A GYANDROMORPH OF *EUREMA HECABE* (LEPIDOPTERA: PIERIDAE) FROM JAPAN

S. Narita, M. Nomura, and D. Kageyama

ABSTRACT: A gynandromorph of a pierid butterfly, *Eurema hecabe*, caught at Mt. Tsukuba in Central Japan is described here. Wings were apparently bilaterally asymmetric, while the external and internal genitalia clearly showed the morphology and genotype of the female. The fact that this individual showed a female genotype excluded the possibility of incomplete feminization due to *Wolbachia* infection. Presence of a spermatophore in the bursa copulatrix and the difference in number of matured eggs between the right and left ovaries indicated that this gynandromorphic individual had copulated and oviposited in the field.

KEY WORDS: *Eurema hecabe*, gynandromorph, Lepidoptera, Pieridae, Mt. Tsukuba, Japan, *Wolbachia*

In wild-caught or laboratory-reared insects, individuals showing a mosaic of morphology of both sexes are found very rarely. They are usually referred to as “gynandromorphs.” The common yellow butterfly, *Eurema hecabe* (L.) (Lepidoptera: Pieridae), is distributed almost all over the Oriental, Australian as well as Afrotropical biogeographic regions, and extends into the cool-temperate zone of the Eastern Palaearctic Region (e.g. the northern part of Honshu, Japan, up to 40°N, Yata, 1995). It was recently recognized that *E. hecabe* consists of two sibling species, tentatively called yellow type and brown type, which are distinctive in various biological and genetic traits.

We captured an abnormal adult of *E. hecabe* (yellow type) with wings of both male (left) and female (right) at Mt. Tsukuba located in the central part of mainland Japan. This individual was examined for morphology and karyotype of the internal and external genitalia. In addition, infection status with endosymbiotic bacteria *Wolbachia* was examined. *Wolbachia* is known to manipulate the reproduction of its host insect in various ways such as feminization, male killing, induction of parthenogenesis or induction of cytoplasmic incompatibility (Bourtzis and Miller, 2003). In Okinawa, a southwestern island of Japan, a part of *E. hecabe* females (ca. 10%) produce progeny with a sex ratio strongly biased in favor of females. These females are infected with two different strains of *Wolbachia* (*w*Hec1 and *w*Hec2), while those producing progeny with a normal sex ratio are infected with *w*Hec1 only. The result of antibiotic treatment (individuals eclosed were all males) as well as of cytogenetic sexing suggested that these females were genetically male but feminized by *Wolbachia* (Hiroki et al., 2002, 2004). Almost all populations of *E. hecabe* in Japanese mainland except...
ing for the northern area, are infected with only one Wolbachia strain, wHec1 (Narita et al., 2006), where the female-biased sex ratio has never been reported.

**Sample:** A gynandromorph of *E. hecabe* was caught at Mt. Tsukuba, Ibaraki Prefecture, Japan in June 23, 2006.

**Sexual phenotype of wings:** Ground color of the left wings was bright yellow (Fig. 1a). On the left forewing underside, the sex-brand which normally appears only in male (Yata, 1995) was present (Figure 1B, 1C). On the contrary, ground color of the right wings was pure white/pale yellow, and the sex-brand was absent in the right forewing (Figure 1). Thus the left wings of this individual were exactly those of the male, while the right wings were exactly those of the female.

**Sexual phenotype of the internal organs and external genitalia:** The internal organs were dissected. They were exactly of the normal female: the bursa copulatrix, seminal receptacle and ovaries (female organs) were present and the testes (male organ) were absent (Table 1; Figure 2). A spermatophore was present in the bursa copulatrix, indicating that this individual had copulated with a male. Matured eggs were significantly fewer in the left ovary than in the right (Figure 2). The external genitalia were macerated in a 10% KOH (potassium hydroxide) for two hours and their scales removed. They were also exactly of the female (data not shown).

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Table 1. Sex-related traits of the gynandromorph of *Eurema hecabe* (yellow type) examined in this study.

<table>
<thead>
<tr>
<th>Sex-related traits</th>
<th>Female</th>
<th>Gynandromorph</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forewing ground color¹ (left/right)</td>
<td>w/w</td>
<td>s/w</td>
<td>s/s</td>
</tr>
<tr>
<td>sex bland (left/right)</td>
<td>-/-</td>
<td>+/-²</td>
<td>+/+</td>
</tr>
<tr>
<td>Internal genitalia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ovary</td>
<td>+</td>
<td>+³</td>
<td>-</td>
</tr>
<tr>
<td>testis</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>seminal receptacle</td>
<td>+</td>
<td>+³</td>
<td>-</td>
</tr>
<tr>
<td>External genitalia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>valva</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>phallus</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ovipositor</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>bursa copulatrix</td>
<td>+</td>
<td>+³</td>
<td>-</td>
</tr>
<tr>
<td>Karyotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malpighian tubule</td>
<td>ZW</td>
<td>ZW</td>
<td>ZZ</td>
</tr>
<tr>
<td>bursa copulatrix</td>
<td>ZW</td>
<td>ZW</td>
<td>ZZ</td>
</tr>
</tbody>
</table>

¹w, weak yellow; s, strong yellow, ²See Figure 1, ³See Figure 2.

