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A PRELIMINARY INVESTIGATION OF EMBRYONIC INBREEDING DEPRESSION IN TWELVE SPECIES OF LEPIDOPTERA

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ABSTRACT. Inbred (sib-sib) matings were made in population cultures of twelve species of Lepidoptera (*Boloria bellona, B. selene, Phyciodes tharos, P. batesii, P. campestris, Junonia coenia, Pararge aegeria, P. megera, Pieris rapae, Arctia caja,* and *Cisseps fulvicollis*) and the resulting loss of egg hatchability was compared with control, outbred matings in the same cultures. A relatively extensive series of inbred matings within a single population of *Phyciodes tharos* revealed that embryonic genetic load is composed of very few, strongly deleterious genetic units. This seems to be true of all of the lepidopteran species tested. Genetic load values for nine of the species seemed homogeneous and varied between 0.5 and 2.0 lethal equivalents per zygote. There was only a single value for the tenth species; this may be anomalous. Two species of the group seem to have significantly lower and higher genetic loads than the other species. This may be a result of differences in population structure.

Genetic variation within populations and species theoretically has a highly adaptive role in enabling the organism to respond to varying environmental stress (e.g., Dobzhansky, 1970). However, a portion of this variation (the "genetic load") consists of genes that, when expressed, result in a loss of fitness for the carrier. Since segregation and recombination in sexual, non-selfing organisms lead inevitably to the production of disadvantageous homozygotes, each population presumably must strike a balance between population size and structure (and thus levels of inbreeding) on the one hand and size of the genetic load on the other. Under conditions such as the laboratory, where close inbreeding suddenly becomes the rule, genetic load is expressed as inbreeding depression. Comparative studies on inbreeding depression in different species and populations within species should provide information on the composition of genetic loads and eventually enable some general inferences to be drawn on the species' natural levels of inbreeding and population structure. This paper represents a preliminary attempt to estimate genetic loads for a small, heterogeneous group of lepidopteran species.

The magnitude of the genetic load in a population is usually estimated by observation of the reduction from normal survivorship during a given sensitive period (i.e., during a time of rapid tissue development and growth) in development of progeny from a series of matings of a known degree of inbreeding. This approach has been used for populations of *Drosophila* (e.g., Dobzhansky et al., 1963), Douglas-fir (Sorenson, 1969), humans (Morton et al., 1956), and domestic animals (Pisani & Kerr, 1961; Sittmann et al., 1966). A second method has been used for *Drosophila.* This uses genetic markers as

a means of estimating the frequencies in a population of chromosomes bearing lethal and sublethal genes. When both methods are used simultaneously on the same *Drosophila* populations (e.g., Dobzhansky et al., 1963; Malogolowkin-Cohen et al., 1964) the former method gives a consistently lower result, indicating synergism among the components of the load. Because of this synergism, estimates of load magnitude made under conditions of intense inbreeding can be considered only as relative values. However, the intensity of inbreeding in the present experiments was less than that necessary to produce significant synergism (Kosuda, 1972) and should reflect actual levels of genetic load.

During the course of breeding work involving maintenance of twelve species of Lepidoptera (*Boloria bellona bellona* Fab., *B. selene* Schiffermüller (ssp. *myrina* Cramer and *sabulocollis* Kohler), *Phyciodes tharos tharos* Drury, *P. tharos pascoensis* Wright (probably best considered a separate species (Oliver, in prep.)), *P. batesii* Reakirt, *P. campestris* Behr (ssp. *montana* Behr), *Junonia coenia coenia* Hübner (all Nymphalidae); *Pararge aegeria* L. (ssp. *tircis* Butler), *P. megera megera* L. (Satyridae); *Pieris rapae rapae* L. (Pieridae); *Arctia caja caja* L. (Arctiidae); and *Cisseps fulvicollis* Hübner (Ctenuchidae)) at various times during a period of ten years, I took the opportunity of making observations on control and inbred broods reared under the same conditions and usually simultaneously. I have made estimates of the magnitude of genetic load in each population culture from a comparison of survivorship of progeny from control and inbred matings. In these species by far the greatest expression of genetic load occurs during embryonic development (Oliver, unpub. data). Data on embryonic survivorship is also much easier to gather than that on later life stages. For these reasons this paper considers only embryonic viability among the twelve species.

