Egg Load Influences Search Intensity, Host Selectivity, and Clutch Size in *Battus philenor* Butterflies

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Numerous studies have documented the influence of environmental factors such as host plant species and host quality on the oviposition behavior of female insects. This paper shows that an internal physiological factor, the number of mature eggs a female carries (egg load), correlates with host selectivity and clutch size in unmanipulated natural populations of the pipevine swallowtail butterfly, *Battus philenor*. In addition, search intensity and host selectivity differed among females whose egg loads were manipulated experimentally before they were released and followed in the field. Females with many eggs searched more intensely for hosts and were less selective when they encountered them.

**KEY WORDS:** Egg load; search intensity; selectivity; clutch size; oviposition behavior.

**INTRODUCTION**

Studies of oviposition behavior in insects have concentrated primarily on documenting environmental factors that affect behavior. For example, it has been shown for butterflies that the probability of oviposition on a plant is influenced by various plant characters, including species identity, size, and quality (e.g., David and Gardiner, 1962; Ma and Schoonhoven, 1973; Singer, 1971, 1983; Chew, 1977; Wiklund, 1975; Jones, 1977b; Rausher, 1978; Courtney, 1981; McDonald and McInnes, 1985). Oviposition response is also affected by the presence of conspecific eggs or larvae (e.g., Rothschild and Schoonhoven, 1977; Rothschild and Schoonhoven, 1977).

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Rausher, 1979a; Shapiro, 1980) or their mimics (Williams and Gilbert, 1981) and by microclimatic variation in oviposition sites (e.g., Singer, 1971; Rausher, 1979b; Williams, 1983; Grossmueller and Lederhouse, 1985). Such environmental factors have also been shown to influence prealighting search behavior (e.g., Jones 1977a; Stanton, 1982). This emphasis on the influence of the oviposition-site environment on female behavior is understandable considering the relationship between environmental quality and offspring survival (e.g., Chew, 1975, 1977; Gilbert and Singer, 1975; Wiklund, 1975; Smiley, 1978; Rausher, 1979a, 1980; Root and Kareiva, 1984; MacKay, 1985).

Considerably less attention has been given to examination of how internal (physiological) factors may influence insect oviposition behavior. While this topic has been explored theoretically (e.g., Jones, 1977b; Iwasa et al., 1984; Courtney, 1986; Chamov and Skinner, 1988), few empirical studies have been attempted. One exception is the work of Singer (1982), who showed that captive Euphydryas editha females become less discriminatory (i.e., female host selectivity changes) as the interval between oviposition events increases. His study suggests that some change in the internal state of a female affects her response to host quality. Pilson and Rausher (1988) describe a similar phenomenon in the pipevine swallowtail, Battus philenor. One possible explanation for these observations is that female response is influenced directly by egg load, which changes over time as eggs mature. One piece of evidence supporting this suggestion is that egg load in Battus philenor affects the type of activities to which a female allocates her time, e.g., whether she searches for oviposition plants, nectars, rests, or lays eggs (Odendaal, 1989).

To ascertain whether egg load plays a role in regulating oviposition behavior, we examined its relationship to host selectivity and clutch size in the pipevine swallowtail, two aspects of oviposition behavior in butterflies that have frequently been shown to be influenced by environmental factors (e.g., Klomp and Teerink, 1962; Stanton, 1979, 1982; Courtney, 1982; Mackay, 1985; McDonald and McInnes, 1985; Singer, 1986; Pilson and Rausher, 1988). The results of Singer (1982) and Pilson and Rausher (1988), as well as theoretical work by a number of authors (Iwasa et al., 1984; Parker and Courtney, 1984; Courtney, 1986; Skinner, 1985; Chamov and Skinner, 1988), suggest that selectivity will increase and clutch size will decrease with increasing egg load. In addition, we examined the influence of egg load on prealighting search behavior. We expected to see in Battus philenor a similar increase in rate of alighting with increasing egg load. This expectation was based on a study by Holling (1966), which showed that in preying mantids the rate of attack on prey items increases as hunger rises, and also on Root and Kareiva's (1984) demonstration that probability of alighting by Pieris rapae is dependent upon a female's egg complement.
MATERIALS AND METHODS

