Egg Phenology of a Host-Specialist Butterfly in the Western Slopes of the Northern Chilean Andes

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Abstract

Phenological studies are especially important in order to understand the ecological process operating at temporal level. The western slopes of the northern Chilean Andes at about 3,500 m asl are a mosaic of arid environments in which precipitations are highly seasonal, mostly concentrated in summer. Teriocollas zelia andina Forbes (Lepidoptera: Pieridae) is one of the most conspicuous and regularly observed butterflies flying in this region; it is a host specialist associated with the native shrub Senna birostris var. arequipensis (Fabaceae). The objectives of this study were (1) to characterize the temporal variations in the relative abundance of eggs of this host-specialist butterfly and (2) to examine the relationship of these variations with leaf phenology. Monthly samplings of eggs were carried out from February 2011 to January 2012. Circular statistical analyses of the relative abundance of eggs indicated clustered distribution along the year with the mean vector in June. Temporal variation in the relative abundance of eggs was correlated (Spearman rank correlation test) with the availability of plant substrate for egg laying and larval feeding.

Introduction

Phenology is the temporal distribution of a phenomenon (Wolda 1988). Phenological studies, in which characterizations of temporal patterns of abundance of organisms are provided and underlying causes are determined, are extremely important in order to adequately understand the ecological process operating at temporal level. This knowledge is useful when forecasting is required for any purpose, either in anthropic or natural environments (Primack et al 2009).

Temporal suitability of the environments is an important factor modelling temporal patterns of animals and plants because successful reproduction and growth may be often limited to a short period each year (Visser & Both 2005). Dealing with insect–plant relationships, the host plant phenology may have a key role as a determinant of many aspects of the ecology of the associated phytophagous insects (Hodkinson et al 2001). This situation could be especially relevant when highly specialized insects are involved. Furthermore, plant phenology is also a strong selective force (Wood et al 1990, Mopper 2005), affecting directly the survival of immature stages or indirectly the success of adult mating (Mopper 2005).

Insect phenology is best known and understood in temperate than in tropical zones (Wolda 1988, Hilt et al 2007). In general, seasonality of tropical insects has been reported for locations with clearly defined dry and wet seasons (Wolda 1988, Kitching et al 2000, Aguirre et al 2011, Muniz et al 2012). However, different patterns may occur in the same location (Pinheiro et al 2002, Hilt et al 2007, Aguirre et al 2011, Silva et al 2011).

Few phenological studies have been carried out on insects of the tropical environments in South America; however, some interesting patterns have been already detected, either at population or at community level. Some of these studies have included immature stages or adults of Lepidoptera, one of the most diverse orders of insects, whose importance as subject of ecological studies has been largely acknowledged (DeVries et al 1997). The most important factors associated

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The western slopes of the northern Chilean Andes at about 3,500 m above sea level (masl) are a mosaic of arid landscapes largely dominated by shrubs (Luebert & Pliscoff 2006, Muñoz 2006). Despite the great biological interest of this area, associated with the aridity, the ecology of invertebrates inhabiting these environments is poorly known. Changes in temperature along the year are minimal in these landscapes, while the water input is determined by the highly seasonal rainfall, which is mostly concentrated between December and February, while a long dry season goes from March to November (Luebert & Pliscoff 2006). Under these conditions, temporal abundance patterns of host-specialist phytophagous insects inhabiting these arid environments are expected to be highly seasonal and mostly determined by food availability. The aim of this study was to characterize the temporal variation in the relative abundance of eggs of one host-specialist butterfly and to examine its relationship with leaf phenology.

Material and Methods

Study site

The work was carried out in the western slopes of the northern Chilean Andes, at about 3,300 m asl, in the surroundings of Socoroma village (18°16′S, 69°35′W), Parinacota Province. This area is characterized by a tropical xeric bioclimate (Luebert & Pliscoff 2006). In general, vegetation cover is seasonal, reaching higher levels after rains, during April–May (Muñoz & Bonacic 2006).

Study species

Teriocolas zelia andina Forbes (Lepidoptera: Pieridae) is one of the most conspicuous and regularly observed native butterflies flying in this area (Peña & Ugarte 1996). It has been reported as a monophagous species in the study site, with egg laying and subsequent larval feeding and development restricted to the shrub Senna birostris var. arequipensis (Fabaceae) (Vargas 2012), which is a very important component of the native flora in this location. Eggs are placed singly on new or mature leaflets; the time elapsed from egg laying to adult emergence is about 45 days.

