

INBREEDING, GENETIC LOAD AND MORPHOMETRIC VARIATION IN NATURAL POPULATIONS OF *Dryas iulia* (LEPIDOPTERA, NYMPHALIDAE)

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ABSTRACT

Because of their wing color pattern homogeneity throughout its range, *Dryas iulia* populations are thought to be large and uniform. In the present paper we attempted to verify this hypothesis, analysing six samples from distinct places in Southern Brazil (state of Rio Grande do Sul) by different approaches. We estimated the mean inbreeding coefficient (F) and the genetic load of populations, using a regression between the rates of non-eclosion of eggs and the inviability of individuals from progenies on f. We also made morphometric analyses, using the forewing length. These approaches revealed that populations show a high level of inbreeding ($0.07 < F < 0.12$), with a genetic load of three lethal equivalents per gamete when egg to adult survival is considered. The wing length means significantly differed among samples from some localities. Males also showed significantly longer forewings than females. Comparing these results with the hypothesis of uniformity of populations, we conclude that they are not as homogeneous as they formerly were thought to be.

INTRODUCTION

The quantification of movements is important for the assessment of the genetic and evolutionary consequences of dispersal patterns (Gilbert and Singer, 1975). However, dispersal can be difficult to measure, because disappearance of individuals from the population under study may be due to predation rather than dispersal (Merrel, 1981).

Heliconian butterflies appear to comprise two very distinct groups, in relation to their dispersal capacity. One, includes more recent species, belonging to the genus *Heliconius*, with limited dispersal capacity. The other group, more dispersive, comprises ancestral species such as *Dryas iulia*, *Agraulis* spp. and *Dione* spp. (Brown Jr., 1981). The ecology, genetics and behaviour of both these groups have been extensively investigated in Southern Brazil (references in Périco and Araújo, 1991; Oliveira and Araújo, 1992). We investigated two main problems associated with *D. iulia*: the first deals with the assumption

of high dispersal of individuals and measurement of its consequences for the genetic structure of the populations in terms of inbreeding and genetic load. Second, if *D. iulia* has great dispersal and gene flow, then the populations are expected to be homogeneous for quantitative traits, namely wing measures, provided that the foodplants for the caterpillars are the same.

MATERIAL AND METHODS

Samples

Samples of adults were collected at six different localities in Rio Grande do Sul (Brazil - Figure 1). The samples came from Estação Agronômica de Águas Belas, Viamão (ABE); Instituto de Pesquisas Hidráulicas, Porto Alegre (IPH); Itapeva, Torres (ITA); Santa Cristina do Pinhal, Parobé (SCP); Parque Estadual do Turvo, Tenente Portela (TUR); and Parque Zoológico, Sapucaia do Sul (ZOO). Three random samples of eggs were also taken from Parque Zoológico (ZOO I, II and III).

Crosses

Adults were reared in an open air insectary and were crossed with different degrees of inbreeding ($f = 0$,

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Figure 1 - Localities in Rio Grande do Sul where samples of *Dryas iulia* were collected for the present study. For abbreviation, see Material and Methods; MAQ and GUA were not included in this analysis due to limited data.

1/4, 3/8, respectively). Eggs of each progeny were maintained individually in vials, at 25°C. After eclosion, the larvae were fed every two days with *Passiflora suberosa* leaves, the most abundant hostplant of *D. iulia* near the University. Estimates of the rate of non eclosion of eggs (NE) and of the inviability at any stage (I) were made for each progeny. The same procedure was adopted for eggs collected in the field, whose average F was to be estimated (see "statistical analyses").

Wing measures

Forewing length was measured in all butterflies collected or reared. Both forewings were carefully removed from the insect, followed by decoloration (Borror and DeLong, 1969); the wings were then placed over a white surface to improve the visualization of the veins. A straight line was taken and measured between the proximal angle of the discal cell and the angle formed by veins R4 and R5 (Figure 2).

