MODIFIERS OF COLOR PATTERN GENES IN *DROSOPHILA POLYMORPHA**

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CONSPICUOUS color polymorphism is not common in the genus Drosophila. However, the work of DA CUNHA (1949, 1955), HEED (1962, 1963) and HEED and BLAKE (1963) showed that the *cardini* group of species to which *polymorpha* belongs, exhibits more externally visible color variants than any other Drosophila group so far studied in this respect. Other species of the *virilis*, *melanica*, *melanogaster* and *victoria* groups also show some genetically determined variability in their pigmentation patterns (PIPKIN 1962, FREIRE-MAIA 1964). It is possible that less evident differences in tergite pigmentation like the one reported by ROBERTSON and Low (1966) in *D. melanogaster* do occur in nature and go unnoticed or are not considered to be genetically determined. As the basis of the developmental and biochemical genetics of Drosophila body pigmentation is being elucidated, knowledge on the naturally occurring variants becomes more important and meaningful to the studies on gene dynamics in populations.

Since 1949 DA CUNHA has established that three phenotypes of *D. polymorpha*, light. intermediate and dark, found in Brazil are due to alleles at the locus *e*, produced by the respective genotypes, $e^l e^l$, $e^l e^d$ and $e^d e^d$. Recently, HEED and BLAKE (1963) discovered a third allele, dominant light e^L in populations in northern South America. These authors found evidence for the action of modifiers in several crosses. A female-limited dominant modifier suppressing the dark phenotype was detected but not firmly established in crosses between strains from Trinidad and Montes Claros (Brazil). Two recessive lethals have also been found by HEED and BLAKE (1963), one being female-limited and the other being linked to the dark allele and not sex-limited. Quite simultaneously NAPP (1963) found that modifiers altered the phenotypic distribution from crosses obtained with material from Rio Grande do Sul.

In this paper we describe the observations that led us to suggest the existence of color modifying alleles that segregate independently from the major locus, *e*. The strength of the effects of these modifiers is such that the darkening allele in homozygous condition, $m^d m^d$, changes the $e^l e^l$ light homozygotes into an intermediate phenotype, and the lightening allele $m^l m^l$ is even stronger. This masking effect makes unreliable the determination of gene frequency by simple phenotypic inspection of individuals from polymorphic populations of this species.

* This paper is dedicated to Professor TH. DOBZHANSKY, the founder of evolutionary genetics in Brazil.

