GENETICAL CONTROL OF PENETRANCE AND EVOLUTION OF DOMINANCE IN *DROSOPHILA*

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1. INTRODUCTION

It is well established that the degree of dominance a character maintains over its allelomorph often depends on the genotype as a whole (for review see Dobzhansky, 1951, p. 104; and Goldschmidt, 1938, p. 107) and there is considerable evidence that genes modifying dominance exist in a variety of organisms, *e.g.* cotton, mice, *Drosophila*, Lepidoptera and poultry, etc. Furthermore, the experiments by Ford (1940) on the dominance of yellow ground colour in the Currant Moth *Abraxas grossulariata*, and by Fisher and Holt (1944) on the *Sd* (short-tail) gene in mice, demonstrate how rapidly dominance relationships can be reversed under artificial selection.

Evidence of an evolutionary nature on dominance modification has been obtained for several species by crossing individuals from a population showing dominance for a certain character with individuals from an unrelated population. If a breakdown in dominance occurs through outcrossing it is attributed to the introduction of an alien background. It is supposed that the highly selected background necessary for dominance to be maintained increased the effect of the major gene so that the same phenotype is produced by either one or two doses of the gene. Removal or disruption of this background by outcrossing destroys the dominance relationships which have evolved.

The breakdown of dominance by outcrossing has been demonstrated in cotton (Harland and Attek, 1941), in the Lesser Yellow-underwing Moth *Triphaena comes* (Ford, 1964, p. 125), in the genus *Papilio* (Clarke and Sheppard, 1960, 1963) and in *Biston betularia* by Kettlewell (Ford, 1964, p. 263). With this background it is not surprising that the theory of dominance modification proposed by Fisher (1930) has gained wide acceptance.

So far studies of evolutionary significance, on the modification of dominance, have been confined to organisms ill-suited for genetical analysis. For this reason Ford (1964, p. 10), has lamented the fact that *Drosophila melanogaster* has not been used in the study of this problem. The purpose of this paper is to describe experiments analysing the genetical control of penetrance and dominance of a character causing abnormal eye development in *D. melanogaster*. The relationship between dosage of the major gene *w⁺* and the modifiers of its dominance and penetrance is discussed. It is intended to demonstrate that genetic systems indicating the evolution of dominance modification have arisen in this species.

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2. MATERIALS AND METHODS

The following chromosome 2 markers were used in the analysis:

41.0	48.5	48.7	55.0	57.5	104.5
Jammed (<i>j</i>)	black (<i>b</i>)	jaunty (<i>j</i>)	witty (<i>wi</i>)	cinnebar (<i>cn</i>)	brown (<i>bw</i>)

Linkage tests show that *wi* is located on chromosome 2 but its precise positioning is made uncertain by incomplete penetrance and partial dominance. Flies homozygous for black (*b*), jaunty (*j*) and witty (*wi*) were crossed to a wild-type strain and the F_1 was backcrossed to *b j wi*. Table 1 shows the phenotypic frequencies in the backcross progenies of reciprocal matings. The presence of "recombinant" classes in the progeny of the cross involving F_1 males indicates incomplete penetrance of the homozygous

TABLE 1

Linkage data for the Witty, Jaunty and Black Loci

	<i>b⁺ j⁺ wi</i>	<i>b⁺ j⁺ wi⁺</i>	<i>bjwi</i>	<i>bjwi⁺</i>
$F_1 \times bjwi^*$	157	2439	1618	581
<i>bjwi</i> $\times F_1$	15	809	404	309

* Unless otherwise stated, the first mentioned in a cross is the female parent.

form as well as occasional expression of the heterozygote. Clearly *wi* is closely linked to *j*. It is not possible to estimate the linkage distance accurately from these results but one can establish 8 map units as an upper limit with 6 units being the most probable value. A similar cross using Jammed (*j*) indicated that *wi* was to the right of *j*. Further evidence will be presented below which is consistent with this map position for the witty locus.

The culture conditions for the experiments outlined in this paper were described earlier (Whitten, 1966).

Witty was first observed "fixed" in a laboratory stock, LL, which was homozygous for jaunty. The prior history of this stock is not known. Under normal laboratory conditions the penetrance of witty in LL is almost zero (see table 2). The other witty lines SL, SH and HH used in this study were derived from LL in a manner to be described under Results.

In the witty lines four phenotypic classes may be observed, (+ +), (L +), (+ R) and (LR) where (+ +) denotes flies wild-type for both eyes, (L +) denotes flies normal for right eye only, etc. That every fly in the LL line is homozygous for *wi* is evidenced by the fact that penetrance increases with temperature. At 25° C. most LL flies are wild-type while at 31° C. very few flies have normal eyes. If it were possible to grow LL flies at a higher temperature, indications are that all flies would be witty. Since it can be shown that all *wi*/+ flies are wild-type at 30° C. it follows that LL flies must be *wi/wi*. Furthermore, at any set temperature the four phenotypic classes (+ +), (L +), (+ R) and (LR) tend towards binomial expectations (see table 2 and Whitten, 1967) which indicates incomplete penetrance rather than segregation of the witty gene is involved.

