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Phenotypic plasticity in the pear psyllid, *Cacopsylla bidens* (Šulc) (Hemiptera, Psylloidea, Psyllidae) in Israel

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ABSTRACT

1. Pear psyllids, belonging to the subgenus *Hepatopsylla*, often exhibit strong seasonal dimorphism. The summer forms are lighter and smaller while the winter forms are relatively darker and larger.
2. We examined whether seasonal polymorphism in *Cacopsylla bidens* is a continuous or discontinuous phenomenon and assessed the role of temperature and photoperiod in its regulation.
3. Adult psyllids were sampled in pear orchards for three years using beating trays. The effects of temperature and photoperiod on induction of phenotyic changes were evaluated in the laboratory. Morphological parameters such as wing size and body coloration of both sexes were monitored.
4. Observation of the wild population indicated that *C. bidens* exhibited continuous phenotypic plasticity during the transition from summer to winter morphs but a step-wise transition from winter to summer morphs.
5. According to laboratory experiments both temperature and photoperiod play a significant role in the regulation of polymorphism in this species.

KEY WORDS: phenotypic plasticity, polyphenism, reaction norm, pear psyllid, *Cacopsylla bidens*.

INTRODUCTION

Polymorphism is a well known phenomenon, though not common in insects. Some insect species maintain distinct seasonal adult phenotypes, which may differ in the proportions of various body parts and may have different food preferences, reproductive characteristics, dispersal capability and colour patterns (Nijhout, 1999; Zera

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and Denno, 1997). Development of radically different morphs of the same species indicates a high degree of phenotypic plasticity. This plasticity can be continuous or discontinuous (polyphenism) (Stearns, 1989). Seasonal polymorphism occurs in representatives of several widely different orders, both holo and hemimetabolous, including hemipterans. Our research focuses on seasonal polymorphism in the jumping plant-lice, in particular the pear (*Pyrus*) feeding psyllids. Pear psyllids belong to the genus *Cacopsylla* (Hemiptera, Psyllidae, Psyllinae) (Burckhardt and Ouvrard, 2012) that constitute two morphologically and biologically characterized groups which were assigned to the subgenera *Hepatopsylla* and *Thamnopsylla* by Burckhardt and Hodkinson (1986). Recently, the subgenera were synonymised (Burckhardt and Ouvrard, 2012). The species of the former subgenus are polyvoltine with a strong seasonal dimorphism, whilst the species of the latter are univoltine (Burckhardt and Hodkinson, 1986). Israeli pear psyllids were originally reported as *Psylla pyricola* Foerster (Swirski, 1954). However, it was later reclassified as *Cacopsylla bidens* (Šulc) by Burckhardt (1994). This species, belongs to the subgenus *Hepatopsylla,* and is distributed in Central and Southern Europe, Central Asia, the Middle East and South America (Chile and Argentina) (Zeidan-Gèze and Burckhardt, 1998). The morphological differences between summer and winter forms, in this species, are so large that they were initially described as different species *C. vasilevi* (Šulc) and *C. bidens* (summer and winter form, respectively) (Burckhardt and Hodkinson, 1986). In contrast to the information accumulated on polymorphism and its regulation on the common European and North American species *Cacopsylla pyricola* and the European *Cacopsylla pyri* L. (Horton, 1999; Strapoulou and Kapatos, 1995), not much has been gathered on *C. bidens*. The above mentioned species are known to display both morphological and physiological seasonal dimorphism. There are small, pale summer forms and larger, dark winter forms that exhibit reproductive diapause. The Israeli population of *C. bidens* also shows seasonal morphological changes in size and wing vein colour (Swirski, 1954; Soroker *et al*., 2003). However, more than two seasonal morphs were observed in this species and about four stages of wing colouration could be distinguished (Soroker *et al*., 2003). It is likely, that the exhibited phenotypic plasticity is continuous, so that phenotypes are changing gradually with graded changes in environmental variables. This type of a phenotypic response to the environment is termed "reaction norm" (Stearns, 1989). The objectives of the present study have been: a) to characterize phenotypic plasticity in this species and b) to test the role of temperature and photoperiod as cues for this phenotypic transition.

MATERIALS AND METHODS

Sampling of the wild population

Adult pear psyllids were collected over two years, 2001 to 2002, in the pear orchards of Kibbutz Zova in the Jerusalem Hills west of Jerusalem. Sampling was carried out every 7 to 14 days in the mornings (when psyllids were inactive due to cool temperatures) using beating trays. Dislodged psyllids were counted, collected, and their sex was determined. The temperature was monitored continuously in the orchards by a meteorological station.

