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STUDIES ON THE GENETICS AND ECOLOGY OF *Heliconius erato* (LEPIDOPTERA; NYMPHALIDAE). IV. EFFECTIVE SIZE AND VARIABILITY OF THE RED RAYLETS IN NATURAL POPULATIONS

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ABSTRACT

Natural populations of the butterfly *Heliconius erato phyllis* from southern Brazil were investigated for effective size and variability of a quantitative trait (the number of red raylets in the hindwings). Three localities were studied, with an effective size of 48, 84, and 56, averaging over all seasons of the year. As for the mean number of red raylets, lower values were found in spring, and higher ones in summer. The frequency distributions of this trait also showed seasonal variation; for one locality the mean and the distribution of raylets were significantly different, according to season. The low effective number and the seasonal variation in the red raylets suggests the possibility of both random genetic drift and natural selection acting on these populations.

INTRODUCTION

Sewall Wright (1931) first called attention to the fact that the population size parameter in models for the evolution of populations should be the "effective size", not the total number of individuals. Since then a large number of mathematical models have incorporated the concept (Crow and Kimura, 1970). However, despite its great theoretical importance few attempts have been made to estimate the effective size of natural populations; this is due, of course, to the practical difficulties in obtaining reliable data,

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the situation being worse in populations with overlapping generations. Begon (1977) and Begon *et al.* (1980) discussed the general requirements for such an estimation and applied them to populations of *Drosophila subobscura*.

We employed an ecological method to investigate the effective size of natural populations of the butterfly *Heliconius erato phyllis* (Nymphalidae). This is a widespread subspecies in the Neotropics, ranging from Eastern Bolivia to Uruguay and Northeastern Argentina, inhabiting tropical and subtropical environments (Brown Jr., 1979). The objectives of the present investigation were: 1. To estimate the effective size of its central and marginal populations in southern Brazil. 2. To analyze the variability of a quantitative trait (the number of red raylets in the hindwings) in the same populations, taking into account the seasons of the year. 3. To relate that variability with the effects of natural selection and genetic drift.

MATERIAL AND METHODS

Three localities in the State of Rio Grande do Sul were selected for the study: Morro Santana (30°01'S, 51°13'W), a hill near the city of Porto Alegre; Parque de Itapuã (30°17'S, 51°01'W), about 60 km from Porto Alegre and Parque do Turvo (27°20'S, 53°10'W), about 500 km away. These localities, with the exception of Morro Santana, have already been subjects of study of other aspects of the biology of *Heliconius erato* (Saalfeld and Araújo, 1981; Pansera and Araújo, 1983). Morro Santana is still within the urban area, with a dense secondary vegetation and plenty of exotic plants like *Eucalyptus* spp.; a road ca. 1500 m long was used for sampling.

Initially the areas were surveyed as if they were subpopulations; however, the amount of butterfly movement among them, both at Itapuã and Morro Santana, was greater than 25% (capture-recapture), so that we treated them as single populations. By the same criterion Parque do Turvo had to be considered as formed by six populations along a road of about 4 km. A capture-recapture program was employed every two weeks at Morro Santana and Itapuã, while for Turvo there were four sampling periods: January/82 (20 consecutive days), May and October/82 and February/83 during six days. All the field work was done from January/82 until June83.

Estimates of the effective population size were made after correction for the excess of males captured, following Merrell (1968) and Begon (1979). The total number of butterflies captured were: 195 at Itapuã, 377 at Morro Santana and 544 at Turvo. Average inbreeding coefficients were estimated by two methods: for Itapuã according to Spiess (1989, p. 266), by using data on esterase polymorphisms previously published for the same area (Lima and Araújo, 1982); for the populations from Turvo the movement of individuals from one population to the other was taken as the migration rate in Wright's equation $F=1/(4\ Nm + 1)$ (Wright, 1931). Both methods of estimation gave very similar results (0.17 and 0.20). These estimates were necessary to calculate the effective size (Ne) as Na/(1+F), (Li, 1976), where Na is the corrected estimate of total population size, which takes into account the biased sex ratio of captures.

