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Effects of temperature and photoperiod on the phenotypic variation of two Pierid Butterfly Species

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Abstract
A study of the effect of temperature and photoperiod on the phenotypic variation of two pierid butterfly species, *Appias libythea* and *Cepora nerissa* was conducted during January to December 2013 in Savar, Dhaka, Bangladesh. Both species of butterflies were found two distinct phenotypic forms i. e. wet season form (WSF) and dry season form (DSF) that alternate according to the season. WSF of both species were observed in wet season (March to October) when photoperiod was >12 hours at relatively high temperature, whereas DSF was observed in dry season form (November to February) when photoperiod was <12 hours at relatively low temperature. The WSF of *A. libythea*, black apical markings of the dorsal side of forewings were darker and broader than DSF and there are black spots streaks at the end of the hind wing veins which were absent in DSF of *A. libythea*. The ventral side of entire forewing and hind wing of WSF veins broadly black margined which were absent in DSF of *A. libythea*. In the WSF of *C. nerissa*, the band of the forewing and small black area became darker and broader than the DSF. Yellow spot of ventral side of WSF was larger than the DSF of *C. nerissa*. Abundance of WSF was much higher than DSF and fluctuation pattern was bimodal for WSF adults of both species. In both species, the turnover of DSF to WSF found to occur around the March when rising temperature and photoperiod as well as increased food plants while the transition of WSF to DSF was found around October when declining temperature and photoperiod as well as decreased food plants.

Keywords: Effect, temperature, photoperiod, phenotypic, variation, butterfly

1. Introduction
Many of the tropical butterflies have distinctive seasonal forms and these forms of the butterflies are called the dry-season form and wet season forms [1]. The dry-season forms (DSF) are usually more cryptic and it has been suggested that the protection offered may be an adaptation. Wet-season form (WSF) shows greater dark colours this may have thermoregulatory advantages by increasing ability to absorb solar radiation [2]. Seasonal polymorphism is an annually repeating pattern of changing phenotypic ratios in successive generations under some kind of environmental factors like photoperiodic effect [3]. Studies on butterfly species under the families Pieridae, Nymphalidae, Lycaenidae and Hesperiidae have generally found that photoperiod acting during some part of the larval period is the predominant environmental factor regulating phenotype [4, 5, 6]. *Bicyclus anynana* butterfly, endemic to Africa, have two distinct phenotypic forms (WSF and DSF) [7]. Similarly, two seasonal distinct forms (DSF and WSF) observed of the butterfly species, *Melanitis leda* and *Mycalesis mineus* (Satyridae) and *Junonia almana* and *Junonia atlites* (Nymphalidae) in Bangladesh [8, 9]. This study is made a first attempt to provide the seasonal phenotypic variation of butterfly wings as well as transitional time of seasonal morph in between dry season morph to wet season morph and reverse in the butterfly species, *Appias libythea* and *Cepora nerissa* (Pieridae) in Bangladesh.

2. Materials and Methods
2.1 Study sites
The study was carried out in three different areas at Savar, the north-west suburbs of Dhaka city. The average temperature of wet season (March to October) was 28.61 °C and dry season (November to February) was 21.63 °C in study areas. Maximum temperature was recorded 34.8 °C in May and minimum temperature was recorded 12.5 °C in January. Day length of wet season was >12 hrs and dry season was less than <12 hrs. Annual rainfall varies between 0 and 791 mm and it is variable from year to year depending on depression and cyclones, which occur mostly in April-October. The pattern of rainfall was bimodal where a period of short rains occurs November to March (average 30 mm) and a period of long rains occurs April

to October (average 397 mm). The study area is composed mixed vegetation lands cultivating for fruit plants, timber plants, flower garden and different types of vegetables. Natural vegetation including small natural forests was mainly covered with different types of grasses, herbs, shrubs and bamboo bushes.