In order to estimate seasonal morphological and physiological changes, the forewing length of about 20 individuals was measured for each collection date, using a stereo microscope Olympus SXZ12. The forewing length was chosen as a size parameter because it was recognized as a seasonally variable character in *C. pyricola* (Wong and Madsen, 1967). The body colour of adults was analysed as described below.

Laboratory rearing

To separate the effects of temperature and photoperiod, psyllids were reared in environmental chambers on caged, two year old Spadona pear trees grafted on quince. To test the effect of photophase on psyllid morphology, feral adults (25 pairs each), collected on December 10th were allowed to lay eggs and the larvae were reared either under a long day photoperiod 14:10 (L:D) or a short day photoperiod (10:14) (L:D) condition, both conditions were at a temperature of 22-25ºC (25 pairs at each condition).

To test the effect of temperature on phenotypic plasticity of psyllids, newly emerged psyllid adults reared at a long photoperiod (22-25ºC), were paired and allowed to lay eggs for 4 days. Subsequently, adults were removed and the larvae developed under the conditions of long day photoperiod at one of 4 temperatures: 15, 20, 25 or 30ºC. The emerging adults were collected every two days, the length of their forewings was measured and their body colour was analysed as described below.

Body colour analysis

To determine body colour, psyllids were photographed individually, via a stereo microscope (X20 magnification) and a Sony DXC 990P Camera. The colours of the dorsal side of the mesothorax were analysed using the ImagePro* program. The analysis was based on the following three parameters: hue, saturation and intensity.

Statistical analysis

Data are presented as means ± SE. Seasonal changes in wing length were compared using General Linear Model, followed by Tukey's Studentized Range (HSD) using the SAS system. The effect of temperature wing length was tested using One way ANOVA, followed by Fisher's PLSD Statistical analyses were performed using StatView for Power PC; version 4.5, Abacus Concepts, Inc. The discriminant analysis was performed using Statistica for windows; version 6.0, Statsoft, Inc. Statistical significance was accepted at significance level of 5%.

RESULTS

Distinctive seasonal changes in female and male *C. bidens* forewing are expressed in Fig 1. Although, males are significantly smaller than females, seasonal changes are similar in both sexes (GLM, respectively for year 2001 and 2002: sex effect $F_{1,923}=64$, $p<0.0001$; $F_{1,1263}=333$, $p<0.0001$, month effect, $F_{8,923}=141$, $p<0.0001$; $F_{11,1263}=310$,

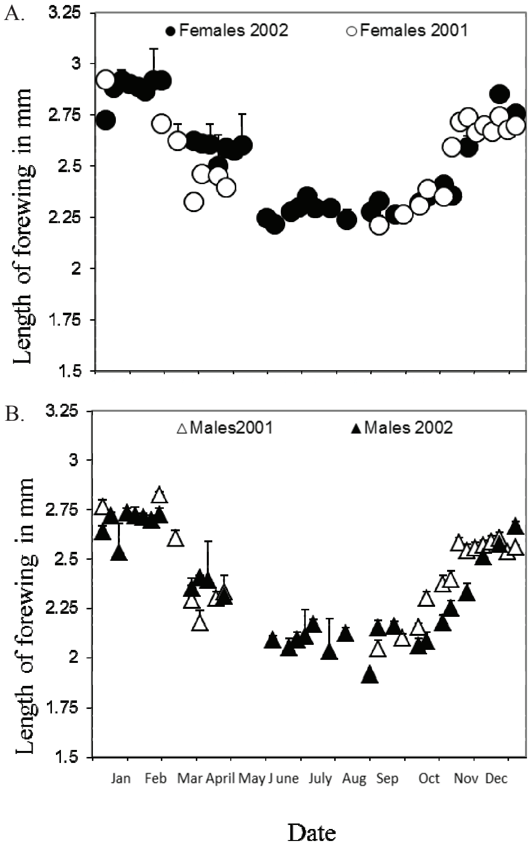

Figure 1. Seasonal changes in forewing length in *C. bidens* females (a) and males (b) Wing length is presented as mm. (mean ± SE) of an average of 20 individuals for each date.