Penetrance of the witty character is variable being determined by background genotype and environmental conditions. The method used in this paper for measuring penetrance was described by Whitten (1966). In

TABLE 2

Effect of selection for increased and reduced penetrance in the wi/wi line LL

	Strain	Sex	(+ +)	(L+)	(+R)	(LR)	$\bar{x}^{**}$	y
Without selection	LL ₁ *	M	156	0	2	0	-3.2	0.7
		F	139	3	5	1	-2.3	0.7
	LL ₂	M	476	4	2	0	-3.2	0.7
		F	476	21	18	4	-2.1	0.7
	LL ₆	M	276	0	0	0	—	—
		F	172	5	6	0	-2.0	0.4
	LL ₁₀	M	528	14	5	0	-2.3	0.4
		F	649	32	22	7	-2.2	0.8
	LL ₁₂	M	120	2	1	0	-3.0	1.0
		F	136	12	8	2	-1.8	0.8
After selection	SH ₆ *	M	416	125	106	64	-0.72	0.4
		F	103	163	124	328	0.51	0.43
	SL ₀ *	M	434	49	31	9	-1.53	0.61
		F	216	115	117	98	-0.30	0.46
	SH ₁	M	102	38	35	35	-0.51	0.75
		F	19	37	29	102	0.66	0.52
	SL ₁	M	250	22	21	2	-1.41	0.1
		F	100	61	51	45	-0.30	0.37
	SH ₂	M	70	44	39	46	-0.18	0.55
		F	18	40	33	114	0.69	0.40
	SL ₂	M	215	33	21	13	-1.38	0.76
		F	68	62	46	88	-0.12	0.61
	SH ₅	M	132	81	72	72	-0.24	0.46
		F	26	53	60	206	0.78	0.34
	SL ₅	M	106	25	37	12	-0.75	0.31
		F	20	27	35	87	0.57	0.52
	SH ₉	M	364	190	181	160	-0.75	0.55
		F	59	118	115	470	0.45	0.46
	SH ₁₂	M	71	52	60	79	-0.03	0.55
		F	11	46	37	253	1.11	0.40
	SL ₁₂	M	182	32	25	6	-1.11	0.22
		F	58	52	53	108	0.27	0.67

* The history of the lines LL, SH and SL is described in the text. $\bar{x}^{**}$ is the mean and y is the standard deviation of the penetrance parameter, ξ . When x is very high or low (e.g. in LL) the estimate of x and y is approximate. Cultures were maintained in the temperature range 24-26° C. SH₁₂ indicates the 12th generation after selection ceased.

addition, the frequencies of the four phenotypic classes or, alternatively, the value of P , the proportion of abnormal eyes, is given. While these latter measurements of penetrance are generally sufficient, it is believed that $\bar{x}$ and s , the mean and standard deviation as determined on an underlying scale give a simpler picture of the action of the modifiers of the witty locus.

3. RESULTS

(a) *Response to selection for increased penetrance*

A selection programme aimed at increasing the penetrance of witty in the homozygous witty line LL was carried out for 26 generations by selecting 2-10 non-virgin females from the previous generation. Penetrance of the gene increased rapidly but never reached 100 per cent. At the 26th generation a sub-line with selection for reduced penetrance was established while in the main line selection for increased penetrance was continued. The reversed selection was effective, at least in the early stages. After 9 generations artificial selection was stopped and the two lines were maintained as the "inbred high" line, SH, and the "inbred low" line, SL (see table 2). A heritability experiment showed that no additive genetic variability remained in the SH and SL lines (Whitten, 1966).

The change in penetrance, whether measured on the underlying scale, or simply as the proportion of individuals with one or both eyes affected, permits one to conclude that the penetrance of *wi* in the homozygous state is dependent on background genotype. Prior to selection the LL strain contained genetic variability which was utilised during the course of selection.

(b) *Generation of the dominant form of witty (Wi)*

F_1 progeny from crosses between LL (the original *wi/wi* population) and wild-type laboratory strains are all wild-type (see table 3). Even after selection has increased the penetrance of *wi/wi* flies to a high level (*i.e.* in the SH line) we find that the F_1 progeny from SH $\times$ wild strains are still wild-type (see tables 4 and 5). Occasionally an F_1 fly exhibits the witty phenotype (see tables 4 and 5). Backcrossing these F_1 's to wild strains produces as few witty as are obtained from backcrosses involving F_1 flies that have a normal phenotype. The presence of the infrequent witty flies in the F_1 will be explained below.

