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Biology of *Pontia daplidice* (Lepidoptera: Pieridae) on its New Host Plant *Lepidium pinnatifidum* from Potohar Region, Pakistan

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Abstract

The butterfly *Pontia daplidice* (L.) was found in large numbers during April to September 2014 in Pothar Region. Its eggs and larvae were found on *Raphanus sativus*, *Brassica napus*, *Coronopus didymus* and *Lepidium pinnatifidum* which were the major part of local flora in the study area. Among host plants *L. pinnatifidum* was found to be a new host plant for *P. daplidice*, where its eggs and larvae were found on young leaves and inflorescence. Life cycle of *P. daplidice* was studied on *L. pinnatifidum* in laboratory from February to September 2014. During study it was observed that temperature affect the life cycle of *P. daplidice* significantly. The local nectar flora of the study area was *Lantana camara*, *Rosa indica*, *Jasminum officinale*, *J. grandiflorum*, *Conyza bonariensis* and *Fumaria indica*.

Keywords: Host plant, *Lepidium pinnatifidum*, *Pontia daplidice*, Biology, Potohar, Pakistan.

1. Introduction

Feeding is considered to be the basic need of an organism for its development, distribution, and abundance in particular habitats^[1], and the host selectivity is shown by almost all the organisms particularly the herbivorous insects^[2]. In butterflies, feeding behaviour is considered significant because food and mode of feeding are different in the larval and adult stages^[1], for food their larvae feed on plant tissues^[3], and adults are dependent on the specific host plants for nectar, therefore, the diversity of butterflies reflects diversity of host plants^[4]. The leaf and inflorescence feeding association of pierid butterflies provides outstanding opportunities for study of their life history phenomenon which influence their population^[5]. The major host plants of family Pieridae belong to Leguminosae, Loranthaceae, Ericaceae and Pinaceae in addition to association with Crucifers^[6]. However, the ancestral host of the family seems to be Fabaceae^[7].

Pierid genus *Pontia* occurs in Europe, temperate Asia and North Africa^[9]. This genus is also common in northwest India extending to Iran, the Middle East, and Asia Minor. In Indian sub-region this genus is common in Ladakh, Jammu and Kashmir and Pakistan. In Pakistan it is common in Balochistan, Chitral and Punjab^[10]. Biogeographically this genus is Afrotropical as well as Holarctic^[11]. In Pakistan genus *Pontia* represents its three species namely *P. daplidice* (L.), *P. glauconome* Klug. and *P. chloridice* (Hübner)^[12]. While the only representative species of Potohar region is *P. daplidice*. This species has a wide range of distribution and found in North Africa, Britain to Southern Europe, India and Japan^[13]. Also have been reported from Europe and North Africa to Abyssinia, extending to North-west India, China, and Siberia^[8]. Common in Asia Minor, Central Asia, Persia, Afghanistan and Pakistan^[14]. From Pakistan reported from Baluchistan to Chitral, Muree, Peshawar, Swat, Attock, Islamabad, and Kashmir^[15, 16, 17, 12].

The reported host plants of *P. daplidice* are *Alyssum*, *Arabidglabra*, *Brassica oleracea*, *Cakile maritime*, *Raphanus sativus*, *Sisymbrium officinale*, *S. altissimum*, *S. irio*, *Sisymbrium* sp., *Lepidium sativum*, *Senebira didyme*, *Sinapis* (Brassicaceae) and *Caylusea*, *Reseda lutea* (Resedaceae)^[8, 12, 18, 19, 20, 21]. However, the reported host plants of *P. daplidice* from Pakistan are *Sisymbrium altissimum*, *S. irio*, *Sisymbrium* sp., (Brassicaceae) and *Reseda* sp. (Resedaceae)^[12, 19].

Limited information is available on biology of *P. daplidice*^[11]. Life cycle of subspecies *P. daplidice moore* (Röber) on *L. ruderala* and recently^[20] on *Lepidium sativum* and *Senebira didymus* have been from India.

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The objective of this study is to explore new host plants for *Pontia daplidice* to increase known host range of this butterfly. Which can help in future conservation of this butterfly.

