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POPULATION BIOLOGY OF TWO SPECIES OF *HELICONIUS* (NYMPHALIDAE: HELICONIINAE) IN A SEMI-DECIDUOUS FOREST IN SOUTHEASTERN BRAZIL

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ABSTRACT. Populations of two species of butterflies, *Heliconius erato* and *Heliconius ethilla*, were studied during 17 months in SE Brazil. For *H. erato*, the number of individuals present per day varied from one to 10. Time of residence was 24.9 ±18.97 days, with the maximum 59 days. Males were recorded traveling distances up to 200 m. A study of wing color patterns of *H. erato* showed results similar to those of previous studies, including the variation in the number of red raylets. For *H. ethilla*, the number of individuals present per day varied from one to 15. Time of residence was 32.6 ± 23.93 days, with the maximum registered of 106 days. Males can travel distances up to 650 m. For both species, the population peaks occurred in March and May of both years. The sex ratio of individuals captured was male biased for most of the months. Age structure was not stable, though intermediate-age individuals dominated in every month. The difference between male and female mean forewing lengths was not significant. These population features agree with patterns previously observed in southern Brazil populations, though with much lower population numbers.

Additional key words: mark-recapture, Heliconiini, red raylets

Although butterflies in the genus *Heliconius* are among the most studied tropical species, with many publications covering diverse aspects of their biology, such as systematics, ecology, genetics and evolution (Turner 1971, Ehrlich & Gilbert 1973, Cook *et al.* 1976, Araujo 1980, Mallet & Jackson 1980, Brown 1981, Ehrlich 1984, Mallet *et al.* 1987, Quintero 1988, Mallet & Gilbert 1995, Ramos & Freitas 1999, Jiggins *et al.* 2001, Gilbert 2003), data from population biology of most species are still unavailable or incomplete (Ramos & Freitas 1999). The work by Ehrlich & Gilbert (1973) brought important information on the biology of *Heliconius ethilla* and is, so far, the only published population report of this species. In contrast, *Heliconius erato* has been studied in many different places in the Neotropics (Turner 1971, Benson, 1972, Araujo 1980, Saalfeld & Araujo 1981, Romanowsky *et al.* 1985, Ramos & Freitas 1999), including studies of genetics and variation on wing color patterns (Saalfeld & Araujo 1981, Pansera & Araujo 1983, Sheppard *et al.* 1985, Oliveira & Araujo 1992, Ramos & Freitas 1999). Most of the published population studies cover only three different regions in the Neotropics (Trinidad, Rio Grande do Sul and coastal São Paulo state) and none in most seasonal semi-deciduous forests or cerrados. In a recent paper, Ramos & Freitas (1999) suggested that *H. erato* is an ecologically plastic species, in use of resources (larval and adults), behavior and population parameters. In this context, the descriptions of different populations of *H. erato* are important to support a broader view about the natural history of this species.

The objective of the present study is to describe and compare population parameters of *H. erato phyllis* (Fabricius) and *H. ethilla narcaea* (Godart) in a small forest fragment of semi-deciduous forest in Southeastern Brazil, reporting also the cyclical annual variation in four color pattern elements in *H. erato*.

STUDY SITES AND METHIDS

The study was carried out in the 250-ha semi-deciduous forest Reserva da Mata da Santa Genebra (22° 49'S, 47° 07'W), a municipal forest reserve in Campinas, São Paulo State, SE Brazil. The annual rainfall is near 1400mm and the average annual temperature is 20.6° C (data from the Campinas Agronomical Institute). The climate of the region is characterized by a dry, cold season (May to August) and a wet, warm season (September to April) (more details and a climatic diagram can be found in Vanini *et al.* 2000). Detailed descriptions and maps of the study area are available in Morellato & Leitão-Filho (1995).