Study Animals and Site

The pipevine swallowtail is distributed throughout the southern United States and Central America. Our study was carried out in the open pine uplands [pine-bluestem savanna of Vogl (1972) and Streng and Harcombe (1982)] of the John Henry Kirby State Forest in Tyler Country, Texas. The two host plants, Aristolochia reticulata (Aristolochiaceae) and A. serpentaria, are small, erect perennial herbs in this habitat. Battus philenor females fly low over the natural vegetation and alight on numerous host and nonhost plants until they encounter a host on which they will lay eggs. After laying one or a few eggs they usually resume searching. At any given time, a female uses one of two distinct search modes distinguishable by the shape of the leaves (broad vs narrow) on which they alight (Rausher, 1978; Rausher and Papaj, 1983). Females searching in the broad-leaf mode tend to alight preferentially on the broad-leaved host, A. reticulata, while those searching in the narrow-leaf mode tend to alight preferentially on the narrow-leaved host, A. serpentaria. During the first half of the season most females use the broad-leaf mode, but during the second half of the season most use the narrow-leaf mode (Rausher, 1979; Papaj and Rausher, 1983). The data reported here were collected during the 1987 field season (March to May).

Note on Dissections

Like other butterflies, Battus philenor typically has eight ovarioles (e.g., Ehrlich and Ehrlich, 1978; Dunlap-Pianka, 1979) coiled up in the posterior abdominal fat tissue, with each ovariole containing a linear arrangement of progressively enlarging follicles. Four ovarioles combine into each of two common tubes that finally form the oviduct that ends in the ovipore. A follicle consists of an oocyte surrounded by nurse cells and enclosed in a follicular envelope. Following completion of oocyte growth, the nurse cells secrete the chorion, and the mature oocytes ovulate from the follicular envelope into one of the eight branches of the oviduct. In the process of ovulation in Battus philenor, the empty follicular envelopes form an orange structure in the ovariole; eggs on the ovipore side of this structure are fully developed, chorionated, ovulated, and ready to be laid. These eggs were counted as mature eggs. Sometimes a few eggs on the other side of the orange structure also appear to have reached their full size, but they are not yet ovulated or fully chorionated and are not counted as mature eggs.
Definitions of Investigated Behavioral Parameters

We define **search intensity** as the number of times a female searching for hosts alighted on any type of plant, hosts and nonhosts, per unit time. **Host selectivity** is defined as one minus the ratio of the number of host plants oviposited on divided by the total number of host plants alighted on in a 5-minute period (see also Stanton, 1982; Courtney, 1982; MacKay, 1985). A low value therefore indicates a low degree of selectivity. Finally, **clutch size** is the number of eggs laid on an individual host plant by a female.

**Butterflies Observed in the Field**

Thirty-eight females encountered in the field were followed by an observer with a hand-held computer that recorded times of all alightings on hosts and nonhosts and all egg-laying events. All butterflies were followed in a 100 × 100 m² area. After 5 min females were collected for dissection. They were immediately killed and put on ice to preserve the ovarian state at the time of capture. Wing length and wing wear, a standard index used to assess butterfly age in the field (e.g., Watt et al., 1977; Iwasa et al., 1983), were recorded. Papaj (1986) reports a good correlation between age and wing-wear conditions in East Texas populations of *B. philenor*. For this particular study, four wear gradations were used, ranging from 1 for fresh individuals to 4 for ragged, scale-bare individuals. Mature eggs were counted and egg load was calculated by adding the number of eggs laid during the 5-min observation period to the number they contained at dissection. Correlations were performed between wing length, age, and egg load. Correlations between egg load and clutch size were performed separately for clutches laid on *Aristolochia reticulate* and *A. serpentaria*, and correlations between egg load and search intensity and host selectivity were performed separately for broad- and narrow-leaf searchers. A female was treated as a broad-leaf searcher when more than two-thirds of her alightings were on broad-leaved plants and as a narrow-leaf searcher when more than two-thirds of her alightings were on narrow-leaved plants (Rausher and Papaj, 1983). The remaining females were classified as intermediate searchers; because there were only three such females, they were not used in the analysis.

**Experimental Procedures**

We supplemented our field data from unmanipulated females by experimentally manipulating the number of mature eggs a female carried. Mated, young females [with fresh and undamaged wings, meaning 1 or 2 days old (Lederhouse and Odendaal, unpublished data)] were collected in the field, kept in cages for about 48 h, and fed twice daily. “Full” and “empty” females were then manufactured by manipulating their access to host plants. Females
in one group were placed on a young, fresh host plant to elicit oviposition. Subsequently each female was offered a series of hosts in succession over a period of about 10 min until oviposition was no longer elicited. A female was regarded as empty, and hence no longer responsive, when she did not start to oviposit within 2 min after being placed on a host. This criterion was less stringent than that previously used by Odendaal (1989); in that study a female was classified as empty only when she did not oviposit after being placed on four successive plants. We used this altered criterion to prevent complete egg depletion because females with no eggs tend to be inactive (Odendaal, 1989) and would yield no data on the three behavioral parameters under investigation. Females in the second group were placed on nonhost plants (to control for handling effects) and they did not lay eggs. They were regarded as full. The mean number of mature eggs was 1.7 times larger for full females (X = 44, n = 10, vs X = 26, n = 7, for full and empty females, respectively). This difference was marginally significant (P < 0.09, Mann–Whitney U test). For more data on egg load see Odendaal (1989).