To test the equality of means by locality and season, a nested analysis of variance was carried out; since the number of observations were different for each locality and season, the ANOVA was made as given by Steel and Torrie (1960). Whenever necessary a Scheffé test for contrasts among means were used (Steel and Torrie, 1980).

RESULTS

Movement among populations at Parque do Turvo

Table I shows the total number of butterflies marked in January/82 and the proportion of migrants from one population to the another (each population was about 500 m apart). As populations three and four had few individuals recaptured they were not included in the estimations of "m". Generally more than 90% of individuals were recaptured in their original population, the exception being population number six, which was located at the edge of the area sampled. Probably butterflies there dispersed to unsampled adjacent areas, decreasing the number of recaptures and further increasing "m". Due to this fact, population six was discarded from the analysis, which resulted in estimates of "m" with very good agreement.

Table I - Number and percentage of butterflies captured in six different populations from Parque do Turvo as well as the proportion of migrants (m) between them.

Population	Butterflies marked	Recaptures (%)	m
1	46	67	0.032
2	35	43	0.067
3	43	12	-----
4	20	20	-----
5	53	34	0.056
6	15	53	0.375

Effective size of populations

Table II shows the estimates of the effective population size (Ne) for Itapuã and Morro Santana, along the seasons of the year after the correction (Na), due to the excess of males captured. The effective size for Itapuã (I) in autumn/82 was unexpectedly low (27), considering that in this season insect populations in southern Brazil generally are at their maximum. In the winter of that year individuals were neither seen nor captured there (population probably extinct and recolonized in the spring). Afterwards the effective size increased until the next autumn (1983), from 25 to 91. At Morro Santana (MS), where no extinction occurred, Ne had greater values, with a maximum in the autumn/83 of 259. The ratio Ne/Na is of the same order of magnitude in both places, being around 85%. When the effective size was estimated by the harmonic mean of the seasons the values were 43 for Itapuã (spring/82, summer and autumn/83) and 44 for Morro Santana (all seasons).

Table II - Estimates of the average population size (Na), corrected for biased sex ratio, and effective population size (Ne) for the populations of Itapuã (I) and Morro Santana (MS).

Season/Year	Locality	Average Na	Ne	Ne/Na
Autumn/82	I	32	27	0.84
	MS	83	69	0.83
Winter/82	I	-	-	-
	MS	34	28	0.82
Spring/82	I	30	25	0.83
	MS	33	28	0.85
Summer/83	I	56	47	0.84
	MS	46	38	0.83
Autumn/83	I	109	91	0.83
	MS	311	259	0.83

Estimates of the effective size for Parque do Turvo were possible only for the summer/82 (Table III). As before, the values were small, ranging from 32 to 92 in the five populations studied; the relationship between Ne and Na, however, was similar to that given in Table II.

Table III - Estimates of the average population size (Na), corrected for biased sex ratio, and effective population size (Ne) for the populations from Parque do Turvo (Summer/82).

Population	Average Na	Ne	Ne/Na
1	110	92	0.84
2	67	56	0.84
3	60	50	0.83
4	38	32	0.84
5	59	49	0.83

Phenotypic variability

The presence of the red raylets, a trait always conspicuous in the hindwings of the subspecies *phyllis* is a useful tool for analysis of changes in the mean value of a polygenic character (estimates of the heritability of this trait can be found in Pansera and Araújo, 1983). Frequency distributions of red raylets are shown in Table IV for the populations of Itapuã and Morro Santana, and in Table V for those from Parque do Turvo (see also Figure 1). For the most part the means for spring were lower (3.8 and 3.7, Table IV) and those for summer, higher (4.6 and 4.3, Table IV). Spring numbers are influenced by the winter bottleneck, when the populations reduce in number, while increasing in size during the summer to attain a maximum during autumn. Summer/83 had higher mean values when compared to summer/82 at Parque do Turvo (Table V). Whatever the parameter used (average temperature, minimum or maximum daily average), January/83 (midsummer) was warmer than January/82.