Butterflies were marked, released and recaptured (MRR) along a trail (1150m long divided into 50m sectors) during 17 months, from December 24, 2001 to May 29, 2003 (1–3 times a week), for a total of 91 days (about 3 hours/day). Butterflies were net-captured, individually numbered on the underside of both forewings with a black permanent felt-tipped pen, and released. Individual characteristics of each individual (age, forewing length, point of capture, sex, food sources and color patterns) were recorded for later analysis (as in Ramos & Freitas 1999). Wing wear, based on six categories (freshly emerged, new, intermediate, old, very old, tattered) was used as an additional measure of age of individual butterflies (following Ehrlich & Davidson 1960, Brussard & Ehrlich 1970, Ehrlich & Gilbert 1973). These six categories were grouped into three (new, intermediate and old) for analysis (as in Freitas 1993, 1996). Age structure was calculated for males only, through the monthly means of daily proportions of each category. Individual vagility was measured as the maximum linear distance from capture to recapture points.

For *Heliconius erato*, four color-pattern variations were recorded: number and shape of the red raylets on the ventral hindwing; presence and color of the cubital spot on the dorsal forewing (more details and pictures in Ramos & Freitas 1999); number of "light yellow squares" on the apical ventral hindwing; and the color of the prolegs (entirely yellow or with tarsal portion red).

The MRR data were analyzed by the Jolly-Seber method for estimating population parameters (software developed by R. B. Francini, UNISANTOS) for obtaining the "estimated numbers". Only males were considered for this analysis due to the low number of females captured. Daily results were recorded as "number of individuals captured per day" (NICD), including recaptures, and "number of individuals present per day" (NIPD). To estimate NIPD, recaptured individuals were considered to be present on all days between the first and last capture.

For *H. erato*, population parameters and residence time were calculated from January to May 2002 and the color patterns data analyzed from December 2001 to December 2002, due to low numbers of captured individuals on other periods. For *H. ethilla* analysis were made form December 2001 to May 2003. The sex ratio was calculated through the monthly means of daily proportions in NIPD.

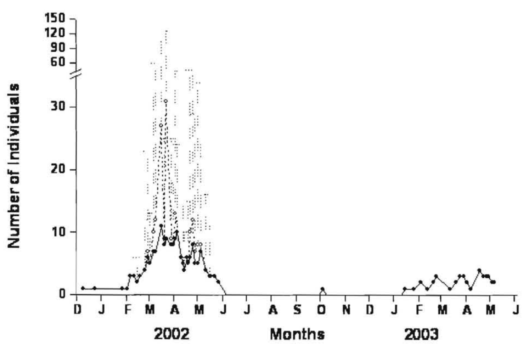

FIG. 1 - Number of males *H. erato* from December 2001 to May 2003 in Santa Genebra, Campinas, SP. Solid circles = NIPD, open circles = estimated number based on Jolly-Seber (bars = 1 standard error).

RESULTS

Heliconius erato phyllis

Population biology. From December 2001 to May 2003, the NICD for males varied from one to five (mean = 1.6; SD = 0.91; n = 52 days). The calculated NIPD varied from zero to 12 (mean = 3.4; SD = 2.03; n = 52 days). Estimated numbers based on Jolly-Seber (Fig. 1) suggests that population numbers are not very much higher than numbers obtained by the NIPD. The population peaks occurred in March and May of both years, although in 2003 the increase in butterfly numbers was about half of that in the same period of 2002. In other months of both years the butterfly numbers remained low and stable (Fig. 1).

Sex ratio. During this study, 47 males and 12 females were captured, giving a male biased sex ratio (χ^2 = 19.59; $p < 0.001$; DF = 1). Males dominated in every month considered (Fig. 2). Males were recaptured from one to six times and females from one to four times; 23 males and three females were recaptured at least once.

Age structure. Even though the proportion of "intermediate" individuals remained greater in every month except for May 2002, the proportions of "new" and "old" individuals were unstable during the months

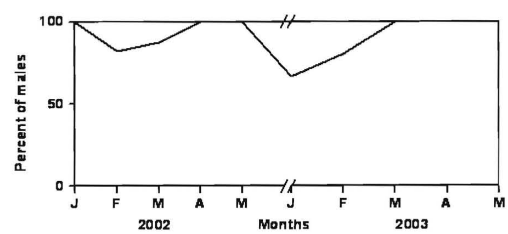

FIG. 2. Sex ratio of *H. erato* from January 2002 to May 2003 in Santa Genebra, Campinas, SP.

considered. Figure 3 shows the proportion of the three age classes from January to May 2002.

