

# EPISTASIS, ASSOCIATED LETHALS AND BROOD EFFECT IN LARVAL COLOUR POLYMORPHISM OF THE PATCH BUTTERFLY, *CHLOSYNE LACINIA*

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## 1. INTRODUCTION

THE polymorphisms exhibited by various lepidopterous insects have been the focus of much research in the field of ecological genetics. However, practically all of this work has involved characters present in the imaginal forms. Recently Jolly *et al.* (1969) reported that the four larval morphs of *Antheraea mylitta* result from two unlinked loci which exhibit epistasis. Work by Clarke *et al.* (1963) indicated that the basic larval colour pattern of *Papilio demodocus* is controlled by a pair of alleles at a single locus. Much earlier Gerould (1926) investigated the inheritance patterns of two mutant colour morphs of the larvae of *Colias philodice* Latreille. Both mutant alleles, which are located at unlinked loci, are recessive to the wild-type colouration with the homozygous recessive of one locus being epistatic to the other locus.

An investigation of the adaptive significance and ecological genetics has been undertaken of a striking colour polymorphism (plate I) which is apparent in the last three larval instars of *Chlosyne lacinia* (Geyer) (Nymphalidae: Melitaeinae). This paper deals primarily with the genetic basis for the polymorphism. First described by Edwards (1893), the three forms are *nigra*, an almost entirely black form, *bicolor*, which is similar to *nigra* but has a prominent mid-dorsal light yellow to orange-red stripe and *rufa*, an all orange to orange-red morph. Gorodenski (1969) has indicated that this polymorphism is controlled by two non-linked loci but his limited progeny broods did not include several of the important expected combinations. In addition, six of the 22 Chi-squared probabilities were significant at the 5 per cent. level. For this reason the authors felt that additional data was needed.

The same three morphs also have been found in local populations of *C. gorgone* (Hübner) and Arizona populations of *C. californica* (Wright). At least one species, *C. nycteis* (Doubleday), produces only black broods. Although *C. californica* is very closely related to *C. lacinia*, *C. gorgone* and *C. nycteis* belong to a different subgenus and at one time these species were placed in the related genus *Melitaea* Fabr. The polymorphism, therefore, may be an ancient one which arose early in the evolution of *Chlosyne*.

The *bicolor* morph is by far the most common morph in field populations reaching a frequency of 70-80 per cent. In most populations the *nigra* morph is somewhat more common than *rufa*. Geographical and seasonal variations in morph frequencies will be the subject of subsequent publications.

## 2. BREEDING AND REARING TECHNIQUES

Details on the rearing and the biology of this butterfly were reported by Drummond *et al.* (1970). Matings were accomplished in large (4 ft. × 2 ft. × 2 ft.) screen cages in which were placed potted host plants (Compositae: *Helianthus annuus* L. and *Ximenesia encelioides* (Cav.)) from which egg masses were collected. Sponges soaked with a honey-water solution (1 : 3) were employed as feeding devices. Four 40-watt Gro-Lux fluorescent light bulbs (Sylvania) were used to illuminate the cages on a 16-hour daylength cycle. The cages were situated in a room with a constant temperature of 29° C. and a relative humidity of 55 per cent.

Hand-pairing (see Platt, 1969) of these butterflies was attempted, but the low percentage of success made it imperative to utilise mass mating techniques. Although this method was extremely productive, there are several inherent disadvantages in analysing the data from mass matings. Two females, for instance, might lay overlapping egg masses. However, it was generally possible to separate these egg masses because of slight variation in the yellow colouration of the eggs. Also a female disturbed during oviposition might fly off and later resume oviposition on another leaf. Thus, the results could include two or more counts on broods laid by the same female.

Multiple matings might also affect the results. Approximately 14.2 per cent. of the field collected females contain two or more spermatophores (unpublished data) indicating a slight tendency toward multiple matings. A few laboratory broods and some field-collected egg masses contained only a few individuals of a certain morph in contrast to large numbers of the other morph(s) which were in a correct ratio. This suggests that sperm precedence is not absolute in this species although Labine (1966) reported that sperm precedence occurs in another melataeinae butterfly, *Eurphydryas editha* Boisduval. She reported a sample in which spermatophore counts indicated that approximately one-third of the females had mated twice. This may be similar to the case reported by Taylor (1967) in which second matings in *Atteva punctella* (Cramer) usually follow an initial infertile mating and that most of the multiple-mated females give rise to progeny fathered only by one male.