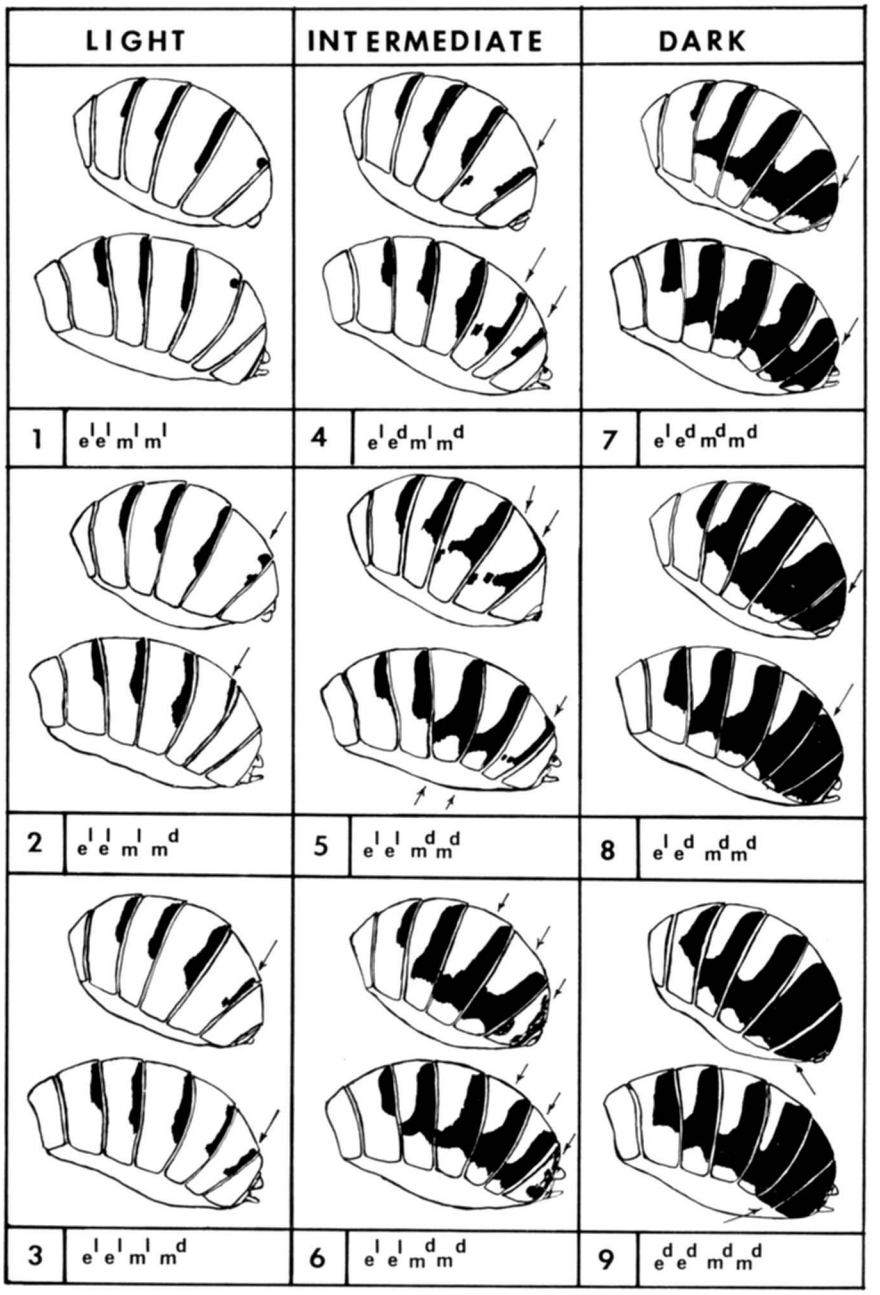

FIGURE 1.—The nine patterns of pigmentation of *Drosophila polymorpha* tergites. The arrows point to spots of increased pigmentation from type **1** to **9**, useful for type determination. Only one of the possible combinations of the major genes, e^l, e^d and the modifiers, m^l, m^d, is presented for each type. The three classes, light, intermediate and dark correspond approximately to DA CUNHA's (1949) classification.

MATERIALS AND METHODS

The strains of *D. polymorpha* DOBZHANSKY and PAVAN used in this work came from a sample collected in the remnants of the tropical rain forest that reaches the northern regions of the

State of Rio Grande do Sul, Brasil, at the locality of Itapeva. The frequency of *polymorpha* was 12.5% in a total of 780 flies collected November 6, 1960. The gravid females isolated in culture vials started the isofemale strains used in this work and maintained until the end of 1964.

Another sample was collected at the locality of Eldorado 40 km South of Porto Alegre, R.G.S. The culture medium we use in our laboratories is quite suitable for this species as well as for most Drosophilae (MARQUES *et al.* 1966). It has been pointed out by DA CUNHA (1949) and HEED and BLAKE (1963), that *D. polymorpha* is not easy to grow in laboratory conditions due partially to the skipping and wandering habit of their larvae. They are also temperature sensitive and start wandering as a result of minor temperature changes. Premature pupation of the wandering larvae decreases the number of adults in each culture. Single-pair cultures give an average of 34 individuals. All cultures have been maintained at 25°C yet the life cycle varied from 15 to 20 days depending on the crowding conditions of the cultures. After sexing, the newly hatched adults were aged in uncrowded culture vials for 8 to 9 days to attain full pigmentation and thus to be scored accurately.

RESULTS

The great variability exhibited by the presumed simple hybrids $e^l e^d$ compelled us to make detailed drawing of the male and female abdominal tergite patterns of pigmentation and score the frequencies of each color form for all crosses. As the data accumulated we chose the nine patterns that allowed the easiest classification of the array of observed forms. These arbitrary nine classes are pictured in Figure 1. These patterns have been arranged in such a way as to allow comparison with the classification presented by DA CUNHA (1949) and adopted by HEED and BLAKE (1963). DA CUNHA considered three major phenotypes only, light, intermediate and dark. From type **1** to **9** there is an increase of the pigmented area of the tergites.

The gravid females collected at Itapeva were isolated in culture vials and their F_1 sexed, aged, and classified according to nine patterns. Single-pair matings were then performed with these individuals. The crosses among the types **1** to **6**, that gave offspring within these limits proved to bear the "light" major allele, e^l, only. However, they appear to be heterogeneous for specific and nonspecific modifiers. Table 1 shows the results of these crosses ordered according to the number of types appearing in the F_1. The males were scored independently from the females. The variability of color patterns of these offspring frequently exceeds the limits of their parents (Table 1). About half of the crosses **1** × **1** gave only type **1** but the other half produced type **2** also. The crosses **1** × **3** produced variable results, twelve of them produced only types **1** and **2** among the females, and twenty-two gave males within these same limits. However, twelve others produced females classifiable from types **1** to **6**, and only eight exhibited males within these same limits (Table 1). The data were obtained for each cross and pooled in Table 1 to illustrate the fact that the males show less variability than the females. The twenty-nine crosses **1** × **4** follow the same trend, which again is seen in the type **2** × **2** crosses. The crosses between darker types usually gave variable results, the males showing lower numbers in the types **5** and **6**. Since the material for these crosses has not been subjected to selection toward stabilization of the phenotypes, this variability is not unexpected. All these results (Table 1) share a common characteristic; they manifest no darker forms than type **6**. The crosses obtained between darker types **7**, **8** and **9** produced types spreading from