Residence time. The residence time varied from three to 59 days (mean = 24.9 days; SD = 18.97). Life expectancy (following Cook *et al.* 1967) was 11.21 days.

Vagility. Only males were analyzed due to the low number of females. The greatest distance recorded along the trail flown by an individual was 200 meters (mean = 65.2 m; SD = 61.12 m; n = 23). Most of the individuals were recaptured at least once in a different

TABLE 1 - Frequency distributions and sample size (N), of the three color patterns recorded in *H. erato* butterflies of both sexes captured in the Santa Genebra from December 2001 to December 2002 (2003 not included due to the low number of captures). For number of red raylets and number of light yellow squares, data are presented as mean ± standard deviation.

Month/year	proleg color		red raylets	Light yellow squares	N
	yellow	red tip			
Dec/01–Jan/02	2	2	5 ± 0	4.25 ± 0.5	4
Feb/02	6	1	4.14 ± 1.07	3.5 ± 0.55	7
Mar/02	8	2	4.3 ± 1.16	3.4 ± 1.35	10
Apr/02	9	3	3.58 ± 1.16	3.17 ± 1.59	12
May/02	6	1	3.71 ± 1.5	3.29 ± 0.49	7
Oct/02–Dec/02	2	0	3.5 ± 2.12	4 ± 1.41	2

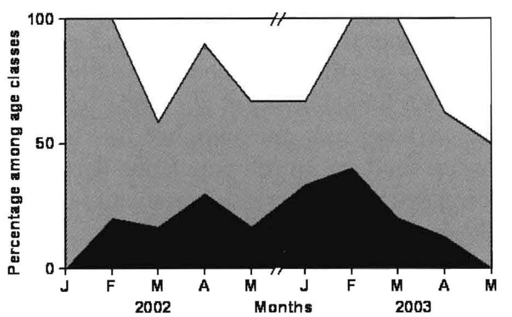

FIG. 3 - Age structure of males *H. erato* from January 2002 to May 2003 in Santa Genebra, Campinas, SP. Black = new individuals, gray = intermediate, white = old.

site from that of first capture (18 out of 23 recaptured individuals).

Wing size. The forewing length of males varied from 30 mm to 39 mm (mean = 35.2 mm; SD = 2.69 mm; n = 47) and of females from 32 mm to 40 mm (mean = 36.1 mm; SD = 2.57 mm; n = 12). This difference was not significant (t = 1,03; DF = 17,7; p = 0,314).

Resource utilization. For 39 visits recorded, 69% were on *Lantana camara L.* (Verbenaceae), 18% on *Chromolaena odorata* (*L.*) R. King & H. Robinson (Asteraceae) and 13% on flowers of other genera, including *Lippia* sp. (Verbenaceae) and *Manettia* sp. (Rubiaceae). In both years, *L. camara* flowers decreased after May, and the visits to other nectar sources increased.

Color patterns. The number of red raylets varied from 1 to 6 in both sexes (mean = 3.88; SD = 1.20; n = 58). Monthly analysis for this trait was done only for 2002, and some months were grouped due to the small number of marked individuals. The average number decreases in colder months (Table 1). The total number of individuals with dot shaped red raylets (41) was greater than the number of individuals with line shaped red raylets (17) (χ^2 = 9.12; p < 0.005; DF = 1) and fits in 3:1 distribution (χ^2 =0.09; p = 0.75; DF=1).

Four categories of cubital spot on the dorsal forewing were recorded with uneven distribution. The predominant category was "yellow" (22 individuals), followed by "absent" (15), "red" (5) and "fusing with the subapical red bar" (5).

The number of "light yellow squares" varied from 0 to 5 in males (mean = 3.0; SD = 1.33; n = 45) and 4 or 5 in females (mean = 4.2; SD = 0.38; n = 12). Monthly analysis for this trait was done only from December 2001 to December 2002, and some months were grouped due to the small number of marked individuals. The average number decreases in colder months (Table 1).

Two color patterns of the prothoracic legs were recorded. The number of individuals with "yellow" legs (33) was greater than the number of individuals with "yellow with red tips" (9) (χ^2 = 13.09; p < 0.001; DF = 1) and also fits in a 3:1 distribution (χ^2 = 0; p=0.99; DF = 1). Monthly analysis for this trait was done only for 2002, and some months were grouped due to the small number of marked individuals (Table 1).

