many insect species to overcome adverse environmental conditions. However, diapause is associated with costs because stored metabolic resources are consumed during diapause, resulting in subsequent influence on post-diapause development and reproduction. I investigated effect of diapause on post-diapause reproductive investment of both males and females in a multivoltine moth, *O. scapulalis*. Post-diapause males and females were smaller and achieved lower mating success than non-diapause individuals. Post-diapause females had lower fecundity and shorter longevity than non-diapause females. However, post-diapause males transferred a similar number of eupyrene and apyrene sperm as non-diapause males. Alternatively, I found that fecundity and longevity of females mated with diapause males and non-diapause males were not significantly different. There was no trade-off between diapause duration (short and long) and post-diapause reproductive investments in both males and females. My results suggest that significant decline in post-diapause female reproductive output was due to her own cost of diapause but not influenced by cost of diapause male.

My thesis shows that males did not increase their reproductive success simply by maximizing the number of females that he can inseminate. Males allocated their resources strategically depending on their own condition, their mate quality and environmental conditions to increase their reproductive success. Such knowledge noted in my thesis should provide many fruitful avenues for further investigations of male reproductive strategy in evolutionary biology.