TABLE 1

Distribution of color-forms' frequencies in F_2

Parents' phenotypes	Number of pair crosses	Phenotypes of the offspring 1	2	3	4	5	6	Total females and males
1 x 1	21(f)	539	-	-	-	-	-	539
	23(m)	552	-	-	-	-	-	552
	20(f)	255	187	-	-	-	-	442
	18(m)	181	57	-	-	-	-	238
	41	1527	274					1771
	44(f)	715	186	-	-	-	-	901
1 x 2	49(m)	632	51	-	-	-	-	683
	6(f)	80	70	23	-	-	-	173
	6(m)	26	2	10	-	-	-	38
	6(f)	40	42	20	26	-	-	128
	1(m)	8	2	-	-	2	-	12
	56	1501	353	53	26	2	-	1935
	12(f)	98	44	-	-	-	-	142
1 x 3	22(m)	141	35	-	-	-	-	176
	8(f)	30	41	12	-	-	-	83
	8(m)	107	26	16	-	-	-	149
	12(f)	37	81	101	34	8	3	264
	2(f)	8	6	-	-	-	8	22
	32	421	233	139	34	8	11	836
	4(f)	25	10	8	-	-	-	43
1 x 4	18(m)	139	47	11	-	-	-	197
	18(f)	105	121	48	68	-	-	342
	3(m)	10	18	-	8	-	-	36
	7(f)	12	45	30	33	6	22	148
	8(m)	53	49	11	3	2	14	132
	29	344	290	108	112	8	36	898
	3(f)	14	33	28	3	10	2	90
1 x 5	3(m)	19	21	1	1	5	3	50
	3	33	54	29	4	15	5	140
	6(f)	129	25	-	-	-	-	154
2 x 2	23(m)	196	58	-	-	-	-	254
	24(f)	124	113	69	95	9	-	410
	7(m)	25	16	10	6	-	-	57
	30	474	212	79	101	9	-	875
	8(f)	41	31	20	26	8	8	141
3 x 2	8(m)	43	23	8	1	2	17	94
	8	84	54	28	27	10	25	235
	8(f)	59	98	51	42	28	8	286
3 x 3	8(m)	99	52	4	9	5	30	199
	8	158	150	55	51	33	38	485
4 x 6	21(f)	7	32	95	53	86	51	324
5 x 6	21(m)	26	40	6	23	30	114	239
6 x 6	21	33	72	101	76	116	165	563
Totals	228	Females, 4610		Males 3128		F+M = 7738		Sex ratio = .596

(f) = females, (m) = males

Pooled data for single-pair matings F_1 from nature; females and males scored separately, and ordered according to the number of types occurring in each F_2 culture.

1 to **9**. This observation indicates that the types darker than **6** in the sample studied contained at least one dark e^d allele.

From the results in Table 1 we inferred that darkening modifiers are frequent in our sample, since 125 in a total of 228 crosses produced type **3** or darker. If we consider that each isofemale strain from Itapeva contributed approximately equal numbers of individuals to these crosses, this result indicates that the frequency of the darkening modifier m^d in this sample is about .50. These strains

TABLE 2

Extremes of pigmentation in homozygotes for the major genes e^l *and* e^d *due to selection of modifiers*

Generation	Homozygotes e^le^l selected toward the relatively darker (**6**) and lighter (**1**) types: Cross types	Offspring types **3**	**4**	**5**	**6**	Cross types	Offspring types **1**	**2**	**3**	*Homozygotes e^de^d selected toward darker type (**9**): Cross types	Offspring types **7**	**8**	**9**
1	♀ **5**	19	21	10	1	♀ **1**	10	22	4	♀ **9**	57	107	23
	♂ **2**	10	14	18	5	♂ **2**	31	12	..	♂ **8**	..	101	35
2	♀ **5**	..	..	51	26	♀ **1**	45	8	..	♀ **9**	..	118	139
	♂ **6**	..	..	21	43	♂ **1**	29	2	..	♂ **8**	..	32	136
3	♀ **6**	..	..	68	19	♀ **1**	47	9	..	♀ **9**	..	17	70
	♂ **6**	..	..	..	67	♂ **1**	43	..	..	♂ **9**	..	..	51
4	♀ **6**	..	..	76	29	♀ **1**	73	15	..	♀ **9**	..	28	79
	♂ **6**	..	..	..	93	♂ **1**	61	..	..	♂ **9**	..	..	118

* Selection favoring lighter types (**7**) was not successful; the cultures have been lost.
♀ = female types crossed and female offspring types observed.
♂ = male types crossed and male offspring observed.
After the 4th generation of selection the lines tended to stabilize around the proportions above, and the e^le^l lighter and darker lines were observed for about 20 generations.

afterwards were maintained by mass culture, and always showed phenotypes varying from **1** to **6**. Other strains exhibited variation between types **7** to **9**. In order to reduce this variability we conducted a simple selection experiment toward the extreme phenotypes appearing in these two sets of strains. This selection progressed rather quickly in lines #117 and #60, which proved to be homozygotes e^le^l, and stabilized around types **1** and **6**, respectively. On the other hand, the line #303, homozygous dark e^de^d, gave a fast response to selection toward type **9** maintaining, however, some variation. The selection of homozygotes e^de^d toward lighter forms (**7**) did not succeed. This failure was probably fortuitous due to the difficulty of raising this fly. New attempts in this direction are underway. As for lines #117, #60 and #303, we have observations during twenty generations showing that they maintained the relative uniformity attained after selection. The males are less variable than the females (see Table 2, Figures 2 and 3). These simple selection experiments suggest also that the color modifier genes are not numerous. It is not easy to assess the precise extent of the variability due to the environmental factors since we have incomplete knowledge of the genetic factors affecting pigment deposition in the tergites. The results described subsequently disclosed a pair of color modifying alleles; the darkening m^d and the lightening m^l modifiers.