Heliconius ethilla narcaea

Population biology. The NICD for males varied from one to six (mean = 2.0; SD = 1.18; n = 90 days). The NIPD varied from one to 15 (mean = 6.4; SD = 4.43; n = 90 days). As in *H. erato*, estimated numbers based on Jolly-Seber (Fig. 1) are not very much higher than actual numbers obtained by the NIPD. The population peaks (based on NIPD) occurred in March and May of both years, and in the remaining months the population numbers remained low and stable (Fig. 4).

Sex ratio. During this study, 123 males and 35 females were captured giving a male biased sex ratio (χ^2 = 47.9; p < 0.001; DF = 1). Males dominated in every month considered, except for December 2002 (Fig. 5).

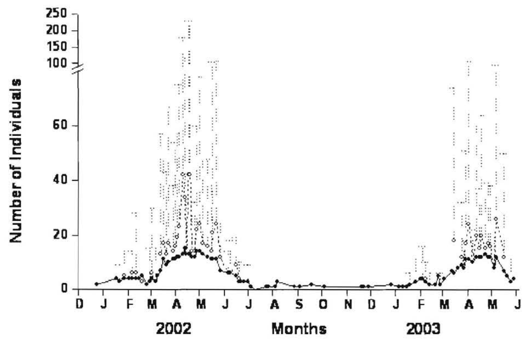

FIG. 4. Number of males *H. ethilla* from December 2001 to June 2003 in Santa Genebra, Campinas, SP. Solid circles = NIPD, open circles = estimated number based on Jolly Seber (bars = 1 standard error).

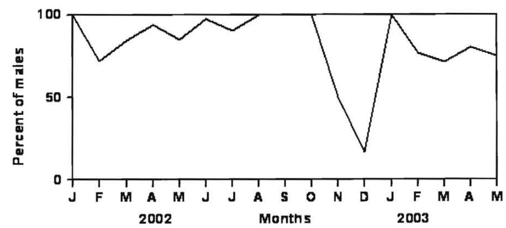

FIG. 5. Sex ratio of *H. ethilla* from January 2002 to May 2003 in Santa Genebra, Campinas, SP.

Males were recaptured from one to 13 times and females from one to three times; 53 males and 11 females were recaptured at least once.

Age structure. The proportion of "intermediate" individuals was greatest in most of the months considered. The proportions of "new" and "old" individuals varied during the months considered. Figure 6 shows the proportion of the three age classes from January 2002 to May 2003.

Residence time. The time of residence varied from two to 106 days (mean = 32.6 days; SD = 23.93). Life expectancy (following Cook *et al.* 1967) was 44.61 days.

Vagility. Only males were analyzed due to the low number of females. The greatest recorded distance along the trail flown by an individual was 650 meters (mean = 83.96 m; SD = 94.45 m; n = 53). Most of the individuals were recaptured at least once in a different site from that of first capture (44 out of 53 recaptured individuals).

Wing size. The forewing length of males varied from 35 mm to 46 mm (mean = 41.5; SD = 2.14; n = 121) and of females from 38 mm to 45 mm (mean = 41.5; SD = 1.77; n = 35). This difference was not significant (t = 0.039; DF = 65.4; p = 0.969).

Resource utilization. For 128 visits recorded, 52% were on *L. camara*, 26% on *C. odorata* and 23% on flowers of other genera, including *Lippia* sp. (Verbenaceae), *Manettia* sp. (Rubiaceae) and *Passiflora suberosa* L. (Passifloraceae). As described for *H. erato*, visitation to other nectar sources increased after May when flower production decreased for *L. camara*.

DISCUSSION

Population biology. The populations showed three distinct phases: growth in January, February and March, peak in April and decline in May and June. The instability in numbers differs from the patterns found in other tropical *Heliconius* studies (Turner 1971, Ehrlich & Gilbert 1973; Ramos & Freitas 1999) that reported stable populations throughout the year, and was to some extent similar to those studied in southern Brazil (Araujo, 1980; Romanowsky et al., 1985), with marked seasonal variation in the number of individuals. However, in southern Brazil, population decline seems to be correlated with a decrease in ambient temperature (Saalfeld & Araujo, 1981; Romanowsky et al., 1985), whereas at Santa Genebra the decline is probably due to decrease in rainfall. The number of individuals in the studied populations was relatively low (up to 60 individuals based on Jolly-Seber estimates in both species) compared with studies in south Brazil, where captures could be as high as 60 individuals in a single day (Romanowsky et al., 1985). In the periods of population peak, the number of captured individuals in both species was equivalent to those reported in other studies in tropical sites (Turner 1971, Ehrlich & Gilbert 1973; Ramos & Freitas 1999).