When the F_1 *wi/+* flies with wild-type phenotype, whether used as male or female parents, are outcrossed, the proportion of witty flies amongst their *wi/+* progeny increases. However, the reciprocal crosses differ in three important characteristics. When the female parent is *wi/+*:

- (i) the progeny contains a higher proportion of witty progeny than occurs in the reciprocal cross (see tables 3 and 4);
- (ii) the majority of witty progeny behave as true dominants whereas no witty progeny from the reciprocal cross behave as dominants (see tables 6 and 7); and
- (iii) the four phenotypic classes ($++$), ($L+$), ($+R$) and (LR) do not conform to binomial expectations, the (LR) class being much more frequent than expected (see tables 3 and 5). In the reciprocal cross, the four phenotypic classes conform to binomial expectations (see table 3 for sample results).

TABLE 3
Incidence of witty phenotype amongst F_1 and back-cross using LL and bwst

Parental cross	F ₁ generation			Back-cross to <i>bwst</i>								
	(++)	(L+)		(LR)	using F ₁ (<i>wi</i> /+) females			using F ₁ (<i>wi</i> /+) males				
		(+R)	(+R)		(LR)	(++)**	(L+)	(+R)	(LR)			
LL × <i>bwst</i>	292	0	0	0	660	12 (6)*	16 (5)	7 (5)	631	1 (0)	4 (3)	1 (1)
<i>bwst</i> × LL	383	0	0	0	463	10 (8)	11 (7)	6 (2)	530	1 (1)	4 (4)	0

* Figures in brackets indicate number of females.

** The actual number of (++) flies is a little more than double this figure but half have been discarded as being +/+ and not wi/+.

As regards the generation of the dominant form it is immaterial whether the *wi/wi* parent is male or female in obtaining the F_1 generation but the sex of the F_1 parent used in the backcross is decisive. This indicates that the difference between reciprocal crosses is not due to sex linked modifiers or maternal effects but may be associated with the absence of crossing over in males. It suggests that *wi* is linked to suppressing modifiers which can only be removed by outcrossing *wi/+* females. Once the modifiers are removed the witty gene becomes sufficiently active in single dose to affect eye development.

TABLE 4

Incidence of Witty* phenotype amongst wi/+ flies in the F_1 and back-cross between SH and a number of unrelated laboratory stocks

Parental cross	F_1 Generation		Back-cross to <i>bust</i>			
			Using F_1 females		Using F_1 males	
	witty	wildtype	witty	wildtype**	witty	wildtype
SH $\times$ <i>bust</i>	4 (4)	645	16 (12)	391	3 (2)	425
<i>bust</i> $\times$ SH	1 (1)	743	17 (13)	526	2 (1)	376
SH $\times$ <i>bw</i>	0	147	8 (6)	327	1 (1)	481
<i>bw</i> $\times$ SH	0	822	9 (8)	449	4 (1)	566
SH $\times$ Canton-S	2 (1)	884	21 (14)	571	3 (3)	401
Canton-S $\times$ SH	1 (1)	1376	17 (10)	493	0	357
SH $\times$ <i>cnbw</i>	0	595	2 (0)	492	0	146
<i>cnbw</i> $\times$ SH	0	942	6 (5)	577	0	389
SH $\times$ <i>bust</i> ***	23 (18)	1109	35 (24)	882	5 (5)	1001
<i>bust</i> $\times$ SH***	25 (21)	1466	37 (24)	1224	3 (3)	1364

* Flies with one or two abnormal eyes are regarded as having witty phenotype. Figures in brackets indicate females.

** The number of wildtype flies scored in the back-crosses was twice the number recorded but it was assumed that half these would be $+/+$ and could therefore be discarded.

*** These crosses were performed several months after the others. Control of environmental variables, especially temperature was not strict and probably accounts for the higher frequency of pseudo-dominants in the F_1 generation in these two cases.

The relatively high proportion of (LR) phenotypes amongst the witty progeny from crosses using *wi/+* mothers—and not fathers—supports this conclusion. Some consideration of this fact, in light of the theory developed earlier (Whitten, 1966) leads to the conclusion that the *wi/+* progeny from the backcross using *wi/+* mothers fall into two classes as regards the probability of their developing an abnormal eye; they either have a high probability or a probability close to zero. It is suggested that those individuals with a high probability have arisen from gametes in which the suppressing modifiers have been removed by a crossover event while the class of individuals with near zero probability still carries the suppressing modifiers.

