Effect of egg size variation on survival rate, development and fecundity of offspring in a migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperiidae)

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Abstract
Life history traits of *Parnara guttata guttata* hatched from large-sized and small-sized eggs were compared. Eggs were grouped into two sizes, larger than 0.19 mm³ (L group) and smaller than 0.16 mm³ (S group). Larvae from these groups were reared individually under a 16L8D photoperiod at 25°C. The survival rate in immature stages was significantly higher in the L group than the S group. Although not significant, the developmental time during immature stages of the L group was shorter than that of the S group. Notably, the pupal period was shortened significantly in the former group. The fecundity of female adults from the L group was higher, but not significantly so. Female adults in the L group laid significantly larger eggs than those in the S group. Phenotypic correlations between egg size and other traits were generally negligible. However, the correlation between the fecundity of females in the L group and the size of eggs they laid was significantly negative. These results suggest that egg size variation under fixed conditions affect fitness of offspring in *P. g. guttata*.

Key words: Small-sized egg; large-sized egg; life history trait; phenotypic correlation; phenotypic plasticity

INTRODUCTION
Egg size in insects is considered to be an adaptive trait responding to changing environments. The optimum size and number of eggs that maximize fitness differ under diverse environmental conditions such as variation in food resources and habitat quality. An adaptive response to a changing environment is commonly observed in insect egg size (Kimura and Masaki, 1977; Braby and Jones, 1995; Ernsting and Isaaks, 1997; Fox et al., 1997).

The migrant skipper, *Parnara guttata guttata*, has typically three generations per year and lays different sized eggs in different generations (Nakasuji and Kimura, 1984). Females of the overwintered and first generations lay smaller eggs on grasses with soft leaves in wet lowlands, while those of the second generation, which migrate in early autumn, lay larger eggs on grasses with tough leaves in dry uplands (Nakasuji, 1982a, 1987). The hatchlings from smaller eggs in the earlier generations cannot survive on grasses with tough leaves (Nakasuji and Kimura, 1984). In addition, a trade-off between the fecundity and egg size in females was observed among these three generations (Nakasuji and Nakano, 1990). Egg size variation among generations in *P. g. guttata* is considered to be an adaptive response (reaction norm) to seasonally different host grasses (Nakasuji, 1987). This variation is mainly determined by day length (Nakasuji and Kimura, 1984) though temperature and larval density also affect egg size (Hareyama et al., 1991).

Under the assumption previously mentioned, many *P. g. guttata* females can produce an optimum egg size in each generation. Therefore, egg size may be constant to some degree within each generation. However, egg size varies not only in the different generations but also among females within each generation considerably in *P. g. guttata* (Hareyama et al., 1991). These findings suggest that egg size in *P. g. guttata* is an important element in the adaptation not only between generations but also within a generation. To test this hypothesis, it is necessary to determine the influence of egg size differences on life history traits under fixed conditions (egg size variation under a fixed condition is regarded as under a generation).
In the present study, we compared the viability and development of offspring and the reproduction of female adults from larger or smaller eggs under fixed conditions in *P. g. guttata*. We also discuss the variation among females within a generation as connected to the variation in egg size among generations in *P. g. guttata*.

**MATERIALS AND METHODS**

The following procedures were designed to test whether the difference in egg size affects other life history traits under fixed conditions. First, eggs laid by females of the parent generation (P) were divided into two groups. Second, the traits related to fitness were compared between the offspring generation (F1) from each group (survival rate, developmental time, body size, F1 fecundity and F2 egg size). Moreover, the phenotypic correlations between traits were examined to clarify the influences of variation in egg size on the life history traits within each group.

Adults of *P. g. guttata* were collected in August and September, 1998, from fields in Okayama, Western Japan. A stock culture was established by the methods of Nakasuji and Honda (1979). Individuals for the experiments were randomly selected from the stock culture that had been maintained for two generations. The stock culture, the parent generation (P) and the offspring generation (F1) were reared under 16L8D at 25°C, which approximates conditions in July when the first generation adults emerge.