To test the equality of means according to locality and season, a nested analysis of variance was performed. The Parque do Turvo population five was used, due to the greater number of individuals and because the surrounding area had a high predictability of resources, making sampling accidents less probable. Results of the ANOVA are in Table VI. Significant differences were obtained only for seasons, the means for localities being statistically equal. A Scheffé's test was applied to the contrast "mean for spring x mean for summer and autumn", since the mean for spring generally was lower than the others. This comparison was significant for the locality Morro Santana ($P < 0.01$). The distributions themselves were also analyzed with the Kolmogorov-Smirnov test. Again, the populations from Morro Santana were different between autumn/82 and spring/82 and between spring/82 and autumn/83 ($P < 0.05$); the remaining distributions were not statistically different. In Figure 1 the shape of the distributions change in the three localities, from autumn/82 to spring/82. A new mode (three raylets) appears in this latter season, which is more obvious at Morro Santana and Parque do Turvo. As temperature rises with the arrival of the summer, the distributions change, with modes at five or six raylets.

Table IV - Frequency distributions of red raylets for Itapuã (1) and Morro Santana (2), according to season of the year.

	Season/Year	No. of butterflies	No. of red raylets						Mean ± s.d.
			1	2	3	4	5	6	
(1)	Autumn/82	36	1	4	7	4	10	10	4.3 ± 1.5
	Spring/82	12	0	1	4	3	4	0	3.8 ± 1.0
	Summer/83	35	0	2	7	4	11	11	4.6 ± 1.3
	Autumn/83	136	1	5	31	24	45	30	4.4 ± 1.2
(2)	Autumn/82	112	4	3	21	19	43	22	4.4 ± 1.3
	Winter/82	41	1	5	7	9	10	9	4.2 ± 1.4
	Spring/82	41	5	0	16	7	7	6	3.7 ± 1.5
	Summer/83	87	2	5	18	16	28	18	4.3 ± 1.3
	Autumn/83	181	0	8	31	37	59	46	4.6 ± 1.2

DISCUSSION

Estimates of effective size are important for an evaluation of the role random process plays in natural populations. The present paper deals with such estimates and with the presumable influence of natural selection on a quantitative trait in the butterfly *H. erato phyllis*. Our data suggest a small effective size for populations of different geographic areas (about 50 individuals, on average) in the State of Rio Grande do Sul. This low value is supported by one aspect of the reproductive biology of *H. erato*: females are monogamic (mean spermatophore count = 0.84 ± 0.37, n = 19, Garcias 1983); further, some males probably do not mate while others do several times, lowering the probability of representation of given genotypes in the following generation, and by consequence, the effective size. Observations made in an insectary and in nature indicate that some males (previously marked) have a greater success in getting females for copulation.

Table V - Frequency distributions of red raylets for the populations from Turvo (1 to 5), according to season of the year.