All larvae from a brood were reared together in a ventilated plastic sandwich box. A semi-artificial diet consisting of a modified Vanderzandt-Adkisson diet (Nutritional Biochemicals Co.; Cleveland, Ohio 44128) plus 8-10 per cent. powdered leaves of the host plant, *Helianthus annuus* L. was used for rearing all crosses (Drummond *et al.*, 1970). Survival rates were high (70-95 per cent.) except for occasional diseased broods which were discarded.

Crosses involving all combinations of the three morphs, including reciprocal crosses, were made. Those broods which contained less than 50 larvae were not considered. Morph determinations of the larvae were generally accomplished in the fourth or fifth (last) instar as it was often difficult to differentiate between the morphs in the third instar.

## 3. RESULTS AND INTERPRETATION OF CROSSES

There appeared to be no indication that the polymorphism is sex-linked as morph frequencies in males and females do not differ significantly (table 1). *Nigra* by *nigra* crosses without exception always gave rise to all *nigra* broods.

This included 13 broods totalling 1825 larvae with brood sizes ranging from 69 to 259 larvae.

TABLE 1  
*Sex ratio of wild population*

	Male	Female	Total	$\chi^2_{(1)}$	P
<i>Nigra</i>	26	34	60	0.816	0.37
<i>Bicolor</i>	53	63	116	0.698	0.40
<i>Rufa</i>	5	8	13	0.308	0.86
Total	86	105	189		

Homogeneity  $\chi^2_{(2)} = 0.294$ ; P = 0.86.

*Bicolor* by *bicolor* crosses on the other hand yielded broods of two types. A number of them consisted of only *bicolor* larvae (31 broods totaling 7568 larvae with brood sizes ranging from 59 to 613) while others yielded both *bicolor* and *nigra* larvae approximating a 3 : 1 ratio (table 2). *Bicolor* and

TABLE 2  
Parental phenotypes: *bicolor* × *bicolor*  
Presumptive parental genotypes: *Bbrr* × *Bbrr*

Brood no.	Phenotypes of progeny (expected ratio—3 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Bicolor</i>	<i>Nigra</i>			
69	181	68	249	0.708	0.40
250	83	35	118	1.367	0.23
320	41	12	53	0.157	0.69
914	115	38	153	0.002	0.96
915	74	27	101	0.162	0.69
916	96	33	129	0.023	0.88
919	45	15	60	0.000	1.00
940	131	40	171	0.236	0.63
	766	268	1034		

Homogeneity  $\chi^2_{(7)} = 2.190$ ; P = 0.95.

*nigra* crosses resulted in broods which were completely *bicolor* (3 broods totalling 456 larvae with brood sizes ranging from 93 to 246) or broods with approximately equal numbers of *bicolor* and *nigra* larvae (table 3). No difference between the reciprocal crosses is evident.

The results of these crosses, therefore, indicated that the *nigra* and *bicolor* phenotypes are controlled at a single locus which has two alleles, *bicolor* (*B*) and *nigra* (*b*). The *bicolor* allele (*B*) is apparently completely dominant to the *nigra* allele (*b*) as efforts to differentiate between homozygous and heterozygous *bicolor* larvae have been unsuccessful.

Crosses involving *rufa* individuals yielded more complicated results than the preceding crosses. *Rufa* by *rufa* crosses gave rise to several types of progeny classes. Nine of the broods were completely *rufa* (a total of 1749 larvae with brood sizes ranging from 50 to 295). In other broods *rufa* and *bicolor* individuals were present in ratios of 3 : 1 and 2 : 1 (tables 4 and 5).