$p<0.0001$, interaction $F_{8,923}=1.64$, $p=0.11$; $F_{11,1263}=3.28$ $p=0002$). Turkey's Studentized Range (HSD) Test reveals over the years 5-6 significantly different wing size groups ($p<0.05$). During the cold months (December to February) the size of adults of both sexes are the largest. Subsequently, no adults are found in the orchard until a new psyllid generation appears around the end of March. The wing size of this generation is significantly shorter than that of the previous one. This generation is still larger than the next group of psyllids that is collected in the field starting from the middle of May. The smallest psyllids were found in June. Subsequently, the size of the forewing of psyllid populations fluctuated but showed a general increase in size at the end of October. From November onwards the forewing size of psyllid populations continuously and steeply increased reaching a maximum size in December.

Comparison of the thorax colour (Fig. 2) showed a significant difference between winter (December) and summer (July) populations (Student's t-test, $p<0.05$ for both hue and intensity).

Average daily temperature fluctuated between 2ºC to almost 35ºC over the two year period (Fig. 3). In general, the lowest temperatures occurred between December and February, while the hottest months were May to September. In both years daily temperature variation was higher during the first half of the year than in the second.

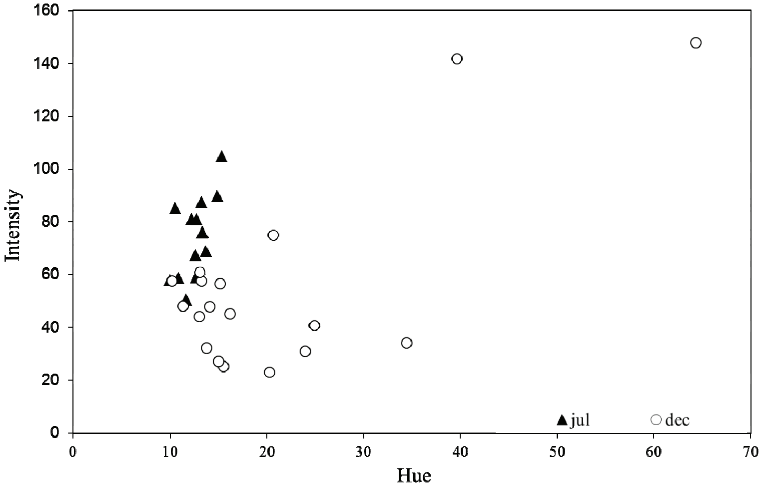

Figure 2. Seasonal changes in thorax colour of *C. bidens*. Discriminant analysis, Wilks lamda =0.498, $F_{2,31}$ =15.6, p<0.001, n=28).

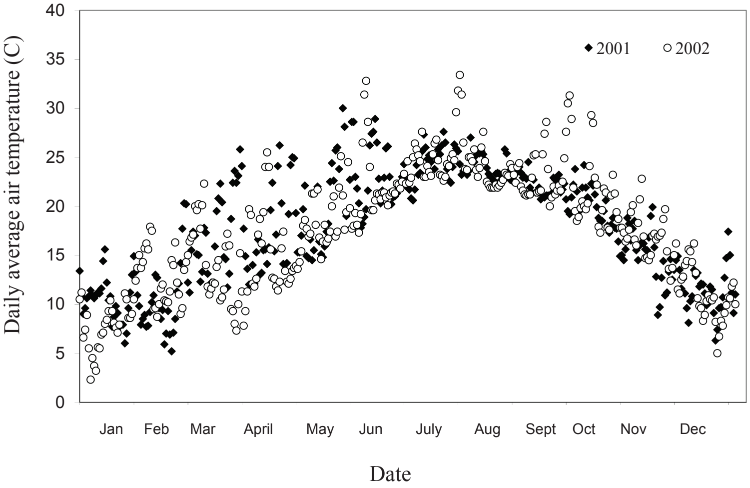

Figure 3. Seasonal changes in air temperature at the experimental site. Data are presented as weekly temperature (mean ± SE) in Centigrade.

In laboratory experiments, varying photoperiodic conditions for larval development led to morphological differences between these populations of psyllids (Fig. 4). Under a long day photoperiod (14L:10D) adults of both sexes had significantly reduced wing lengths relative to psyllids reared under short day photoperiod (10L:14D). (Student's t-test, assuming unequal variances, $P<0.05$). Moreover psyllids reared under short day regime, tend to be darker than the adults reared under long day regime (Fig 5).

