A NEW COLOR ALLELE AT THE e LOCUS OF *DROSOPHILA* *POLYMORPHA* FROM NORTHERN SOUTH AMERICA

WILLIAM B. HEED AND PATRICK R. BLAKE

Department of Zoology, University of Arizona, Tucson, Arizona

Received October 10, 1962

THE general phenomenon of polymorphism and its advantages for understanding evolutionary processes have recently been reviewed by HUXLEY (1955). Color polymorphism is not common in *Drosophila* but when it does occur, in species that may be bred in the laboratory, it affords excellent material for genetic analysis. In 1949 BRITO DA CUNHA established that the three phenotypes of *Drosophila polymorpha* DOBZHANSKY and PAVAN in Brazil are controlled by two alleles. The three morphs are distinguishable by the color and pattern of the abdomen, the heterozygote being intermediate to the light and dark forms. The frequencies of the three morphs in collections from seven localities in southern Brazil in different seasons of the year show that the allele for the dark abdomen fluctuates in frequency from 60 to 80 percent. Most assuredly this is a case of polymorphism as defined by FORD (1953), and in all probability it is due to balanced selective forces, even though they are not known.

Recent collections of *Drosophila* throughout the Caribbean area by the Genetics Foundation of the University of Texas have established that *D. polymorpha* ranges as far north as Colombia, Venezuela, Trinidad and the small island of Grenada, in the West Indies 92 miles north of Trinidad. The species does not inhabit Central America, even, apparently, in the lower Darien Province of the Republic of Panama (HEED 1962). Interestingly enough, the species is almost completely monomorphic for the light abdomen in the northern limit of its range. Our field records indicate that this is so, and all but one of the 11 laboratory populations from northern South America are true breeding for the light form. The exceptional laboratory population was kindly sent to us from El Colegio, Colombia, by DR. ALICE HUNTER while the manuscript was in preparation. This population is analysed in another paper (HEED 1963).

Hybridization tests between three light northern and two dark southern populations were initiated by the junior author in order to investigate their fertility relationships. In the first generation it was discovered that the northern light phenotype from Trinidad is effectively dominant to the dark phenotype from Brazil. The purpose of the present report is to present data clarifying two questions which immediately present themselves. First, what is the genetic mechanism underlying the dominance and second, how widespread is the effect?

The results indicate the dominance effect is caused by a third allele at locus e , as established by DA CUNHA, and that two of the three northern populations tested, Trinidad, West Indies, and Santa Marta, Colombia, are homozygous for

it, while the third population, Villavicencio, Colombia, is heterozygous for the dominant light allele and the light allele which gives an intermediate phenotype with the dark gene. The questions of the origin and possible adaptive significance of the new allele are discussed.

MATERIALS AND METHODS

The stock numbers and origins of the five laboratory populations used in the tests are: No. H334.3, Arima, Trinidad, April 1958, collected by M. WASSERMAN, apparently several individuals; No. H186.49, El Recuerdo, at 3500 feet in the Sierra Nevada de Santa Marta near Santa Marta, Colombia, August 1956, collected by M. WASSERMAN, H. L. CARSON and W. B. HEED, at least three individuals; No. H194.12, Villavicencio, Colombia, September 1956, collected by M. WASSERMAN, many individuals; No. 2374.8, Montes Claros, Minas Gerais, Brazil, received at the University of Texas, January 1956, many individuals; No. 2099.9, Pirassununga, Sao Paulo, Brazil, received at Columbia University, 1951, sent by A. B. DA CUNHA.

The five localities form the corners of a five-sided kite, the apex being located at Santa Marta. Villavicencio is located east of the Cordillera Oriental in the geographical center of Colombia and is approximately 480 miles south of the Sierra Nevada de Santa Marta and 950 miles southwest of the island of Trinidad. Pirassununga is approximately 3000 miles southeast of Santa Marta and Montes Claros is 2,200 miles southwest of Trinidad. The two Brazilian localities are about 425 miles apart.

All matings were maintained on regular banana, malt, karo, agar, yeast media in large shell vials. The mold inhibitor was propionic acid. Kleenex tissues dipped in a suspension of live Fleishmann's yeast were regularly supplied to the larvae for food and pupation space. The temperature in the laboratory varied from 69° to 74°F. All flies were aged at least one week before being scored. The total number of progeny in the majority of tests was quite low. A partial explanation may be found in the skipping and wandering habit of the larvae. Many larvae migrate away from the media even when yeasted Kleenex is added, and pupate prematurely and die in the cotton stopper. The proportion that regularly do this has not been determined. Also when matings were attempted with single pairs there was a high proportion that produced no progeny. Of the few sterile pairs dissected, it was found that the female was never inseminated. There are no systematic data on the point however.

RESULTS

Phenotypes: The five laboratory populations are designated by two letters: Trinidad, TR; Santa Marta, SM; Villavicencio, VC; Montes Claros, MC and Pirassununga, PA. The first three populations breed true for the light phenotype and they are indistinguishable among one another. The last two populations from Brazil breed true for the dark phenotype but they are usually distinguishable. The black pigment of PA is usually less extensive on the fifth tergite. The

two dark strains have been hybridized and they breed true to black in the F_1 and F_2 . The three alleles at the e locus are designated as e^L , e^I and e^d . Of the six kinds of genotypes possible with three alleles there are four major phenotypes. The *light* abdomen class may be of the genotype e^L/e^L , e^L/e^I or e^I/e^I . The second phenotype is named "*light*" abdomen class, e^L/e^d . There are usually enough diffuse markings on the posterior tergites to enable one to distinguish this class from the former one with practice. However, when the e^L allele is from the Villavicencio population, there is a strong overlap with the light class. In other words, dominance is more effective. The class scored as *intermediate*, e^I/e^d , is variable, in part depending upon the origin of the e^d allele. The *dark* class, e^d/e^d , is distinct and constant within each mating.

Figure 1 illustrates the abdominal pattern of the female for the various combinations of alleles. The first three abdomens show the effectiveness of the dominant light allele, and that the one from Villavicencio is the most potent. The original light phenotypes from the three northern populations are identical or very similar to the $e^{L\ VC}/e^{d\ PA}$ phenotype. In the latter case these populations lack the dot on the seventh segment and the posterior band on the fifth.