TABLE 3

Parental phenotypes: *bicolor* × *nigra*Presumptive parental genotypes: *Bbrr* × *bbrr*

Brood no.	Phenotypes of progeny (expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Bicolor</i>	<i>Nigra</i>			
233	29	28	57	0.018	0.90
281	53	52	105	0.010	0.92
282	117	139	256	1.891	0.17
344	56	56	112	0.000	1.00
351	100	94	194	0.186	0.67
352	56	61	117	0.214	0.64
353	33	38	71	0.352	0.55
354	43	50	93	0.527	0.47
496	78	93	171	1.316	0.25
508	70	60	130	0.769	0.38
513	152	180	332	2.361	0.12
528	76	73	149	0.060	0.81
539	40	42	82	0.049	0.83
542	39	43	82	0.195	0.66
554	29	25	54	0.296	0.59
577	90	115	205	3.049	0.08
	1061	1149	2210		

Homogeneity  $\chi^2_{(15)} = 7.788$ ; P = 0.93.

TABLE 4

Parental phenotypes: *rufa* × *rufa*Presumptive parental genotypes: *BBRr* × *--Rr*

Brood no.	Phenotypes of progeny (expected ratio—3 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Bicolor</i>			
1	117	33	150	0.720	0.40
2	268	86	354	0.094	0.76
4	168	62	230	0.470	0.49
9	78	22	100	0.480	0.49
11	75	18	93	1.581	0.21
12	234	74	308	0.156	0.69
15	142	53	195	0.494	0.48
16	167	48	215	0.570	0.45
256	74	17	91	1.938	0.16
259	78	26	104	0.000	1.00
315	67	22	89	0.004	0.95
391	38	16	54	0.617	0.43
587	174	61	235	0.115	0.73
590	190	55	245	0.850	0.36
591	220	76	296	0.072	0.79
597	101	37	138	0.242	0.63
601	174	53	227	0.330	0.57
606	205	64	269	0.209	0.65
	2570	823	3393		

Homogeneity  $\chi^2_{(17)} = 8.017$ ; P = 0.97.

TABLE 5

Parental phenotypes: *rufa* × *rufa*  
 Presumptive parental genotypes: *BBRr* × *--Rr*

Phenotypes of progeny  
 (expected ratio—2 : 1)

Brood no.	<i>Rufa</i>	<i>Bicolor</i>	Total	$\chi^2_{(1)}$	P
8	124	56	180	0.400	0.53
392	63	32	95	0.005	0.94
715	102	50	152	0.013	0.91
716	99	48	147	0.031	0.86
727	155	75	230	0.054	0.82
	678	331	1009		

Homogeneity  $\chi^2_{(5)} = 0.437$ ; P = 0.99.

TABLE 6

Parental phenotypes: *rufa* × *rufa*  
 Presumptive parental genotypes: *bbRr* × *BbRr*

Phenotypes of progeny  
 (expected ratio—4 : 1 : 1)

Brood no.	<i>Rufa</i>	<i>Bicolor</i>	<i>Nigra</i>	Total	$\chi^2_{(2)}$	P
716	99	26	22	147	0.472	0.80

TABLE 7

Parental phenotypes: *rufa* × *rufa*  
 Presumptive parental genotypes: *BbRr* × *BbRr*

Phenotypes of progeny  
 (expected ratio—12 : 3 : 1)

Brood no.	<i>Rufa</i>	<i>Bicolor</i>	<i>Nigra</i>	Total	$\chi^2_{(2)}$	P
14	171	44	19	234	1.309	0.52
745	91	18	5	114	1.520	0.47
934	72	19	7	98	0.177	0.92
	334	81	31	446		

Homogeneity  $\chi^2_{(4)} = 2.445$ ; P = 0.66.

TABLE 8

Parental phenotypes: *rufa* × *rufa*  
 Presumptive parental genotypes: *BbRr* × *BbRr*

Phenotypes of progeny  
 (expected ratio—8 : 3 : 1)

Brood no.	<i>Rufa</i>	<i>Bicolor</i>	<i>Nigra</i>	Total	$\chi^2_{(2)}$	P
715	102	36	14	152	0.249	0.88
727	155	53	22	230	0.788	0.67
	257	89	36	382		

Homogeneity  $\chi^2_{(2)} = 0.028$ ; P = 0.99.

