[SHORT COMMUNICATION]

Effects of Fertilization Status and Age of Gravid Females on the Egg Size in *Tetranychus urticae* Koch

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**INTRODUCTION**

Two-spotted spider mite, *Tetranychus urticae* Koch, is an arrhenotokous mite, in which females emerge from diploid eggs but males emerge parthenogenetically from haploid eggs (Helle and Pijnacker, 1985). Adult females produce haploid and diploid eggs after mating, and they also produce haploid eggs even if they do not mate. In the case of arrhenotokous hymenopteran insects, the sex is determined primarily by maternally controlled fertilization, and the combination (or single if not fertilized) of alleles of the sperm and the oocyte determines to start development of sexual characters in each egg under the CSD model, which was proposed as a genetic mechanism of sex determination (Whiting, 1939, 1943). The diploid males in the inbreeding experiments was not derived from the cytoplasmically sex-determined eggs, but developed from the cytoplasmically homogenous eggs with the combination of homogenous alleles by fertilization (Bull, 1983; Cook, 1993).

In *T. urticae*, on the other hand, diploid males did not appear in the inbred lines, on the contrary the sex ratios in some inbred lines increased compared with the original population (Overmeer and Harrison, 1969). While they control the sex ratio of their offspring in relation to the environmental condition if inseminated (Young et al., 1986), the sex seems to be determined primarily cytoplasmically (Wrensch, 1992). The function of sperm is thus for something other than sex determination (Wrensch, 1992), and the genetic mechanism of sex determination is indistinct. To understand the sex determination in *T. urticae*, sexual difference of the quality and quantity in the eggs and/or oocytes must be detected as an evidence of maternal control of offspring sex. Sexual dimorphism in egg size was illustrated in the pseudo-arrhenotokous...
phytoseiid mites although the mechanism of sex determination has not been clarified yet (Toyoshima and Amano, 1998). Therefore, the size of eggs just laid was investigated as a first step to elucidate the maternal control of sex in *T. urticae*, and the possibility of cytoplasmic sex determination just before the genetic sex determination by fertilization is discussed.

MATERIALS AND METHODS

Thirty females of *T. urticae* were sampled from apple trees (*Malus domestica* L.) in the experimental field (Apple Research Station, National Institute of Fruit Tree Science located in Morioka, Japan: lat. 39.76°N; long. 141.13°E) to establish the stock culture. Before experiments, they have been reared for more than 3 years on the kidney bean, *Phaseolus vulgaris* L., in the chamber controlled at 20°C with a 15L9D photoperiod.

Virgin females for the experiment were prepared from eggs laid by 20 gravid females in the stock culture. The gravid females were transferred from the stock culture onto a bean leaflet (4 x 4 cm) and were reared at 20°C and 90% RH with a 15L9D photoperiod. The bean leaflet was kept on the cotton soaked with water. After 24 hr of oviposition, the gravid females were removed from the leaflet and eggs laid on the leaflet were reared. Sixty teleiochrysalid females were transferred individually onto a small bean leaflet (2.5 x 2.5 cm), and 40 adult females molted at the same day were obtained.

Twenty virgin females were reared without mating, and other 20 females were mated individually for 24 hr with a young male. Both virgin and fertilized females were reared individually and observed daily until they died.

All eggs laid on the leaflet were transferred individually onto the small leaflet and reared to determine their sexes. Eggs, laid at the 4-5th days and at the 14-15th days after emergence, were measured their size before individual rearing. In order to determine their size, the eggs were transferred individually into a slide glass by a fine brush, and the diameter of the eggs was measured 4 times with changing the angles at 45° under a phase-contrast microscope (at 400× magnitude, Nikon ECLIPSE E600).

An anova was applied and a *t*-test was used to examine differences in the reproductive characters and the longevity between virgin and fertilized females. For the size of eggs laid by fertilized females, the generalized linear model (GLM) was applied to analyze the effect of the females, the age of the females and the sex of offspring on the size of eggs. Parameters of the GLM were assumed with maximum likelihood technique, based on the poisson distribution. The GLM was also applied to analyze the effect of females and the age of females on the size of eggs laid by virgin females. These statistical analysis was computed with JMP® software (version 8.0.2, from SAS Institute Inc., Cary, NC, USA).

The eggs developing to adult females and those to adult males are called, in short, as female-eggs and male-eggs, respectively, in this study.

RESULTS AND DISCUSSION

Total fecundity, oviposition period, and adult longevity were not different between virgin
and fertilized females, but the daily fecundity on average during whole period of oviposition was significantly different (\(t\)-test, \(p<0.05\)) (Table 1). The daily fecundity of virgin females at the peak of fecundity between 5th and 9th days after emergence (9.10±0.34) was also significantly different from that of fertilized females (10.63±0.20) (\(t\)-test, \(p<0.05\)).

The effect of females on the egg size (Chi-sq., \(p=0.96\)) was not significant but that of the age of the females (Chi-sq., \(p=0.009\)) and the sex of offspring (Chi-sq., \(p=0.004\)) was significant. The interactions (female \(\times\) age, age \(\times\) sex, female \(\times\) sex, female \(\times\) age \(\times\) sex) were not significant (\(p>0.05\)). The size of eggs laid by fertilized females was various in responses to the age of the females and the sex of offspring. Female eggs laid by fertilized females at 4-5th days after emergence were larger than male eggs at the same timing (Table 2, Fig. 1). The difference in size between female and male eggs was maintained at 14-15th days after emergence of fertilized females (Table 2).