**Parent generation (P).** Larvae in the parent generation (P) were randomly selected from the stock culture and reared in a plastic cage at a density of 20 individuals per cage (35x25 cm, 35 cm in height) under 16L8D at 25°C. In the cage, emerged adults were fed a 10% honey solution supplied in artificial vinyl flowers. The adults were allowed to mate and lay eggs on rice seedlings (Nakasuji and Honda, 1979). Female adults that began to lay eggs were isolated in a cylindrical cage (8 cm in diameter, 18 cm in height) with a honey solution and rice seedlings. The 25 females were then mated and fifty eggs were randomly sampled for each female on the first or second day after mating. The diameter (d) and height of the eggs (h) were measured with a video-micrometer (OLYMPUS, VM-60). The volume (V) of eggs was calculated by the formula $V = \frac{\pi d^2 h}{6}$ on the assumption that shape is a half ellipsoid. The eggs were divided into two groups, i.e. those larger than 0.19 mm$^3$ (L group) and smaller than 0.16 mm$^3$ (S group). To clarify the influence egg size may exert, all eggs between 0.16 mm$^3$ and 0.19 mm$^3$ were discarded. The mean egg size was 0.149 mm$^3$ for the S group and 0.202 mm$^3$ for the L group. These values correspond to the size of eggs laid in the field by overwintered or first generation adults (0.15 mm$^3$) and by the second generation adults (0.20 mm$^3$) (Nakasuji and Kimura, 1984). Eggs in the L group accounted for 23.7% and eggs in the S group for 22.2% of all eggs measured.

**Offspring generation (F1).** The eggs were selected and placed on a piece of wet filter paper until they hatched. The hatching rate of both groups was about 90%. A nearly random sample of the hatchlings was individually introduced into a transparent plastic cup (4.5 cm in diameter and 3.5 cm in height) and fed rice seedlings. A piece of wet filter paper was placed at the bottom of the cup. Food was replenished at one- or two-day intervals. The developmental time (immature stage and pupal stage) and survival rate of immature-stage hatchlings (during first instar stage and from hatching to emergence) were measured through the course of individual rearings. The pupae were weighed on the day after pupation with a balance (A and D, FX-300N). The female adults that emerged were marked individually on the wings with a felt pen and allowed to mate with male adults of the same group in the plastic cage. Thirty-one females in the L group and 33 in the S group mated. The mated females were isolated in a cylindrical cage and allowed to lay eggs. The fecundity of each female and the size of fifty randomly selected eggs were measured on the first or second day after mating.

**RESULTS**

The survival rate of offspring (F1) in the immature stages was compared between the groups of large-sized eggs (L) and small-sized eggs (S) (Table 1). The survival rate of the first instar larvae did not differ between the two groups. In contrast, the survival rate to emergence was significantly higher in the L group (p<0.05, $\chi^2$-test). The developmental time in the immature stages and pupal
weight were compared between the two groups (Table 2). The larval period was shorter in the L group, although the difference was not significant in either sex. However, the pupal period of the L group was significantly shorter than that of the S group for both sexes (Table 2, *p* < 0.01 in males and *p* < 0.05 in females, Mann-Whitney *U*-test). The period through immature stages (the total of larval and pupal period) was significantly shorter in the L group than the S group in males. Pupal weight did not differ between the two groups. The fecundity of females was higher in the L group, but not significantly so. Eggs laid by females in the offspring generation (F₁) were significantly larger in the L group than the S group (Table 3, *p* < 0.001, Cochran-Cox’s test).

Phenotypic correlations between the life history traits of parents and offspring were measured in each group (Fig. 1; Table 4). The correlation coefficient between the size of eggs laid by parent females (F₁ egg size) and offspring females (F₂ egg size) was significantly positive when both groups were considered together (Fig. 1; Table 4, *p* < 0.001). However, the correlation between F₁ egg size and F₂ egg size was not significant in either group (Table 4). The correlation between fecundity of F₁ females (F₁ fecundity) and F₂ egg size was significantly negative in the L group (Table 4, *p* < 0.05), but not significant in the S group or in both groups together (Table 4). No significant correlations between F₁ egg size and pupal weight of the offspring generation (F₁ pupal weight) were found for each group and in both groups together (Table 4). No significant correlations between F₁ pupal weight and the size of F₂ eggs laid by F₁ females were found either (Table 4).