Rainfall is considered as an important factor limiting population numbers for *Heliconius*, since it is closely

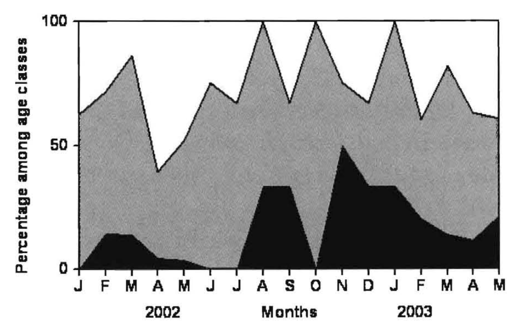

FIG. 6 - Age structure of males *H. ethilla* from January 2002 to May 2003 in Santa Genebra, Campinas, SP. Black = new individuals, gray = intermediate, white = old.

linked with adult resource availability (see Ehrlich & Gilbert, 1973 and Gilbert, 1984). The seasonal variation in rainfall probably explains the population fluctuation patterns reported in the present paper, which is quite different from stable populations reported in tropical or coastal areas where rainfall is constant throughout the year. The accentuated decline and low population numbers in the Santa Genebra populations can also be a consequence of migration of individuals to surrounding subpopulations in more suitable environments (as proposed in Saalfeld & Araujo 1981) outside our sampling area.

High values of residence time agree with other studies of *Heliconius* (Turner 1971, Benson 1972, Ehrlich & Gilbert 1973, Araujo 1980, Quintero 1988, Ramos & Freitas 1999). These numbers may be related to restricted dispersion of adults and to several visits to resources patches. For both species studied here, sex ratio was male biased, agreeing with field results from several butterfly studies (Ehrlich & Gilbert 1973, Mallet & Jackson 1980, Ehrlich 1984, Ehrlich *et al.* 1984, Freitas 1993, Ramos & Freitas 1999). Differential behavior in the sexes may cause this deviation.

The data on resource utilization by both species suggests that they are using all available flowers in the study site. Flowers of *L. camara* were the most visited by both *Heliconius* in Santa Genebra. Previous studies showed that *Lantana* is a major pollen source for some species of *Heliconius* in several tropical sites (Boggs *et al.* 1981, Ramos & Freitas 1999). We suggest that intense use of *Lantana* and other flowers with small pollen grains could be related with more generalist edge species of *Heliconius*.

Comparing both species in the study site, *H. ethilla* is more abundant and persistent than *H. erato*, but both species show the same seasonal fluctuation pattern of population. Both species are suggested as plastic species (Ramos & Freitas, 1999 and this paper), but there are more data available for *H. erato* than for *H. ethilla* at the moment. Additional studies of *H. ethilla*, including host plant use, larval performance and population dynamics in different habitats are needed.

Color patterns in *Heliconius erato*. The decline in the mean number of red raylets in colder months agrees with Pansera & Araujo (1983), Oliveira & Araujo (1992) and Ramos & Freitas (1999). The mean number of “light yellow squares” also seems to decline in colder moths, and the population becomes virtually absent during winter and spring. For this reason it is difficult to perform any analysis to help to clarify the direction and origin of the variation of this and other traits.

The three-to-one proportion between the two colors of prothoracic legs may be a hint about the heritability of this trait. However, due to the low number of *H. erato* captured, it is difficult to infer much about the biology of the color traits considered in this study.

Further studies could investigate the influence of seasonal climate pattern on the availability of resources for long-life butterflies such as *Heliconius*, and how these factors restrain population numbers. Also, the understanding of temporal and spatial fluctuation patterns of color patterns can elucidate ecological processes related to natural selection and genetic variability.

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