2.2 Collecting methods and seasonal form identification

The adult butterflies (*Appias libythea* and *Cepora nerissa*) were collected from three selected areas at Savar through the year in 2013. The capturing of butterflies using sweep net was done once sunny day per month from those selected areas. Collected butterflies were immobilized and brought back to the Laboratory. Insect stretching board was used to stretch their wings and properly dried overnight in an oven at 50 °C. Seasonal forms were identified based on the characteristics of their wing shapes (angular/regular) and patterns (presence or absence of eye spots, colour and band pattern as well as vein markings).

3. Results

We have identified two butterfly species (*Appias libythea* and *Cepora nerissa*) from Pieridae family as good candidates for seasonal morphs viz. dry season form (DSF) and wet season form (WSF) according to the season when they appeared as adults.

Fig 1: Dorsal (left side) and ventral (right side) views of WSF *Appias libythea* adult

3.1 Description of WSF of *Appias libythea* adult

The dorsal side of the forewing white with dentate black streaks along the veins two a depth of 8 mm at the apex, tapering to very small on vein 1. There was a small black streak at the end of the hind wing veins. The apex of the ventral side of the forewing and the entire hind wing was little cream with gray scaling. Slightly yellow narrow basal streak, lies along the costa on each hindwing. Ventral side of the hind wing there was a white discal band and a more diffuse row of postdiscal spots (Figure-1).

Fig 2: Dorsal (left side) and ventral (right side) views of DSF *Appias libythea* adult

3.2 Description of DSF of *Appias libythea* adult

Dorsal side of both wings is white and narrow black apical markings were produced in the forewing. There are no black spots or streaks at the end of the hind wing veins. The ventral side of both wings is creamy white and there are no spots or streak entire forewing and hind wing (Figure- 2).

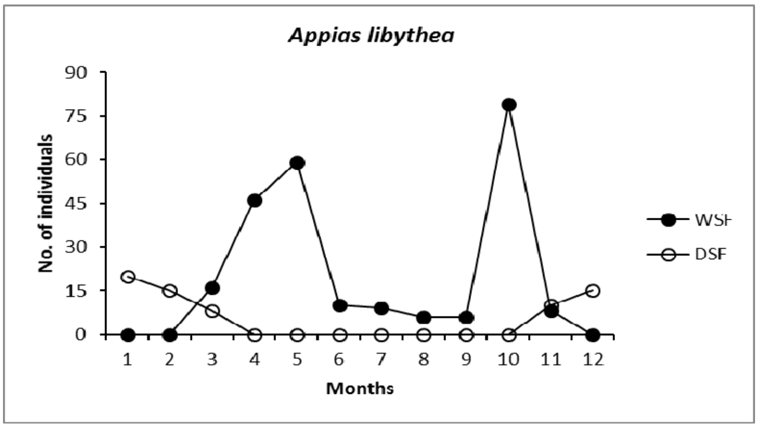

Fig 3: Seasonal abundance and transition period of DSF and WSF adult of *Appias libythea*

3.3 Seasonal abundance and transition period of DSF and WSF adult of *Appias libythea*

DSF adults appeared in October and formed a small peak in January. WSF adults appeared in March and showed a first peak in May. After May its number began to decline strikingly and very few number of WSF was found in the month of June to September thereafter population began to rise again and formed second peak in October. The second peak of WSF was higher than first peak of WSF which occurred in May. The peak of the DSF which occurred in January was far lower compared to the first and second peak of WSF adults. The transition period of WSF to DSF was October and the transitions of DSF to WSF were found to occur in March (Figer-3).

Fig 4: Dorsal (left side) and ventral (right side) views of WSF *Cepora nerissa* adult

3.4 Description of WSF *Cepora nerissa* adult

Dorsal side of both wings is white but veins broadly margined by pale, markings more extensive and veins mostly darkened, especially margin of cells. Forewing bears an additional black spot on vein 1b which is absent in DSF of *C. nerissa*. Forewing apex bearing two small white spot. Subterminal series of dusky spots in interspaces 1 to 6; more often than not the spot in 5 entirely absent. Ventral side of both wings as in the dry-season specimens, but basal black area became darker and border than DSF. Subterminal series of dusky spots in interspaces 1 to 6 became more prominent than DSF (Figure-4).

