論 文 の 内 容 の 要 旨

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論文題目　**Studies on the reproductive alteration induced by *Wolbachia* infection in the genus *Ostrinia* (Lepidoptera: Crambidae)**

（アワノメイガ属における *Wolbachia* 感染による生殖異常に関する研究）

Wolbachia is a group (genus) of cytoplasmic endosymbiotic bacteria found in arthropods and nematodes. *Wolbachia* is known to manipulate the reproduction of arthropod hosts to facilitate the spread of infection into the host population. From these reasons, *Wolbachia* is considered as a selfish genetic element in arthropods. The actions of *Wolbachia* infection on the arthropod hosts include feminization of genetic males, male–killing, cytoplasmic incompatibility (CI) and induction of parthenogenesis.

Ostrinia (Lepidoptera: Crambidae) is a group (genus) of small moths represented by the Asian corn borer moth *O. furnacalis,* the major pest of corn in the East Asia. Eight *Ostrinia* species found in Japan are divided into *O. palustralis* group, *O. latipennis* group and *O. furnacalis* group based on the morphological similarity. In the genus *Ostrinia*, *Wolbachia*-infection was found in *O. furnacalis*, *O. scapulalis*, *O. orientalis*, and *O. zaguliaevi* (these four species belong to *O. furnacalis* group), and *O. ovalipennis*

(this species belongs to *O. latipennis* group). The sequences of a cell-cycle gene (*fts*Z) as well as a *Wolbachia* surface protein gene (*wsp*) of *Wolbachia* that infect the four species of *O. furnacalis* group were identical, indicating that *Wolbachia* strain(s) in these host species are the same or very closely related. In the *O. furnacalis* group, *Wolbachia* infection was found only in females at a low frequency in the field. *Wolbachia* infecting *O. furnacalis* group causes production of all-female offspring. The all-female production was first interpreted as feminization of genetic males, since elimination of *Wolbachia* by antibiotic treatment resulted in the production of all-male offspring. However, a detailed analysis of the sexual genotype (male = ZZ, female = ZW) of individuals based on observations of the sex chromatin (W) and the comparative genomic hybridization of sex chromosomes led Kageyama & Traut (2004) to conclude that the all-female production in infected *O. scapulalis* is the consequence of male-specific death provoked by the feminizing effect of *Wolbachia* (*w*Sca). They also indicated that the all-male production after antibiotics treatment is caused by female-specific death, suggesting that *w*Sca is indispensable for the survival of infected females. In contrast to *O. furnacalis* group, *Wolbachia* infection was found in both females and males of *O. ovalipennis* at a high frequency in the field. Because of the difficulty in rearing of *O. ovalipennis* in the laboratory, the action of *Wolbachia* in this species has not been clarified in detail.

In the present study, I first investigated the phylogenetic relationship of *Wolbachia* infecting *O. ovalipennis* and those infecting *O. furnacalis* group to infer the origin of *Wolbachia* strain that causes all-female production in *Ostrinia*. Secondly, I investigated the relative importance of *Wolbachia* and host genotypes in determining the type of reproductive alteration in the host. Thirdly, I tried to obtain a clue for the mechanism of all-female production by the examination of the sexual mosaics produced by antibiotics treatment of *Wolbachia*-infected adult females.

Characterization of *Wolbachia* strain infecting *O. ovalipennis*

I determined partial sequences of *fts*Z and *wsp* gene of *Wolbachia* infecting *O. ovalipennis*. These sequences were the same as those of *Wolbachia* infecting *O. furnacalis* group. These results indicate that the same or a very close strain of *Wolbachia* infect *O. ovalipennis* and species of *O. furnacalis* group. Since the *Wolbachia* strain in *O. ovalipennis* does not cause all-female production in the hosts, these results may suggest that the ability to cause reproductive alteration was acquired by the *Wolbachia* infecting *O. furnacalis* group in the recent past in the evolutionary time scale.