A few broods contained all three morphs, *rufa* : *bicolor* : *nigra*, in proportions of 4 : 1 : 1, 12 : 3 : 1 and 8 : 3 : 1 (tables 6, 7 and 8).

TABLE 9

Parental phenotypes: *rufa* × *bicolor*  
 Presumptive parental genotypes; *Bbrr* × *BBRr*; *BBrr* × *--Rr*

Phenotypes of progeny  
 expected ratio—1 : 1)

Brood no.	Phenotypes of progeny expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Bicolor</i>			
411	72	86	158	1.241	0.27
412	115	95	210	1.905	0.17
413	146	110	256	5.063	0.02
414	21	36	57	3.947	0.05
415	127	112	239	0.941	0.33
449	101	110	211	0.384	0.54
450	76	85	161	0.503	0.48
451	43	43	86	0.000	1.00
452	94	89	183	0.137	0.71
453	67	70	137	0.066	0.80
454	45	58	103	1.641	0.20
467	33	44	77	1.571	0.21
469	35	31	66	0.242	0.62
473	177	180	357	0.025	0.87
475	115	126	241	0.502	0.48
477	175	195	370	1.081	0.30
479	136	139	275	0.033	0.86
480	57	65	122	1.525	0.47
481	100	106	206	0.175	0.68
524	112	116	228	0.070	0.79
526	107	100	207	0.237	0.63
	2001	1949	3950		

Homogeneity  $\chi^2_{(20)} = 19.603$ ;  $P = 0.48$ .

The *rufa* by *bicolor* crosses yielded two progeny classes. Some broods showed approximately equal numbers of *rufa* and *bicolor* individuals (table 9) while one brood exhibited all three morphs in a *rufa* : *bicolor* : *nigra* relationship of 2 : 1 : 1 (table 10).

TABLE 10

Parental phenotypes: *rufa* × *bicolor*  
 Presumptive parental genotypes: *bbRr* × *Bbrr*

Phenotypes of progeny  
 (expected ratio—2 : 1 : 1)

Brood no.	Phenotypes of progeny (expected ratio—2 : 1 : 1)			Total	$\chi^2_{(2)}$	P
	<i>Rufa</i>	<i>Bicolor</i>	<i>Nigra</i>			
586	51	16	25	92	2.848	0.23

The *rufa* by *nigra* crosses produced broods of various morph combinations. Some had approximately equal numbers of *rufa* and *nigra* (table 11) while others had approximately equal numbers of *rufa* and *bicolor* (table 12). A number of broods contained all three morphs in a *rufa* : *bicolor* : *nigra* combination of 2 : 1 : 1 (table 13).

The diverse progeny classes which resulted from crosses involving *rufa* adults can be easily explained if they are considered on a *rufa* and non-*rufa* basis. Using this criterion, the results of the majority of the *rufa* by *rufa*

TABLE 11

Parental phenotypes: *rufa* × *nigra*  
Presumptive parental genotypes: *bbRr* × *bbr*

Phenotypes of progeny  
(expected ratio—1 : 1)

Brood no.	Phenotypes of progeny (expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Nigra</i>			
405	180	177	357	0.025	0.87
406	139	120	259	1.394	0.24
407	147	151	298	0.054	0.82
408	177	139	316	4.570	0.03
416	106	119	225	0.751	0.39
422	53	49	102	0.157	0.69
429	169	176	345	0.142	0.71
441	43	33	76	1.316	0.25
444	80	72	152	0.421	0.52
457	123	128	251	0.100	0.75
461	145	137	282	0.227	0.63
	1362	1301	2663		

Homogeneity  $\chi^2_{(10)} = 7.758$ ; P = 0.65.

crosses were the basic Mendelian ratio of 3 : 1 (*rufa* : non-*rufa*) with the exception of those broods in tables 5, 6 and 8 which are to be discussed below. The 3 : 1 ratio can be explained by postulating a *rufa* allele (*R*) which is

TABLE 12

Parental phenotypes: *rufa* × *nigra*  
Presumptive parental genotypes: *BBRr* × *bbr*

Phenotypes of progeny  
(expected ratio—1 : 1)