The results of single-pair matings between the lightened light $e^le^l\ m^lm^l$ line #117 and the darkened light line #60, $e^le^l\ m^dm^d$, are pooled and presented in Table 3. The F_1 show in two-way crosses the types **1**,**2**, and **3** in both females and males. These three types are of course $e^le^l\ m^lm^d$. The lightening modifier acting upon the homozygous e^le^l is almost completely dominant. The type **3** is light

TABLE 3

Segregation of the lightening and darkening modifiers from single-pair crosses between selected lines, #117 ($e^le^lm^lm^l$) *and #60* ($e^le^lm^dm^d$).

Phenotypes and genotypes of the P_1	Generation and number of crosses		Color forms 1 Light†	2	3	4 Intermediate†	5	6	Totals	
♀ **5** and ♀ **6**	33	F_1 ♀	4	269	371	...	...	...	644	
$e^le^lm^dm^d$		♂	120	250	53	...	...	...	423	
×		♀ + ♂	124	519	424	...	...	...	1067	
♂ **1**										
$e^le^lm^lm^l$	33	F_2 ♀	120	271	205	61	90	54	801	$\chi^2 = .15$
			obs. = 596	exp. = (600.75)		obs. = 205	exp. = (200.25)			df = 1, P = .70
		♂	205	130	34	3	16	73	461	$\chi^2 = 6.25$
			obs. = 369	exp. = (345.75)		obs. = 92	exp. = (115.25)			P > .01
♀ **1** and ♀ **2**	16	F_1 ♀	18	215	132	...	...	...	365	
$e^le^lm^lm^l$		♂	158	96	7	...	...	...	261	
×		♀ + ♂	176	311	139	...	...	...	626	
♂ **6**										
$e^le^lm^dm^d$	39	F_2 ♀	148	213	238	34	87	97	817	$\chi^2 = 1.23$
			obs. = 599	exp. = (612.75)		obs. = 218	exp. = (204.25)			P > .20
		♂	206	100	12	6	12	90	426	$\chi^2 = .22$
			obs. = 318	exp. = (319.50)		obs. = 108	exp. = (106.50)			P > .50
		♀	obs. = 1195	exp. = (1213.50)		obs. = 423	exp. = (404.50)		1618	$\chi^2 = 1.13$
										P > .20
F_2 totals		♂	obs. = 687	exp. = (665.25)		obs. = 200	exp. = (221.75)		887	$\chi^2 = 2.84$
										P > .05
		♀ + ♂	obs. = 1182	exp. = (1878.75)		obs. = 623	exp. = (626.25)		2505	$\chi^2 = .022$
										P > .90*

† Category of color forms according to Da Cunha (1949)
m^l = lightening modifiers; m^d = darkening modifier.

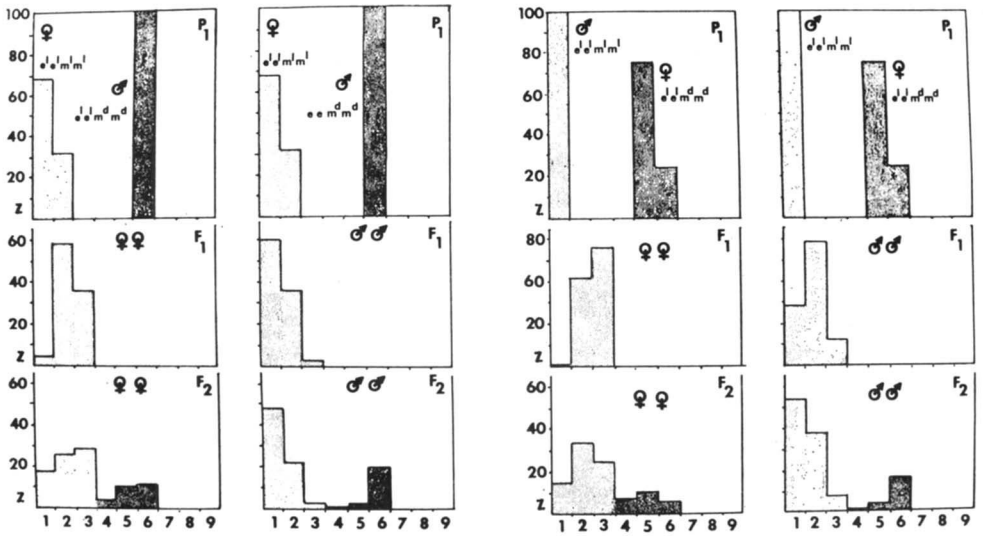

FIGURE 2.—Frequency distribution (in percent) of the phenotypic frequencies of homozygotes e^le^l with lightening modifiers (m^lm^l) or darkening modifiers (m^dm^d) and their offspring. The lightening modifiers are quasi-dominant over the darkening ones (see Table 3 also). (The numbers **1** to **9** correspond to those of Figure 1.)