The majority of individuals from Pirassununga are not as dark as those of the Montes Claros population and this contrast shows up nicely in the heterozygotes carrying the e^I allele from Villavicencio (the fourth to seventh abdomens, Figure 1). The variation *within* each of the two genotypes, $e^{L\ VC}/e^{d\ PA}$ and $e^{I\ VC}/e^{d\ MC}$, may have a direct genetic basis since the intermediate classes of many of the families, each derived from a different pair mating, are sometimes more similar to one another than to another family. The point would be exceedingly difficult to establish however, since the differences are usually slight. Also some families are quite variable within themselves. Moreover, DA CUNHA, personal communication, attempted to determine a genetic basis for different grades of intermediates derived from the *same* population but was unable to do so. The two abdomens in the lower right corner in Figure 1 illustrate the average difference between the PA and MC populations.

Hybridization tests: The hybridization tests may be divided into three categories: (1) the tests in which TR and SM are mated to PA and MC which usually give straightforward F_2 ratios of 3:1 or 1:2:1 depending on the effectiveness of the dominance and the experience of the scorer, and testcrosses giving 1:1 ratios (Tables 1 through 4). There are also some modified ratios in the tests and they are discussed. (2) The tests in which VC is mated to MC and PA give F_1 results dependent on the genotype of VC in which variously modified F_2 and testcross ratios are illustrated (Tables 5 through 9). (3) The matings designed to test for the presence or absence of epistasis include the VC, TR and MC populations (Tables 10 through 13). In the majority of tables, chi-square values are calculated for individual matings and are added to give the sum of the chi-squares. A pooled chi-square is calculated from the totals for each table and a heterogeneity chi-square is derived by subtracting the pooled from the sum of the chi-squares.

Trinidad and Santa Marta: A total of 14 of the 20 possible heterogamic mating

FIGURE 1.—Abdominal patterns of *Drosophila polymorpha* females. Letters in superscript indicate origin of allele: VC, Villavicencio, Colombia; SM, Santa Marta, Colombia; TR, Arima, Trinidad; PA, Pirassununga, Brazil; MC, Montes Claros, Brazil. The e^L/e^d genotype is the "light" phenotype, the e^I/e^d genotype is the intermediate phenotype, and the e^d/e^d genotype is the dark phenotype. The light phenotype ($e^L/e^L, e^L/e^I, e^I/e^I$) is equivalent to or lighter than the $e^L VC/e^d PA$ abdomen.

combinations were made among the five populations. Table 1 produced progeny of the light class only. The matings in Tables 2 and 3 make up the earlier tests and due to lack of experience no attempt was made to distinguish the e^L/e^d heterozygote. In Table 4 the heterozygous class, e^L/e^d , was scored and the data

TABLE 1

*Hybridization results of the three northern light populations:
Villavicencio (VC), Santa Marta (SM) and Trinidad (TR)*

No.	Female	Male	Type mating	No. hybrids	Phenotype
1.	VC	× TR	1 pr/vial (15/20 vials fertile)	849	light
2.	TR	× VC	1 pr/vial (5/20 vials fertile)	250	light
3.	SM	× TR	20 prs/vial	193	light
4.	TR	× SM	20 prs/vial	233	light
5.	VC	× SM	20 prs/vial	64	light
6.	SM	× VC	20 prs/vial	73	light
7.	VC/TR	× VC/TR	20 prs/vial (15 vials)	2000+	light
8.	TR/VC	× TR/VC	20 prs/vial (5 vials)	500+	light
9.	SM/TR	× SM/TR	mass	147	light
10.	TR/SM	× TR/SM	mass	378	light
11.	VC/SM	× VC/SM	mass	71	light
12.	SM/VC	× SM/VC	mass	138	light

TABLE 2

Montes Claros females (e^d/e^d) × Trinidad males (e^L/e^L)

No.*	Female × Male	Light and "light"	Dark	χ^2	d.f.	P
1.	MC/TR × MC/TR	61 (60.75)	20 (20.25)	0.004	1	0.95
2.	MC/TR × MC/TR	109 (95.25)	18 (31.75)	7.36	1	<.01
	[(males only of No. 2)	47 (45)	13 (15)	0.36	1	>.50]
3.	MC/TR × MC/TR	113 (105)	27 (35)	2.40	1	>.10
4.	MC/TR × MC/TR	150 (135.75)	31 (45.25)	5.57	1	>.01
5.	MC/TR × MC/TR	83 (78)	21 (26)	1.03	1	>.30
6.	MC/TR × MC/TR	160 (151.5)	42 (50.5)	1.70	1	>.10
7.	MC/TR × MC/TR	61 (54)	11 (18)	3.12	1	>.05
8.	MC/TR × MC/TR	88 (83.25)	23 (27.75)	0.86	1	>.30
9.	MC/TR × MC/TR	76 (69)	16 (23)	2.45	1	>.10
10.	MC/TR × MC/TR	18 (14.25)	1 (4.75)	3.56	1	>.05
11.	MC/TR × MC/TR	91 (93.75)	34 (31.25)	0.45	1	.50
12.	MC/TR × MC/TR	88 (80.25)	19 (26.75)	0.21	1	>.50
13.	MC/TR × MC/TR	40 (33.75)	5 (11.25)	3.92	1	>.02
14.	MC/TR × MC/TR	35 (36)	13 (12)	0.50	1	>.30
15.	MC/TR × MC/TR	25 (21.75)	4 (7.25)	1.38	1	>.20
16.	MC/TR × MC/TR	58 (51.75)	11 (17.25)	2.56	1	>.10
	Total	1303	309	37.43	16	<.01
	Expected	1209	403	29.23	1	<.01
	Heterogeneity			8.20	15	>.90

* Six of 15 pair matings fertile producing 312 hybrids (e^L/e^d , "light"). F_1 of the six pairs inbred in small mass cultures (five females, five males), Numbers 1 to 6, and in pair matings 7 to 16. Expected phenotypes in parentheses.