Sampling

Eggs were collected at monthly intervals from February 2011 to January 2012. Fifty plants were randomly selected each time, and a careful examination was carried out to determine the total number of eggs on each. Phenological studies on the host plant are unknown for the study area; thus, the number of plants with new or mature leaves was monthly recorded in order to estimate the availability of substrate for egg laying and larval feeding. New leaves were those not completely expanded or that have just completely expanded, typically dark green and lightly flexible. Mature leaves were completely expanded, whitish green, and coriaceous. Additionally, the number of plants with old (light yellow or light brown) leaves was also determined.

Statistical analyses

A chi-square test was performed to determine if eggs were randomly distributed among new and mature leaves. Descriptive circular analyses were performed to characterize the monthly relative abundance of eggs. The Rayleigh uniformity test was conducted to verify if the eggs were uniformly distributed along the year. Circular statistics is a tool of great importance for data analysis in studies of temporal variation of biological phenomena, although these procedures have not been frequently employed in published contributions dealing with phenology (Hudson 2010). However, these tools have been successfully used in studies of temporal variation in the Neotropics, either with plants (Morellato et al. 2000) or insects (Pinheiro et al. 2002, Silva et al. 2011), including butterflies (Ribeiro et al. 2010).

In order to determine if the relative abundance of eggs is related with leaf phenology and/or some abiotic factor, Spearman rank correlation analyses were conducted with the following variables: maximum and minimum temperature (in degrees Celsius), precipitation (in millimeters), number of plants with new leaves, number of plants with mature leaves, and number of plants with old leaves. Following Pinheiro et al. (2002), analyses were performed with the variables measured in the respective sampling month and the variables measured in the previous month (delayed variable), because the time elapsed from egg laying to adult emergence of this butterfly is about 45 days. Climatic information was obtained from the Instituto de Investigaciones Agropecuarias, INIA-URURI (Fig. 1). Statistical analyses were performed with the software BioEstat 5.0 (Ayres et al. 2007).

Results

Relative age of leaves used for egg laying

Overall, 274 eggs were counted during the sampling period, 233 of which were found on new leaves, while the remaining 41 were on mature leaves. No eggs were found on old leaves.
Then, relative ages were differentially used for egg laying ($\chi^2=134.54; df=1; p<0.0001$).

**Phenological pattern**

The 274 eggs counted during the sampling period were found between March and December 2011 (Fig 2). These were not uniformly distributed throughout the year (Rayleigh test, $p<0.001$; Table 1); the mean vector (127.3555°, Table 1) was located in June. Interestingly, 88.7% of the eggs were concentrated in autumn ($n=141$) and winter ($n=102$) samplings, whereas no eggs were found in summer months February 2011 and January 2012.

**Relationships with biotic and abiotic factors**

The relative abundance of eggs was positively and significantly correlated with the two variables associated to substrate availability in the collecting month: number of plants with new leaves ($p=0.0048$; Table 2) and number of plants with mature leaves ($p<0.0001$; Table 2). A similar situation was observed with resource availability measured in the previous month, but it was restricted to the number of plants with new leaves ($p<0.0001$; Table 2). Availability of plants with old leaves was not significantly correlated with the relative abundance of eggs, either in the sampling month or in the previous month (Table 2). The only climatic variable significantly associated with the relative abundance of eggs was minimum temperature measured in the sampling month, but this relationship was negative ($p=0.0250$; Table 2).

**Discussion**

The differential use of leaf relative age suggests that females search and select new leaves for oviposition. This specialized behavior could be underlying the temporal pattern here detected. Then, as expected for a highly host-specialist butterfly living in a location with highly seasonal rain distribution, eggs were not uniformly distributed throughout the year (Table 1 and Fig 1), and it was strongly correlated with the level of availability of host plant substrate for egg laying and larval development (Table 2). The high relative abundance of eggs during the first half of the dry season could be understood as a cumulative effect of leaf production prompted by the wet season. Surprisingly, the scant precipitations during the wet season, in January and February 2011 (77.3 mm; Fig 1), were enough to stimulate leaf growth (Fig 2). Interestingly, the 7 months previous to January 2011 were extremely dry, with just 0.2 mm precipitation in November 2010. During 2011, “unusual” rains were recorded during the (normally) dry season (Fig 1), in April (14.4 mm) and July (4.4 mm), which could have contributed, at least partially, in maintaining the active leaf growth, because a great quantity of plants with new leaves was found until August, decreasing quickly from September (Fig 2). Thus, the subsequent decrease in the relative abundance of eggs during the second half of the dry season could be understood as the inevitable effect of reduction in the availability of adequate leaves. The absence of significant correlation between the relative abundance of eggs and old leaves, either in the sampling month or in the previous month (Table 2), was expected because these leaves are not used for egg laying or larval feeding. The negative relationship between the relative abundance of eggs and minimum temperature in the collecting month (Table 2) is not well understood.