(see Figure 1) according to the classification of DA CUNHA (1949). The F_2 shows besides the above phenotypes the ones classified as **4**, **5** and **6**. These three types can be considered as e^le^l m^dm^d. The type **4** which does not appear in the darkened line #60 may be explained by the effect of some minor lightening gene present in the line #117, besides the m^l gene. The agreement with the 3:1 ratio is good except for the distribution of the males of the first set of crosses. The totals for the males and for the females conform with this hypothesis (see Table 3). The overall total fits the hypothesis of a pair of modifying alleles with a 3:1 segregation pattern. Figure 2 presents graphically the phenotypic distributions of the parental lines compared to their offspring (F_1 and F_2) appearing in Table 3. These results clearly demonstrate a simple Mendelian basis for the color modifiers. We may call attention to the fact that the darkening modifiers contained in line #60 suffice to convert the light homozygotes e^le^l into intermediate phenotypes.

Crosses between e^le^l and e^de^d, homozygotes for the major genes, gave very diverse results depending on the modifiers present. If the darkening modifiers are prevalent, the F_1 exhibited color forms from **6** to **8** as shown in Table 4, which summarizes the crosses between line #60 (e^le^l m^dm^d) and line #303 (e^de^d m^dm^d). Consequently the heterozygote, e^le^d m^dm^d, for the major alleles shows about 87% of females and 88% of males among the dark phenotypes **7** and **8**. In the F_2, the type **4** that was not present in the parental line #60 now appears. This fact further supports our observations that line #303 bears one weak lightening modifier with low penetrance. In this segregation type **4** occurred in 1% of the females only (Table 4). From these crosses and others that will be described subsequently, it is noticeable that type **9** occurred only when the geno-

TABLE 4

Frequencies of color forms in the F_1 and F_2 from single-pair matings between $e^le^lm^dm^d$ *(line #60) and* $e^de^dm^dm^d$ *(line #303)*

Phenotypes and genotypes of the P_1	Generation and number of crosses		Color forms* 4	5	6	7	8	9	Totals
			Intermediate			Dark			
	23 F_1	♀	.....	.....	66	222	220	.....	508
					(12.99)	(43.70)	(43.31)		
		♂	.....	.....	36	95	164	.....	295
					(12.21)	(32.30)	(55.59)		
♀ **5** and ♀ **6**					102	317	384		803
$e^le^lm^dm^d$									
×									
♂ **8** and ♂ **9**	24 F_2	♀	5	38	88	172	164	30	497
$e^de^dm^dm^d$			(1.00)	(7.64)	(17.71)	(34.61)	(33.00)	(6.04)	
		♂	.....	31	35	102	92	63	323
			.....	(9.60)	(10.84)	(31.58)	(28.48)	(19.50)	
			5	69	123	274	256	93	820
	31 F_1	♀	.....	.....	64	263	212	.....	543
			.....	.....	(11.79)	(39.78)	(48.43)	.....	
		♂	.....	.....	66	147	139	.....	352
♀ **8** and ♀ **9**					(18.75)	(41.76)	(39.94)	.....	
$e^de^dm^dm^d$									
×			.....	.....	130	410	351	.....	895
♂ **5** and ♂ **6**									
$e^le^lm^dm^d$	1 F_2	♀	9	49	125	319	244	62	808
			(1.11)	(6.07)	(15.47)	(39.48)	(30.20)	(7.67)	
		♂	.....	15	86	120	164	108	493
			.....	(3.04)	(17.44)	(24.34)	(33.27)	(21.91)	
			9	64	211	439	408	170	1301

* Category of color form according to DA CUNHA (1949)

In parentheses are the frequencies in percent for each class of phenotype.

type $e^d e^d$ $m^d m^d$ was expected. Several individual crosses produced type **9** only in some generations, type **8** recurring, however, especially among the females in other generations. This may indicate that environmental variations are partially responsible for this variation. Nevertheless, some genetic variability is most certainly present since different strains present diverse degrees of variation.

As can be seen in Table 4 the F_1 distribution of phenotypes overlaps both parental lines. Assuming that the frequencies of heterozygotes for the major genes in the F_2 can be deduced from their relative frequencies in classes **6**, **7** and **8**, we found that the ratios differ greatly from the expected 1:2:1 with an excess of "heterozygotes". This surplus of heterozygotes is more likely due to the masking effect of the darkening modifiers and not to their superior fitness.