TABLE 3

Montes Claros females (e^d/e^d) × Trinidad males (e^L/e^L)

No.*	Female × Male	Light and "light"	Dark	χ ²	d.f.	P
1.	F ₁ light × F ₂ dark	11 (9)	7 (9)	0.52	1	>.25
2.	F ₁ light × F ₂ dark	23 (21)	19 (21)	0.22	1	>.50
3.	F ₁ light × F ₂ dark	42 (27.5)	13 (27.5)	14.20	1	<.01
	[(males only of No. 3)]	15 (13)	11 (13)	0.34	1	>.50]
4.	F ₁ light × F ₂ dark	14 (13)	12 (13)	0.04	1	>.75
5.	F ₁ light × F ₂ dark	28 (24)	20 (24)	1.02	1	>.25
6.	F ₁ light × F ₂ dark	22 (16.5)	11 (16.5)	3.02	1	>.05
7.	F ₂ light × F ₂ dark	34 (30)	26 (30)	1.06	1	.30
8.	F ₂ light × F ₂ dark	25 (25.5)	26 (25.5)	0.02	1	>.80
9.	F ₁ light × F ₂ light	38 (30.75)	3 (10.25)	6.84	1	<.01
10.	F ₁ light × F ₂ light	118 (118)	3 pairs	...	.	..
11.	F ₂ light × F ₂ light	63 (58.5)	15 (19.5)	1.10	1	>.30
12.	F ₂ light × F ₂ light	167 (167)	4 pairs	...	.	..
Total		199	134	20.10	8	.01
Expected		166.5	166.5	12.70	1	<.01
Heterogeneity				7.40	7	>.25

* Various testcrosses and F₂ inbred pair matings. The sum, pooled and heterogeneity chi squares are for the 1:1 ratio only. Expected phenotypes in parentheses.

TABLE 4

Santa Marta (e^L/e^L) × Pirassununga (e^d/e^d)

No.*	Female × Male	Light	"Light"	Dark	χ ²	d.f.	P
1.	SM/PA × SM/PA	7 (8.25)	25 (16.5)	1 (8.25)	9.47	2	<.01
2.	SM/PA × SM/PA	26 (20.25)	35 (40.5)	20 (20.25)	2.40	2	.30
3.	SM/PA × SM/PA	51 (45.5)	90 (91)	41 (45.5)	1.12	2	>.50
4.	SM/PA × SM/PA	10 (12.75)	22 (25.5)	19 (12.75)	3.94	2	>.10
5.	PA/SM × PA/SM	36 (37.5)	72 (75)	42 (37.5)	0.72	2	>.50
6.	PA/SM × PA/SM	23 (39.5)	85 (79)	50 (39.5)	10.14	2	<.01
7.	PA/SM × PA/SM	25 (32.25)	79 (64.5)	25 (32.25)	6.52	2	>.02
Total		178	408	198	34.31	14	<.01
Expected		196	392	196	2.31	2	>.25
Heterogeneity					32.00	12	<.01

* Ten of 43 pair matings fertile producing 502 hybrids (e^L/e^d, "light") when SM was female parent. Seven of 20 pair matings fertile producing 405 hybrids when PA was female parent. Hybrids inbred in small mass cultures from selected F₁ pairs as indicated. Expected phenotypes in parentheses.

illustrate that Santa Marta behaves in a similar manner to Pirassununga as Trinidad does to Montes Claros, except for the presence of some modifying genes in the latter tests which will be discussed below. The two northern populations are homozygous for a dominant light allele at the *e* locus.

Although the ratios are fairly good in matings of Montes Claros with Trinidad

TABLE 5

Villavicencio (VC) pair matings with Montes Claros (MC) and Pirassununga (PA)

No.	Female × Male	No. pairs mated	No. fertile pairs; e^L/e^L	genotype of VC partner e^L/e^L	VC partner e^l/e^l
1.	MC × VC	69	24	8	1
2.	VC × MC	69	18	7	0
3.	PA × VC	24	0	2	0
4.	VC × PA	68	18	2	0
Total		230	60	19	1

TABLE 6

*Villaviencio pair matings with Montes Claros and Pirassununga that produced 18 or more hybrids having both "light" and intermediate phenotypes.**Expected phenotypes in parentheses*

No.	Female × Male	"Light"	Intermediate	χ^2	d.f.	P
1.	MC × VC	22 (22.5)	23 (22.5)	.02	1	.90
2.	MC × VC	22 (30.5)	39 (30.5)	4.74	1	>.02
3.	MC × VC	17 (16)	15 (16)	0.12	1	>.50
4.	MC × VC	18 (16.5)	15 (16.5)	0.28	1	>.25
5.	MC × VC	23 (17.5)	12 (17.5)	3.46	1	>.05
6.	VC × MC	25 (22)	19 (22)	0.82	1	>.25
7.	VC × MC	28 (38)	48 (38)	5.26	1	>.01
8.	VC × MC	30 (34)	38 (34)	0.94	1	>.25
9.	VC × MC	14 (13.5)	13 (13.5)	0.04	1	>.75
10.	VC × MC	18 (20.5)	23 (20.5)	0.60	1	>.25
11.	VC × MC	14 (15)	16 (15)	0.14	1	>.50
12.	VC × MC	18 (16.5)	15 (16.5)	0.28	1	>.50
13.	PA × VC	29 (25.5)	22 (25.5)	0.96	1	>.25
14.	VC × PA	18 (17.5)	17 (17.5)	0.04	1	>.75
15.	VC × PA	10 (9)	8 (9)	0.22	1	>.50
Total		306	323	17.92	15	>.25
Expected		314.5	314.5	0.46	1	.50
Heterogeneity				17.46	14	>.20

(Tables 2 and 3), there are also some modified ratios many of which are not noticeable in the tables. The cause of the modified ratios may be classified into two types, dependent upon the expected color ratios and/or the expected sex ratio. The first type is a significant, or nearly significant, low number of females in the sex ratio but a normal color ratio in both sexes; this is called the *female semilethal effect*. It is especially noticeable because the females are nearly always in slight excess of the males in normal crosses. The semilethal effect is found in the following tests: Mass mating 6, Table 2 (85 females:117 males); Pair mating 11, Table 2 (44 females:81 males); Pair mating 9, Table 2 (36 females:53 males) and Pair mating 2, Table 3 (14 females:28 males). There was no significant departure from an equal sex ratio in the F_1 progeny from the six original pair matings (Table 2) and the effect must be considered recessive in the hybrid

TABLE 7

F₂ phenotypes of Villavicencio females × Montes Claros males in ratio of 2:1:1 (light: intermediate: dark) when F₁ parents are "light" (e^L/e^d) and intermediate (e^l/e^d)