The (LR) phenotype is very infrequent amongst witty flies in the F_1 generation and in backcrosses involving *wi/+* fathers. This suggests that all *wi/+* flies in these progenies have a very low probability of producing an abnormal eye. Occasionally developmental-noise, probably aided by the general environment, must cause the level of some morphogenetic substance (m.s.) to surpass the threshold in one side of a fly causing

TABLE 5
Incidence of witty phenotype amongst *wi*/+ flies in the *F*₁ and back-cross using *SH*, *bwst* and *cnbw*

Parental cross	<i>F</i> ₁ generation			Back-cross* to <i>bwst</i> using <i>F</i> ₁ females			Back-cross* to <i>cnbw</i> using <i>F</i> ₁ females		
	(+ +)	(L +)	(+ R)	(LR)	(+ +)	(L +)	(+ R)	(LR)	(LR)
<i>bwst</i> × <i>SH</i>	1459	12 (11)	15 (12)	1 (1)	1192**	72 (36)	56 (32)	37 (24)	—
<i>cnbw</i> × <i>SH</i>	1530	1 (1)	1 (1)	0	—	—	—	—	11 (9)

* The witty male progeny from these backcrosses were used to study distribution of crossing-over during the generation of dominant forms. Figures in brackets indicate number of females.

** The actual number of (+ +) flies is a little more than double this figure but half are discarded as being +/+ and not *wi*/+.

asymmetrical development. Such an argument would explain why these witty individuals behave no differently to wild-type $wi/+$ flies on further outcrossing (see table 6).

TABLE 6

Incidence of witty phenotype amongst $wi/+$ offspring from cross between $bwst$ and pseudo-dominants*

Cross	(+ +)	(L +)	(+ R)	(LR)
$bwst \times "wi"$	385	2 (1)	4 (1)	0

* The pseudo-dominants are $wi/+$ flies with witty phenotype arising in the F_1 generation or in the back-cross which involved F_1 males (see tables 4 and 5).

Once the dominant form (Wi) has been generated it can be outcrossed either as the male or female parent with comparable results (see table 7). Penetrance is consistently high but as will be shown it is affected in much the same way as the recessive wi by background genotype and the environment. Dominance of the witty character is retained despite repeated backcrosses between $Wi/+$ males and $+/+$ females.

TABLE 7

Incidence of witty phenotype amongst $Wi/+$ offspring using the "true" dominant form*

Cross	(+ +)	(L +)	(+ R)	(LR)
$bwst \times Wi/+$	787	201 (98)	184 (89)	181 (110)
$Wi/+ \times bwst$	694	144 (82)	127 (70)	190 (144)

* Figures in brackets indicate number of females.

It should be pointed out that Wi is not dominant in sensu strictu because, as is shown in (e), Wi/Wi and $Wi/+$ are easily distinguishable. Wi is simply not recessive but since this terminology leads to confusion, I am referring to Wi as the dominant form. The relationship between Wi and wi is considered more fully in the discussion.

(c) Location of dominance modifiers

If true dominants can only be generated from the recessive wi by the removal of modifiers on chromosome 2, then the proportion of Wi flies produced by the backcross $wi/+ \times +/+$ must depend on the number and position of the dominance modifiers. The frequency with which dominants are generated is inversely related to the degree of linkage between the dominance modifiers and the witty locus. Unfortunately, the variable penetrance of the dominant form and the occurrence of pseudo-dominants prevent the direct use of the phenotypic frequencies of witty as a measure of the linkage between wi and its modifiers. However, an approximate idea of the distribution of crossing-over in chromosome 2 during the generation of the dominant form can be determined from the markers present in these stocks. Witty male progeny from the crosses:

$$(1) \frac{j \ wi \ +}{+ \ + \ bw} \times \frac{bw \ st}{bw \ st}$$

$$(2) \frac{j \ wi \ + \ +}{+ \ + \ cn \ bw} \times \frac{bw \ st}{bw \ st}$$

were crossed to *j* and *cn bw* females respectively. If the modifiers are located between *j* and *wi*, we would expect the witty progeny from (1) to be $+/+$ at the jaunty locus. A similar argument holds for the witty progeny from (2) if the modifiers are located between *wi* and *cn*. In this case we would expect the *wi*-bearing chromosomes in the witty progeny to carry *cn* since initially *cn* and *wi* were in repulsion. Of the 14 witty males from (2) that were examined, none carried *cn* and of 60 witty males from (1) 50 were still carrying *j*. A further 222 witty males from (1) were examined and, of these, 69 (*i.e.* 31 per cent.) showed recombination between *wi* and *bw*. The map distance between *wi* and *bw* is 50 units and, according to Dr P. A. Parsons (personal communication), recombination between these two loci should be about 35 per cent. which is in fairly good agreement with the observed value (31 per cent.). Since dominant males may be formed without the loss of *j* or the acquisition of *cn* in these two crosses, we can conclude that no dominance modifiers of major significance occur between *j* and *cn*. The frequency of recombinant types between *wi* and *bw* appeared normal but the frequency of recombinant types between *wi* and *j* (10 out of 60) was significantly higher than normal (16.7 per cent. instead of 6 per cent.). An increased recombination between *wi* and *j* of the magnitude observed would occur if a modifying region is situated some 30 units to the left of the witty locus.