The crosses of females $e^l e^l$ $m^l m^l$ (line #117) with males $e^d e^d$ $m^d m^d$ (line #303) produced an F_1 ranging from types **1** to **6** as can be seen in Table 5. The extreme light types **1** to **3** were not expected. Some of the single-pair crosses pooled in Table 5 did not produce these types. We believe that line #303 bears a weak lightening modifier which in conjunction with m^l reinforces its suppressing effect. This hypothesis will be tested in further work. Recalling that the known modifiers m^l and m^d segregated in a ratio 3:1 (Table 3), and that the major alleles e^l and e^d should give a 1:2:1 ratio, it clearly impossible to establish the limits of a 6:3:3:2:1:1 ratio from data presented in Table 5, because of the phenotypic overlap. The segregation of the major genes is masked by the effects of the modifiers. Reducing the nine classes to three—light, intermediate and dark—the corresponding frequencies do not agree with the 1:2:1 ratio, due to an excess of light phenotypes. This certainly results from quasidominance of the lightening modifiers (see Table 3 also).

Evidence favoring the view that line #303 bears a weak lightening modifier, that may also be present in the lightened light line # 117, was mentioned already. Further support for the view that dark lines with types **8** and **9** only may conceal this weak lightening modifier is presented in the pooled data of Table 6. The crosses **1** × **8** and **1** × **9** should give intermediate types only; however, in 70% of the cases the unexpected light types **1**, **2** and **3** appeared together with the intermediate and frequently with the dark types also. We never observed the exclusive occurrence of light types in these crosses and thus the possibility that the dominant light allele described by HEED and BLAKE (1963) was present is excluded. The results presented in Table 6 are properly accounted for by the presence of a weak modifier in both lines (#117 and #303). The crosses between **8** × **9** and **9** × **9** differ significantly in their F_1 (Table 6), which validates the distinction between these classes and points to the possibility that type **8** harbors the weak lightening modifier more frequently than type **9** individuals.

Figure 3 shows the known genotypes of homozygotes and F_1 heterozygotes obtained in this work. The relative frequency of each phenotype is shown for each genotype. Only the very dark type **9** appears to correspond unequivocally to one genotype, $e^d e^d$ $m^d m^d$, both in males and in females. It is clear from Tables 4, 5 and 6 and from the results of our crosses that the type **9** appears only when the above mentioned genotype can be predicted.

TABLE 5

Frequencies of color forms in the F_1 and F_2 from single-pair matings between $e^le^l\ m^lm^l$ *(line #117) and* $e^de^d\ m^dm^d$ *(line #303)*

Phenotypes and genotypes of the P_1	Generation and number of crosses	Color forms: Light* 1	2	3	Intermediate* 4	5	6	Dark* 7	8	9	Totals
♀ **1** and ♀ **2**	71 F_1 ♀	19	182	326	615	52	24	..	..	..	1218
$e^le^l\ m^lm^l$		(1.56)	(14.94)	(26.77)	(50.49)	(4.27)	(1.97)				
×											
♂ **9**	♂	59	69	345	311	142	43	..	..	..	969
$e^de^d\ m^dm^d$		(6.09)	(7.13)	(35.60)	(32.09)	(14.65)	(4.44)				
		78	251	671	926	194	67				2187
	45 F_2 ♀	64	148	44	237	75	13	31	79	14	705
		(9.08)	(20.99)	(6.24)	(33.62)	(110.64)	(1.84)	(4.40)	(11.21)	(1.98)	
	♂	96	33	62	97	150	37	6	11	101	593
		(16.19)	(5.56)	(10.46)	(16.36)	(25.29)	(6.24)	(1.01)	(1.86)	(17.03)	
		160	181	106	334	225	50	37	90	11	1298

* Category of color form according to DA CUNHA (1949)
In parentheses are the frequencies, in percent for each class of phenotype.

TABLE 6

Distribution of phenotypes of the offspring of single-pair crossings between types

Cross	Number of crosses	Phenotypes of the offspring 1	2	3	4	5	6	Totals ♀ ♂
1 × 8	2	6	7	13	15	..	..	19/21
	5	13	32	47	115	26	..	135/97
	3	14	21	55	64	10	6	105/65
	Total	33	60	115	193	36	6	260/183
	Expected	14.8	54.5	135.1	187.3	37.6	13.9	443
1 × 9	4	21	29	114	122	26	..	162/150
	8	..	94	225	294	42	..	364/291
	3	..	15	38	73	33	44	112/91
	Total	21	138	377	489	101	44	638/532
	Expected	39.2	143.6	357.0	494.7	99.4	36.3	1170
		$\chi^2 = 30.2$		df = 4		P < .001		

Cross	Number of crosses	Phenotypes of the offspring 7	8	9	Totals ♀ ♂
8 × 9	2	2	106	16	53/71
	4	8	99	25	85/48
	7	..	210	64	157/117
	17	..	436	216	364/288
	Total	10	851	321	(658/524)
	Expected	5.3	649.6	527.1	1182
9 × 9	2	2	89	15	58/48
	16	..	358	291	300/249
	13	..	177	392	325/244
	6	..	.	178	96/82
	Total	2	624	876	879/623
	Expected	6.7	825.4	669.9	1502
		$\chi^2 = 263.0$	df = 1	P < .001	

DISCUSSION

Our results clearly show that color modifiers are present in the southernmost natural populations of *Drosophila polymorpha* which confirms the suspicion of DA CUNHA (1949). HEED and BLAKE (1963) observed that some crosses "produced intermediates" that are "variable depending upon the origin of the e^d allele." These variations suggest the presence of modifiers at different frequencies in Villavicencio, Pirassununga (S. Paulo) and Montes Claros (Minas Gerais). The importance of these modifiers in determining color patterns is shown in Figures 1, 2 and 3 and in the F_1 and F_2 distributions presented in our tables. Consequently it will be necessary to prepare special analyzer strains to determine the gene frequencies in natural populations, since the genotypes cannot be deduced from simple inspection of the phenotypes.