No.*	Light female × Int. male	Type mating	Light, "light"	Inter.	Dark	χ ²	d.f.	P
1.	VC/MC × VC/MC	4 prs.	20 (15.5)	7 (7.75)	4 (7.75)	2.40	2	>.10
2.	VC/MC × VC/MC	1 pr.	27 (22)	13 (11)	4 (11)	4.96	2	>.05
3.	VC/MC × VC/MC	2 prs.	23 (26.5)	21 (13.25)	9 (13.25)	5.37	2	>.05
4.	VC/MC × VC/MC	4 prs.	30 (24)	12 (12)	6 (12)	3.78	2	>.10
5.	VC/MC × VC/MC	1 pr.	53 (43.5)	15 (21.75)	19 (21.75)	4.50	2	>.10
Total			153	68	52	21.01	10	>.01
Expected			136.5	68.25	68.25	5.86	2	>.05
Heterogeneity						15.15	8	>.05

* Expected phenotypes in parentheses. The brother-sister Matings 1 to 3 originate from three different parental pairs (A, B and C). Matings 4 and 5 are derived from two separate parental pairs. The F₁ females came from Pair D and her sibs were all light. The F₁ males came from Pair C and his sibs were light and intermediate.

TABLE 8

Villavicencio tests with Montes Claros and Pirassununga

No.	F × M	Light, "light"	F ₂	Dark	χ ²	d.f.	P
1.	MC × VC	31 (32.25)	12 (10.75)	0.20	1	>.50	
2.	MC × VC	11 (9.75)	2 (3.25)	0.64	1	>.25	
3.	MC × VC	39 (36)	9 (12)	1.00	1	>.25	
4.	MC × VC	18 (15.75)	3 (5.25)	0.77	1	>.25	
5.	MC × VC	37 (34.5)	9 (11.5)	0.72	1	>.25	
6.	MC × VC	53 (43.5)	5 (14.5)	8.29	1	<.01	
7.	MC × VC	31 (27.75)	6 (9.25)	1.52	1	>.10	
8.	MC × VC	69 (60)	11 (20)	5.35	1	>.01	
9.	MC × VC	35 (32.25)	8 (10.75)	0.63	1	>.25	
10.	MC × VC	41 (39.75)	12 (13.25)	0.16	1	>.50	
11.	VC × MC	56 (48)	8 (16)	4.69	1	>.025	
12.	VC × MC	60 (56.25)	15 (18.75)	0.75	1	>.25	
13.	VC × MC	35 (33.0)	9 (11.0)	0.27	1	>.50	
14.	VC × MC*	35 (34.5)	11 (11.5)	0.02	1	.90	
15.	VC × MC*	74 (59.25)	5 (19.75)	14.70	1	<.01	
16.	VC × MC	45 (43.5)	13 (14.5)	0.21	1	>.50	
17.	VC × PA*	27 (24.0)	5 (8.0)	1.04	1	>.25	
18.	VC × PA*	97 (90.75)	24 (30.25)	1.72	1	>.10	
19.	VC × PA*	28 (26.25)	7 (8.75)	0.47	1	>.25	
20.	VC × PA*	69 (69)	23 (23)	...	1	>.99	
Total		891	197	43.15	20	<.01	
Expected		816	272	27.57	1	<.01	
Heterogeneity				15.58	19	>.50	

* F₁ hybrids ("light") inbred in pair and small mass matings.

Matings 11, 12 and 13 are two pooled pairs each. Mating groups 1 to 7, 8 to 10, 11, 12, 13, and 14 to 20 each originate from different parental pairs. Expected phenotypes in parentheses.

TABLE 9

F₂ phenotypes from Villavicencio × Montes Claros. F₁ hybrids test mated and inbred in the combinations indicated

No.*	Female × Male	No. of pairs	Light, "light"	Intermediate	Dark	χ ²	P
1.	MC/VC(lt) × MC(dk)	3	34 (32)		30 (32)		
2.	VC/MC(lt) × MC(dk)	4	28 (30)		32 (30)		
3.	MC(dk) × VC/MC(lt)	2	9 (6.5)		4 (6.5)		
4.	VC/MC(int) × VC/MC(int)	4	14 (11.5)	25 (23)	7 (11.5)	2.47	>.25
5.	MC/VC(int) × MC/VC(int)		17 (20.25)	41 (40.5)	23 (20.25)	0.90	>.50
6.	MC(dk) × VC/MC(int)	5		43 (37)	31 (37)		
7.	F ₃ of No. 6		6 (6.13)	39 (36.75)	53 (55.12)	0.24	>.75

* Expected phenotypes in parentheses. Number 5 mating is derived from the only mating of Villavicencio producing all intermediate F₁, which is a small mass mating of 11 individuals. Number 7 is derived from three pairs from Number 6. The expected ratio at equilibrium is 1:6:9 (light:intermediate:dark).

TABLE 10

Test to show absence of epistasis in Trinidad × Montes Claros hybrids

No.*	F ₁ testcross Female × Male	No. F ₂ pairs	F ₃ light	F ₄ light
1.	MC/VC(int) × TR(A)	4	64	119
2.	MC/VC(int) × TR(B)	1	26	34
3.	MC/VC(int) × TR(A)	3	163	173
4.	MC/VC(int) × TR(A)	1	44	..
5.	MC/VC(int) × TR(B)	1	36	8
6.	TR(A) × MC/VC(int)	1	96	162
7.	TR(B) × MC/VC(int)	1	35	18
8.	TR(A) × VC/MC(int)	1	28	..
9.	TR(B) × VC/MC(int)	1	122	105
10.	TR(A) × VC/MC(int)	2	76	52
Total		16	690	671

* F₂ pairs are heterozygous for the same alleles ($e^L/e^l \times e^L/e^l$). Expected F₃ and F₄ phenotypes all light. Numbers 1 and 2 originate from P₁ pair 1; two TR males tested. Number 3 originates from P₁ pair 5; one TR male tested. Numbers 4 and 5 originate from P₁ pair 10; two TR males tested. Numbers 6 and 7 originate from P₁ pair 1; two TR females tested. Numbers 8 and 9 originate from P₁ pair 3; two TR females tested. Number 10 originates from P₁ pair 1; 20 TR females tested in mass culture.

genotypes. All the pair matings agree quite well with a 3:4 ratio (female:male) whether they are derived from parents who are both F₁ hybrids, or from backcrosses. The ratio is based on a recessive lethal that segregates freely from the color genes. The sex ratio from the mass mating agrees with a 3:4 ratio but also with a 27:29 ratio (female:male) which would be more likely to occur from a multiple mating where lethal-free parents would mate with one another also.