If the modifying region is located 30 map units from the major gene, one might expect the dominant form to arise more frequently than it appears to. However, incomplete penetrance would prevent detection of a proportion of the dominants that are produced. Allowing for incomplete penetrance it can be shown that 20-30 per cent. of the offspring from crosses of the type $\frac{wi}{+} \times \frac{+}{+}$ are in fact *Wi/+* which agrees with the estimated map position of the modifiers. Incomplete penetrance and its dependence on other factors presumably explains the variable frequency of dominants in the progenies of the different backcrosses (see tables 3, 4 and 5).

(d) *Modifiers controlling the penetrance of the dominant form, Wi*

Two stocks were constructed in both of which all the males carried one identical *Wi*-bearing chromosome. In both stocks the homologous chromosome was derived randomly from the *bw st* line. In one of the stocks, D36, all the males carried one identical chromosome 3 (labelled 3-*cn bw*) which was taken from the *cn bw* line. This line possesses a background producing low penetrance of the witty character (tables 11 and 12). In the other stock, D38, all the males carried one identical chromosome 3 (labelled 3-*bw*) derived from the *bw* line known to have a background enhancing penetrance (see tables 11 and 12). In both D36 and D38 the homologous chromosome 3 was derived randomly from *bw st*. Both lines were maintained by backcrossing females from the *bw st* line to males with genotypes as indicated below:

$$\text{for D36} \quad \frac{bw \ st}{bw \ st} \times \frac{Wi \ +}{+ \ bw} \frac{st}{+ \ (3-cn \ bw)}$$

$$\text{and for D38} \quad \frac{bw \ st}{bw \ st} \times \frac{Wi \ +}{+ \ bw} \frac{st}{+ \ (3-bw)}.$$

All $Wi/+$ progeny in D36 and D38 have either scarlet or wild-type eye colour. Those individuals with wild-type eye colour must also be carrying intact, $3-cn\ bw$ in D36 and $3-bw$ in D38. Thus both lines can be maintained indefinitely using $Wi/+$ males with wild-type eye colour in the backcross.

Penetrance was measured in $Wi/+$ individuals from D36 and D38 over a number of generations and was found to be the same for scarlet flies in both lines. However, in flies with wild-type eye colour, the penetrance was higher in D38 than in D36 (see table 8 for the results from a typical generation). All the comparisons have been made using the penetrance parameter rather than the actual proportion of abnormal flies, although an analysis based on percentage abnormality leads to the same general conclusions.

TABLE 8
Effect of chromosome 3 on penetrance of Wi

Strain	Sex	Scarlet eye colour st/st^*				Wildtype eye colour $st/+$			
		Sample size	x	y	p	Sample size	x	y	p
D38	M	141	-0.81	0.73	0.26	181	-0.24	0.37	0.43
	F	158	-0.57	0.95	0.34	172	+0.27	0.28	0.61
D36	M	238	-0.78	0.25	0.22	285	-0.81	0.1	0.21
	F	191	-0.51	0.49	0.32	301	-0.63	0.1	0.26

* The st bearing chromosome in both D38 and D36 was randomly drawn from the *bwst* line, whereas the st^+ bearing chromosome in D38 was taken from a line which enhanced penetrance; in D36 the st^+ chromosome came from the *cnbw* line which is noted for its reducing effect on penetrance (see table 10). p is the proportion of abnormal eyes in the respective populations.

Scarlet individuals in both D36 and D38 carry one identical chromosome 2 while the homologous chromosome and the remaining complement in both lines are drawn more or less at random from *bw st*. It is not surprising, then, that penetrance is the same in both lines for scarlet individuals; $st/+$ individuals, on the other hand, carry $3-cn\ bw$ in D36 and $3-bw$ in D38. Therefore, the difference in penetrance between these lines in $st/+$ individuals can only be attributed to differences in their third chromosome. It follows that the genetic constitution of chromosome 3 influences the action of the warty locus.

Furthermore, it can be shown that the *bw st* strain is heterozygous for the modifiers it carries on chromosome 3. Because the number of flies scored in each mating is small, the data in table 8 represents the pooled results for 10 single pair matings in line D38 and 14 in D36. Penetrance in both st/st and $st/+$ individuals varied somewhat from cross to cross. This variation permitted a short-term two-way selection experiment to be performed in which D38 and D36 males, from crosses where penetrance was notably high or low, were backcrossed to *bw st*. There was a response to selection in both $st/+$ and st/st progeny although it was substantially higher amongst the st/st class (see table 9).