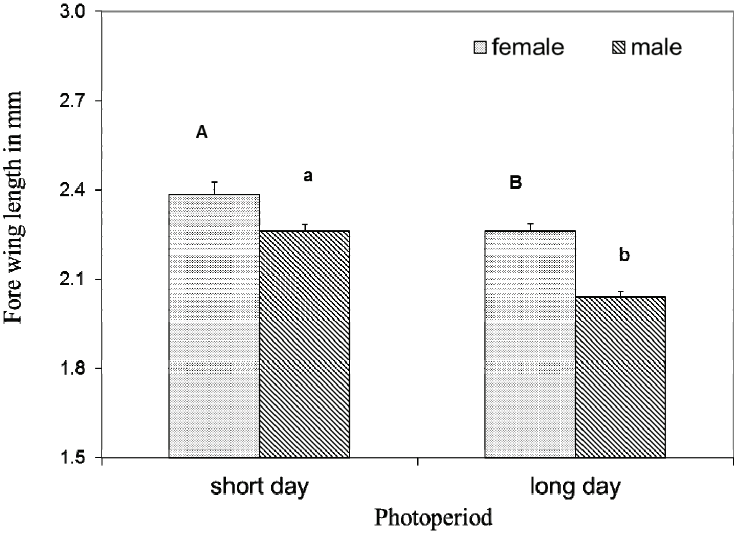

Figure 4. The effect of photoperiod on the forewing length of emerging adults of larvae reared on a short – and long-day photoperiod. Within each sex columns indicated by different letters are significantly different (t_{56}, $p<0.001$ and t_{53}, $p=0.012$ for females and males respectively, at short photoperiod: $n=25$ and $n=37$ and at long photoperiod: $n=17$ and $n=33$).

The temperature regime also had a significant effect on psyllid morphology. The body size was inversely affected by temperature (Fig. 6), (ANOVA, $F_{3,128}=32$, $p<0.0001$ and $F_{3,84}=39.6$, $p<0.0001$ for females and males respectively; followed by Fisher's PLSD at significance level of 5%). The temperature effect on males was more profound. Some difference in body colour was also visible (Fig. 7). With the decline in rearing temperatures the body colour tend to be darker in both sexes. However no significant differences were found in hue in both sexes. Intensity differed significantly between females reared in 15 and 30 degrees (ANOVA, $F_{3,44}=2.9$, followed by Fisher's PLSD, $p<0.05$), but not males.

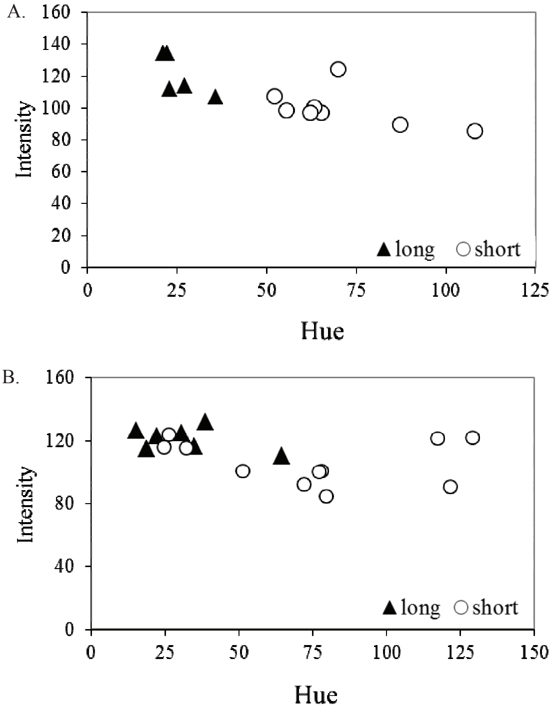

Figure 5. The effect of photoperiod on the body colour of emerging adults: female (a) and males (b). Discriminant analysis, for males, Wilks lamda =0.469, $F_{6,52}$ =3.99, p<0.003. For females Wilks lamda =0.581, $F_{2,15}$=5.408, p<0.02.

