Comparison of oviposition and larval development of Leptosia nina Fabricius (Lepidoptera: Pieridae) on two different food plants in different seasons

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Comparison of oviposition and larval development of *Leptosia nina* Fabricius (Lepidoptera: Pieridae) on two different food plants in different seasons

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**Abstract**

Information on food plant preference and seasonal variation in life cycle attributes of butterflies are vital in their role for environmental conservation. The food plant range of the psyche butterfly, *Leptosia nina* Fabricius, is comprised of *Capparis*, *Crateva* (Capparaceae) and *Cleome* (Cleomaceae) species in its distribution range comprising Oriental, east Palearctic and Mediterranean regions. However, in northeastern parts of India which lie on the western flank of the Indo-Myanmar biodiversity hotspot, where *Capparis* and *Crateva* plants are absent, *L. nina* uses two food plant species, *Cleome monophylla* (Cleomaceae) and *Rorippa indica* (Brassicaceae), only for oviposition and larval development in different proportions in different seasons; females used *C. monophylla* in higher proportions during the summer and rainy seasons but used *R. indica* in higher proportions in autumn and winter. Here *R. indica* is recorded as a novel host record of *L. nina* from its distribution range. Between the two plant species, development time did not show variation when host transfer experiments were carried out. Host transfer experiments showed that both *C. monophylla* and *R. indica* have the same value as larval food. Results suggested that *L. nina* larvae showed adaptation to a narrow range of hosts in the area of this study. This pattern seems to be consistent with the patch dynamic hypothesis of host selection proposed by Thomson in 1988 according to which geographic variation in host use follows geographic variation in the relative abundance of potential hosts. This has important implications for the association of hosts and butterflies and their conservation.

**Key words**
Larval food plants, larval development, oviposition, Psyche butterfly, seasonal variations.

**Introduction**

The selection of proper food plants for growth and development of insect larvae by ovipositing females is crucial to establishment of phytophagous species and speciation (Tabushnik, 1983; Nylin et al., 2009; Dennis, 2010). This involves availability of taxonomically correlated plants (Thorsteinson, 1960), appearance (minimum density per unit area) of acceptable hosts (Bernays and Chapman, 1994) and tradeoffs between preference by females for oviposition and performance by developmental stages of insects (van Nouhuys et al., 2003). The choice of food plants by a species is often limited to a few habitats where selected food plants occur in abundance (Gilbert and Singer, 1975; Wiklund, 1977). Butterflies are very selective in the choice of their habitats for food plants for maximum fitness (Wiklund, 1977; Rausher, 1979; Bonebrake et al., 2010). Ovipositing females prefer habitats in which larval growth and development are good and avoid habitats and food plants in which growth and development are poor (Gilbert and Singer, 1975). It is established that, given a choice of several potential food plants, a female is likely to lay most eggs on the most preferred food plant, fewer eggs on the next preferred food plant, and so on (Jaenike, 1990; Lytan and Firake, 2012). Selection of food plants also vary with geographical regions (Nylin et al., 2009). For example, the recorded larval food plants of the psyche butterfly, *Leptosia nina* Fabricius (Lepidoptera: Pieridae) are *Capparis rheedii* L., *Capparis spinosa* L., *Capparis zeylanica* L., *Crateva adansonii* DC (family: Capparaceae), *Cleome viscose* L. and *Cleome monophylla* L. (family: Cleomaceae) in its distribution range comprising tropical and sub-tropical parts of the Oriental region, parts of eastern Europe, and the Mediterranean region (Kunte, 2000). Its preferred habitats are green and moist lands comprising of farms, other open fields, edges of drains and ditches covered with herbage, moist deciduous forests, and it occurs throughout the year (Agarwala et al., 2010). However, on the tropical western flank of the Indo-Myanmar biodiversity hotspot in Tripura, a hot and humid province in northeast India, larvae of *L. nina* are recorded only from *C. monophylla* and *Rorippa indica* (L.), Hiern, (family: Brassicaceae). Both the plant species are wild

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herbs with short rhizomes and erect stems, occur on well drained surfaces, open land with grasses and herbs, farms and roadside drains in urban and rural areas in north east India (Deb, 1981, 1983), and are endemic to the Indo-Malayan region, Madagascar and other Indian Ocean Islands (Grubb and Denton, 2004).