Statistical analyses

A linear regression of transformed values (arcsin) of NE and I against the f values of progenies was performed. Values of NE and I obtained from the random samples of eggs (ZOO I, II, III), were then used in the regression equation, solving for F , the average inbreeding

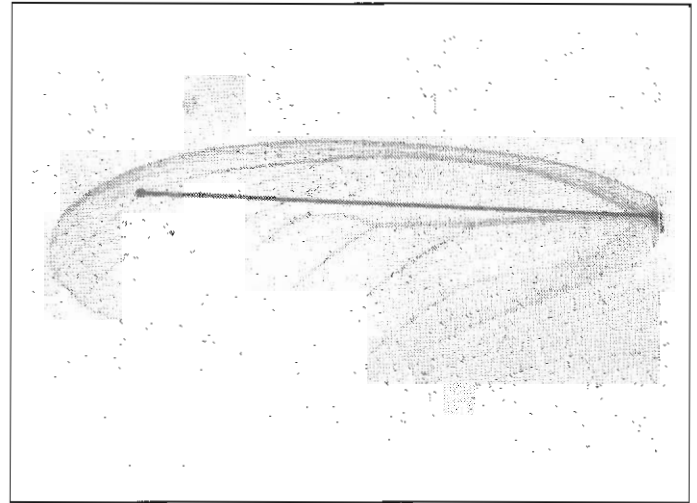


Figure 2 - *Dryas iulia* decolorized wing, showing the line taken to measure.

coefficient of the ZOO population. The complements $(1 - NE)$ and $(1 - I)$ were also used in another regression to estimate the genetic load, according to the method of Morton *et al.* (1956).

Hypotheses about sample means for wing length were tested by a two-way ANOVA; origin and sex, or f value and sex were the factors. Multiple comparisons were made by Duncan's test. Tests were run using the NCSS statistical package.

RESULTS

Ten progenies with different values of f were followed during development from egg to adult (Table I). In three of these progenies, the sex ratio deviated significantly from 1:1 (D, F and J; $p < 0.01$). Generally, there was an increment in the rate of non-eclosion of eggs (NE) and inviability (I), as the degree of inbreeding increased. Regression equations were, respectively, $Y = 0.3176 + 1.2571X$ ($p < 0.01$) and $Y = 0.5664 + 1.3692X$ ($p < 0.01$). Using a weighted mean of NE and I of the three random samples of eggs (bottom of Table I), the average inbreeding coefficient obtained for ZOO was 0.1747 for NE and 0.1337 for I. We found 1.46 to 1.58 lethal equivalents per gamete for the rate of eclosion $(1 - NE)$ and 2.92 to 3.26 for survival $(1 - I)$.

The analysis of variability of forewing length in adult butterflies revealed that both right and left means from the offspring of insectary crosses were slightly lower than those from adults handled in the field (Table II). The variance of the mean of wing lengths was otherwise greater in the group of butterflies obtained by crosses. This strongly suggests that the underlying quantitative genetic system involves dominance rather than additivity.

A two-way ANOVA showed that progenies with different values of inbreeding coefficient had significantly

Table I - Number (n) of eggs from crosses with different degrees of relationship (f), proportion of males (M), non eclosion of eggs (NE) and inviability (I).

Progeny	n	f	M	NE	I
A	92	0.000	0.45	0.12	0.35
B	54	0.000	0.43	0.06	0.32
C	50	0.000	0.46	0.08	0.18
D	43	0.250	0.83	0.33	0.72
E	119	0.250	0.56	0.49	0.71
F	87	0.250	0.29	0.37	0.45
G	133	0.375	0.43	0.40	0.71
H	103	0.375	0.46	0.34	0.76
I	109	0.375	0.53	0.60	0.82
J	105	0.375	0.39	0.50	0.70
ZOO I	19	-	0.58	0.26	0.37
ZOO II	37	-	0.38	0.19	0.57
ZOO III	28	-	0.35	0.36	0.39

Letters A to J were used to identify progenies derived from different females. ZOO I, II and III are random samples of eggs.

different means of both right and left wing lengths (Table III). Sexes also differed in their wing length means, though for the right wing this was not statistically significant ($0.06 > p > 0.05$). However, both wings were consistently shorter, as higher the value of f (coefficient of inbreeding), and larger in males than in females (Table IV).