Unfortunately, there is little hard data on the population structure of these species. *P. t. tharos* has a wide range of habitats and is generally abundant; there is good evidence for near panmixis over the eastern United States (Vawter & Brussard, 1975). *P. t. pascoensis* is more habitat-restricted and tends to show significant population differentiation (Oliver, in prep.); *P. c. montana* probably has a similar population structure. *P. batesii* in the northeastern United States occurs in small, highly isolated populations (Oliver, 1979). My field observations indicate that *P. megera* (Oliver, 1972b) and *P. aegeria* show little individual motility and tend to occur in localized populations, as do *B. selene*, *B. bellona*, and *C. fulvicollis*. *A. caja* is common and general in central Europe; *P. rapae* is abundant in almost all open areas in the eastern United States. *J. coenia* is a highly vagile

species that tends to form large colonies which may often be founded by single fecundated females (e.g., Shapiro, 1978).

Almost nothing is known about the composition of the genetic load in Lepidoptera. If the heterozygosity involved in genetic loads is similar to the great genic variation that has been observed in natural populations of most organisms (Lewontin, 1974; Richmond, 1972), including butterflies (Burns & Johnson, 1967), then the chances are very small of picking at random even two parents having a level of variation differing significantly from the population average. The data of Dobzhansky et al. (1963) seem to support this view; no correlation was found between viability and pedigree in a long series of inbred *Drosophila* cultures. However, when lethals or semilethals are involved, very high levels of inbreeding depression may be due to the actions of only a few genes.

METHODS

One to six cultures were maintained for each species. Detailed information on culturing techniques has been given in earlier papers (Oliver, 1972a, 1972b, 1977, 1978, 1979). Each culture was begun from parents caught in a separate locality. Embryonic survivorship in the progeny from inbred matings between siblings (coefficient of inbreeding, $F_x = .250$) was compared with that from control, outbred intrapopulation matings ("combined controls" in Table 1). In each case the siblings used were the progeny of an outbred (usually wild) intrapopulation mating having normal hatchability.

Each of the cultures was begun with a comparatively small number of wild parents, from 2 to 22. Because of uncertainty regarding the reliability of data from very small population samples, the program was carried out in two parts. The first (Part A) consisted of observations on cultures of all species maintained from 1968 to 1978, the second (Part B) of a more systematic set of inbred matings made during 1973 and 1975 using a population of *Phyciodes tharos* from western Pennsylvania. In this part six isofemale families were established and 9 to 14 successful sibling pairings made within each family. An additional two families (73-23 and 73-31) were derived by outcrossing the F_1 progeny of the original wild parents of these families. Sib matings were then made within each of these two additional families.

Cultures were begun from individuals caught at the following localities:

Boloria b. bellona—MASSACHUSETTS: Acton, Middlesex Co.; PENNSYLVANIA: Forward Twp., Allegheny Co.
B. selene myrina—MASSACHUSETTS: Acton, Middlesex Co.
B. s. sabulocollis—SOUTH DAKOTA: Custer Park, Custer Co.

TABLE 1. Mean hatchability of eggs and embryonic genetic loads (L. E./zygote) in control and inbred lines of Lepidoptera. Coefficient of inbreeding (F) = 0 for controls (c); (F) = .250 for inbred (i) broods.