*Leptosia nina* is oligophagous (feeding on host plants of closely related genera or families) and shows a wide choice of food plants in its distribution range; however the food plant choice is found to be narrow in the study area. This butterfly might show a local adaptation to *R. indica* because *Capparis* and *Crateva* hosts are absent from the Indo-Malayan region of Asia (Deb, 1981, 1983). This study was aimed at recording the performance of *L. nina* on the two food plant species, *C. monophylla* and *R. indica*, especially oviposition by females and development time of immature stages in different seasons. It was predicted that natural populations of *L. nina* should show a similar preference for oviposition by females and larval performance to two different food plant species if these are essential for its development and reproduction (Tabashnik, 1983; Price, 1997; Tiple et al., 2011). However, there were no experimental studies to show that both *C. monophylla* and *R. indica* have the same value as larval food.

Materials and methods

Study site

The study area, Trishna wildlife sanctuary is a moist deciduous forest (area: 194.70 km2) located between 23°29'04.31"N, and 91°17'13.19"E adjacent to the international border with Bangladesh. A study site of 1.5 km2 was selected in the protected zone of the sanctuary for this study. The study site comprises open fields with low herbage, clumps of bamboos and deciduous trees, grasslands, ditches and canals which are characteristic of a large part of the Indo-Myanmar hotspot region.

Distribution of eggs on food plants in field

In the field, eggs of *L. nina* which were laid singly on the undersides of leaves could be easily identified under a hand-held magnifying lens (10x) by their spindle shape. The individual egg stands on one end and, on an average (mean ± SE), measures 1.23 ±0.05 mm long and 0.56 ± 0.02 wide (n=40). Freshly laid eggs appear bluish green in the first 24 hours which, gradually turn to pale yellowish green in the next 24-60 hours, and finally to deep yellowish green as the eggs near hatching time. Walk censuses for distribution of eggs on *C. monophylla* and *R. indica* were held along two 500 m long parallel line transects separated by an average distance of about 20 m every seven days from March 2007 to February 2009. The censuses were done between the hours of 8 am to 11 am in the rising phase of the sun when the maximum number of butterflies visit their hosts for food collection and oviposition. The walking pace was kept slow but at uniform speed in order to maximize the records of food plants and eggs of *L. nina*. In addition, after the census hours, some of the females were trailed to their oviposition sites to confirm the host selection. Specimens of all the food plants selected by females for oviposition were collected, preserved and identified by comparing the specimens in the herbarium of Plant Taxonomy and Biodiversity laboratories of the Tripura University. *Cleome monophylla* and *R. indica* were found to be the only hosts of *L. nina* for oviposition and larval development in the area of this study. Numbers of plants of the two species present and numbers of those found with one or more psyche eggs were recorded during walk censuses in the two years of study. Ten leaves, selected at random, of each *C. monophylla* and *R. indica* plant encountered in walk censuses were individually examined for eggs of *L. nina* and the average number of eggs per ten leaves of the two plant species were determined based on the average of the two years.

Development of immature stages

Leaves of the two food plant species with bluish green eggs of approximately 0-24 hrs age were collected from the field and brought to the laboratory. In the absence of information on their exact age, a median value of 12 hrs was used for determining the development time of the egg. Single eggs were kept in 10 cm diameter paired Petri dishes lined with water-soaked tissue papers. These were observed at 6-8 hours intervals between 8am and 8pm to record the development time of eggs, larvae, and pupae. Mortality in development, if any, was recorded. This was repeated 20 ~ 30 times on each of the two plant species to record any variation in different seasons, once in December (winter: December – February; average temperature = 19.23°C, average humidity = 46.67%, rainfall = 32.80 mm), in March (spring: March – April; average temperature = 27.88°C, average humidity = 65.25%, rainfall = 44.80 mm), in May (summer: May – June; average temperature = 30.70°C, average humidity = 75.75%, rainfall = 647.50 mm), in August (rainy: July – September; average temperature = 30.43°C, average humidity = 65.50%, rainfall = 1066.90 mm), and in October (autumn: October – November; average temperature = 25.10°C, average humidity = 58.75%, rainfall = 288.00 mm), respectively. Daily records of climate data were obtained from the nearest meteorology station of the Agriculture department, Government of Tripura at Udaipur, about 3 km from the study site.
Host transfer experiments

The bluish green eggs of approximately 0-24 hrs age were collected from C. monophylla and R. indica leaves in fields and were subjected to reciprocal transfer of hosts to record the development of larvae in a new food environment. Single eggs were securely placed in the laminar area of fresh leaves of potted plants of similar age on the field host (control) and alternate host (treatment). Two treatments were set up simultaneously using eggs laid on leaves of C. monophylla and R. indica. This was repeated twelve times for each treatment once in December, in March, in May, in August, and in October, respectively, to confirm the effect of seasonal variation. Egg hatching, larval molt, pupation and adult emergence were recorded at 8am, 2pm and 8pm during the period of development. All experiments were set up at room temperature in the semi-natural environment of the wildlife sanctuary.