Immediately following the above procedure, females were put carefully on vegetation in the field. As soon as they began searching, they were followed for 5 min by observers. We were able to follow and recapture 13 full and 11 empty females. Search intensity, host selectivity for narrow-leaf searchers, and clutch size on A. serpentaria were analyzed by one-way ANOVA, but the small sample size for empty females precluded analysis of host selectivity for broad-leaf searchers and for clutch size on A. reticulata.

RESULTS

For unmanipulated females (Table I), the search intensity of broad- and narrow-leaf searchers and the host selectivity of broad-leaf searchers were not significantly correlated with egg load. We note, however, that the signs of these correlations are all as would be expected if increasing egg load leads to increased search intensity and increased selectivity. Host selectivity in narrow-leaf searchers was correlated significantly with egg load, and butterflies became less selective as egg load increased. Clutch size was significantly correlated with egg load for both narrow- and broad-leaf searchers and became larger as egg load increased. Age, size, and egg load were not correlated significantly inter se for unmanipulated females.

Search intensity of manipulated females was first analyzed by a two-way ANOVA with experimental treatment and search mode (broad-leaf, narrow-leaf, or intermediate searchers) as main independent effects. Search mode was included as an effect because Rausher and Papaj (1983) report that search intensity is higher for narrow-leaf searchers. Although treatment showed a significant effect on search intensity (F = 4.18, P = 0.0550), search mode did not (F =
Table I. Correlations (Spearman Rank) Between Behavioral Parameters, and Egg Load for Unmanipulated Females

<table>
<thead>
<tr>
<th>Statistic</th>
<th>X</th>
<th>n</th>
<th>SE</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Search intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broad</td>
<td>36</td>
<td>17</td>
<td>2.65</td>
<td>0.1090</td>
<td>0.6770</td>
</tr>
<tr>
<td>Narrow</td>
<td>45</td>
<td>18</td>
<td>3.47</td>
<td>0.3436</td>
<td>0.1627</td>
</tr>
<tr>
<td>Selectivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broad</td>
<td>0.84</td>
<td>23</td>
<td>0.06</td>
<td>0.2690</td>
<td>0.2144</td>
</tr>
<tr>
<td>Narrow</td>
<td>0.67</td>
<td>19</td>
<td>0.07</td>
<td>0.5269</td>
<td>0.0204</td>
</tr>
<tr>
<td>Clutch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broad</td>
<td>2.42</td>
<td>7</td>
<td>0.68</td>
<td>0.9337</td>
<td>0.0021</td>
</tr>
<tr>
<td>Narrow</td>
<td>1.84</td>
<td>14</td>
<td>0.27</td>
<td>0.6786</td>
<td>0.0076</td>
</tr>
</tbody>
</table>

*X = mean; n = sample size; SE = standard error; r = correlation coefficient; P = significance level. Search intensity = No. of alightings/5 min; selectivity = 1 - No. of hosts oviposited on/total No. of hosts alighted on.

2.06, \( P = 0.1546 \)), and no interaction existed between search intensity and search mode (\( F = 0.06, P = 0.8114 \)). We therefore decided to analyze search intensity by one-way ANOVA. Table 2 shows that there was again a significant overall effect of treatment ("full" vs "empty") on search intensity.

Host selectivity in narrow-leaf searchers was also significantly affected by the treatment (Table II), but clutch size on *A. serpentaria* did not differ significantly for full and empty females, although the trend was in the expected direction (i.e., full females produced larger clutches). Full narrow-leaf searchers encountered more hosts (mean = 2.5 hosts, \( n = 4, \) range = 1–6) than empty females (mean = 1.6 hosts, \( n = 9, \) range = 0–6) in a period of 5 min.

**DISCUSSION**

Our results suggest that females modify their searching intensity, host selectivity, and clutch size in response to a change in egg load or some internal factor correlated with egg load. All nine relationships between egg load and behavioral variables examined (Tables I and II) were in the expected direction. Five were statistically significant. These results, as well as those of Odendaal (1989), suggest that changes in egg load, or some factor associated with it, may also have caused the changes in clutch size and host selectivity in *Euphydryas editha* observed by Singer (1982) and *Battus philenor* observed by Pilson and Rausher (1988).