	Season/Year	No. of butterflies	No. of red raylets 1	2	3	4	5	6	Mean ± s.d.
(1)	Summer/82	41	0	2	9	7	19	4	4.3 ± 1.1
	Autumn/82	7	0	0	1	3	2	1	4.4 ± 1.0
	Spring/82	13	0	0	6	1	4	2	4.2 ± 1.2
	Summer/83	22	0	2	2	2	10	6	4.7 ± 1.2
(2)	Summer/82	35	2	1	7	5	13	7	4.3 ± 1.4
	Autumn/82	22	1	2	5	4	5	5	4.1 ± 1.5
	Spring/82	13	1	4	2	1	3	2	3.5 ± 1.7
	Summer/83	30	0	1	4	2	16	7	4.8 ± 1.1
(3)	Summer/82	35	1	1	8	5	13	7	4.4 ± 1.3
	Autumn/82	37	0	3	10	8	12	5	4.1 ± 1.2
	Spring/82	11	0	1	3	3	2	0	3.5 ± 0.9
	Summer/83	25	0	1	3	3	12	7	4.9 ± 1.0
(4)	Summer/82	19	0	1	6	2	6	4	4.3 ± 1.3
	Autumn/82	18	2	5	4	4	3	0	3.0 ± 1.3
	Spring/82	20	0	4	8	2	6	0	3.5 ± 1.2
	Summer/83	13	0	1	2	1	6	3	4.6 ± 1.3
(5)	Summer/82	53	1	3	7	6	25	11	4.4 ± 1.6
	Autumn/82	11	0	0	1	1	6	3	5.0 ± 0.9
	Spring/82	21	0	2	9	1	5	4	4.0 ± 1.4
	Summer/83	21	0	2	6	1	4	8	4.5 ± 1.5

Mallet (1986) estimated the "neighborhood deme size" of *H. erato* from Costa Rica by two different methods, obtaining values of 55 and 147. The first utilized capture-recapture of adult insects and the second estimate, by marking newly-eclosed individuals (butterflies were marked just after wing expansion).