### DISCUSSION

The survival and success of offspring depends upon the initial biomass given to the young, which can be typically measured as the initial size of the propagule (Roff, 2002). Larger eggs and/or offspring have a higher survival rate, particularly under harsh conditions (Fox and Mousseau, 1996; Fox and Czesak, 2000; Fischer and Fiedler, 2001). Egg size also affects the development of larvae and the reproduction of adults after emergence (Braby, 1994; Tanaka, 1995; Fox et al., 1997; Fox and Czesak, 2000). The present study indicates that differences in egg size relate to viability, development and reproduction in *P. g. guttata*.

First, the survival rate throughout the immature stages was higher in the L group than the S group (Table 1). The survival rate of the first instar larvae that hatched from larger eggs did not differ from that of the larvae from smaller eggs (Table 1). Conversely, the survival rate of first instar larvae that hatched from smaller eggs (the larvae in the second generation) was markedly lower than larvae from large eggs.

#### Table 1. Comparison of survival rate between individuals hatched from large-sized (L) and small-sized (S) eggs

<table>
<thead>
<tr>
<th>Egg size</th>
<th>No. of hatchlings</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>During 1st instar stage</td>
</tr>
<tr>
<td>L (&gt;0.190 mm³)</td>
<td>167</td>
<td>95.8 n.s.</td>
</tr>
<tr>
<td>S (&lt;0.160 mm³)</td>
<td>183</td>
<td>94.5 n.s.</td>
</tr>
</tbody>
</table>

n.s. *p* > 0.05, *p* < 0.05, *χ²*-test.

#### Table 2. Comparison of developmental time and pupal weight between individuals hatched from large-sized (L) and small-sized (S) eggs (mean ± S.D.)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Egg size</th>
<th>n</th>
<th>Developmental time (days)</th>
<th>Pupal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Larvae</td>
<td>Pupae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>L¹</td>
<td>58</td>
<td>17.5 ± 1.5 n.s.</td>
<td>7.5 ± 0.8 *</td>
</tr>
<tr>
<td></td>
<td>S²</td>
<td>68</td>
<td>18.5 ± 2.8 n.s.</td>
<td>7.9 ± 0.6 **</td>
</tr>
<tr>
<td>Female</td>
<td>L³</td>
<td>81</td>
<td>19.0 ± 1.7 n.s.</td>
<td>7.4 ± 0.5 *</td>
</tr>
<tr>
<td></td>
<td>S⁴</td>
<td>62</td>
<td>19.4 ± 2.2 n.s.</td>
<td>7.6 ± 0.6 *</td>
</tr>
</tbody>
</table>

n.s. *p* > 0.05, *p* < 0.05, **p* < 0.01, Mann-Whitney *U*-test.
Mean egg size, ¹0.204; ²0.147; ³0.207; ⁴0.147.
The significance of pupal weight was tested using Student’s *t*-test.
larger eggs (those in the overwintered generation) as shown by Nakasuji and Kimura (1984). The difference between that study and the present study may be due to a difference in conditions of the host plants used in these experiments. The larvae were fed on cogon grass with tough leaves in the study by Nakasuji and Kimura (1984), whereas the larvae were fed on rice seedlings with soft leaves in the current study. In *P. g. guttata*, the influence of egg size on survival of young larvae appears under harsher environmental conditions (the hardness of the leaf in this case).