Data analysis

Average values of field data on proportion of food plants of the two species used by L. nina for laying eggs and their frequency per leaf in different seasons based on records of two years were used to draw population curves and these were compared between the two food plant species using a non-parametric test and regression analysis. For this purpose weekly data were pooled on a monthly basis. Means of development time (in days) of larvae on the two plant species were compared by Student t-test and between different seasons by one way ANOVA and Tukey’s multiple comparison tests. Data on egg distribution on two host species in the field were subjected to regression analysis for understanding the relationship between oviposition sites and host utilization. Data on development time were also correlated with climatic factors of temperature and relative humidity. Best fit linear curves were used to get the regression equations and correlation values. Average of minimum and maximum data of temperature and humidity, and quantity of rainfall each day of the two years were used for each season. The computer program Origin 7 (www.originlab.com) was used for statistical analysis.

Results

Distribution of eggs on food plants

Figures 1a and b, respectively, shows the average numbers of two years of C. monophylla and R. indica plants and those with one or more eggs of L. nina recorded month-wise at the study site. Figure 2 shows the average number of eggs per ten leaves of C. monophylla and R. indica recorded in different months in the two years of study.

The two food plant species, C. monophylla and R. indica, are annual herbs which grow in the wild and show seasonality in vegetative growth and flowering. Both the plant species in vegetative stage were found throughout the year in the study site. Between the two species, C. monophylla occurred in greater abundance in the summer and rainy months whereas R. indica occurred in greater abundance in the autumn, winter and spring months.

![Figure 1](image-url)
Oviposition and larval development of *Leptosia nina* (Figs 1a and b). Females laid eggs in singles at the edges and on the undersides of tender or young leaves of *C. monophylla* and *R. indica* throughout the year; 65% to 92% of *C. monophylla* (n=345) and 45% to 82% of *R. indica* plants (n=294), respectively, were found with one or more eggs. Between the two food plant species, higher numbers of *C. monophylla* plants were selected for oviposition in summer (May-June) and rainy (July-September) seasons and the maximum number of egg-bearing plants was recorded in July (Fig. 1a, Mann-Whitney: *Hc* = 8.001, p < 0.001). In comparison, *R. indica* plants were selected in higher numbers for oviposition in autumn and early winter months with the maximum number of egg-bearing plants recorded in May (Fig. 1b, Mann-Whitney: *Hc* = 8.017, p < 0.001).

Average number of eggs per ten leaves recorded on the two plant species in the two years did not show significant difference (mean ±SE: *C. monophylla*: 1.39 ±0.33 eggs, *R. indica*: 1.25 ±0.16 eggs, *t* = 0.99, *df* = 637, *p* = 0.32). However, the frequency of egg distribution on the two plant species was somewhat different (Fig. 2). The average number of eggs per ten leaves on *C. monophylla* gradually increased from January to August and decreased in the following months.

In comparison, the average number of eggs laid per ten leaves on *R. indica* showed an increasing trend from January to October but with two sharp dips noticed in April and September, respectively. Regression analysis

![Fig. 2. Average number based on two years of sampling of *L. nina* eggs recorded per ten leaves of *C. monophylla* and *R. indica* in different months.](image)

![Fig. 3. Relationships between the number of eggs recorded and number of leaves observed in field based on average of two years of study. (a) *C. monophylla*, (b) *R. indica*.](image)
between numbers of eggs counted and number of leaves observed of the two food plant species showed positive linear relationships (Fig. 3a and b). The relationship was found to be stronger in the case of _C. monophylla_ (Fig. 3a: \(r = 0.96\), errors= 4.53) than that of _R. indica_ (Fig. 3b: \(r = 0.47\), errors=15.82).