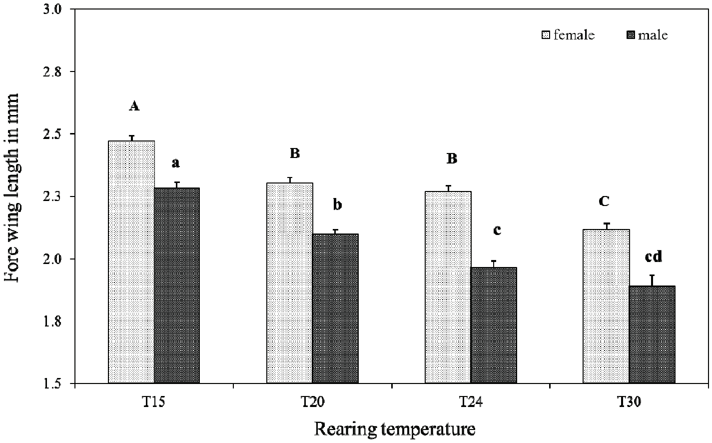

Figure 6. The effect of temperature on the forewing length of emerging adults of both sexes. Wing length is presented as mm. (mean ± se) of at least 18 individuals per treatment. Within each sex, different letters represent groups that are significantly different (One Way ANOVA, followed by Fisher's PLSD at significance level of 5%).

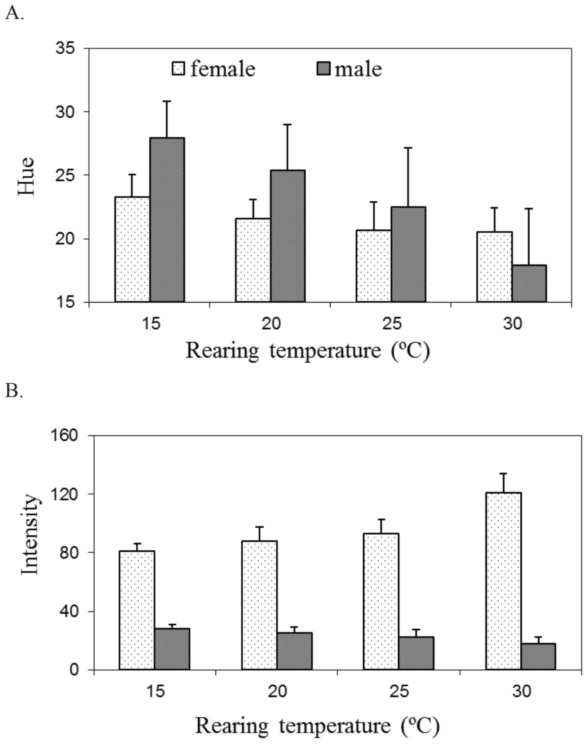

Figure 7. The effect of temperature on the body colour of emerging adults. The data are as mean ± se. of at least five individuals (psillids raised at 30ºC) or up to 21 individuals for females at 15ºC: hue (a) saturation (b).

DISCUSSION

Phenotypic plasticity in insects has many manifestations among which morphological, physiological and behavioural characteristics (Nijhout, 1999). Monitoring morphological changes in the feral population of pear psyllids for two years revealed that different seasonal morphotypes in the *C. bidens* populations are clear examples of phenotypic plasticity. Morphological changes not only include the wing-lengths and wing-vein-colouration but also changes in body-colouration. In general, this phenomenon is similar to that observed in other pear psyllids such as *C. pyri* and *C. pyricola*. However, unlike the latter species, more than two forms are visible in *C. bidens*. The first two generations are clearly morphologically distinguishable in terms of wing-length. Moreover, segregating the wild population of *C. bidens* into classes according to their forewing-lengths revealed 3-5 size classes. The transition from summer to winter forms was gradual while clear step-wise decreases in size was visible in the transition from winter forms to the first, spring generation. The next step is from first to the second generation.