The second type of modified ratio is a significantly low number of the dark female class but a normal color ratio in the males and a normal sex ratio. It is named the *dark female suppressor effect*. Examples are: Mass mating 2, Table 2 (62 light:5 dark females) and four pair matings in Table 3, where a 1:1 color

TABLE 11

Test to show absence of epistasis in Trinidad × Montes Claros hybrids

No.*	F ₁ Testcross Female × Male	No. F ₂ pairs	F ₃		χ ²	d.f.	P
			Light	"Light" Int.			
1.	MC/VC(int) × TR(A)	5	170(164)	88(82)	70(82)	2.42	2 >.25
2.	MC/VC(int) × TR(A)	1	22(19)	9(9.5)	7(9.5)	1.16	2 >.50
3.	MC/VC(int) × TR(B)	1	14(13.5)	4(6.75)	9(6.75)	1.89	2 >.25
4.	MC/VC(int) × TR(C)	2	114(101.25)		21(33.75)	6.41	1 >.01
5.	MC/VC(int) × TR(A)	4	121(109.5)	62(54.75)	33(54.75)	11.52	2 <.01
6.	MC/VC(int) × TR(B)	10	247(230.5)	121(115.25)	93(115.25)	5.77	2 >.05
7.	TR(A) × MC/VC(int)	1	44(44.5)	21(22.25)	24(22.25)	0.22	2 >.75
8.	TR(B) × MC/VC(int)	1	33(31)	15(15.5)	14(15.5)	0.29	2 >.75
9.	TR(A) × VC/MC(int)	1	29(31)	14(15.5)	19(15.5)	1.06	2 >.25
10.	TR(B) × VC/MC(int)	1	23(24)	10(12)	15(12)	1.12	2 >.25
11.	TR(C) × VC/MC(int)	1	9(12)	10(6)	5(6)	3.59	2 >.10
12.	TR(D) × VC/MC(int)	3	118(154.4)	99(77.25)	92(77.25)	17.84	2 <.01
13.	TR(A) × VC/MC(int)	3	27(23)	10(11.5)	9(11.5)	1.12	2 >.50
Total		860	463	390	48.00	24	<.01
Expected		856.5	428.25	428.25	6.24	2	>.025
Heterogeneity					41.76	22	>.005

* F₂ pairs heterozygous for different alleles (e^L/e^l × e^L/e^d). Expected F₃ ratio 2:1:1 (light:"light":intermediate). In Number 4, dominance was too effective to distinguish the lighter phenotypes and the χ² tests for a 3:1 ratio. Number 1 originates from P₁ pair 5; one TR male tested. Numbers 2 to 4 originate from P₁ pair 10; three TR males tested. Numbers 5 to 8 originate from P₁ pair 1; two TR males and two TR females tested. Numbers 9 to 12 originate from P₁ pair 3; four TR females tested. Number 13 originates from P₁ pair 2; 15 TR females tested in mass culture.

ratio is expected, Number 1 (7 light:1 dark female), Number 3 (27 light:2 dark females), Number 5 (17 light:6 dark females) and Number 6 (15 light:5 dark females). Notice that three of the four pair matings produce good 3:1 color ratios which would be expected if the suppressor were dominant and segregated independently from the color alleles. An approximate 6:1 ratio (light:dark) would be expected in the former mass mating if the first generation hybrids were equally represented by the presence and absence of the suppressor, as they appear to have been. The ratio is compounded from a 3:1 ratio (no suppressors present), two 7:1 ratios (suppressor present in one parent), and a 15:1 (suppressor present in both parents).

A third type of modified ratio appears to be a combination of the female semi-lethal and the dark female suppressor effects. However, when they are combined, only the suppressor effect shows a significant difference. Examples are: Mass mating 4, Table 2 (74 light:12 dark females; 86 females:95 males). The color ratio conforms to an expected 6:1 and the sex ratio conforms to an expected 27:29. Another example is Pair mating 8, Table 2 (43 light:5 dark females; 48 females:63 males). The color ratio conforms to a 7:1 and the sex ratio conforms to a 3:4. There is one more example that comes from the only mating that is not recorded in a table, TR females × MC males. Only one pair mating out of 15 produced progeny, and they were all light. The sex ratio was seven females to 15 males.

TABLE 12

Test to show absence of epistasis in Trinidad × Montes Claros hybrids

No.*	F ₁ Testcross Female × Male	No. F ₂ Pairs	F ₃			χ ²	d.f.	P
			Light	"Light"	Dark			
1.	MC/VC(int) × TR(A)	4	31 (57.5)	141 (115)	58 (57.5)	17.53	2	<.01
2.	MC/VC(int) × TR(A)	1	30 (24.5)	43 (49)	25 (24.5)	1.99	2	>.25
3.	MC/VC(int) × TR(C)	1	26 (31.5)		16 (10.5)	3.94	1	>.025
4.	MC/VC(int) × TR(C)	1	45 (47.25)		18 (15.75)	0.43	1	>.50
5.	MC/VC(int) × TR(B)	1	16 (16.5)		6 (5.5)	0.01	1	>.90
6.	MC/VC(int) × TR(A)	2	24 (14)	29 (28)	3 (14)	15.78	2	<.01
7.	MC/VC(int) × TR(B)	1	48 (32)	62 (64)	18 (32)	14.18	2	<.01
8.	MC/VC(int) × TR(B)	1	21 (17)	41 (34)	6 (17)	9.49	2	<.01
9.	TR(A) × MC/VC(int)	1	23 (21.5)	43 (43)	20 (21.5)	0.21	2	.90
10.	TR(A) × MC/VC(int)	1	47 (36.75)	64 (73.50)	36 (36.75)	4.09	2	>.10
11.	TR(C) × VC/MC(int)	1	9 (8.75)	20 (17.5)	6 (8.75)	1.23	2	>.50
12.	TR(D) × VC/MC(int)	1	7 (11.75)	29 (23.5)	8 (11.75)	3.49	2	>.10
13.	TR(A) × VC/MC(int)	2	8 (9.75)	23 (19.5)	8 (9.75)	1.25	2	>.50
Total		248		495	188	69.24	20	<.01
Expected		232.75		465.5	232.75	11.47	2	<.01
Heterogeneity						57.77	18	<.01

* F₂ pairs heterozygous for same alleles (e^L/e^d × e^L/e^d). Expected F₃ ratio 1:2:1 (light:"light":dark). Mating Number 1 gives χ²=∞.01 and P>.90 for a 3:1 ratio. Numbers 3 to 5 are tested for a 3:1 ratio because dominance was too effective to distinguish the lighter phenotypes. Number 1 originates from P₁ pair 5; one TR male tested. Numbers 2 to 5 originate from P₁ pair 10; three TR males tested. Numbers 6 to 10 originate from P₁ pair 1; two TR males and one TR female tested. Numbers 11 to 12 originate from P₁ pair 3; two TR females. Number 13 originates from P₁ pair 1; 20 TR females tested in mass culture.

