

Ecophysiological study on the alternative life cycles of males in the Japanese common grass yellow *Eurema mandarina*.

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Introduction

Most insects in the Temperate Zone have evolved diverse strategies to survive winter. Reproductive diapause is usually important for adult overwintering in both sexes. However, males can reproduce without overwintering and reproductive diapause by pre-overwintering copulation, indicating two fundamental ways to fertilize eggs in males when females overwinter as adults: One is pre-overwintering copulation, and the other is the combination of reproductive diapause with post-overwintering copulation.

The Japanese common grass yellow, *Eurema mandarina*, shows a seasonal polyphenism consisting of non-diapause summer-form and diapause autumn-form adults. Short day conditions and low temperature induce emergence of autumn-form adults. However, approximately a half of males are insensitive to these conditions, and summer-form males emerge with autumn-form adults in late autumn. Autumn-form females mate with summer-form males before winter, and re-mate with autumn-form males in the following spring. Summer-form males can reproduce only when autumn-form females have sexual receptivity before overwintering. In the present study, I aimed to reveal adaptive significance of pre-overwintering copulation of females and ecological factors affecting the frequency of summer-form males in late autumn.

Two hypotheses have been proposed as adaptive significance of pre-overwintering copulation. One is the nuptial gift hypothesis that females obtain nutrients for overwintering from male ejaculates. The other is the risk hedge hypothesis assuming pre-overwintering copulation as preparation for less chances of mating in the following spring. These hypotheses were tested by dissection of field-caught females and a laboratory experiment.

Sperm competition between summer- and autumn-form males and overwintering success of autumn-form adults are potential factors affecting the frequency of summer-form males in late autumn. Intensity of sperm competition was estimated by dissection of field-caught females. Overwintering success was estimated by a mark-release-recapture study and an outdoor rearing experiment. Furthermore, frequencies of summer-form males in late autumn and pre-overwintering copulation were examined at various localities from the subtropical zone to cool-temperate zone in Japan.

Materials and Methods

Reproductive phenology of autumn-form females was investigated to test the risk-hedge hypothesis and to estimate intensity of sperm competition. Autumn-form females were collected in pre- and post-overwintering periods in central Japan, and they were dissected under a stereoscopic microscope. The number of eggs in the ovary was counted to detect the onset time of oviposition. The number and morphological shape of spermatophores in the bursa copulatrix was examined to know the time of re-mating in spring. Furthermore, to test the nuptial gift hypothesis, survival times under winter-like conditions were compared between mated and unmated autumn-form females in the laboratory.

The mark-release-recapture experiment was conducted from November to May in 2013-2014. In the outdoor rearing experiment, I recorded survival time of field-caught autumn-form adults in the outdoor cage. Furthermore, frequency of summer-form males was examined for several populations located from the subtropical to temperate zone in Japan by a field survey, and a laboratory rearing experiment under short day-conditions at low temperature. Frequency of pre-overwintering population of females was also investigated by counting spermatophores in field-caught autumn-form females.

Results and Discussion

Most autumn-form females mated with summer-form males in autumn, and re-mated with autumn-form males before the onset of oviposition in the following spring. This indicates that the females had sufficient chances for re-mating in spring. In the laboratory experiment, mated females showed higher longevity than unmated females at low temperature, supporting the nuptial gift hypothesis. Therefore, I suggest importance of nuptial gifts in the evolution of pre-overwintering population of autumn-form females.

The estimated reproductive phenology of autumn-form females also indicates that the fitness of both forms of males strongly depends on sperm competition between summer- and autumn-form males. Summer-form males had significantly longer eupyrene sperm bundles than autumn-form males. This might be an adaptation of summer-form males for the sperm competition.

From the mark-release-recapture and outdoor rearing experiments, I estimated an overwintering success of approximately 5% for both sexes. Pre-overwintering populations of females have been reported in some other butterfly species showing low overwintering success.

Nuptial gifts must be important for overwintering females especially under low overwintering success. On the other hand, no remarkable geographic variation was detected in the proportion of summer-form males in late autumn, indicating that the emergence of summer-form males is not direct adaptation to low overwintering success. This lack of remarkable geographic variation can be explained by the fact that most females mated before winter in all populations examined.

Conclusion

Low overwintering success have enhanced the evolution of pre-overwintering copulation of females, and the frequency of summer-form males in late autumn depends on sperm competition rather than overwintering success.