Species & provenance	No. of broods	No. of wild parents	No. of eggs	Colored/laid[1,2]	Hatched/laid[2]	L. E./zygote
B. bellona						
Pennsylvania (c)	1	2	33	1.000	1.000	
Massachusetts (c)	1	2	173	.954	.954	
Combined c					$\bar{x}$ = .977	
Pennsylvania (i)	3	2	403	.916 ± .074	.816 ± .106	0.721
B. selene						
Massachusetts (c)	7	10	1160	.960 ± .037	.896 ± .056	
Massachusetts (i)	5	2	692	.775 ± .118	.612 ± .068	1.525
South Dakota (i)	4	2	741	.830 ± .221	.685 ± .211	1.074
						$\bar{x}$ = 1.300
P. tharos						
Florida (c)	1	2	272	.864	.860	
Virginia (c)	4	4	1011	.990 ± .011	.988 ± .011	
West Virginia (c)	1	2	362	.983	.956	
Massachusetts (c)	1	2	648	1.000	.998	
Connecticut (c)	11	10	1606	.995 ± .006	.985 ± .024	
Combined c					$\bar{x}$ = .978	
Florida (i)	3	2	945	.941 ± .061	.635 ± .078	1.726
Texas (i)	2	2	137	.856 ± .144	.798 ± .174	0.813
West Virginia (i)	9	2	2412	.962 ± .048	.858 ± .081	0.525
						$\bar{x}$ = 1.021
P. pascoensis						
Montana (c)	5	8	945	.983 ± .028	.976 ± .027	
Alberta, Canada (c)	2	4	113	.965 ± .035	.965 ± .035	
Combined c					$\bar{x}$ = .973	
Montana (i)	2	2	305	.788 ± .181	.778 ± .192	0.895
Alberta, Canada (i)	3	2	751	.730 ± .146	.484 ± .016	2.793
						$\bar{x}$ = 1.844
P. campestris						
California (c)	2	4	562	1.000 ± .000	1.000 ± .000	
California (i)	1	2	406	.788	.749	1.404

P. batesii						
New York (c)	5	8	2533	.938 ± .099	.929 ± .098	
New York (i)	1	2	100	.950	.240	5.412
J. coenia						
Florida (c)	1	2	208	1.000	1.000	
Florida (i)	3	2	1373	.998 ± .002	.968 ± .026	0.130
P. aegeria						
Hampshire, United Kingdom (c)	2	4	33	.931 ± .069	.931 ± .069	
Hampshire, United Kingdom (i)	3	2	106	.655 ± .406	.655 ± .406	1.406
P. megera						
Oxford, United Kingdom (c)	3	4	161	.973 ± .019	.938 ± .092	
Boulogne, France (c)	2	4	146	.910 ± .025	.910 ± .025	
Combined c					$\bar{x}$ = .927	
Oxford, United Kingdom (i)	2	2	307	.870 ± .119	.590 ± .090	1.808
Boulogne, France (i)	2	2	102	.938 ± .062	.604 ± .146	1.715
						$\bar{x}$ = 1.762
P. rapae						
Pennsylvania (c)	3	6	334	.956 ± .008	.920 ± .010	
Pennsylvania (i)	28	6	2186	.894 ± .105	.704 ± .151	1.070
C. fulvicollis						
Connecticut (c)	7	14	546	.984 ± .030	.953 ± .054	
Massachusetts (c)	1	2	171	1.000	.988	
Texas (c)	1	2	223	.996	.991	
Iowa (c)	6	12	1417	.985 ± .015	.963 ± .029	
South Dakota (c)	6	12	367	.914 ± .091	.883 ± .090	
Combined c					$\bar{x}$ = .939	
Connecticut (i)	14	12	2185	.924 ± .057	.516 ± .253	2.394
Massachusetts (i)	3	2	516	.975 ± .006	.088 ± .121	9.469
Texas (i)	9	2	1673	.963 ± .047	.489 ± .148	2.610
Iowa (i)	11	4	2415	.962 ± .027	.735 ± .179	0.980
South Dakota (i)	1	2	248	.911	.827	0.508
						$\bar{x}$ = 3.192
A. caja						
N.W. Germany (c)	5	?	4628	.936 ± .055	.888 ± .104	
N.W. Germany (i)	11	?	10,587	.861 ± .159	.522 ± .287	2.125

[1] Showing visible evidence of embryonic development.
[2] Mean ± standard deviation given if no. of broods >1.