TABLE 13

Summary of tests proving absence of epistasis in Trinidad × Montes Claros hybrids

		No. Pairs Obs. Exp.		Light	"Light"	Inter- mediate	Dark	χ ²	P
Table 10	light × light	16	18	Obs. 690 Exp. 690					
Table 11	"light" × light	38*	36	Obs. 860 Exp. 856.5	463 428.25	390 428.25		6.24	>.025
Table 12	"light" × "light"	18	18	Obs. 248 Exp. 232.75	495 465.5		188 232.75	11.4	<.01

* Only 34 pairs were recorded in Table 11; the other four pairs produced less than 16 individuals each.

They were inbred in mass culture and the next generation yielded 100 light:13 dark individuals. The color ratio of the females was again the modified one (47 light:3 dark) and the total sex ratio was 50 females to 63 males. The color ratio is not significantly different from a 6:1 light:dark. The sex ratio corresponds to a 27:29, female:male.

The effects just described are not found at random in the mating schemes of MC × TR, but tend to run in families. All the pair matings in Tables 2 and 3 originate from three of the six original pair matings of Montes Claros female ×

Trinidad male (Numbers 1, 2 and 4 in Table 2). Of all the six mass matings in Table 2, Numbers 1, 3 and 5 were not affected by either the semilethal or suppressor effect. The color ratios and sex ratios are normal. Also Pair Mating 12, Table 2, which originated from the same pair mating as did Mating 1 (Table 2), was normal in color and sex ratio. Pair Matings 13 to 16 in Table 2 originate from Parental Pair 2 (female suppressor effect) but they were all normal. Pair Matings 7 to 11 in Table 2 originate from Parental Pair 4 (both effects) and two showed female semilethal and one showed both effects. The backcross Pair Matings 1 to 6 in Table 3, also originate from the Number 4 parental pair. Of these six backcrosses, four showed the suppressor effect, one showed the semilethal effect and one was normal. This indicates inheritance nicely but it does not tell us from which strain the two effects originated. However, it would appear logical that the suppressor for the dark female phenotype would originate from the light population. Pair Matings 7, 8 and 11 (Table 3), which are made by F_2 progeny, were normal in all respects and they originated from Pair Mating 9 in Table 2. This mating is classified as having the female semilethal effect. The normal ratios in the F_3 indicate the recessive lethal was not present in both F_2 parents in each case.

The possibility that the female-limited dominant suppressor gene and recessive lethal are synthetic in the hybrids does not appear to be the case because the effects carry through in later generations. Neither effect is present in the matings of Villavicencio $\times$ Montes Claros. The sex ratios in Table 1 and the remainder of the tests, including the ones for epistasis, are normal or nearly so.

Villavicencio: In the mating of Villavicencio with Montes Claros (Table 5) it was soon discovered that the light partner must be of several different genotypes, for although the majority of pair matings produced only light progeny, several pairs produced light and intermediate classes in equal frequencies. This indicated that the Villavicencio population carried the e^l allele as well as the dominant light allele. The number of matings of Villavicencio with the dark southern populations was then increased in order to find a pair that predictably would produce only hybrids of the intermediate class and thus illustrate a homozygous e^l/e^l individual. Table 5 shows that only one mating of this type appeared in 80 fertile pairs. A total of 11 individuals of the intermediate class were recovered from the cross. When they were inbred in mass culture they produced the 1:2:1 ratio expected of two alleles with no dominance (17 light:41 intermediate:23 dark).

Table 6 presents the data for 15 of the 19 pairs which produced equal progeny of the light and intermediate classes in the Villavicencio tests. When the light and intermediate classes are crossbred for another generation the expected 2:1:1 ratio is in the main fulfilled (Table 7). Table 8 shows that the dark class is recovered in matings of all heterozygous light individuals irregardless of whether they were derived from P_1 pair matings that produced all light hybrids or light and intermediate hybrids. The "light" class, e^l/e^d , is pooled with the light class, since the majority of individuals are indistinguishable. Various testcross and inbred ratios with Villavicencio are presented in Table 9. Notice the fulfillment of expectation for the 9:6:1 ratio (Mating 7) when the frequency of the e^l allele

is approximately $\frac{1}{4}$ and the frequency of the e^d allele is approximately $\frac{3}{4}$ in several culture vials of the previous generation. These tests prove that the Villavicencio population is heterozygous for the two alleles, e^L and e^l .

Epistasis: The third category of matings tests for the presence or absence of epistasis. There is a possibility that epistasis could be the factor responsible for making the Trinidad and Santa Marta populations light. For instance Trinidad may in reality be homozygous for the dark allele from Brazil, this fact being masked by another dominant light gene in double dose. A simple test would have been to mate light individuals from Brazil with light individuals from Trinidad. The F_2 should segregate out intermediate and dark classes if epistasis of this kind were present. Since no light stocks from Brazil were available, and none forthcoming, tests were made utilizing the e^l allele from the Villavicencio population. Figure 2 illustrates the mating scheme which makes use of the three populations Montes Claros, Trinidad and Villavicencio. In the second generation, the light and "light" classes were brother-sister mated in pairs at random. Each pair of parents was then classified, after mating, according to one of the two possible genotypes, e^L/e^l or e^L/e^d , as though no epistasis were present. The parents were then rechecked, if they were still alive, when it became obvious to which of the three groups their progeny belonged, and this in turn determined to what geno-

FIGURE 2.—Breeding scheme to indicate whether the dominant gene for the light yellow abdomen from Trinidad is allelic to the e^d gene from Montes Claros and the e^l gene from Villavicencio. The F_2 was mated in 76 pairs at random.

types the parents must belong. In some lines the scoring of the parents was perfect. In other lines the scoring was wrong most of the time or questionable from the start. The reasons for this are explained below.