The higher relative abundance of eggs during the dry season recorded for *T. zelia andina* resembles patterns reported for lepidopteran larval abundance in the Brazilian Cerrado, with peaks mostly reached in the first half of the dry season (Morais *et al* 1999, Diniz *et al* 2007). In the same biome, a similar pattern was documented for larvae of the bivoltine micro-moth *Chlamydasistis platyspora* (Meyrick).
number of plants with mature leaves, temperature, PP with old leaves, nonsignificant correlation, from February 2011 to January 2012. Dental slopes of the Andes, Parinacota Province, northernmost Chile, a Significant correlation. Teriocolias zelia andina (Fabaceae) are collected in the field in different months of the year without presence of new or mature leaves in the study site. This fact suggests that diapause could be involved at some stage of the life cycle. However, more than 50 individuals have been reared until adult stage in laboratory from eggs or larvae collected in the field in different months of the year without observation of diapause at the egg, larval, or pupal stages (HAV, unpublished data). At adult stage, reproductive diapause has not been studied for T. zelia andina, although this is a strategy used by some tropical seasonal butterflies (Braby 1995, Canzano et al. 2003, Pieloor & Seymour 2001). However, although adult abundance was not determined in this study, adults were searched each month in the same day of the egg samplings in order to verify their active presence in the study site. As a result, these were found in all sampling dates. Therefore, the absence of eggs in the samplings is not due to the absence of adults.

Furthermore, apparently reproductive diapause was not affecting these butterflies, because during the last days of November, when the relative abundance of eggs and frequency of plants with new or mature leaves were drastically reduced in the sampling site, one plant with abundant new and mature leaves was found in Socoroma village, at a distance about 3 km from the sampling site. This “anomalous” phenological stage was triggered by accidental irrigation from a nearby canal. The plant was carefully examined, and 17 eggs were found. This fact is suggesting an

Table 1 Descriptive circular statistics and Rayleigh one-sample test for egg abundance of Teriocolias zelia andina (Lepidoptera: Pieridae) on Senna birostris var. arequipensis (Fabaceae) in the neighboring area of Socoroma village (18°16′S, 69°35′W), at about 3,300 m asl, in the occidental slopes of the Andes, Parinacota Province, northernmost Chile, from February 2011 to January 2012.

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<sup>a</sup> Significant correlation.

Table 2 Spearman rank correlation analysis between egg abundance of Teriocolias zelia andina (Lepidoptera: Pieridae) on Senna birostris var. arequipensis (Fabaceae) and some biotic and abiotic variables measured in the collecting month and previous (delayed) month in the neighboring area of Socoroma village (18°16′S, 69°35′W), at about 3,300 m asl, in the occidental slopes of the Andes, Parinacota Province, northernmost Chile, from February 2011 to January 2012.

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<sup>a</sup> NS nonsignificant correlation, NPML number of plants with new leaves, NPNL number of plants with mature leaves, NPOL number of plants with old leaves, MAXT maximum temperature, MINT minimum temperature, PP precipitation, -D “delayed” variable.
opportunist behavior of the females, which would be able to find plants in the adequate phenological stage for egg laying. Unfortunately, phenological studies on the host plant in the western slopes of northern Chile were not found in literature. However, considering that precipitation is not always evenly distributed in space, under natural conditions, some plants could be expected to be out of the general phenological pattern, offering egg laying and food substrate for the butterfly throughout the year.

Based on the available data, it is evident that the most important proximate cause, as defined by van Schaik et al. (1993), for the temporal pattern of egg abundance of *T. zelia andina* in the western slopes of the northern Chilean Andes is the availability of substrate for egg laying and larval feeding. Thus, it is expected that any factor modifying the plant phenology will be reflected in the butterfly phenology.

Besides the geographical variation, precipitation may also vary year after year, with the subsequent effect on plant phenology. Thus, future studies should assess the possible variation in phenology of the host plant–butterfly system at geographic and interannual scale.

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