Materials and Methods

The Potohar Region is located at 32.5°N to 34.0° N latitude and from about 72° E to 74°E longitudes with an area of 23161 sq. km in Rawalpindi Division. This region presents one of its best developments of a singular geomorphic unit known as Potohar Plateau, a highly dissected plateau that covers about 18,000 sq. km in the central part of the Potohar Region [22]. The plateau situated with Punjab Province in the North and along the Himalaya's foot hills of Azad Kashmir to the West [23] with Indus River in the West and Jhelum River in the East, Kala Chitta Range and Margalla Hills in the North and Salt Range in the South. Prior to the eggs collection of *P. daplidice* from the field, egg laying behavior of 10 gravid females were observed for two days in the field from 10 am to 3 pm. Twenty (20) freshly laid eggs of *P. daplidice* on its new host plant *L. pinnatifidum* were collected from different areas of Islamabad District. The eggs were collected by detaching the whole leaf, on which eggs were laid. Petiole of each leaf were rapped with socked cotton and kept in Petri dishes (15cm x 2.5cm). Two replicates were prepared with five fresh eggs containing leaves on *L. pinnatifidum*. Both replicates were brought to the laboratory of National Insect Museum, National Agricultural Research Center (NARC) Islamabad for study, during February – September 2014, at different adjusted average minimum and maximum field temperatures given in Table-1. The month wise average temperature i.e. from January to September of the field area is given in Table-2.

Table 1: Average minimum and maximum Temperature (°C) in lab.

| Experimental Months | Min. Tem. (°C) | Average (°C) | Max. tem (°C) | Average (°C) |
|---------------------|----------------|--------------|---------------|--------------|
| Feb-March | 8.3-13.1 | 10.7 | 18.5-25.7 | 22.1 |
| April-May | 17.3-22.8 | 20.05 | 29.9-37.0 | 33.45 |
| June-July | 25.4-25.8 | 25.6 | 38.3-34.6 | 36.45 |
| August-Sep | 24.4-22.8 | 23.6 | 32.3-32.4 | 32.35 |

Replicates were placed in separate wooden cages of size (15x15x15 mm) covered with wire mesh in the laboratory at the field adjusted average max. and min. temperature of months given in Table-1 i.e. February to September respectively.

Eggs were examined daily at 3 hours intervals to record incubation period. After hatching 1st instars larvae were keenly observed and supplied with fresh leaves. Each larval instar (1st – 5th) was examined after 12 hours interval. The time of ecdysis for each instar was recorded in hours. The morphological characters (size, shape and colour) of eggs, larval instars, pupae and adults were carefully observed and noted. Mortality rate was found by using formula;

$$\text{Mortality rate} = \frac{\text{No of Larvae died during replica}}{\text{Total number of larvae used in Replica}} \times 100$$

Results and Discussion

During the study of host plants diversity of pierids butterfly from Potohar Region, different new host plants from family Brassicacea were explored (under preparation). One of these was *L. pinnatifidum*, which was found as a new host plant for *P. daplidice*. Eggs of *P. daplidice* were found on young leaves and inflorescence while larvae were found feeding on young leaf including inflorescence. During surveys of Potohar

Region this plant was found in all districts of Potohar. While adults butterflies were found nectaring on flora such as *Lantana camara*, *Rosa indica*, *Jasminum officinale*, *J. grandiflorum*, *Conyza bonariensis*, and *Fumaria indica*.

Egg Laying Behaviour: During two days 10 gravid females in the field were observed prior to egg laying female flies very close to the ground in search of a suitable host plant. After selection of the suitable host plant female sit for 10 seconds on leaf or inflorescence and started laying eggs. Eggs were laid singly at the apical end of young leaf either dorsally or ventrally also found laid within inflorescence. Egg laying duration was between six to ten seconds. Female lays eggs on three to four plants at one place.

Morphological Characters

Egg: The average size of egg was about 0.89 ± 0.02 mm. width 0.33 ± 0.02 mm. Eggs were flask shaped, with 12 fine longitudinal ribs and series of transverse lines between longitudinal ribs, wider in the middle with an apical rim. Freshly laid egg was yellowish white, became brownish later on turned orange.