Fig 5: Dorsal (left side) and ventral (Right side) views of DSF *Cepora nerissa* adult

3.5 Description of DSF *Cepora nerissa* adult

Dorsal side of both wings is white. Forewing with outer marginal black border especially broad in the apical area; a black spot in the middle of area 2, separated from the black border by a white spot; costal darkened for its proximal half. Forewing apex bearing two small white spots. Dorsal side of the hind wing, veins 4 to 7 with outwardly-dilated broad black edgings and form an anterior, irregular, black, terminal margin to the wing. Ventral side of the forewing is white, the veins broadly margined by dusky black; costal margin broadly and apex suffused with yellow; there are two subterminal black spots, those are between veins 1 and 2, and 3 and 4. Ventral side of the hind wing is yellow and entirely suffused with the veins diffusely bordered with black, subterminal series of dusky spots in interspaces 1 to 6; more often than not the spot in 5 entirely absent (Figure-5).

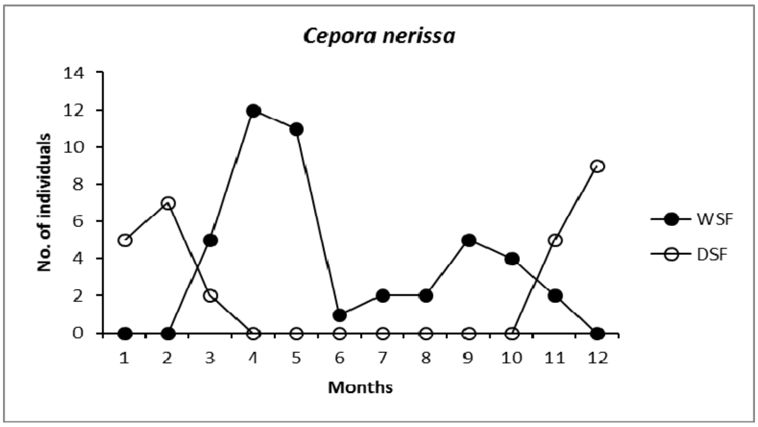

Fig 6: Population abundance and transition period of WSF and DSF adult of *Cepora nerissa*

3.6 Seasonal abundance and transition period of WSF and DSF adult of *Cepora nerissa*

DSF adults of *C. nerissa* appeared in November and formed a peak in December and another small peak formed in February. While WSF adults appeared in March and showed a first peak in April and population drastically decreased from May and few number of WSF was found in the month of June to August thereafter population increased and formed second peak in September that was far smaller than first peak which occurred in April. The result also mentioned that the first peak of WSF was higher compared to the peak of DSF adults. The transitions of WSF to DSF were found to occur around October in contrast the transition period of DSF to WSF was found around the month of March (Figure-6).