Second, the period through the immature stages was significantly shorter in the L group than in the S group in males, and the between-group differences in the pupal period were significant in both sexes (Table 2). In some species, the developmental time of individuals hatched from larger eggs is shorter (Stewart et al., 1991; Braby, 1994; Ernsting and Isaaks, 1997; Fox et al., 1997). In *Drosophila melanogaster*, the body size tends to be larger and the feeding rate tends to be higher among first instar larvae in females that lay larger eggs (Azevedo et al., 1997). In *P. g. guttata*, the head width of the first instar larvae hatched from larger eggs is large (Mitamura, 1956), and the feeding rate in the first instar larvae hatched from larger eggs appears to be higher. The higher feeding rate may shorten the developmental time by the accumulation of more energy in an initial larval stage. In *P. g. guttata*, the mortality caused by several parasitoids tends to in-
crease rapidly during the older larval or pupal stages (Nakasuji, 1982b; Matsumura, 1992). If the shorter developmental time can reduce attacks by parasitoids, the difference of egg size in *P. g. guttata* may also influence viability. For example, in the damselfly *Lestes sponsa*, the developmental time is shorter when the predator (perch) exists (Brodin and Johansson, 2002). No difference in pupal weight was found between the two groups (Table 2). This species may have a system by which the optimum body size is determined genetically and puation is induced at a certain body size. The long developmental time of the pupal stage in the S group may be to accumulate resources to achieve the body size determined genetically (Fox, 1997).

Third, the difference in egg size also affected the reproduction of adults. The fecundity between groups was not significant, but female adults from larger eggs laid significantly larger eggs than did females from smaller eggs in *P. g. guttata* (Table 3). Moreover, the phenotypic correlation between 

\[ F_1 \text{ egg size and } F_2 \text{ egg size was significantly positive when putting both groups together (Fig. 1; Table 4). Two factors may account for a positive correlation between parent and offspring. The first is a non-genetic factor such as a maternal effect. The difference in the accumulation of energy in the younger larval stages can influence the reproduction of adults after emerging (Falconer and Mackay, 1996; Fox et al., 1999; Heath et al., 1999; Potti, 1999). A female hatched from a larger egg may have more energy than one hatched from a smaller egg, and thus this may increase the allocation of resources per egg. The second is a genetic factor. There is a possibility that egg size variation in *P. g. guttata* is heritable. The high positive correlation between parent and offspring may indicate that egg size in *P. g. guttata* is primarily genetically determined. However, life history traits that are directly connected to fitness, such as egg size tend to have lower heritabilities because the genetic variance of these traits decreases with selection (Mousseau and Roff, 1987). It is necessary to verify whether egg size variation in *P. g. guttata* is heritable.

The results suggest that the difference in egg size affects fitness not only between generations but also within a generation. Females that lay larger eggs may be more adaptive because their survival rate was higher, the developmental time shorter and the size of the eggs larger. However, the results suggest that a significantly negative correlation, i.e. trade-off, existed between *F*1 fecundity and *F*2 egg size in the L group (Table 4). Under such conditions, there is an optimum egg size in each generation (Smith and Fretwell, 1974; Kishi, 1978). If the difference in egg size influences only the viability during the young larval period in *P. g. guttata*, it may be adaptive to lay many small eggs in the first generation and a few larger eggs in the second generation. However, many factors correlate with the optimum egg size, since egg size differences affects several traits correlated with fitness in the first generation of *P. g. guttata*. Moreover, when an environmental condition within a generation changes, the optimum egg size will also change. A change in leaf toughness can affect the survival rate of the first instar larvae in *P. g. guttata*, even if the host plant species is the same within a generation (Matsumura, 1992). If the leaf of the host plant is tougher than in normal years, an individual hatched from a large egg may have a better chance survival. Moreover, when the parasitoids of the older larvae or pupae are more plentiful than usual, the individual hatched from a large egg may be able to avoid parasitoids by hastening the developmental time as compared with that of larvae hatched from the small egg. Under these conditions, laying many small eggs may not necessarily be adaptive in females of the first generation. Thus, egg size variation may be maintained among females within a generation because many factors correlate with the optimum egg size and environmental conditions within a generation changes unpredictably.

Egg size variation among females within a generation can be maintained not only in the first generation but also the second or overwintered generation. Several factors that maintain egg size variation in each generation may affect not only the optimization of egg size but also the reaction norm of egg size among generations (i.e. phenotypic plasticity of egg size). Egg size variation among females within a generation may reflect various patterns of the reaction norm of egg size among generations (i.e. the possibility that the phenotypic plasticity of egg size among generations in *P. g. guttata* varies). To verify this hypothesis, it is necessary to establish pure lines with the same geno-
type and compare the reaction norms among the lines (Via, 1984; Falconer and Mackay, 1996; Lynch and Walsh, 1998).

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