The genetic basis of apparently very complex quantitative variation can be relatively simple as has been demonstrated by THODAY (1961) and SPICKETT and THODAY (1966) for genes controlling the number of bristles in *D. melano-*

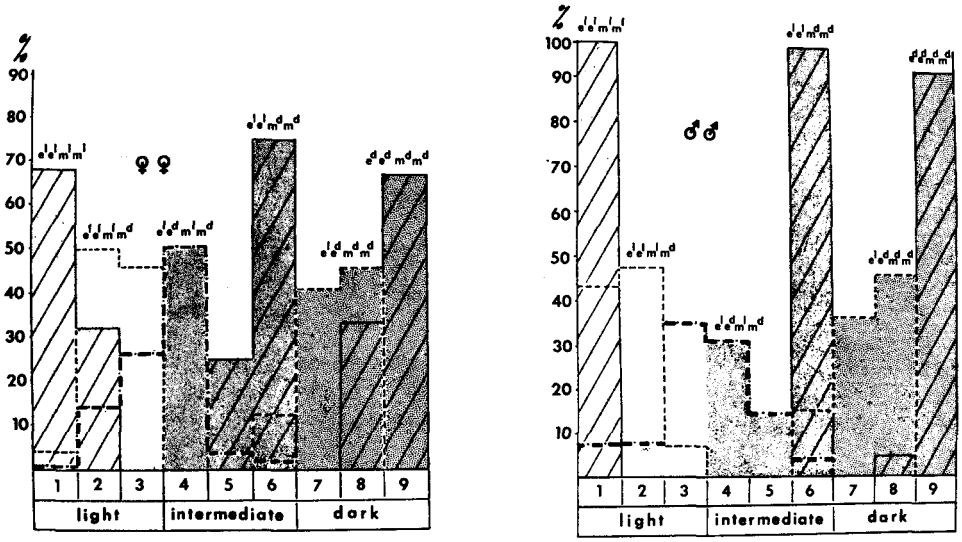

FIGURE 3.—Frequency distribution (in percent) of the pigmentation phenotypes of *Drosophila polymorpha* with known genotypes. Females and males were scored separately. The shades correspond to the three phenotypic classes (light, intermediate and dark), and the numbers **1** to **9** to the types shown in Figure 1.

gaster. Marker genes are not as yet available for a similar analysis of *D. polymorpha* color genes; however, our data indicate that most of the genetic variance in color patterns is due to a few loci.

The full understanding of the genetic basis of Drosophila tergite pigmentation depends on the elucidation of developmental and biochemical processes. We may consider very briefly the evidences on these grounds and their bearing on our problem. The origin of the imaginal hypoderm of the thorax and the abdomen differ greatly, and this may be the basis of why many Drosophila species have quite different color in the thorax and the abdomen, and also the reason why some polymorphisms are restricted to one of these areas. *D. lebanonensis* shows thorax polymorphism and *D. polymorpha* and many others show abdomen polymorphism. The thorax imaginal hypoderm, which secretes the cuticle, is a product of proliferation and fusion of the peripheral primordia cells. The abdomen hypoderm originates from one dorsal and one ventral pair of cells that appears in each segment a few hours after pupation in *D. melanogaster* (ROBERTSON 1936). These cells divide and actively displace the larval hypoderm, except in the last segment that is formed by the genital disc.

Cells from the lateral larval spiracles contribute to this process. The complete fusion of these growing sites occurs at the 34 hr pupal stage. The completely formed hypoderm starts secreting the imaginal cuticle at about the 50 hr pupal stage. Coincidentally the sensitive period for the induction of color phenocopies by temperature shock is, according to MITCHELL (1967), between 67 to 68 hr of pupation. This occurs when there is an increase in the phenol oxidase production

(GEIGER and MITCHELL 1966). As MITCHELL (1967) pointed out, it is important to remember that the phenol oxidases "are involved more vitally in epicuticle formation, epinephrine formation and perhaps resilin formation than in production of melanin. The lack of pigmentation (in insects) would probably never be due to the total absence of this complex enzyme as this would most certainly be a lethal condition since the fly has no substitute for the hardening of the cuticle." Recent findings of MITCHELL and WEBER (1965) and MITCHELL (1966) indicate that at least five protein components participate in the dopa oxidase conversions, and that although the A tyrosinase component is always present in all stages from late larvae to adult flies, melanization occurs in an orderly fashion that starts with thoracic bristles and follows to the abdominal bristles and tergite plates (*D. melanogaster*). Studying intersex mosaicism in *D. melanogaster* (*2X 3A*) tergite color patterns, STERN (1966) demonstrated that there is no change in the chromosome numbers but that the maleness or femaleness of pigmentation is "caused by processes similar to developmental differentiation in which the genetic content of different tissues is considered to be alike, although the differentiation activity of various genes may have been different." Apparently the antagonistic factors present can be favored alternatively at the so-called "turning points" of the developmental process. The *D. polymorpha* heterozygotes $e^l e^d\ m^l m^d$ show rather frequently asymmetrical color patterns already observed by DA CUNHA (1946, 1949) in the F_1 obtained by crossing the dark and light true breeding strains.