The response amongst $st/+$ progeny, if it is real, cannot be explained in terms of modifiers on either chromosome 2 or 3 since all these individuals possess one chromosome 2 and one chromosome 3 identical by descent, the

homologues being randomly taken from the *bw st* line *after* selection. Response to selection, in this case, must be due to modifiers on chromosome 1 and/or 4. The pronounced response amongst *st/st* progeny must be due, at least in part, to genetic differences between *st* chromosomes from the

TABLE 9

Response to selection for increased and reduced penetrance amongst st/st and st/+ individuals in the dominant strains D36 and D38

Strain	Sex	Direction of selection	Scarlet eye colour (<i>st/st</i>)			Wildtype eye colour (<i>st/+</i>)		
			Sample size	<i>x</i>	<i>y</i>	Sample size	<i>x</i>	<i>y</i>
D38	M	+	86	-0.45	0.49	97	+0.24	0.40
	M	-	110	-0.96	0.61	100	+0.21	0.49
	F	+	82	-0.30	0.37	85	+0.81	0.52
	F	-	109	-0.54	0.28	112	+0.78	0.73
D36	M	+	114	-0.6	0.55	159	-0.57	0.55
	M	-	101	-1.02	0.1	135	-0.84	0.40
	F	+	124	-0.15	0.55	139	-0.6	0.49
	F	-	51	-0.50	0.1	125	-0.81	0.1

matings with high and low penetrance. It follows that *bw st* is heterozygous for modifiers on chromosome 3 and perhaps on chromosome 1 and/or 4 which affect the penetrance of the dominant form.

(e) *Further evidence on location of the witty gene*

In Materials and Methods, evidence was presented indicating that the witty gene was located about 55 units from the left end of chromosome 2. Further information on the position of *wi*, in relation to *j*, can be obtained from the following type of cross:

$$\frac{Wi\ j}{++} \times \frac{Wi\ j}{++}$$

The *j/j* progeny from this cross will be *Wi/Wi* or *Wi/+* depending on whether a cross-over has occurred between *wi* and *j*. Thus, if we can distinguish between *Wi/Wi* and *Wi/+*, we can measure the map distance between *wi* and *j*. Fortunately, it is possible to distinguish between these two genotypes since all *Wi/Wi* individuals are (LR) and have very extreme expression. (The HH line was derived from similar *Wi/Wi* flies.) *Wi/+* individuals, on the other hand, show incomplete penetrance, and the expression is milder. Supporting evidence for this statement is derived from the fact that lines established from these extreme (LR) individuals maintain complete penetrance and pronounced expression indefinitely. It follows that the jaunty progeny which are not (LR), are *Wi/+* and have resulted from crossing-over between *wi* and *j*.

Two crosses of the type outlined above were made and the percentage of phenotypes amongst the *j/j* progeny which were not (LR) was found to be 5.6 per cent. (see table 10). This value is a slight underestimate of the map distance between *wi* and *j* because a few *Wi/+* individuals would be (LR) and therefore wrongly classified as *Wi/Wi*. However, the value is in good agreement with the earlier estimate (6.8 units).

(f) *The relationship between modifiers of dominant and recessive forms of witty*

The above evidence indicates that the penetrance of both the dominant and recessive forms of witty are under polygenic control. Data are presented in this section which suggest that the modifiers controlling penetrance of the dominant form, *wi*, also control penetrance of the recessive form, *wi*.

TABLE 10

Frequency of the witty phenotype amongst j/j progeny from crosses involving j Wi/+ + parents

Cross	Sex	(+ +)	(L +)	(+ R)	(LR)
D38 × D38	M	7	0	1	219
	F	6	5	5	234
D36 × D36	M	6	1	1	155
	F	9	2	1	139

TABLE 11

Frequency of the witty phenotype amongst F₂ wi/wi offspring from crosses between SH and four unrelated laboratory strains

Background introduced	Sex	(+ +)	(L +)	(+ R)	(LR)	<i>x</i>	<i>y</i>
<i>bw</i>	M	53	25	29	112	+0.6	1.5
	F	18	13	17	146	+1.8	1.6
<i>bwst</i>	M	127	25	28	58	-0.7	1.6
	F	62	29	18	93	+0.4	1.6
<i>cnbw</i>	M	155	25	28	25	-1.1	1.2
	F	79	27	28	49	-0.3	1.2
Canton-S	M	101	7	10	23	-2.2	2.6
	F	88	14	15	42	-0.9	2.1

TABLE 12

Frequency of the witty phenotype amongst the Wi/+ offspring from crosses between the dominant form (Wi/+) and the same strains mentioned in table 11