Larvae

1st Instar: Average body length was 2.07 ± 0.03 mm. Width 0.37 ± 0.03 mm. It was shiny pale yellowish with numerous fine black setae positioned laterally and dorsally. Emergence occurred from the micropyle end of the egg. Newly hatched larvae first feed on egg shell before eating young leaves.

2nd Instar: Average body length is 4.6 ± 0.06 mm. width 0.81 ± 0.02 mm. Head black, body light brownish yellow with two fine grey lateral lines, body consists of uniform 12 segments and covered with fine black setae. Use to feed upon young leaves and inflorescence.

3rd Instar: Average body length 8.1 ± 0.04 mm. width 1.62 ± 0.03 mm. Light green with fine, long, numerous black setae, two fine grey dorsal lines, and lateral grey wider lines having numerous black setae. Use to feed upon young leaves and inflorescence.

4th Instar: Average body length 12.75 ± 1.23 mm. Width 1.21 ± 0.21 mm. Head light green covered with numerous white setae and speckled with black dots. Body yellowish green with light grey longitudinal lines, marked with black spots, as body was covered with numerous fine white setae. Use to feed upon host leaves and also on green stem.

5th Instar: Average body length 23.04 ± 1.00 mm. width 3.11 ± 0.18 mm. Morphologically very similar to 4th instar, however, it was quite sluggish and inactive. Head greyish green, to yellow covered with numerous white, fine setae sprinkled with black dots. Dorsal plate pointed, covered with numerous white, fine setae, marked with black dots.

Pupa: The average body length 16.5mm with width about 3.99mm. Greyish white to greenish colour, sometime brownish white having green to grey longitudinal lines speckled with black specks, both ends were tapering and pointed with dark tipped horns.

Adult

Male: Has white upper and under surfaces. There are marked differences in upper and lower side of forewings and hind

wings. The apex of fore wing basal half of costa dusted with black scales, at apex of cell there was larger black spot, above vein 3 the apex and termen are largely black with a series of the ground colour sustained as fine lines to the terminal margin. Hind wing homogeneous, the markings of the underside showing through, there was black base of both fore and hind wings. Under side, of fore wing has apical markings with the base of cell was dusted with green scales, the cell-spot has extensions up to the costa marking with green, having dark spot in inter space 1. Hind wings were green having three

curved series of white spots. Apically body appears to be black and basally white.

Female: Has the dusky black markings having more extensions and are very much broad, with a black spot in interspace 1 on fore wing. On the hind before apex the wing has large costal black spot, a black sub terminal curved b and a terminal series of black marks. The broader markings of the underside are the unique feature of female.

Table 2: Effect of Temperature on duration of different life stages of *Pontia daplidice*.

| Months | Feb-March | | April-May | | June-July | | Aug-Sept. | |
|-------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Replica 1 | Replica 2 | Replica 1 | Replica 2 | Replica 1 | Replica 2 | Replica 1 | Replica 2 |
| Average Temp. °C | 16.4°C | | 26.7 °C | | 31°C | | 27.9°C | |
| Egg | 68 (hr.) | 67.05 (hr.) | 63 (hr.) | 62.10 (hr.) | 59 (hr.) | 58.56 (hr.) | 61 (hr.) | 60.23 (hr.) |
| Larval Instars | | | | | | | | |
| 1 st | 52 (hr.) | 51.49 (hr.) | 49 (hr.) | 48.57 (hr.) | 46 (hr.) | 45.16 (hr.) | 47 (hr.) | 46.58 (hr.) |
| 2 nd | 38 (hr.) | 37.55 (hr.) | 33.31 (hr.) | 32.45 (hr.) | 32 (hr.) | 31.21 (hr.) | 32 (hr.) | 31.12 (hr.) |
| 3 rd | 39.28 (hr.) | 38.50 (hr.) | 35 (hr.) | 34.59 (hr.) | 32.30 (hr.) | 31.12 (hr.) | 33 (hr.) | 32.01 (hr.) |
| 4 th | 37.15 (hr.) | 36.35 (hr.) | 36.09 (hr.) | 35.25 (hr.) | 33.05 (hr.) | 32.11 (hr.) | 34.5 (hr.) | 33.45 (hr.) |
| 5 th | 80 (hr.) | 79.12 (hr.) | 76.13 (hr.) | 75.11 (hr.) | 74.17 (hr.) | 73.10 (hr.) | 75.22 (hr.) | 74.11 (hr.) |
| Total larval duration (hours) | 246.43 (hr.) | 246.37 (hr.) | 229.53 (hr.) | 227.28 (hr.) | 217.52 (hr.) | 213.16 (hr.) | 221.27 (hr.) | 218.05 (hr.) |
| Pupa duration (hours) | 163.59 (hr.) | 162.55 (hr.) | 162.30 (hr.) | 161.09 (hr.) | 156 (hr.) | 155.09 (hr.) | 158 (hr.) | 157.41 (hr.) |
| Total Hours | 478.07 | 476.37 | 455.38 | 450.47 | 432.52 | 426.35 | 440.27 | 436.15 |
| Total period (days) | 19.9 1 (days) | 19.84 (days) | 18.97 (days) | 18.76 (days) | 18.02 (days) | 17.76 (days) | 18.34 (days) | 18.17 (days) |