A similar pattern can be seen in a two-way ANOVA for butterflies from nature (Tables V and VI). For progenies with known f, the sexes differ in their wing length means, and here this disagreement is statistically significant for both wings ($p < 0.04$ and $p < 0.01$). In a two-way ANOVA for the two groups of butterflies (progenies and nature) the interaction between sources of variation was not significant (Tables III and V). This means that wing lengths of the sexes are not differentially influenced by inbreeding and sample origin. Males always have longer wings than females. Samples differed significantly in wing length means (Table VII).

DISCUSSION

The estimated values for the inbreeding coefficient of *D. iulia* from the population of Parque Zoológico (ZOO) 0.1747 and 0.1337 were surprisingly high (they do not differ greatly from those estimated for *Heliconius erato*;

Table II - Means, upper (UL) and lower (LL) limits of the mean estimate, standard deviation (SD), variance (VAR) and coefficient of variation (CV) of right and left forewing lengths for individuals from the field and from crosses made in the insectary.

	Wing	Mean*	UL	LL	SD	VAR	CV
Field n=177	Right	1.21	1.22	1.20	0.0560	0.0031	0.0463
	Left	1.21	1.22	1.20	0.0548	0.0030	0.0453
Crosses n=147	Right	1.19	1.20	1.17	0.0787	0.0062	0.0663
	Left	1.19	1.20	1.18	0.0756	0.0057	0.0634

*In log cm.

Table III - Two-way ANOVA for right and left forewing lengths, according to the inbreeding coefficient and sex, for individuals from the crosses.

	Source	DF	SS	MS	F	PROB
Right wing	Coefficient	2	0.1248	0.0624	14.11	< 0.0001
	Sex	1	0.0168	0.0168	3.80	< 0.0600
	Interaction	2	0.0021	0.0011	0.24	> 0.7000
Left wing	Coefficient	2	0.0819	0.0410	8.70	< 0.0001
	Sex	1	0.0269	0.0269	5.72	< 0.0200
	Interaction	2	0.0018	0.0009	0.19	> 0.8000

Table IV - Means and standard deviations (SD) of the right and left forewing lengths for individuals with different values of inbreeding coefficient and for sexes of individuals from crosses.

		Groups	n	Mean*	SD
Right wing	Coefficient of inbreeding	0.000	36	1.25	0.0110
		0.250	16	1.18	0.0166
		0.375	71	1.16	0.0079
	Sex	Males	52	1.20	0.0042
		Females	71	1.18	0.0079
	Left wing	Coefficient of inbreeding	0.000	36	1.24
0.250			16	1.19	0.0172
0.375			71	1.17	0.0081
Sex		Males	52	1.21	0.0095
		Females	71	1.18	0.0081

n = Number of individuals analysed; *in log cm.

Table V - Two-way ANOVA for right and left forewing lengths according to the sample origin and sex. Individuals captured in nature.

		Source	DF	SS	MS	F	PROB
Right wing	Sample	5	0.0409	0.0082	3.33	< 0.007	
	Sex	1	0.0107	0.0107	8.65	< 0.040	
	Interaction	5	0.0080	0.0016	0.71	> 0.600	
Left wing	Sample	5	0.0415	0.0101	3.75	< 0.004	
	Sex	1	0.0310	0.0310	14.00	< 0.010	
	Interaction	5	0.0100	0.0022	0.90	> 0.400	

Di Mare and Araújo, 1986). How could these estimates be explained for a species with a presumably high vagility? We believe the estimates are reliable because we obtained a similar value using a different methodology (enzyme electrophoresis in Haag *et al.*, 1993).

Though the average inbreeding coefficient of populations of *D. iulia* is not much different from that of *H. erato*, the same is not true for their estimated genetic load. Taking into account the survival rate in the period egg-adult, the number of lethal equivalents per gamete for *D. iulia* is between 2.92 and 3.26; for *H. erato* the corresponding values are 0.28 and 1.96 (Di Mare and Araújo, 1986). The population structure of *H. erato* (island type) together with small population size (Oliveira and Araújo, 1992) facilitates the removal of deleterious genes;

Table VI - Means and standard deviations (SD) of the right and left forewing lengths for the different locations and divided by sex.