Brood no.	Phenotypes of progeny (expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Nigra</i>			
402	39	47	86	0.744	0.39
430	49	63	112	1.750	0.19
433	116	111	227	0.110	0.74
436	99	115	214	1.196	0.28
439	43	48	91	0.275	0.60
445	61	50	111	1.090	0.30
462	98	87	185	0.654	0.42
464	22	26	48	0.333	0.56
	527	547	1074		

Homogeneity  $\chi^2_{(7)} = 5.780$ ; P = 0.57.

dominant to a non-*rufa* allele (*r*). Again dominance, as far as larval colour is concerned, appears to be complete as no distinguishing characters have been found between homozygous (*RR*) and heterozygous (*Rr*) *rufa* individuals. If either or both of the *rufa* parents were homozygous (*RR*), then all resulting

progeny were *rufa*. If both of the *rufa* parents were heterozygous (*Rr*), then one-fourth of the progeny were non-*rufa*.

The composition of the non-*rufa* portion was quite variable. Our results show that this portion can be completely *bicolor* or have broods consisting of *bicolor* and *nigra* larvae in either a 1 : 1 or a 3 : 1 ratio, respectively. Thus, it seems that the *bicolor-nigra* locus is segregating normally without influence from the *rufa*-non-*rufa* locus but is not expressed when the dominant *rufa* allele (*R*) is present. Thus, the *rufa* allele (*R*) is epistatic to the *bicolor-nigra* locus.

TABLE 13

Brood no.	Parental phenotypes: <i>rufa</i> × <i>nigra</i>			Total	$\chi^2_{(2)}$	P
	Presumptive parental genotypes: <i>BbRr</i> × <i>bbr</i>					
	Phenotypes of progeny (expected ratio—2 : 1 : 1)					
	<i>Rufa</i>	<i>Bicolor</i>	<i>Nigra</i>			
401	74	46	34	154	2.104	0.35
403	54	28	30	112	0.214	0.90
404	71	32	38	141	0.518	0.77
410	156	78	77	311	0.010	0.99
417	90	42	37	169	1.012	0.60
419	54	30	32	116	0.621	0.73
420	66	47	35	148	3.676	0.16
421	109	33	53	195	6.815	0.03
428	168	86	105	359	3.485	0.18
438	55	23	27	105	0.543	0.76
456	49	25	17	91	1.945	0.38
463	38	26	37	101	8.584	0.01
466	28	16	23	67	3.269	0.20
505	101	56	56	213	0.568	0.75
	1113	568	601	2282		

Homogeneity  $\chi^2_{(26)} = 31.034$ ; P = 0.23.

This interpretation is further bolstered by a consideration of the *rufa* and non-*rufa* crosses. If the *rufa* parent were heterozygous (*Rr*), then half of the progeny were heterozygous *rufa* (*Rr*) while the other half would be (*rr*) with non-*rufa* phenotypes. Again, the non-*rufa* portion could assume various combinations of *bicolor* and *nigra* larvae. In the *rufa* by *nigra* crosses the non-*rufa* portion might contain all *bicolor* (*BBRr* × *bbr*), all *nigra* (*bbRr* × *bbr*) or approximately equal numbers of those two morphs (*BbRr* × *bbr*). The results of the *rufa* by *bicolor* crosses would be the same combinations with the addition of a 4 : 3 : 1 (*rufa* : *bicolor* : *nigra*) ratio (*BbRr* × *Bbrr*). The lack of significant deviations from normal Mendelian ratios in the non-*rufa* portions of these broods indicated that there is no linkage between these two loci. This is not unexpected because of the relatively large number of chromosomes in this species ( $n = 31$ ) (Emmel, personal communication). The authors feel that the data presented here leave no doubt of the inheritance of this polymorphism.

This form of epistasis is similar to that found controlling fruit colour in summer squash (*Cucurbita pepo* L.) (Sinnot and Durham, 1922) and petal colour in the sweet pea (*Lathyrus odoratus* L.) (Beale *et al.*, 1939) with white :



yellow : green and purple : red : salmon, respectively, being analogous to *rufa* : *bicolor* : *nigra*. The only identical examples in animals found by the authors are the inheritance of vertebral stripe colour (green : red : grey analogous to *rufa* : *bicolor* : *nigra*) in *Acris crepitans* (cricket frog) (Pyburn, 1961) and the inheritance of dorsal background colour (red : brown : green analogous to *rufa* : *bicolor* : *nigra*) in *Pseudacris triseriata* (chorus frog) (Matthews, personal communication).