Background introduced	Sex	(+ +)	(L +)	(+ R)	(LR)	<i>x</i>	<i>y</i>
<i>bw</i>	M	170	18	11	3	-1.5	0.4
	F	129	51	43	35	+0.55	0.6
<i>bwst</i>	M	496	76	69	22	-1.2	0.5
	F	353	108	106	62	-0.7	0.5
<i>cnbw</i>	M	563	18	10	2	-2.7	1.0
	F	477	69	47	31	-1.4	0.8
Canton-S	M	412	3	4	1	-3.0	0.8
	F	373	21	21	5	-1.7	0.4

Penetrance was measured in F₂ *wi/wi* offspring from crosses between the SH line and various laboratory stocks, *i.e.* after the introduction of background from these different sources into the *wi/wi* line. Penetrance in each case was compared with that for *Wi/+* flies after the introduction of the same background (see tables 11 and 12). A similar phenotypic manifestation

was obtained for both the dominant (Wi) and recessive (wi) forms. Penetrance was highest with a background from bw and decreased with backgrounds from $bw\ st$, $cn\ bw$ and Canton-S, in that order.

There is no reason to suppose that $bw\ st$, $cn\ bw$ or Canton-S were ever subject to selection for their effect on penetrance of the witty gene. Hence, if the modifiers affecting Wi are distinct from those affecting wi , it is remarkable that both classes of modifiers have a corresponding effect in four unrelated laboratory stocks. These results may be explained simply by assuming that the penetrance of both the dominant and recessive forms of witty are controlled by the same polygenes.

4. DISCUSSION

(a) *The relationship between dosage of the witty gene and its modifiers*

Results from the experiments described above give some understanding of the relationship between dosage of the witty gene and its modifiers. This relationship is easier to follow if we return to the concept of the underlying

FIG. 1.—Distribution of m.s. for the four major genotypes.

variable as the distribution of some morphogenetic substance (m.s.). Suppose that the witty locus controls the production of some substance in each eye disc which, if present in excess of a certain level, causes the eye to develop abnormally. The general method for characterising the distribution of morphogenetic substance (m.s.) was outlined by Whitten (1966). Figure 1 depicts the distribution of m.s. for one and two doses of the major gene with and without the dominance modifiers, *i.e.* for what might be regarded as the four major genotypes, $wi/+$, wi/wi , $Wi/+$ and Wi/Wi . In each case, it is assumed that the general background is the same and the environment is uniform so that the differences in m.s. can be attributed to differences at the major locus and differences in dominance modifiers. In a background where two doses of wi produce flies with intermediate penetrance (*i.e.* the amount of m.s. is distributed about the threshold) we find that penetrance of $wi/+$ flies is effectively zero (m.s. in this case is distributed below the threshold and only very occasionally does it surpass the threshold) (see fig. 1).

When the dominance modifiers are removed the witty gene increases in activity so that only one dose of *Wi* is necessary to produce the same amount of m.s. as two doses of *wi* (fig. 1). A double dose of the active form (*Wi/Wi*) produces a level of m.s. well above the threshold since penetrance of *Wi/Wi* individuals is complete and expression is extreme.

Thus we may conclude that the action of the witty gene is essentially additive when considered in terms of the underlying scale. The phenomenon of dominance is a consequence of the presence of a threshold. This is in agreement with the well established principle that dominance is a property of the phenotype and not of the gene. It follows that dominance of the witty character is controlled by genes at other loci since it is dependent on the level of morphogenetic substance relative to the position of some threshold.

The dominance modifiers are situated on the same chromosome as *wi*, and are probably restricted to one region and have a major effect on the witty gene. In contrast, the penetrance modifiers are scattered throughout the genome and behave as typical polygenes, each with a small effect. Nevertheless, it should be remembered that the dominance modifiers affect penetrance of the homozygous form (*e.g.* in the HH line) just as the penetrance modifiers affect the heterozygote, etc., and consequently the difference between the two classes of modifier is rather one of degree than of type. Undoubtedly the witty system illustrates the model relating dominance, penetrance and expressivity proposed by Goldschmidt (1938) and summarised by Rendel (1962).

Although it is not known at what level between gene and phenotype the modifiers act, circumstantial evidence suggests they may operate at the chromosome level. If one orders the witty strains according to increasing expression, *i.e.* LL, SL, SH and HH, one finds a corresponding increase in the degree of jauntiness. HH flies resemble the mutant *curly* while LL flies are almost wild-type. This correlation may reflect pleiotropy or it may indicate that a region of chromosome 2, including *j* and *wi* has been so altered to impair gene activity in the region.