The progeny from a total of 76 matings could be grouped into the three classes that one would expect if no epistatic factor were present in the Trinidad population. Moreover the frequencies of the three classes of offspring in the F_3 are what one would predict on the basis of the random mating of equal numbers of the two F_2 genotypes, e^L/e^l and e^L/e^d , in that the heterogamic matings were twice as frequent as the homogamic matings. The data are presented in Tables 10, 11, and 12, according to the three categories of the F_3 progeny and they are discussed in that order.

In the mating of $e^L/e^l \times e^L/e^l$, all progeny are of the light class (Table 10). Most of the 16 pairs were carried still another generation and no other phenotype emerged. Notice that five males and four females from Trinidad were tested individually for epistasis. If a light epistatic factor were present in Trinidad, one would expect at least a few intermediate and dark phenotypes in the F_3 and F_4 in Table 10.

In the cross $e^L/e^l \times e^L/e^d$, a 2:1:1 ratio (light:"light":intermediate) is expected (Table 11). In Mating 4, the first two classes could not be distinguished and so the test was for a 3:1 ratio. An explanation for this effect is offered below. In general the agreement between the expected and observed values is good in Table 11, although in Mating 5 the intermediate class is much lower than expected (this is accounted for below), and in Mating 12 the light class is much lower than expected. Notice that six males and six females from Trinidad were tested individually for epistasis. If a light epistatic factor were present in the Trinidad population one would expect a 3:3:1:1 ratio (light:"light":intermediate:dark) in Table 11.

In the mating $e^L/e^d \times e^L/e^d$ a ratio (light:"light":dark) is expected whether epistasis is present or not (Table 12). However there are some modified ratios that demand an explanation. In Tests 3 and 4, dominance was too effective to score the "light" phenotype as a separate class. This effect is derived from the same pair mating (10C) in the F_1 ($e^l/e^d \times e^L/e^L$) as was Mating 4 in Table 11, which also showed strong dominance. A reasonable explanation is the presence of a "weak" allele, or possibly a closely linked modifier in the Montes Claros population that works in the presence of either the e^L allele from Trinidad or the e^l allele from Villavicencio. The intermediate class in Number 4 (Table 11) is lighter than usual and this indicates the effect is introduced through the dark parent, Montes Claros, which must have been heterozygous from the modifier or isoallele, since the effect does not appear in the other two lines (10A and 10B), which are also derived from the original pair mating of Montes Claros (e^d/e^d) $\times$ Villavicencio (e^L/e^l). In Mating 5 (10B) in the table, no attempt was made to distinguish the two light classes because of the low yield.

In Matings 6, 7, and 8 (Table 12), the low yield of the dark class is actually a lethal effect in both sexes. As proof of this, the flies that did emerge and later darken, lived only a few days and easily became stuck in the food. The two light

classes of Mating 7 were bred together for another generation and this time no representatives of the dark class appeared at all, even though approximately ten of the dark class would be expected from the subsequent total of 109 individuals. The count was 87 light:21 "light." Since both Matings 1A and 1B show the effect, the cause most likely can be traced to the dark partner of the original pair mating (1) of MC $\times$ VC. However, the two reciprocal test matings derived from the identical parental pair, do not show the effect (Numbers 9 and 10 [Table 12]). The data suggest that the original Montes Claros female was heterozygous for a lethal gene closely linked to the dark allele. There is not sufficient evidence, however, to rule out the possibility that the lethal is a synthetic one or that it is a newly arisen mutation. The lethal effect appears to be partially dominant in hybrid combinations since the "light" class (e^L/e^d) is low in yield also, in the sense that this class is not twice the frequency of the e^L/e^L class (Numbers 6 and 7). Also in Table 11, Matings 5 and 6, the same allele lowers the expected values in the intermediate class (e^L/e^d), but not the "light" class (e^L/e^L).

Interestingly enough, in three of the four matings of 1A and 1B, that had the lethal in Table 12, the "light" class was more variable than usual and generally there was at least one individual very close to the intermediate class. In fact one female had to be so classified. She was subsequently mated to an e^L/e^d brother and produced seven individuals of the more typical "light" class. They were in turn bred together and produced four females of the light class and five females and three males of the typical "light" class. The results, although not conclusive, suggest that the original exceptional female was of the e^L/e^d genotype. Perhaps the release of closely linked modifiers keeping the lethal in an inactive state in the original MC female also affected the phenotype of the e^L/e^d individuals. The further tests with the exceptional female, however, do not support this, since no intermediate class appeared in later generations.

There remains one other nonconforming ratio to account for in Table 12. There is an unusually high frequency of the "light" class in Mating 1. The progeny of the four pair matings included here were scored according to the three phenotypes light, "light" and dark, as follows: (1) 3:47:17 (2) 3:45:17 (3) 11:13:6 and (4) 14:36:18. Since all the crosses check out well for a 3:1 ratio, many of the light class individuals must have been misclassified. Part of the confusion arises from the original dark Montes Claros female (Pair Mating 5) which contributed an allele, or modifier, that is lighter than usual in combination with the e^L from Trinidad and the e^L from Villavicencio, as in Matings 3 and 4. However, the e^L allele from Trinidad did not always behave normally either, since individuals known to be e^L/e^L or e^L/e^L , from crosses giving only light progeny, sometimes had large enough paramedian spots on the fifth tergite to be scored as the "light" class (e^L/e^d). The result is that most of the e^L/e^L , e^L/e^L and e^L/e^d genotypes in Mating 1 had a phenotype intermediate to the light and "light" classes.

Table 13 summarizes the results for the tests for the presence or absence of

epistasis. It is concluded there is no major epistasis giving the dominant light phenotype in the three northern populations.

DISCUSSION

The evidence is good that there exists another major color gene at the e locus in *D. polymorpha*. There are undoubtedly many minor alleles at the locus also (Figure 1) but they are difficult to separate from normal variability and environmental effects. The new light allele is dominant, but with practice one may usually distinguish most of the individuals heterozygous with the dark allele. The dominant light gene from the Villavicencio population, however, is too strong for this to be done effectively. We know that the difference between the Villavicencio population and the Trinidad and Santa Marta populations is that the latter two are homozygous for the dominant allele but that Villavicencio has the e^l gene also. Constant pairing of different alleles may make each one more effective. However there also could be a reason for the more effective dominance in this particular population that concerns population genetics rather than physiological genetics.