4. Discussions

Many species of butterflies are known to exhibit a seasonal polymorphism, where one form predominates at one time of year and an alternative form occurs in a different season. Seasonal polymorphism of butterfly's species showed different phenotypic pattern in both dry and wet season by showing cryptic form to eye spot form, regular wing shape to angular wing shape and light melanic scale to deep melanic scale. In this study was attempted to be acquainted with seasonal polymorphism in two butterfly species, *Appias libythea* and *Cepora nerissa* (Pieridae). Both species of butterflies clearly exhibits two distinct phenotypic forms that alternate according to the season. The WSF of *A. libythea*, black apical markings of the dorsal side of forewings were darker and broader than DSF. The ventral side of entire forewing and hind wing of WSF veins broadly black margined which were absent in DSF of *A. libythea*. In the WSF of *C. nerissa*, the band of the forewing and small black area became darker and broader than the DSF. Yellow spot of ventral side became larger than the DSF and no change occurred in the wing shape. The DSF wings of both species showed dull colored and more cryptic as a result they were beautifully camouflaged as dry leaves and brown brush which has been suggested that the protection offered may be an adaptation. In contrast, the WSF wings of both species showed dark colored and black area was darker and broader which could not easily rely on cryptic coloration for protection from vertebrate predators because the background vegetation is green. These results mentioned that the phenotypic variation of WSF and DSF of *A. lybithea* and *C. nerissa* species is triggered by environmental cues specially photoperiod (day length) and temperature. The WSF induced by long day length (>12 hours photoperiod) at higher temperature (23.8 °C to 34.7 °C) whereas DSF induced by short day length (<12 hours photoperiod) at lower temperature (14.5 °C to18.4 °C). *Eurema hecabe* is one of the old world pierid butterfly species, it has a darker summer adult morph, triggered by a long day exceeding 13 hours in duration, while the shorter diurnal period of 12 hours or less indices a paler morph in the post-monsoon period [10]. [11] Reported that the temperature is of primary importance in regulating wing phenotype of several Lepidoptera [12]. Also found that the different temperature in final instar larvae of *Bicyclus safitza* induced different seasonal forms. [13] Showed the temperature interact with photoperiod to phenotypic response in tropical pierid butterflies. But [14, 15, 16] found the polymorphism of a Japanese butterfly *Polygonia c-aureum*, is environmentally controlled through the neuroendocrine system of the larvae. They demonstrated that the longer day length at higher temperatures activated the brain-corpus cardiacum system to release ecdysone hormone could induce the development of reproductively active summer morph, while the brain-corpus cardiacum system registers the shorter day length and lower temperature to prevented ecdysone secretion then appears reproductively inactive autumn form of adult *Polygonia c-aureum*.

The dark colored markings in the WSF of both butterfly species might be the high melanin deposition in the wing scale and it could be increased in wet season when temperature was high and photoperiod was longer than 12 hours. It is indicating that the change in seasonal markings of both species were correlate with temperature and photoperiod, in theses instance it is believed that one color form can provide more rapid or effective solar heating through a larger proportion of darker pattern. In other cases, the seasonal forms are believed to be connected to a more effective cryptic ability according to the nature of available background surfaces [17]. Worked on pierid butterfly, *Nathalis iole* and they demonstrated that the higher density of melanic scales increases the efficiency of absorption of thermal energy more quickly when they alight on leaves. Presumably this facilitates a higher intensity and longer duration of activity [18]. [2] Have shown similar result and they suggested that the dark colored in the WSF may have thermoregulatory advantages by increasing ability to absorb solar radiation.

A libythea was less abundant and *C. nerissa* was very less abundant during study period. Peak season of WSF adults of *A. libythea* was May and October. The first peak which occurred in May was smaller than second peak which occurred in October. Whereas WSF of *C. nerissa*, first peak formed in April that was much larger than second peak which formed in September. The DSF adult of *A. libthea* and *C. nerissa* was little higher in December and February during dry season (November to February). In this result suggested that WSF population was much higher than DSF population and fluctuation pattern was bimodal for WSF adults of both species. It mentioned that the WSF was more active which produced by long day length at higher temperature than those in the DSF and also suggested the reproductive success was optimized in each season by an interaction of phenotype and behavior [2]. The longer day length at higher temperatures activated the brain-corpus cardiacum system to release ecdysone hormone could induce the development of reproductively active summer morph in *Polygonia c-aureum* [15]. The transition of DSF to WSF coincided with rising temperature when increased food plants while the transition of WSF to DSF was found at declining temperature when decreased food plants. It is evident from this study, that the seasonal polymorphism is linked with seasonal changes in breeding status which associated with changes in habitat favorably. The overall results revealed that, the action of temperature and photoperiod (day length) may be the cause of seasonal polymorphism or it may be hereditary characteristic of polymorphic species by which butterfly can adapt performing camouflage and given threat to the predator.

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