Our data imply that the environmental cues regulating the phenotypic plasticity are most probably not operating uniformly all year around. Both biotic (changes in host plant) and climatic factors are known to induce seasonal phase transition in insects, amongst these photoperiod and temperature are the most common. As a monophagous species the life history of the pear psyllid must be synchronized with that of its host. As a deciduous tree, pear passes through an annual cycle that contains two major phases: dormancy and growth. Upon completion of dormancy, it blooms, and undergoes intensive vegetative growth and fruit development, which is followed by mild vegetative growth and ends up by leaf shedding and dormancy. The timing of these events is in turn regulated by a number of abiotic factors, consisting mainly of temperature and photoperiod (Westwood, 1993). Synchronization between the psyllid and the pear annual cycle is especially clear during the winter and the spring. Leaves are shed mostly during November-December. Between January and February psyllids can be found either as winter adults or as eggs which are preferentially laid on flower buds (Horton, 1999; Soroker personal observation). According to Swirski (1954) only these winter adults are able to feed on the branches of the dormant pears. The hatching of the first psyllid generation is generally synchronized with bud burst. Later in the season, when the pear is full of leaves, separation of the psyllid generations becomes less clear (Soroker, unpublished observations). A correlation between the climatic conditions at the experimental site and the morphological changes observed in psyllid populations reveals that summer forms emerge during a 12:12 h photoperiod with the temperatures increasing. With regard to winter forms, and assuming a generation time of one month, we conclude that the transition towards winter morph starts in mid September when the day length is around the equinox but the temperatures are still rather high, ranging between 15 and 33ºC but generally in decline.

Results of laboratory manipulation of temperature and photoperiod on psyllid phenology show that eggs of *C. bidens* are not predetermined as to their morphs. Thus, larvae from the same origin, developing at different temperatures and photoperiod conditions, develop into morphologically distinct adults. Psyllids that developed under a short photoperiod or colder temperatures are significantly larger and darker than those that developed under a long photoperiod and higher temperature. A similar phenomenon was previously observed in *C. pyricola* (Oldfield, 1969; Mustafa and Hudson, 1984). The sensitivity to environmental cues may, however, change from generation to generation. For example, in *C. pyricola*, progeny derived from winter forms is less sensitive to short photoperiods than those derived from the summer forms (Mustafa and Hudson, 1984).

Concerning the mechanisms operating on colour composition in *C. bidens*, homochromy does not seems to be the key factor. The colour differences were apparent in the laboratory experiments despite similar background of fully leaved pear that were provided to the psyllids. Colour changes, such as found in the pear psyllid may be regulated by abiotic factors. Developmental temperature is known as an important factor in pigment development and thus in the colouration of many insects. The temperature-colour relationship in *C. bidens* fits a general tendency for insects reared under very high temperatures to be pale, and those developing at low temperatures to be dark (Chapman, 1998).

Although, the significance of these colour changes is unknown, these are expected to be adaptive. Seasonal colour change has been implicated as a mechanism for thermoregulatory adaptation by affecting the body temperature (Chapman, 1998). Darker insects reflect relatively little of the radiation that falls on them, while lighter coloured reflect more. De Jong *et al.* (1996) present experimental data that, dark insects absorb more radiation than pale ones and become active earlier than would be the case if they had remained pale. As regards to pear psylla, these colour changes may have further impact on camouflage and thereby contribute to the ability of psyllids to avoid predation. Dark winter forms are much less conspicuous on the bark of a defoliated pear tree, while pale green/yellowish summer forms are more cryptic on the green leaves in the summer time.

Although, similar effects of temperature and photoperiod on colour polymorphism have been described already in other species, the effect of these factors on body size has been less explored. Changes in body size towards smaller adults under hotter conditions may also have thermoregulatory adaptation, given that metabolically produced heat can be lost more rapidly by smaller insects with larger surface to body volume ratio (Chapman, 1998). On the other hand, the smallest sizes, in this case at 30˚C, may also indicate that these high temperatures are less favourable to this Palaearctic insect species.

Taking into consideration the response of psyllids to both photoperiod and temperature, with the understanding of its annual cycle, suggests a differential phenotypic plasticity, which occurs stepwise, from winter to summer, while gradually from summer to winter. We hypothesize that both temperatures and photoperiod affect the size and colour of the adult psyllid in a reaction norm pattern. The phenotype is also likely to be affected in a cumulative manner during the developmental period. It was previously suggested by Nijhout (2003) that some kind of integration of environmental cues occur during the sensitive developmental period. Thus, a long term exposure to certain temperature or day length regime is expected to have a stronger effect than a partial exposure. The transition from the winter generation to the first spring generation is synchronous with its host plant phenology. Since its transition is rather abrupt, the larvae of this generation are exposed to a uniform photoperiod and other abiotic conditions at analogous stages leading thus to the appearance of a homogeneous and a distinct phenological morph. However, subsequent generations are less homogeneous as the populations face changing environmental conditions that lead to gradual changes in the phenotype, thereby generating a continuous polymorphism later in the season. Whether this phenotypic plasticity is limited to morphological characteristics or is also expressed physiologically remains to be evaluated.

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