While our results do not permit us to identify changes in egg load definitively as the main factor causing changes in behavior, such a conclusion is reasonable for several reasons. First, simple mechanisms involving stretch
**Table II.** (A) Data and Results of One-Way ANOVA for Manipulated Butterflies; (B) Analysis of Variance for the Above Variables

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intensity</th>
<th>Selectivity (narrow)</th>
<th>Clutch (narrow)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Narrow</td>
<td>Broad</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Empty</td>
<td>X</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>3.67</td>
<td>5.01</td>
</tr>
<tr>
<td>Full</td>
<td>X</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>3.03</td>
<td>7.26</td>
</tr>
</tbody>
</table>

### B

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Treatment</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>SS</td>
</tr>
<tr>
<td>Intensity</td>
<td>1</td>
<td>1647.21</td>
</tr>
<tr>
<td>Selectivity (narrow)</td>
<td>1</td>
<td>0.85</td>
</tr>
<tr>
<td>Clutch (narrow)</td>
<td>1</td>
<td>9.54</td>
</tr>
</tbody>
</table>

*Search intensities (No. of alightings/5 min) for "full" and "empty" narrow, broad, and intermediate searchers are presented. Selectivity (1 - No. of hosts oviposited on/total No. of hosts alighted on) for narrow-leaf searchers and average clutch size on *A. serpentina* are also represented. X = mean; n = sample size; SE = standard error.*

Receptors could allow females to detect abdominal distension associated with eggload in *Battus philenor*. Such mechanisms exist in the bug *Dipetalogaster maximus* (Nijhout, 1984), where stretch receptors detect abdominal distension associated with taking a blood meal. By analogy, we suggest that stretch receptors detect abdominal distension caused by increasing egg load in *Battus philenor*.

Second, female fitness should be increased by modifying behavior in response to egg load, as several models have demonstrated. Iwasa *et al.* (1984) and Parker and Courtney (1984) showed that when mortality occurs between oviposition events, clutch size should increase and selectivity should decline as the number of eggs available to be laid increases. Parker and Courtney (1984) concluded that clutch size should increase with increasing search costs for oviposition sites. Skinner (1985) showed by computer simulation that a female should lay all currently mature eggs on an oviposition site, up to (but no exceed-
ing) the optimal clutch size. Insects with larger egg loads should thus lay larger clutch sizes. Charnov and Skinner (1988) explicitly linked egg load and egg maturation to previous models predicting clutch size and selectivity.

Although no one has, to our knowledge, addressed theoretically how search intensity should be affected by egg load, we expect a female with a large egg load to search more intensely than one with a small load. By increasing her search intensity, a female presumably increases her host-finding rate and her chances to lay eggs. If the proportion of hosts to nonhosts stays constant, she would obviously encounter more hosts as the small data set in our results indicate. Presumably, the reason that females with small egg loads do not search with maximal intensity is that there is also a cost. A likely candidate for such a cost is an increased susceptibility to predation, since in general, increased movement by insects renders them more susceptible to predation [(Edwards and Wratten, 1982; Schultz, 1983a,b); for predation on Battus philenor on our study site see Odendaal et al. (1987)].

The effect of egg load on oviposition behavior may also explain two further observations on our field site. On one occasion we observed a female butterfly moving at great speed from plant to plant, apparently attempting to oviposit on hosts and nonhosts alike. After capture it was established that her abdomen was greatly extended with a massive egg load but her ovipositor damaged, with a cluster of eggs blocking the ovipore and preventing her from laying any eggs. We also commonly observed bursts of searching and egg-laying activity at the onset of changes in good weather after a rainy period (see also Gossard and Jones, 1977).

This study supports the theoretical expectation (see above) that internal mechanisms play an important role in mediating female insect behavior. Together with the empirical work by Singer (1982), Pilson and Rausher (1988), and Odendaal (1989), it suggests that studies of egg load or associated factors may help explain insect behavioral responses. Furthermore, since environmental conditions affect egg development (e.g., Stern and Smith, 1960; Rankin, 1974; Barker and Herman, 1976; Gossard and Jones, 1977; Pouzat, 1978), egg load and associated physiological factors may form a mechanistic link between environmental stimuli and female behavior. Studies on vertebrates have already shown that the internal physiological state commonly mediates the response of individuals to external stimuli (for review, see Farner et al., 1971–1983; Carter, 1974; Lofts, 1974; Rankin et al., 1983; Van Tienhoven, 1983; Pitcher, 1986). We expect similar studies on insects to lead to similar conclusions.

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REFERENCES