Phyciodes t. tharos—FLORIDA: 4 mi. E of Cedar Key, Levy Co.; TEXAS: San Antonio, Bexar Co.; WEST VIRGINIA: Spruce Knob, elev 4500 ft, Pendleton Co.; PENNSYLVANIA: Upper Tyrone Twp., Fayette Co.
P. t. pascoensis—MONTANA: 1 mi. S of Hall, Powell Co.; ALBERTA, CANADA: 6 mi. E of Nordegg, Red Deer.
P. campestris montana—CALIFORNIA: Lang Crossing, elev 4500 ft, S. Fork Yuba R., Nevada Co.
P. batesii—NEW YORK: Syracuse, Onondaga Co.
Junonia c. coenia—FLORIDA: Wauchula, Hardee Co.
Pararge aegeria tircis—ENGLAND: Pamber Forest, Hampshire.
P. m. megera—ENGLAND: Oxford, Oxfordshire; FRANCE: Boulogne-sur-Mer.
Pieris r. rapae—PENNSYLVANIA: Upper Tyrone Twp., Fayette Co.
Arctia c. caja—Northwest GERMANY.
Cisseps fulvicollis—CONNECTICUT: Woodbridge, New Haven Co.; MASSACHUSETTS: Littleton, Middlesex Co.; TEXAS: San Antonio, Bexar Co.; IOWA: Dubuque, Dubuque Co.; SOUTH DAKOTA: Custer Park, Custer Co.

Observations on embryonic survivorship included counts of eggs showing evidence of embryonic tissue differentiation (a darkening of egg color from pale yellow or green to brown or black) and of eggs hatching. An increase in failure to show embryonic development in the inbred broods was taken as evidence of very early embryonic mortality. There is a possibility that there was lowered fertilization rate of eggs in the inbred matings, but the males used in these matings were of the same age and condition as those used for laboratory control matings. Eggs from these control matings showed fertility equal to that from wild-fecundated females. Both visible fertility and incidence of egg hatch are combined under the term "hatchability."

The Wilcoxon Two-Sample Statistic (Owen, 1962) was used to compare differences in hatchability of broods in Part B. Calculations of load magnitude were made using the equations presented by Freire-Maia (1964), which are a simplification of those of Morton et al. (1956). Since the comparison is between inbred and control broods with F = 0, it is possible to use Freire-Maia's equation

$$B = \frac{\log(S_i/S_c)}{-0.4343F_i}$$

where B is the magnitude of the genetic load in lethal equivalents per zygote; S_i and S_c denote the mean survivorship of the inbred and control broods, respectively; and F_i is the coefficient of inbreeding of the inbred group. Morton et al. (1956), who used the term "mutant" to refer to any gene deleterious when homozygous, defined lethal equivalent (L. E.) as "a group of mutant genes of such number that if dispersed in different individuals, they would cause on the average one death, e.g., one lethal mutant, or two mutants each with a 50 per cent probability of causing death, etc."

RESULTS

The results of Part A are shown in Table 1. In every species inbreeding caused a significant reduction in hatchability, but the amount varied greatly from one population and species to another. In the control broods hatching failure was due in the great majority of cases to infertility rather than to embryonic inviability. In the inbred broods, however, there were usually substantial reductions in both the incidence of visible embryonic development and in late embryonic viability. There was interspecific and interpopulation variation in the inbred broods in the proportion of mortality occurring before and after visible evidence of embryonic development. Variation in inviability was many times higher for the inbred broods than for the controls. The amount of variation was not a function of the magnitude of inbreeding depression. There were large differences between species and between populations in the size of the genetic load (L. E./zygote calculated from total hatchability) and of the standard deviation in viability observed for each series of broods.

The results of Part B are shown in Table 2. Hatchability within each family line of this population of *Phyciodes tharos* varied greatly, but only the family showing the least inbreeding depression (75-3) differed significantly ($P < .005$) from those showing the greatest (73-23, 73-31, 75-4).

DISCUSSION

The degree of interbrood variation in hatchability (indicated by standard deviation) shown within each family line of *P. tharos* and the other Lepidoptera used here reveals that genetic loads in these species consist mainly of relatively few genetic units with strongly deleterious effects when homozygous. These genetic loads do not seem directly to reflect the genic variation that has been shown to occur in Lepidoptera.

Fig. 1 shows the distribution of L. E./zygote values for the 85 inbred *P. tharos* broods summarized in Table 2. The graph consists of four main peaks, with values clustered at or near 0, 1, 2, 3, and 4 L. E./zygote. A distribution of this type could result from the action of only several strongly deleterious (i.e., lethal) recessive genes in each family, perhaps along with a few much weaker sublethals.

In Table 2 there is a considerable spread in L. E. values, even though only the very lowest value is significantly different from the highest. Thus, there is a spread on either side of the mean of about 0.7 L. E./zygote that is not significant here. If we assume for the

TABLE 2. Mean embryonic genetic loads expressed in F_1 progeny of sib-sib matings in 8 lines of *Phyciodes tharos* from Upper Tyrone Township, Pennsylvania.