Table 3: Mortality rate of larvae during the study period.

| Months | Feb-March | | April-May | | June-July | | Aug-Sept. | |
|------------------------|-----------|---------------|---------------|-----------|---------------|-----------|-----------|---------------|
| | Replica 1 | Replica 2 | Replica 1 | Replica 2 | Replica 1 | Replica 2 | Replica 1 | Replica 2 |
| Average Tem. °C | 16.4°C | | 26.7 °C | | 31°C | | 27.9°C | |
| No. of Eggs taken | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Larval instars | | | | | | | | |
| 1 st | 5 | 4 | 5 | 5 | 4 | 5 | 4 | 5 |
| 2 nd | 4 | 4 | 5 | 4 | 3 | 3 | 4 | 4 |
| 3 rd | 4 | 3 | 4 | 4 | 3 | 3 | 3 | 4 |
| 4 th | 4 | 3 | 3 | 4 | 2 | 3 | 3 | 4 |
| 5 th | 4 | 3 | 3 | 4 | 2 | 2 | 3 | 4 |
| Pupa | 4 | 3 | 4 | 3 | 2 | 1 | 3 | 4 |
| Adult (survived) | 3 3♂ | 3 2♂ 1♀ | 3 1♂ 2♀ | 3 3♀ | 2 1♂ 1♀ | 1 1♂ | 2 2♀ | 4 2♂ 2♀ |
| Mortality Rate | 40% | 40% | 40% | 40% | 60% | 80% | 60% | 20% |

Average height of egg was about 0.89 ± 0.02 mm while width was 0.33 ± 0.02 mm which is quite different from earlier studies (for example [11]; on subspecies *P. daplidice moore* and *P. daplidice* L. They recorded egg height was about 0.87 ± 0.03 mm and 0.80 ± 0.05 mm while width was 0.34 ± 0.02 mm and 0.50 ± 0.05 mm. Variations were also recorded in the length of instars e.g. [20] reported the length of 1st instar 2.16 ± 0.70 mm and width 0.37 ± 0.05 mm. However, in another study [11] reported 2.05 ± 0.04 mm and width was 0.36 ± 0.04 mm. During present study the body length was recorded as 2.07 ± 0.03 mm and width 0.37 ± 0.03 mm. The length of 2nd instar recorded by [20] 3.07 ± 0.58 mm and width 0.5 ± 0.03 mm while [11] reported it 4.46 ± 0.07 mm and width 0.83 ± 0.03 mm. However, in present study the length of body was recorded as

4.6 ± 0.06 mm and width 0.81 ± 0.02 mm. The length of 3rd instar measured [20] was 7.05 ± 0.92 mm and width 1.08 ± 0.1 mm. While [11] measures the length 7.6 ± 0.12 mm and width 1.64 ± 0.17 mm. In the present study the body length 3rd was 8.1 ± 0.04 mm. While width were 1.62 ± 0.03 mm. Significant difference was also recorded in the length of 4th instar larva which was 11.33 ± 3.21 mm and width 1.41 ± 0.1 mm reported by [20]. While according to [11] the length of 4th instar was 23.0 ± 0.47 mm and width as 3.3 ± 0.12 mm. It seems that [11] has mistakenly mentioned its length. However, the specimen in his collection needed to be re-examined. The average length and width of 4th instar larvae reported by [20] were 11.33 ± 3.21 mm and width 1.41 ± 0.1 mm and during current study 12.75 ± 1.23 mm and width 1.21 ± 0.21 mm [11].