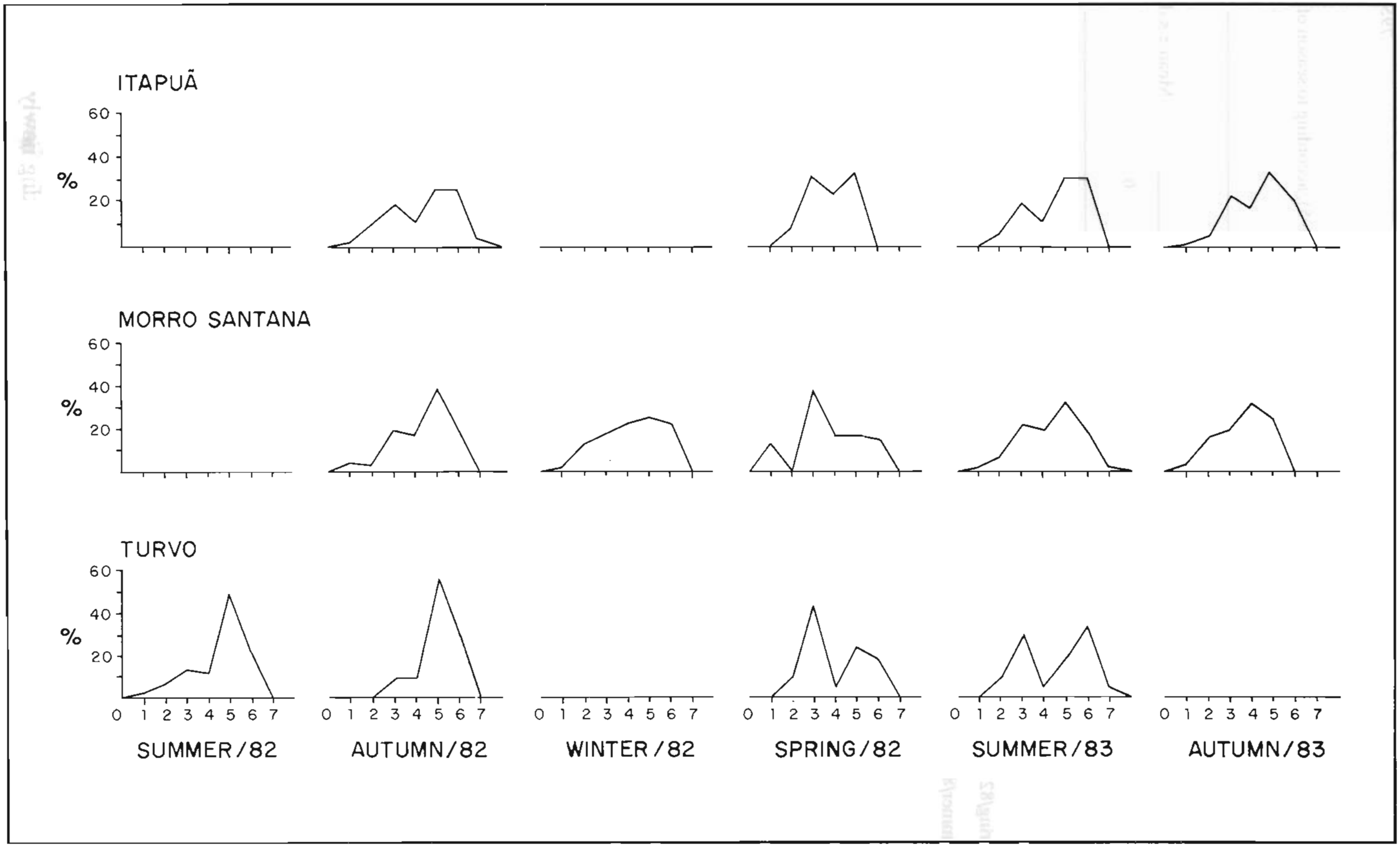

Figure 1 - Frequency distributions of red raylets in the three sampled localities, showing seasonal variation.

Table VI - Result of the nested analysis of variance for the number of red raylets (log transformed) in the three localities studied.

Source of variation	SS	DF	MS	F'(a)	P
Between localities	0.1597	2	0.0799	0.9422	n.s.
Seasons within loc.	1.4397	6	0.2400	2.9083	< .01
Observations within seasons	32.9193	399	0.0825		
Total	34.5187	407			

(a) - F'was calculated after Steel and Torrie (1960).

On the other hand, an analysis of the population structure of *H. erato* by F-statistics, based on the polymorphism for three enzymatic systems, showed significant values of the component Fst. For five different populations belonging to two close geographic areas in Rio Grande do Sul that value was 0.0798 (Silva, 1989). This is suggestive of action of random genetic drift.

Other factors, possibly related to small effective size are the fidelity to communal roosting sites and the restricted home range (Di Mare and Araújo, in preparation). Another evidence of drift in *H. erato* is related to the red raylets and was reported by Pansera and Araújo (1983). Three populations along a road with 9 km long (part of which was sampled in this study) showed significant differences in the frequency distributions of raylets, hardly attributable to selection, since no clear differences in the environment were detected. In our study, evidence for the action of natural selection was detected. When the same place was followed along different seasons of the year, the mean number of raylets as well as the frequency distribution changed as if butterflies with a high number of raylets survived better in the summer and those with a low number in the spring or just after the winter bottleneck. On the basis of such findings one could expect a higher frequency of individuals with seven raylets (the maximum number already seen in *H. e. phyllis*) in warmer regions of the country. This indeed occurred in samples from Natal, Rio Grande do Norte, in tropical Brazil (Lima, unpublished results). We are not able at the moment to ascribe any direct action of natural selection on this trait. Experiments are in progress, however, to test the survival of larvae from strains with high and low number of raylets under different temperatures.

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RESUMO

Populações naturais da borboleta *Heliconius erato phyllis* do Rio Grande do Sul foram investigadas quanto ao seu tamanho efetivo e variabilidade em relação a uma característica quantitativa (o número de "red raylets" das asas posteriores). Nas três localidades estudadas, as médias para tamanho efetivo foram 48, 84 e 56, considerando todas as estações do ano. Em relação ao número médio de "red raylets", os valores para a primavera foram os mais baixos e os para o verão, os mais altos. As distribuições de freqüência para a mesma característica também se modificaram com as diferentes estações do ano. Tanto as médias quanto as distribuições, mostraram diferenças significativas para um dos locais. O baixo tamanho efetivo e a variação sazonal na característica quantitativa, sugerem a possibilidade da ação da deriva genética e da seleção natural sobre estas populações.

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