The sexual size dimorphism was shown in eggs at the beginning of post-embryonic development in \(T.\ urticae\). There are much review of the trade-off between progeny size and number (e.g., Fox and Czeask, 2000) and the sexual size dimorphism in immature and adult insects (e.g., Stillwell et al, 2010). But the sexual dimorphism in eggs was known only in scale insects (Brown and Bennett, 1957) and phytoseiid mites (Toyoshima and Amano, 1998). The dimorphism seems to have a relation to the sex determination in the abnormal reproductive system (paternal genome loss) of both animal groups (Brown, 1965). However, it has not been investigated whether both animals develop the dimorphism before or after fertilization because they lay eggs only after copulation.

In this study, on the other hand, the size of eggs laid by virgin females was compared to investigate the effect of fertilization on the sexual size dimorphism in eggs in \(T.\ urticae\). The effect of females (Chi-sq., \(p=0.93\)) and the age of females (Chi-sq., \(p=0.69\)) on the egg size were not significant (Table 3). If the size of eggs among three categories (the female and male eggs by fertilized females, and the male eggs by virgin females) was computed, the difference of size was significant each other at 4-5th and 14-15th days (Tukey-Kramer’s HSD test, \(p<0.05\)), respectively. The male eggs laid by fertilized females were smaller than those by virgin females at 4-5th days, and the egg of fertilized females was not different from that of virgin females at 4-5th days (\(t\)-test, \(p>0.05\), Fig. 2).

It is suggested from the result of egg size at 4-5th days that \(T.\ urticae\) develops sexual size dimorphism in eggs regardless of fertilization. Virgin females seem to produce cytological female eggs, which were fundamentally fated to female but developed to male if not fertilized. On the other hand, the concealed sexual size dimorphism in eggs laid by virgin females at 14-15th

<p>| Table 1. Comparison of reproductive characters between virgin and fertilized females of (Tetranychus urticae) |
|----------------------------------------|-------|----------------|-----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Fertilization status</th>
<th>(n^1)</th>
<th>Total fecundity</th>
<th>Daily fecundity*</th>
<th>Oviposition period (days)</th>
<th>Adult longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin</td>
<td>16</td>
<td>184.6±13.87</td>
<td>6.83±0.248</td>
<td>27.4±2.62</td>
<td>38.9±3.88</td>
</tr>
<tr>
<td>Fertilized</td>
<td>18</td>
<td>209.1±12.19</td>
<td>8.19±0.323</td>
<td>24.4±1.53</td>
<td>36.1±2.19</td>
</tr>
</tbody>
</table>

Mean±SE. * indicates the characters with significant difference between virgin and fertilized females (\(t\)-test, \(p<0.05\)).

1 : Female died accidentally during the experiment were removed from the statistical analysis.

2 : Daily fecundity in this column is the mean fecundity in days in the whole period of oviposition.
days was not detected probably because nutritional condition of fertilized females progressed at that time more than that of virgin females. Although the difference of daily fecundity between virgin and fertilized females was significant (Table 1), the experimental result was not enough to interpret the relationship of egg size among three categories at 14-15th days. The most important point of this study that the cytological investigation of sex determination in *T. urticae* must be

Table 2. Comparison of diameters of eggs laid by fertilized females of *Tetranychus urticae* at 4-5th and 14-15th days after emergence

<table>
<thead>
<tr>
<th>Sex of eggs</th>
<th>4-5th days</th>
<th>14-15th days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>132.3±0.16 (265)</td>
<td>135.0±0.26 (112)</td>
</tr>
<tr>
<td>Male</td>
<td>126.6±0.42 (69)</td>
<td>132.5±0.31 (166)</td>
</tr>
</tbody>
</table>

Mean diameter (μm)±SE. The number in the parentheses are replicates (Data of each female are pooled).

The generalized linear model estimated that the effect of the females on the size of eggs was not significant (*p*=0.96) but the effect of the age of the females (*p*=0.009) and the sex of offspring (*p*=0.004) was significant. Interactions between each combination and among three factors were not significant (*p*>0.05).

Table 3. Diameters of eggs laid by virgin females of *Tetranychus urticae* at 4-5th and 14-15th days after emergence

<table>
<thead>
<tr>
<th>Sex of eggs</th>
<th>4-5th days</th>
<th>14-15th days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>130.8±0.22 (252)</td>
<td>130.5±0.19 (299)</td>
</tr>
</tbody>
</table>

Mean diameter (μm)±SE. The number in the parentheses are replicates (Data of each female are pooled).

The effects of the female (*p*=0.93) and the age of females (*p*=0.69) on the size of eggs were not significant.

Fig. 1. Frequency distribution of egg-size laid by fertilized females of *Tetranychus urticae* at 4-5th days after emergence. Female eggs (132.3±0.16, *n*=265. Data of each fertilized female are pooled.) are larger than male eggs (126.6±0.42, *n*=69. Data of each fertilized female are pooled.) (*t*-test, *p*<0.05).
conducted in advance to the investigation of genetic sex determination. The cytological female eggs would be proved if the difference of membrane structure on oocytes to interrupt sperm penetration was distinguished as a maternal control of fertilization, and/or if the disappearance of penetrated sperms in male oocytes was observed as evidence in which *T. urticae* females do not control sperm penetration.

**REFERENCES**