#### 4. EXPLANATION OF DEVIATIONS FROM EXPECTED RESULTS

A number of the *rufa* by *rufa* crosses contained *rufa* and non-*rufa* larvae in a 2 : 1 ratio instead of the expected 3 : 1 ratio (tables 5, 6 and 8). Ford (1965) has pointed out that recessive lethals and semi-lethals will tend to become closely linked to a dominant allele which is at low enough frequency so that homozygous individuals are very rare. These lethals would be sheltered from selection in the heterozygous individuals. When the allele becomes common enough so that the homozygotes are more common, they would be selected against in those individuals although they would still be sheltered in the heterozygotes. Thus, if the homozygous *rufa* individuals in these crosses were carrying closely linked recessive lethals which they inherited from both of their *rufa* parents, they would be inviable and the result would be a 2 : 1 ratio (*rufa* : non-*rufa*). All of the *rufa* individuals in these broods would be heterozygous (*Rr*). The fact that only nine of the 33 *rufa* by *rufa* crosses exhibited this deficiency indicates that these postulated lethals are by no means present on all chromosomes containing a *rufa* allele.

Tables 14, 15 and 16 are taken from table 13 to illustrate what the authors have termed "brood effect". Tables 14 and 15 are from crosses which utilised *bicolor* males and *rufa* females while table 16 is from the reciprocal cross. It is important to note that some of the *rufa* individuals used as parents in all three tables came from the same brood, and that the same is true for the *bicolor* individuals. These mating cages were operating at the same time and the males and females of the two morphs were separated and placed in their respective cages.

It is interesting to note that both table 15 and table 16 have very high homogeneity probabilities and have a disproportionate number of broods with an excess of *bicolor* individuals over the expected 1 : 1 ratio. A sign test reveals a significant number of broods with excess *bicolor* larvae ( $\chi^2_{(1)} = 7.0$ ;  $P = 0.01$ ) in table 16 while table 15 is not quite significant ( $\chi^2_{(1)} = 2.66$ ;  $P = 0.10$ ). The authors feel that this indicates that the individuals which were the parents of the broods in table 15 were closely related to each other as were the parents of the broods in table 16. In neither table 15 ( $\chi^2_{(1)} = 0.821$ ;  $P = 0.37$ ) nor table 16 ( $\chi^2_{(1)} = 1.899$ ;  $P = 0.17$ ) is the summed  $\chi^2$  value significant. The egg masses from each cage were laid on two successive days but separated by a four-day interval. Table 14 reveals a very low homogeneity (significant heterogeneity) and a non-significant sign test ( $\chi^2_{(1)} = 0.166$ ;  $P = 0.68$ ). These egg masses were laid on three successive days but were apparently not from related parents since the deviations are at random. The total  $\chi^2$  for table 14 significant ( $\chi^2_{(7)} = 14.910$ ;  $P = 0.04$ ) because the deviations in this table are quite large and it includes two significant deviations (brood nos. 413 and 414). The probable reason

TABLE 14

Parental phenotypes: *bicolor* × *rufa*Presumptive parental genotypes: *BBrr* × *B-Rr*; *B-rr* × *BBRr*

Brood no.	Phenotypes of progeny (expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Bicolor</i>			
411	72	86	158	1.241	0.27
412	115	95	210	1.905	0.17
413	146	110	256	4.063	0.02
414	21	36	57	3.947	0.05
415	127	112	239	0.941	0.33
467	33	44	77	1.571	0.21
469	35	31	66	0.242	0.62
	549	514	1063		

Homogeneity  $\chi^2_{(6)} = 13.758$ ; P = 0.03.