**Cytogenetic Sexing:** In many species of Lepidoptera including *E. hecabe*, the sex chromosome constitution is female-heterogametic (ZZ in males/ZW in females), and the W chromosome is conspicuous as a condensed sex-chromatin body in interphase nucleus (Traut and Marec, 1996). In this study, the Malpighian tubules and bursa copulatrix were removed and fixed in methanol : acetic acid (3:1) for ca. 1 min, transferred to slides, stained and mounted in lactic acetic orcein and examined under a light microscopy as described in Kageyama and Traut (2004). Sex chromatin bodies were present in every nucleus of Malpighian tubule and bursa copulatrix cells, indicating that at least a part of the body was genetically female (Table 1).

**Detection and Identification of Wolbachia:** *Wolbachia*-specific PCR amplifying wsp gene (ca. 600 bp) gave a positive result. The primers used were wsp81F 5' TGGTCCAATAAGTGATGAAGAAAC -3' (forward) and wsp691R 5'- AAAAAATTCAAACGCTACTCCA -3' (reverse) (Zhou et al., 1998). To characterize the *Wolbachia* strains detected, the PCR product of wsp gene were cloned and subjected to DNA sequencing. The sequences of 27 clones examined were all identical to those of cytoplasmic-incompatibility-inducing *Wolbachia* seen in *E. hecabe* from the mainland of Japan (wHec1, accession number AB210826).
DISCUSSION

Abnormal individuals that are comprised of morphologically male and female tissues are usually referred to as “gynandromorphs.” Strictly speaking, however, “gynandromorph” is defined as chimeric individual of genetically both male and female. Gynandromorphs usually result from either loss of a sex chromosome during early embryogenesis, or from fertilization of a bi-nucleated egg (Laugé, 1985). In Lepidoptera, sex chromosome constitution is heterogametic (ZW) in females and homogametic (ZZ) in males. The most frequent origin of a gynandromorph in the silk moth, *Bombyx mori* (L.), is double-fertilization of a bi-nucleated egg (Z+W), resulting in an individual with ZZ and ZW cell karyotypes (Goldschmidt and Katsuki, 1927). In *Drosophila*, gynandromorphs are typically produced by the loss of an X chromosome during the first cleavages of an XX zygote (Morgan, 1914). In the honeybee, *Apis mellifera* L., where males are haploid and females are diploid, the male tissues of gynandromorphs are of maternal origin and the female tissues are of biparental origin (Rotenbuhler et al., 1952).

Similarly, intersexes are individuals that are comprised of phenotypically male tissues and female tissues. But they are essentially different from gynandromorphs in that an intersex is genetically either purely male or purely female. In *Drosophila*, various mutants that produce intersexes have been isolated and contributed to the understanding of the sex-determining mechanism (e.g. Morgan et al., 1943; Sturtevant, 1945; Watanabe, 1975; Hildreth, 1965). In the gypsy moth, *Lymantria dispar*, a crossing between geographic strains resulted in production of intersexes having purely male karyotype (Goldschmidt, 1934). In some of the strains of the adzuki bean borer, *Ostrinia scapulalis* (Lepidoptera: Crambidae) and *E. hecabe* are infected with endosymbiotic bacteria *Wolbachia* that have an ability to feminize their genetically male hosts (Kageyama and Traut, 2004; Hiroki et al., 2002). In *O. scapulalis*, incomplete elimination of *Wolbachia* resulted in production of intersexes which were genetically purely male (Kageyama et al., 2003; Kageyama and Traut, 2004).

The individual of *E. hecabe* examined here was morphologically female except for the left wings. Cytogenetic observation of this individual revealed that the internal organs and external genitalia were genetically female. Unfortunately, however, we could not examine the sexual genotype of wings, the only tissue showing the male phenotype. Thus we could not determine whether this individual was an intersex or not. Although *Wolbachia* was detected, the fact that the bursa copulatrix and Malpighian tubules were genetically female indicated that this was not the case of feminization of genetic males caused by *Wolbachia*.

It is very likely that this individual had copulated with a male and laid eggs in the field, since one spermatophore was present in the bursa copulatrix and matured eggs in the left ovary were apparently fewer than in the right one (Figure 2).

ACKNOWLEDGEMENTS

This study was financially supported in part by Japan Society for the Promotion of Science (JSPS) fellowship for Young Scientists to S. N.