Transinfection reveals the crucial importance of *Wolbachia* genotypes in determining the type of reproductive alteration in the host

*w*Sca induced male-specific death, while another strain of *Wolbachia* (*w*Kue) infecting the Mediterranean flour moth *Ephestia kuehniella* induces CI in the resident host. Transinfection of *Wolbachia* can be a powerful tool to elucidate the relative importance of *Wolbachia* and the host in determining the type of reproductive alterations. Recently, male-killing was shown to occur in *E. kuehniella* transinfected with *w*Sca. Here, I transferred *w*Kue to *O. scapulalis* by embryonic microinjection. In the *O. scapulalis* transinfected with *w*Kue, not male killing, but CI occurred. Thus, in addition to *w*Sca, *w*Kue was shown to induce the same type of alteration in a foreign host as in its natural host. These results demonstrate the crucial role of *Wolbachia* genotype in determining the type of reproductive alteration. However, the present study also revealed the involvement of host factors. First, the degree of incompatibility induced by *w*Kue in *O. scapulalis* was stronger than that in *E, kuehniella*, indicating the host factors can affect the level of CI. Second, the vertical transmission rate of *w*Kue in *O. scapulalis* was generally low, suggesting that host affects the dynamics of *Wolbachia* transmission.

The mode of male-specific death in *Wolbachia*-infected *O. furnacalis*

To clarify the mode of sex-specific death, I observed sex chromatins of individuals derived from *Wolbachia*-infected and cured females of *O. furnacalis* through the larval development. Both genetic males and females were included in the hatched larvae in the broods derived from infected females. Likewise, both genetic males and females were found in the hatched larvae in most broods derived from cured females. In contrast, the last instar larvae derived from infected females were in the all-female condition, and those derived from cured females were in the all-male condition. These results clearly indicated that opposite sex specific lethality occurred during the larval development in the broods of *Wolbachia*-infected and cured females of *O. furnacalis*.

The interaction of *Wolbachia* and host occurs specifically during the embryonic stage

In *O. scapulalis* and *O. furnacalis*, the ability of *Wolbachia* to feminize genetic males was evidenced by the finding that antibiotics treatment of infected female adults led to the production of sexual mosaics, the genetic sex of which was male. Examination of organ(s) in the sexual mosaics feminized and not feminized by *Wolbachia* would allow

us to estimate the mode and limitation of feminizing activity of *Wolbachia*. I observed sexual mosaics produced by tetracycline treatment of *Wolbachia*-infected *O. furnacalis* female adults. A small proportion of them had female wing pattern in the entire area. None of these mosaics had ovary in spite of their complete female-like wing pattern and having the bursa copulatrix. Surprisingly, none of the sexual mosaics were found to harbor *Wolbachia*. Then, I checked the presence of *Wolbachia* in the eggs and hatched larvae derived from tetracycline-treated *Wolbachia*-infected females. None of the larvae hatched from eggs laid after two days of tetracycline treatment harbored *Wolbachia* in spite of the occurrence of male-specific death. These results demonstrate that *Wolbachia*-infected genetic males are destined at the early embryonic stage to die later in the larval development, and that *Wolbachia* is indispensable for females only during the embryonic developmental stage.

W chromosome in *Wolbachia*-infected *O. furnacalis* is differentiated from that in normal females

In *Wolbachia*-infected insects, one of the Z chromosomes in the male (ZZ) may aggregate and appear like sex chromatin. To examine this possibility, I amplified microsatellite locus on W chromosome in *O. furnacalis* by PCR. The band found in normal females were not observed in any of the *Wolbachia*-infected females. Surprisingly, I found a band specific to females of *Wolbachia*-infected *O. furnacalis*, which is different from the band of normal females in size by 300 bp. The band was found in cured females, but not found in the all-male progeny produced by the cured females. The appearance of the band thus coincided with the presence of sex chromatin, indicating that sex chromatin in the *Wolbachia*-infected female is W chromosome. These results suggest that females in the *Wolbachia*-infected matrilines of *O. furnacalis* group have a W chromosome different from normal females.

In conclusion, this study clarified the uniqueness of reproductive alteration caused by *Wolbachia* infecting *O. furnacalis* group. I showed that the *Wolbachia* strain infecting *O. furnacalis* causes male-killing, and has the ability to feminize genetic males. Heretofore, male-killing and feminization of genetic males have been considered as entirely independent phenomena. However, the present study clearly demonstrated that feminization underlies the phenomenon of male-killing. This finding would help us to understand the mechanisms commonly underlying various types of *Wolbachia*-induced reproductive alterations in insects.