Reported only four instars instead of five which is very common in pierids and papilionids butterflies. The 5th instar was very much similar to 4th instar with body length and width which was $22.33 \pm 2.51\text{mm}$, $2.73 \pm 0.25\text{mm}$ [20]. During study it was measured as $23.04 \pm 1.00\text{ mm}$ and width was $3.11 \pm 0.18\text{mm}$. In pupae length and width variations were also

recorded by [20]. The pupal length was $15.50 \pm 0.70\text{ mm}$ and width $4.75 \pm 0.35\text{mm}$. However, [11] reported length $18 \pm 1\text{mm}$ and width $4.2 \pm 0.13\text{mm}$. During this study, the pupal maximum length was recorded as $16.75 \pm 0.05\text{mm}$ while width was $3.99 \pm 0.03\text{ mm}$. The mortality rate of larvae was during the months of June-August due to parasitoid attack.

Table 4: Comparison of length and width of *Pontia daplidice* during life stages.

| Life stages | | Present study | [20] | [11] |
|------------------------|--------|-----------------------------|-----------------------------|-----------------------------|
| Egg | Length | $00.89 \pm 00.02\text{ mm}$ | $00.87 \pm 00.03\text{ mm}$ | $00.80 \pm 00.05\text{mm}$ |
| | Width | $00.33 \pm 00.02\text{ mm}$ | $00.34 \pm 00.02\text{ mm}$ | $00.50 \pm 00.05\text{mm}$ |
| 1 st instar | Length | $02.07 \pm 00.03\text{ mm}$ | $02.16 \pm 00.70\text{ mm}$ | $02.05 \pm 00.04\text{mm}$ |
| | Width | $00.37 \pm 00.03\text{ mm}$ | $00.37 \pm 00.05\text{mm}$ | $00.36 \pm 00.04\text{mm}$ |
| 2 nd instar | Length | $04.06 \pm 00.06\text{ mm}$ | $03.07 \pm 00.58\text{mm}$ | $04.46 \pm 00.07\text{mm}$ |
| | Width | $00.81 \pm 00.02\text{ mm}$ | $00.05 \pm 00.03\text{mm}$ | $00.83 \pm 00.03\text{mm}$ |
| 3 rd instar | Length | $08.01 \pm 00.04\text{ mm}$ | $07.05 \pm 00.92\text{mm}$ | $07.06 \pm 00.12\text{mm}$ |
| | Width | $01.62 \pm 00.03\text{ mm}$ | $01.08 \pm 00.01\text{mm}$ | $01.64 \pm 00.17\text{mm}$ |
| 4 th instar | Length | $15.34 \pm 00.03\text{ mm}$ | $11.33 \pm 03.21\text{mm}$ | $23.00 \pm 00.47\text{mm}$ |
| | Width | $02.14 \pm 00.03\text{ mm}$ | $01.41 \pm 00.01\text{mm}$ | $03.03 \pm 00.12\text{mm}$ |
| 5 th instar | Length | $23.04 \pm 01.00\text{ mm}$ | $22.33 \pm 02.51\text{ mm}$ | Not reported |
| | Width | $03.11 \pm 00.18\text{ mm}$ | $02.73 \pm 00.25\text{mm}$ | |
| Pupa | Length | $18.00 \pm 01.00\text{ mm}$ | $15.50 \pm 00.70\text{mm}$ | $18.00 \pm 01.00\text{ mm}$ |
| | Width | $04.02 \pm 00.13\text{ mm}$ | $04.75 \pm 00.35\text{mm}$ | $04.02 \pm 00.13\text{mm}$ |





Fig 1: Different Life stages of *P. daplidice* on *L. pinnatifidum*

Conclusion

It is concluded that significant differences in biological parameters such as egg, larva, pupa indicate that environmental factors such as temperature, climate and host plant can affect the life cycle and to some extent the morphological characters such as color of eggs, larvae and pupae. Furthermore, this study can be helpful in future conservation of *P. daplidice* if needed in the area.

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