Sex ratio distortion by a *Spiroplasma* symbiont in the small brown planthopper, *Laodelphax striatellus*

Sanada-Morimura S.[*1], Matsumura M.[*1] & Noda H.[*2]

[*1]: National Agricultural Research Center for Kyushu Okinawa Region, Koshi, Kumamoto 861-1192
[*2]: National Institute of Agrobiological Sciences, Owashi, Tsukuba, Ibaraki 305-8634, Japan
sanadas@affrc.go.jp

The small brown planthopper (SBPH), *Laodelphax striatellus (*Fallén) (Hemiptera, Delphacidae), is one of the most important pests of rice and wheat crops across a wide area of East Asia. SBPH can act as a vector for the *Rice stripe virus*. However, the feeding activities of SBPH do not cause serious damage to the plants unlike those of migratory rice planthoppers, such as the brown planthopper, *Nilaparvata lugens* (Stål), or the whitebacked planthopper, *Sogatella furcifera* (Horváth). SBPH overwinters in temperate regions in Japan, Taiwan, and China.

Intracellular symbiotic bacterial species, for example, *Wolbachia*, are present in SBPH populations in East Asia, including Japan and Taiwan. Such intracellular symbionts are common in many invertebrate organisms such as insects, mites, and nematodes. Some of these symbionts manipulate the reproductive system of their host to produce increased numbers of females, at the expense of the number of males, because the bacteria are maternally inherited through the egg cytoplasm (Moran & Wernegreen, 2000). This strategy of manipulation of the host's reproductive system has evolved in phylogenetically diverse bacteria, including *Wolbachia*, *Rickettsia*, *Arsenophonus*, *Spiroplasma*, and *Cardinium* (Moran et al. 2008). In SBPH, *Walbachia* also causes cytoplasmic incompatibility, resulting in sterile breeding between infected males and uninfected females (Noda, 1984; Noda et al., 2001). Through cytoplasmic incompatibility, *Wolbachia*-infected populations have increased their distribution in Japan (Hoshizaki & Shimada, 1995). Symbionts can produce other effects in their host species, such as male killing, parthenogenesis, or feminization. However, to date, the only effect on host reproduction identified in planthoppers is cytoplasmic incompatibility.

We found female-biased sex ratios in Taiwanese (TW) and Japanese (JP) SBPH strains collected in 2006; the TW strain had only 30% of males, while the JP strain had 43% males. Furthermore, both strains were found to carry another symbiont species, *Spiroplasma*. *Spiroplasma* infections are well known to cause male-killing in other insects. We carried out three analyses to determine whether *Spiroplasma* in SBPH manipulated host reproduction. First, the frequency of *Spiroplasma* and *Wolbachia* infection was determined in the TW and JP strains by PCR using appropriate specific primers. Second, we established female-biased maternal lines (FB lines) and non-biased maternal lines (NB lines) from the TW and JP strains. Third, antibiotic treatment was carried out on females of FB lines to eliminate the effect of any symbionts. Sex ratios and survival rates in the progeny were then monitored for three generations.

We found a *Spiroplasma* infection rate of 0.86 (N = 50) in the TW strain and of 0.10 in the JP strain (N = 50). The *Wolbachia* infection rate was 1.0 in both the TW (N = 50) and JP (N = 50) strains. Two FB lines and three NB lines were successfully established from females from the TW and JP strains, indicating that the trait of female-biased sex ratio was maternally inherited. The FB lines were found to be doubly infected with *Spiroplasma* and *Wolbachia*, whereas the NB lines were only infected with *Wolbachia*. This analysis indicates that *Wolbachia* infection alone does not influence the sex ratio. Antibiotic treatment restored the normal sex ratio of the progeny, strongly suggesting that symbiont manipulated host reproduction. The antibiotic treatment also enabled us to establish maternal lines infected only with *Spiroplasma*, a situation that has not been observed in the field. The *Spiroplasma*-single infection lines showed a strongly female-biased sex ratio in their third generation

progeny. This is the first report of *Spiroplasma*-mediated sex ratio distortion in hemimetabolous insects.

References

Hoshizaki, S., Shimada, T. (1995) PCR-based detection of *Wolbachia*, cytoplasmic incompatibility microorganisms, infected in natural populations of *Laodelphax striatellus* (Homoptera: Delphacidae) in central Japan: has the distribution of *Wolbachia*(italic) spread recently? Insect Mol. Biol. 4: 237–243.

Moran, N. A., Wernegreen, J. J. (2000) Lifestyle evolution in symbiontic bacteria: insights from genomics. Trends Ecol. Evol. 15: 321–326.

Moran, N. A., McCutcheon, J. P., Nakabachi, A. (2008) Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet. 42: 165–190.

Noda, H. (1984) Cytoplasmic incompatibility in a rice planthopper. J. Hered. 75: 345–348.

Noda, H., Koizumi, Y., Zhang, Q., Deng, K. (2001) Infection density of *Wolbachia* and incompatibility level in two planthopper species, *Laodelphax striatellus* and *Sogatella furcifera*. Insect Biochem. Mol. Biol. 31: 727–737