Assuming that the light phenotype has a definite selective advantage in the northern populations of *D. polymorpha*, then any mechanism that would protect the populations from the influx of the dark allele should also have an advantage. Dominance for the light phenotype would be one kind of protection. An epistatic gene suppressing the dark phenotype, although not as effective because it would not compete at the color locus, would be another mechanism. It is not possible to establish from our records whether the Villavicencio population contains the dark allele. In any event the population may be geographically closer to an area where the allele does exist, causing selection constantly to choose the more effective dominant allele (HALDANE 1930) in order to protect against the immigration of the e^d gene.

The discussion has been in terms of discrete alleles or a single modifier such as a suppressor because there is little evidence that the dominance in the northern populations has been established by the accumulative action of many modifiers. The point is difficult to make however, since the dominant light allele may also exist, although in low frequency, in the populations of southern Brazil. If this be true, then it may appear difficult to discuss not only the origin of the dominance but also its genetic character, since the new allele may be as highly modified in the proposed southern part of its range as in the north (FISHER 1958). However, the great distances involved in this case, 2,200 miles between Montes Claros and Trinidad, which includes the intervening Amazon and Orinoco River systems, demands that the modifiers either themselves be different or at least act in different ways, and such differences should appear in the hybrids (FORD 1955). Except for the uncovering of the female-limited dominant suppressor and two recessive lethals, one being female limited and the other being closely linked to the dark allele and not sex limited, the hybrids segregate as expected if one allele were dominant light and the other recessive dark. It

seems reasonable to suppose that the dominance arose as a single mutation (HALDANE 1939) at least once, perhaps at the margin of the population as it expanded northward from Brazil, if that country were its center of origin. The frequency of *polymorpha* among all species in the genus *Drosophila* is highest in southern Brazil.

FORD (1953), discusses the relation of lethal genes to polymorphism by saying that lethals may accumulate near the locus of an allele which remains rare in a population and hence is almost always present as a heterozygote. One of the lethals in our tests is associated with the dark allele that traces back to one pair mating of Montes Claros $\times$ Villavicencio. The lethal may not have been newly arisen because of its association with the most severe breakdown of phenotypic classes in all of the tests. The release of modifiers for the lethal, closely linked to the color locus, by crossing over, may account for the activity of the lethal in the e^d/e^d class as well as the variability of the phenotype in the e^L/e^d class. However one exceptional female, of an e^L/e^d genotype with an intermediate phenotype, was tested by mating her to an e^L/e^d brother and then inbreeding their progeny. Since only the light and "light" classes appeared in later generations, the explanation of the release of modifiers is not verified. In any event, the e^d gene in southern Brazil varies in frequency between 60 to 80 percent of the color locus (DA CUNHA 1949) and would not be expected to harbor lethals near it. There must be a point in the northern part of South America, however, where the e^d allele indeed becomes very rare and where the lethal could have become established.

The evidence for the presence of the light dominant suppressor is not on firm ground since it was discovered in the data and not specifically tested for. It is submitted, however, as further evidence that the light phenotype in the north is being protected from the dark allele further south. Genetic dominance is here given a slightly different value on the level of the population than that with which it is sometimes assigned. For instance, BIRCH (1960) lists dominance as a method for storing variability in diploid organisms. It may not be too difficult to explore the possibility that there exists a high frequency of genes, located on the periphery or near the periphery of their pool, that are more dominant than those centrally located. Given equal genetic variability in both areas, gene flow from the center to the periphery of the population would not immediately increase phenotypic variability, whereas gene migration in the opposite direction would be felt at once by the central population. One of the advantages of a smaller number of phenotypes presented to the environment at the margin of a population could be to reduce competition between closely related forms which overlap there. Several members of the *cardini* species group of *Drosophila* exhibit monomorphism at the margin of their distributions and polymorphism in the center (HEED 1963).

SUMMARY

Evidence is presented that a third major color gene exists at the *e* locus in *Drosophila polymorpha*. It is effectively dominant and makes the abdomen light yellow. The new allele, designated e^L , may be distributed throughout the range of the species, but it is much more frequent in the northern part of South America. Of the three northern populations tested, a Trinidad population and a Sierra Nevada population from Colombia were found to be homozygous for the new allele. A Villavicencio population from Colombia, although also monomorphic for the light abdomen, is heterozygous at locus *e* for the new allele and the recessive light allele, e^l . The two homozygous dark populations (e^d/e^d) used as testers are from Pirassununga and Montes Claros in southern Brazil.

Two recessive lethals appeared in several of the tests. One is female limited and the other is closely linked to the dark allele from Montes Claros. A female-limited dominant modifier that suppresses the dark phenotype appeared in the tests of Trinidad $\times$ Montes Claros. The interaction of these modifiers and the dominance are discussed in relation to the population structure of *D. polymorpha*.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to DR. A. B. DA CUNHA and DR. HAMPTON CARSON for reading the manuscript and making valuable suggestions. The work is supported by the National Science Foundation.

LITERATURE CITED

- BIRCH, L. C., 1960 The genetic factor in population ecology. *Am. Naturalist* **94**: 5-24.
- DA CUNHA, A. B., 1949 Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. *Evolution* **3**: 239-251.
- FISHER, R. A., 1958 *The Genetical Theory of Natural Selection*. Dover Publishing Co., New York.
- FORD, E. B., 1953 The genetics of polymorphism in the Lepidoptera. *Advan. Genet.* **5**: 43-87.
- 1955 Polymorphism and taxonomy. *Heredity* **9**: 255-264.
- HALDANE, J. B. S., 1930 A note on Fisher's theory of the origin of dominance and on a correlation between dominance and linkage. *Am. Naturalist* **64**: 87-90.
- 1939 The theory of the evolution of dominance. *J. Genet.* **37**: 365-374.
- HEED, W. B., 1962 Genetic characteristics of island populations. *Studies in Genetics II. Research Reports on Drosophila Genetics, Taxonomy and Evolution*. Edited by M. R. WHEELER. Univ. of Texas Publ. **6205**: 173-206.
- 1963 Density and distribution of *Drosophila polymorpha* and its color alleles in South America. *Evolution*. (In press.)
- HUXLEY, J., 1955 Morphism and evolution. *Heredity* **9**: 1-52.