		Groups	n	Mean*	SD	
Right wing	Location	SCP	15	1.19	0.0128	
		ITA	22	1.19	0.0106	
		ZOO	42	1.24	0.0076	
		ABE	39	1.22	0.0128	
		TUR	15	1.21	0.0116	
		IPH	37	1.18	0.0082	
	Sex	Males	117	1.22	0.0046	
		Females	54	1.19	0.0068	
	Left wing	Location	SCP	15	1.19	0.0122
			ITA	22	1.19	0.0100
			ZOO	42	1.24	0.0073
			ABE	39	1.21	0.0075
TUR			15	1.20	0.0122	
IPH			37	1.18	0.0076	
Sex		Males	118	1.22	0.0043	
		Females	53	1.18	0.0075	

n = Number of individuals analysed; *in log cm. Location abbreviations defined in Material and Methods.

Table VII - Results obtained by Duncan's test, when applied to the differences in mean forewing length in males. Localities with the same number are not statistically different for an alpha of 0.05.

Samples	SCP	ITA	IPH	TUR	ABE	ZOO
SCP	1	1	1	1		
ITA	1	1, 2	1, 2	1, 2	2	
IPH	1	1, 2	1, 2	1, 2	2	
TUR	1	1, 2	1, 2	1, 2	2	
ABE		2	2	2	3, 2	3
ZOO					3	3

Dryas, on the contrary, with an isolation by distance type of population structure should keep deleterious alleles in heterozygosity. Under experimental conditions, as in the approach used here, a higher genetic load would appear. Finally, even with the limitations in the interpretation of the B/A ratio in the studies of genetic load (Wallace, 1968) the case for *D. iulia* (B/A = 8.6 for survival and 12.2 for egg eclosion) suggests a mutational instead of a segregational load. It must be borne in mind also that

heliconian females do not show crossing-over at meiosis (Suomalainen *et al.*, 1973; Turner and Sheppard, 1975). Therefore linked deleterious alleles would not be disrupted, facilitating their spread in the population.

The populations sampled were not homogeneous for wing length (Tables V, VI and VII). The populations from ABE and ZOO showed the largest means, while IPH showed the lowest. It is difficult to explain the differences on the basis of the foodplant utilized by larvae; in all localities, with one exception (TUR), *Passiflora suberosa* is the main source of food. This species assures a high growth rate for *D. iulia* (Périco and Araújo, 1991). These three localities have similar vegetation, with plenty of *Eucalyptus* spp., mixed with native plants. ABE and ZOO, on the other hand are unstable habitats due to human activity (removal of trees, burning). Greenwood (1988) argues that wing polymorphism is related to habitat stability. Mousseau and Roff (1989) reported that some species of crickets with wing polymorphism showed populations with larger means in those places where favorable conditions occurred for short periods. At present we are not able to discriminate clearly among these possibilities to explain our results.

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RESUMO

Devido à homogeneidade quanto ao padrão de coloração das asas ao longo de sua área de distribuição, acredita-se que as populações de *Dryas iulia* são grandes e uniformes. No presente artigo procurou-se responder esta questão, analisando seis amostras de distintos locais do sul do Brasil (estado do Rio Grande do Sul) através de diferentes abordagens, como as estimativas do coeficiente médio de endocruzamento e da carga genética das populações, utilizando a regressão entre as taxas de não-eclosão de ovos e inviabilidade de indivíduos de progênies sobre f. Realizaram-se ainda análises morfométricas, utilizando o comprimento das asas anteriores. Estas abordagens revelaram que as populações apresentam um alto grau de endocruzamento ($0.07 < F < 0.12$), e sua carga genética equivale a 3 equivalentes letais por gameta, considerando a sobrevivência de ovo a adulto. Amostras de alguns locais diferiram significativamente quanto

às médias de comprimento das asas. Machos também apresentaram asas anteriores mais longas do que as fêmeas. Confrontando esses dados com a hipótese de uniformidade das populações, concluiu-se que elas não são tão homogêneas quanto pareciam.

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