### Development time of immature stages

Development time, period (in days) of development of eggs in the bluish green stage to eclosion of pupae, was found to vary in different seasons on both the food plant species (Table 1; ANOVA, _C. monophylla_ : \(F=2822.00\), \(df=4\), \(p=0.001\); _R. indica_: \(F=4624.64\), \(df=4\), \(115\), \(p=0.001\)). It was longest in December and shortest in August. The development times on the two plant species were not significantly different in other months (Table 1). Regression analysis of development time with temperature and relative humidity recorded in different months of the year showed inverse relationships (temperature: _C. monophylla_: \(y=54.58-1.06x\), \(r=-0.76\); _R. indica_: \(y=54.32-1.05x\), \(r=-0.74\); relative humidity: _C. monophylla_: \(y=60.15-0.48x\), \(r=-0.81\); _R. indica_: \(y=60.33-0.48x\), \(r=-0.80\)). Development time of eggs, larvae and pupae on the two host plant species showed an almost similar trend (Table 1). Egg hatching time varied in different seasons (ANOV A: _C. monophylla_: \(F=46.87\), \(df=4\), \(115\), \(p<0.001\); _R. indica_: \(F=30.42\), \(df=4\), \(115\), \(p<0.001\)) but did not show a difference between the two food plant species in any one season. The egg period was longest in December and shortest in August. First instar larvae were relatively immobile and chewed on soft leaf tissues in the immediate vicinity of oviposition sites. Second instar and older larvae (third, fourth and fifth) showed limited movements and fed actively from edge to centre of the leaves. Likewise, the development time of larvae and pupae showed significant differences in different seasons (ANOVA: _C. monophylla_: \(F=426.75\), \(df=4\), \(115\), \(p<0.001\); _R. indica_: \(F=401.81\), \(df=4\), \(115\), \(p<0.001\)) but did not show a difference between the two host species in any one season (Table 1).

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**Table 1** Mean values of development time (in days) of _L. nina_ on _C. monophylla_ and _R. indica_. Values in parenthesis following means under column 'Total' represent SE. The same letter following mean values in each row denote insignificant differences between the means by Student’s t-test \((p>0.05)\); different number in a column denote significant differences between means by Tukey’s multiple comparison test \((p<0.05)\).

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th><em>C. monophylla</em></th>
<th></th>
<th><em>R. indica</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Egg</td>
<td>Larva</td>
<td>Pupa</td>
<td>Total</td>
</tr>
<tr>
<td>December</td>
<td>30</td>
<td>4.2±1</td>
<td>12.1±1</td>
<td>18.1±1</td>
<td>34.4(0.1)±1</td>
</tr>
<tr>
<td>March</td>
<td>20</td>
<td>4.3±1</td>
<td>12.1±1</td>
<td>17.5±1</td>
<td>33.9(0.2)±1</td>
</tr>
<tr>
<td>May</td>
<td>20</td>
<td>3.5±2</td>
<td>10.5±2</td>
<td>9.4±2</td>
<td>23.4(0.5)±2</td>
</tr>
<tr>
<td>August</td>
<td>30</td>
<td>3.1±3</td>
<td>9.2±3</td>
<td>8.8±3</td>
<td>21.1(0.2)±3</td>
</tr>
<tr>
<td>October</td>
<td>20</td>
<td>4.4±1</td>
<td>11.1±4</td>
<td>15.1±4</td>
<td>30.6(0.4)±4</td>
</tr>
</tbody>
</table>

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**Table 2** Mean (SE) values of total development time of all stages of _L. nina_ on field host (control) and on transferred host (treatment) recorded in different seasons. The same letter following mean values in each row denote insignificant differences between the means by Student’s t-test \((P>0.05)\); different number in a column denote significant differences between means by Tukey’s multiple comparison test \((p<0.05)\).

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th><em>C. monophylla</em></th>
<th>to <em>R. indica</em></th>
<th><em>R. indica</em></th>
<th>to <em>C. monophylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>(treatment)</td>
<td>Control</td>
<td>(treatment)</td>
</tr>
<tr>
<td>December</td>
<td>30</td>
<td>34.4(0.1)±1</td>
<td>34.9(0.2)±1</td>
<td>34.6(0.2)±1</td>
<td>34.9(0.3)±1</td>
</tr>
<tr>
<td>March</td>
<td>20</td>
<td>33.9(0.2)±1</td>
<td>34.0(0.1)±1</td>
<td>33.9(0.2)±1</td>
<td>34.0(0.1)±1</td>
</tr>
<tr>
<td>May</td>
<td>20</td>
<td>23.4(0.5)±2</td>
<td>23.8(0.4)±2</td>
<td>23.7(0.6)±2</td>
<td>23.1(0.4)±2</td>
</tr>
<tr>
<td>August</td>
<td>30</td>
<td>21.1(0.2)±3</td>
<td>21.1(0.3)±3</td>
<td>21.0(0.2)±3</td>
<td>21.5(0.2)±3</td>
</tr>
<tr>
<td>October</td>
<td>20</td>
<td>30.6(0.4)±4</td>
<td>31.9(0.2)±4</td>
<td>30.5(0.4)±4</td>
<td>31.9(0.5)±4</td>
</tr>
</tbody>
</table>
Host transfer experiments

Results of development time of *L. nina* fed on food plant species in the field and in the laboratory in the two treatments are presented in Table 2. In both the experiments, the larvae accepted the alternate host and the development time did not differ significantly by host plants in any month.