(b) *Evolutionary considerations*

As mentioned earlier, the witty gene was first discovered in a laboratory stock, LL, as a recessive but with near zero penetrance under normal laboratory environments. By outcrossing this stock to any of four laboratory stocks, it was found possible to construct lines with any degree of penetrance and even to generate dominant forms of witty. Thus, unlike the four laboratory stocks, the background of LL ensures that the witty gene has no visible effect on eye development. Presumably the witty phenotype is at a selective disadvantage to wild-type. Hence we must assume that the witty gene exists in a well adapted genetic background. It follows that at some point in the history of the strain, whether in the natural state or in the laboratory, natural selection operated to suppress the action of *wi* at least as regards its effect on eye development.

The chain of events may have been as follows: A mutant occurred which, in the heterozygous condition disrupted eye development. However, the beneficial effect of this mutant on some other aspect of development ensured its survival and spread in the population. This necessitated the suppression of its effect on eye development which was achieved by the

linkage of a suppressing element or dominance modifying system to the *wi* locus. The role of these modifiers was to reduce the activity of *wi* to a level such that one dose of *wi* had no phenotypic effect, *i.e.* the witty character became recessive.

With the continued spread of *wi*, the homozygous form became more frequent. A double dose of witty, even in the presence of the dominance modifiers, was sufficiently active to disrupt development and so natural selection, operating on the polygenic background, reduced penetrance to a low level for the range of environments which the insect was likely to encounter. In the course of the experiments reported in this paper we have more or less reversed the course taken by natural selection and recreated the dominant and primitive form of the witty character. It is possible that modification of the effect of the *Wi* gene did not occur till the witty locus had become fixed for *Wi*. This is considered unlikely because it would mean that *Wi/Wi* flies were common at one stage and since vision in flies with this phenotype must be very restricted if not totally distorted such flies would have little chance of surviving under natural conditions.

A study of other eye mutants may reveal a similar story. One mutant, scarp, affects eye development in much the same way as witty and it may be considered to impair the vision of flies in which it is expressed. Three wild-type laboratory stocks, two of which are completely unrelated, have been shown to be homozygous for scarp (Hanson and Gardner, 1962). As with witty, scarp does not express itself under normal laboratory conditions but requires a temperature of 30° C. for high penetrance. Thus the scarp gene appears to be widespread amongst wild populations of *D. melanogaster* and yet its penetrance is zero.

5. SUMMARY

1. The major gene, *wi*, controlling *witty*, an eye mutant in *D. melanogaster*, has been located on chromosome 2, 55.
2. The penetrance of the *witty* character is shown to be controlled by a major factor on chromosome 2 as well as polygenes on chromosome 3 and probably 1 or 4.
3. The major factor on chromosome 2 destroys the recessiveness of *witty*.
4. Evidence is presented which indicates that *wi* occurs in a well-adapted background such that it is a recessive with zero penetrance. It is argued that, in its initial state, *wi* was not a recessive but that its recessiveness has evolved as a result of natural selection.
5. The relationship between dosage of the major gene, *wi*, and its modifiers is discussed.

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6. REFERENCES

- CLARKE, C. A., AND SHEPPARD, P. M. 1960. The evolution of mimicry in the butterfly *Papilio dardanus*. *Heredity*, 14, 163-173.
- CLARKE, C. A., AND SHEPPARD, P. M. 1963. Interactions between major genes and polygenes in the determination of mimetic patterns of *Papilio dardanus*. *Evolution*, 17, 404-413.

- DOBZHANSKY, T. 1951. *Genetics and the Origin of Species*. 3rd Ed. Columbia University Press.
- FISHER, R. A. 1930. *The Genetical Theory of Natural Selection*. Oxford University Press.
- FISHER, R. A., AND HOLT, S. B. 1944. *Ann. Eugen.*, 12, 102-120.
- FORD, E. B. 1940. Genetic research in the Lepidoptera. *Ann. Eugen.*, 10, 227-252.
- FORD, E. B. 1964. *Ecological Genetics*. Methuen and Co., London.
- GOLDSCHMIDT, R. 1938. *Physiological Genetics*. McGraw-Hill, New York.
- HANSEN, A. M., AND GARDNER, E. J. 1962. A new eye phenotype in *Drosophila melanogaster* expressed only at temperatures above 25° C. *Genetics*, 47, 589-598.
- HARLAND, S. C., AND ATTECK, O. M. 1941. The genetics of cotton. XIX. Normal alleles of the crinkled mutant of *Gossypium barbadense* differing in dominance potency, and an experimental verification of Fisher's theory of dominance. *J. of Genetics*, 42, 21-48.
- RANDEL, J. M. 1962. The relationship between gene and phenotype. *J. Theoret. Biol.*, 2, 296-308.
- WHITTEN, M. J. 1966. The quantitative analysis of threshold characters using asymmetry. *Genetics*, 54, 465-483.
- WHITTEN, M. J. 1967. Quantitative measurement of the effect of temperature on the penetrance of the eye mutant, witty, in *D. melanogaster*. *D.I.S.*, 42, 110.