Another phenomenon observed by DA CUNHA (1949) and confirmed by us is that the phenotypic variability greatly increases among the hybrids in comparison with that of their parents. This fact is apparent in our results as can be seen particularly in Table 5 and Figure 3.

Environmental effects may play a greater role in increasing phenotypic variability of heterozygotes than of the homozygotes. We observed that heterozygosis for modifier genes increases the phenotypic variability and apparently its dependence on environmental conditions. This effect *per se* might well be of importance for mimetic adaptation. This dependence on environment could account partially for the contradictory results that have been reported by STALKER (1953) and HEED (1962) on the polymorphism of *D. acutilabella* as well as for the failure of STURTEVANT (1921) to determine the genetic basis for the inheritance of color pattern in *D. cardini*. His conclusion, that the conditions under which the larvae develop determine the phenotype of the adults, may have a similar basis.

DA CUNHA (1955) found two geographic races of *D. neocardini* that are uniform in their ranges but differ from each other; *D. n. mourensis* having tergites with very narrow dark bands and *D.n. itambacuriensis* exhibiting broader dark bands that reach the anterior margins of the 3rd, 4th and 5th tergites. Their hybrids display an array of F_1 and F_2 forms classified in five types by the author. These facts were interpreted by DA CUNHA as indicating that each race is homozygous for the major gene or genes determining their color patterns but each race bears genes that act as color modifiers in the interracial hybrids only. We would like to call attention to the similarity of these results with those reported in our Table 5 in which two relatively uniform lines (#117 and #303),

one light the other dark, produced an F_1 with types **6** and **9** in the F_2. Each line was homozygous for the two known loci, however the heterozygotes displayed a quite surprising variability.

The natural populations of this species in northern South America are monomorphic in several collecting places studied by HEED and BLAKE (1963) and by HEED (1963). These authors found that these populations contain another allele, dominant light e^L, that decreases in frequency southward. To explain the excess of the homozygotes observed by DA CUNHA (1949) in the southern populations, HEED (1963) made the assumption that this allele might be present at low frequencies in these populations. This interesting hypothesis cannot be excluded by the fact that we have not detected this allele in our sample. As HEED suggested, this allele follows a geographic trend decreasing southward and our sample is the southernmost so far studied. All those problems have to wait for a genetic analysis of the natural populations with appropriate marker strains.

On the other hand we can only speculate that if the heterozygotes $e^l e^d$ have some superior physiological characteristic in relation to homozygotes, the relatively high frequency of the extreme phenotypes observed in nature could be produced by disruptive selection acting upon the modifiers and providing a high frequency of $e^d e^l m^l m^l$ and $e^d e^l m^d m^d$, phenotypically light and dark, respectively. Consequently, both the physiological advantages of heterozygosis for the major genes and the fitness value of the extreme phenotypes are obtained. The extreme phenotypes can be fitted in two opposing ecological niches or favored alternatively in time by cyclic selection (THODAY 1959).

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SUMMARY

The southernmost natural populations of *D. polymorpha* from Brazil proved to carry a locus with at least two alleles that greatly alter the phenotypes determined by the e^l and e^d alleles responsible for pigment deposition in the abdominal tergites. The lightening modifier (m^l) is quasi dominant over the darkening modifier (m^d) at least on a homozygous background, $e^l e^l$, and shows variable degrees of epistasis over the major genes in heterozygotes, $e^l e^d$. The darkening modifier in homozygous condition transforms the $e^l e^l$ genotype (light) into intermediate types, and most heterozygotes $e^l e^d$ into dark types. There are indications that a weaker pair of modifiers is present in this material and is capable of interacting with $e^l e^d$ $m^l m^d$ and $e^d e^d$ $m^d m^d$ to produce lighter than expected phenotypes. Therefore, the exact frequencies of the major alleles e^l and e^d cannot be deduced from phenotypic frequencies determined by direct inspection of the individuals. —Some aspects of the development of pigmentation are discussed to call attention to this side of the problem.—Despite the uncertainty of the data so far obtained on gene and genotype frequencies in the southern natural populations of *D.*

polymorpha, a working hypothesis is advanced to explain the excess of the presumed "homozygotes," and the apparent balanced nature of the color polymorphism exhibited by this species. Assuming that the heterozygotes $e^l e^d$ are physiologically most fit, a disruptive selection can favor the modifiers to produce the extreme phenotypes with the constitutions $e^l e^d\ m^l m^l$ and $e^l e^d\ m^d m^d$ sharing the double advantage of being heterozygotes for the major genes and phenotypically extreme. This suggestion and the one advanced by HEED (1963) are not mutually exclusive and will be tested in further work.

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