Family line	No. of broods	No. of wild parents	No. of eggs	Hatched/laid[1]	L. E./zygote
controls	12	24	3037	.993 ± .011	
73-1	14	2	5884	.765 ± .154	1.043
73-4	9	2	2459	.803 ± .184	0.850
73-23	9	4	2663	.596 ± .272	2.041
73-31	9	4	2595	.591 ± .146	2.076
75-1	12	2	3749	.779 ± .132	0.971
75-2	11	2	3046	.683 ± .172	1.494
75-3	12	2	3021	.888 ± .113	0.448
75-4	9	2	2571	.671 ± .190	1.623
					$\bar{x}$ = 1.318

[1] Mean ± standard deviation.

moment that this amount of variation applies also to the other species in Table 1, all except *P. batesii, J. coenia,* and *C. fulvicollis* probably fall within the normal range of *P. tharos* and form a fairly homogeneous group. The single value for *P. batesii* falls at the upper limit of values for *P. tharos* (above 98.81% of values in Fig. 1), but this single sample cannot be considered significant. All three values of *J. coenia* are at the lower level for *P. tharos;* there is a less than .001 chance of any three randomly picked values of *P. tharos* from Fig. 1 all falling this far to one end of the graph. The overall mean L. E./zygote value for *C. fulvicollis* falls at the upper end of the graph. From this it seems likely that *C. fulvicollis* and at least this population of *J. coenia* carry genetic loads that are, respectively, larger and smaller than those of the other species. A very small genetic load would seem adaptive for the inbred populations resulting from the sort of colonization events that occur in *J. coenia.* Too little is known about the population structure of *C. fulvicollis* to speculate on the comparatively large genetic load it carries.

The lethal equivalent values calculated here are generally in line with those estimated for other insects. One to two L. E./zygote have been estimated (by survival from egg to adult) for *Drosophila* (Diptera) (Dobzhansky et al., 1963; Stone et al., 1963; Malogolowkin-Cohen et al., 1964) and for *Tribolium* (Coleoptera) (Levene et al., 1965). For humans a value of 3 to 5 L. E./zygote (by survival from birth to sexual maturity) has been established by Morton et al. (1956), whereas embryonic loads in Douglas-fir average about 10 L. E./zygote (Sorenson, 1969). In none of these organisms has a definite relationship been shown between magnitude of load and population structure. The general similarity of load magnitude among the very different

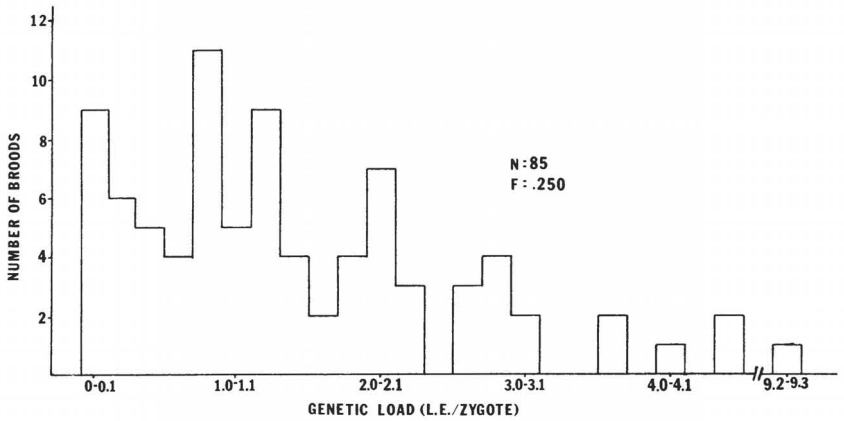

FIG. 1. Distribution of embryonic genetic loads in lethal equivalents per zygote in 85 inbred broods of *Phyciodes tharos* from Upper Tyrone Township, Pennsylvania.

insects thus far examined may indicate that genetic loads are more related to some internal genetic balancing responsible for the metabolic integration of the individual organism than to population structure. However, there are surely situations (as in species colonizing by single fecundated females) where adjustment of load magnitude is highly adaptive.

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