TABLE 15

Parental phenotypes: *bicolor* × *rufa*Presumptive parental genotypes: *BBrr* × *B-Rr*; *B-rr* × *BBRr*

Brood no.	Phenotypes of progeny (expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Bicolor</i>			
477	175	195	370	1.081	0.30
479	136	139	275	0.033	0.86
480	57	65	122	0.525	0.47
481	100	106	206	0.175	0.68
524	112	116	228	0.070	0.79
526	107	100	207	0.237	0.63
	687	721	1408		

Homogeneity  $\chi^2_{(5)} = 1.299$ ; P = 0.9.

TABLE 16

Parental phenotypes: *rufa* × *bicolor*Presumptive parental genotypes: *B-Rr* × *BBrr*; *BBRr* × *B-rr*

Brood no.	Phenotypes of progeny (expected ratio—1 : 1)		Total	$\chi^2_{(1)}$	P
	<i>Rufa</i>	<i>Bicolor</i>			
449	101	110	211	0.384	0.54
450	76	85	161	0.503	0.48
451	43	43	86	0.000	1.00
452	89	94	183	0.137	0.71
453	67	70	137	0.066	0.798
454	45	58	103	1.641	0.20
473	177	180	357	0.025	0.87
475	115	126	241	0.502	0.48
	713	766	1479		

Homogeneity  $\chi^2_{(7)} = 1.358$ ; P = 0.99.



*Plate I*

*Chlosyne lacinia*. A, Adult female; B, *Rufa* larva (fifth instar); C, *Bicolor* larva (fifth instar)  
D, *Nigra* larva (fifth instar).

for these deviations will be discussed below. Thus, it seems that an excess of one morph over the expected number is in some broods hereditary although the mechanism involved is unknown.

Several of the broods in table 9 (brood nos. 413 and 414), table 11 (brood no. 408) and table 13 (brood nos. 421 and 463) have significant deviations from expected ratios ( $P < 0.05$ ). In at least three of these (brood nos. 408, 413 and 414) the deviations may be due in part to diapausing larvae. When larvae of this species go into diapause they decrease in size and alter their colour. Black colouration becomes brownish-black, and orange colouration becomes burnt orange. It is sometimes quite easy to confuse the morphs in this condition. No reason for the deviations in brood nos. 421 and 463 is known as complete laboratory records were not kept for these broods.

Despite the large number of crosses made during this study some of the theoretically possible progeny classes did not appear. The absence of all *rufa* broods in the *rufa* by *bicolor* and *rufa* by *nigra* crosses reflects the scarcity of homozygous *rufa* (*RR*) individuals. This is not only due to the low frequency of the *rufa* allele as mentioned above, but also to the presence of recessive lethals as discussed above. No 3 : 1 (*rufa* : *nigra*) broods were obtained from *rufa* by *rufa* crosses. Such a brood results only if both parents were heterozygous for the *rufa* allele and homozygous for the *nigra* allele (*Rrbb*). Using field frequencies this cross should result in approximately 5 per cent. of the *rufa* by *rufa* crosses. We, apparently, failed to get this combination solely by chance. The fourth progeny class which did not appear was a 4 : 3 : 1 (*rufa* : *bicolor* : *nigra*) ratio from a *rufa* by *bicolor* cross. In this case both parents must be heterozygous for the *bicolor* allele (*Bb*) while the *rufa* parent would have to be heterozygous (*Rr*). Apparently the *bicolor* individuals used in this cross were homozygous (*BB*).

## 5. SUMMARY

1. The striking polymorphism exhibited by the larvae of *Chlosyne lacinia* (Geyer) is described and illustrated.
2. The morphs are present in similar proportion in both males and females.
3. Data presented indicate that the *bicolor* and *nigra* morphs are controlled by a simple one-locus, two-allele system with *bicolor* (*B*) completely dominant to *nigra* (*b*).
4. Crosses involving *rufa* individuals indicate that another locus which is epistatic to the *bicolor-nigra* locus controls the *rufa* morph with *rufa* (*R*) being completely dominant to non-*rufa* (*r*).
5. The results of several broods indicate that some of the chromosomes which carry the *rufa* (*R*) allele have recessive lethals which are probably closely linked to the *rufa* (*R*) allele.
6. Results of some crosses indicate that an excess of one morph over the expected number may be inherited.

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