Discussion

Natural populations of phytophagous insects including butterflies frequently encounter a wide choice of food plants of differing suitability according to habitats and resource sizes (Hooks and Johnson, 2001; Badeness et al., 2004; Dennis et al., 2006). Geographical variation in host plant utilization has been recorded in several butterfly species (Tabashnik, 1983; Dennis, 2010). However, the dominant strategy among herbivorous insects involves specialization on a set of closely related plants that will maximize offspring survival and fitness (Ehrlick and Murphy, 1988; Ward and Spalding, 1993; Gibbs et al., 2006). Against this background, it is evident that *L. nina* has adopted two plant species, *C. monophylla* and *R. indica*, in the present area of study. The latter species is a new food plant record for this butterfly from its entire distribution range of Oriental, east Palearctic and Mediterranean regions (Kunte, 2000, 2005). *Rorippa* plants show a phylogenetic affinity with other recorded hosts in the Capparaceae, Cleomaceae and Brassicaceae, all of which belong to the order Brassicales.

The results of this study suggest that in the western flank of its Indo-Myanmar geographic range, *L. nina* females did not use the two plant species in direct proportion to their natural abundance. Females used *C. monophylla* in higher proportions during the summer and rainy seasons but used *R. indica* in higher proportions in autumn and winter. Seasonal preference in host use by *L. nina* may be partly attributed to seasonal differences in their relative abundance (Fig. 1) and partly to seasonal differences in chemical components which induce oviposition behavior, a factor which was not evaluated in this study because results from laboratory experiments showed that larval performance on the two plant species did not differ in any season. Thus, larvae show local adaptation to *R. indica* in the area of study just as this species is adapted to *Capparis* and other hosts in other parts of its distribution range (Kunte, 2000, 2005).

This pattern of host use by *L. nina* has consequences for its population dynamics (Hanski and Singer, 2001). Unlike monophagism, where food plant-herbivore association is based on resource size and optimal synchronization of their respective life-cycles, phytophagous populations living on short-lived patchy food sources usually show oligophagism or polyphagism (Price, 1997; Dixon, 1998; Nylin *et al*., 2009). Such populations must find adequate numbers and kinds of food resource to sustain their survival, development and reproduction in both lean and peak periods (Thomson, 1988). Optimization theory predicts that food width of a species should match its life history requirements in time and space (Begon *et al*., 1996; Scheirs and Bryn, 2002). Smaller or bigger food width could be disadvantageous in terms of inter- or intra-specific competition for food resource except in extreme circumstance of scarcity (Quinn *et al*., 1998). This appears to be true for *L. nina* which uses only two host plants in the study area. In the present study, no other insect species was found to feed on these plants except for a small population of the mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae), which sucked plant juices of *R. indica* in summer and rainy seasons (Agarwala and Das, 1998; Agarwala *et al*., 1998). Thus, *L. nina* did not face direct competition with other phytophagous insect species on the two food plants. Both the plant species shared a phylogenetic relationship, are relatively abundant and show similar habitat preference (Deb, 1981, 1983; Hall *et al*., 2002). Kuussaari *et al*., (2000) showed that this pattern of host plant use is consistent with the interaction between host plant distribution and spatial variation of adult butterfly preference. This pattern also vindicates the patch dynamic hypothesis of host selection which envisages that geographic variation in host use follows geographic variation in the relative abundance of potential hosts (Thomson, 1988). The results of this study have enriched the available knowledge of larval food plants of *L. nina* and strengthened the thesis that expanding populations of herbivores often seek new hosts of the same or closely related taxa in a new environment (Tabashnik, 1983).

Acknowledgments

We are thankful to the Indian Council of Agricultural Research, New Delhi for partly financial assistance; to Dr V.V. Ramamurthy, IARI, New Delhi for encouragements, to Dr D. Ghosh, Head, Department of Zoology for the laboratory facilities, and to Dr Badal Datta, Department of Botany, Tripura University for help in identification of plants. Helpful suggestions and corrections offered by two anonymous reviewers for improvement of the paper are gratefully acknowledged.
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Oviposition and larval development of *Leptosia nina*

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[Received February 12, 2014. Accepted June 8, 2014]

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