THE GENETIC BASIS OF NATURAL VARIATION. II. ANALYSIS OF A POLYGENIC SYSTEM IN *DROSOPHILA MELANOGASTER*¹

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MUCH natural variation has a polygenic basis. An investigation of such natural variation leads one to the ground lying between the analysis of single-locus variation and the analysis of complex phenotypes without investigating specific genes.

Several attempts have been made to carry the techniques of classical genetic analysis into the complex realm of naturally occurring polygenes (WADDINGTON 1953; BARIGOZZI and DI PASQUALE 1956; MILKMAN 1956, 1958, 1960a, b). The work reported here is a continuation of such an effort.

The crossveinless (*cve*) complex has been described (MILKMAN 1960b) as a group of common, widespread, naturally occurring polygenes, distributed over the major chromosomes of *D. melanogaster*, and interacting to affect the ability of the developing animal to make posterior crossveins. This is in agreement with the various conclusions reached as a result of previous or contemporary studies of the *cve* genes (WADDINGTON 1953; MOHLER 1955; BATEMAN 1955 and 1956). These attributes of the *cve* genes, together with their susceptibility to various lines of investigation, make them a likely choice for detailed analysis.

MATERIALS AND METHODS

General materials and methods, as well as the results obtained from the basic analysis of the crossveinless complex, may be found in the first paper of this series (MILKMAN 1960b). In brief, a true-breeding, polygenic crossveinless strain was obtained from wild Massachusetts flies by selection. The criterion for selection was, in the early stages, susceptibility to production of crossveinless phenocopies, a trait shown to be one aspect of low crossvein making ability (MILKMAN 1960b). The *cve* genes cause defects of the posterior crossveins of the wings, and this effect is more pronounced at 18°C than at 25°C. It was possible to rate the phenotype quantitatively in terms of the crossvein fraction missing. Each wing can be rated from 0 (normal; no sixths missing) to six (crossvein

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completely absent; six sixths missing). A fly may therefore be rated from 0 to 12, by adding the individual wing ratings.

With the findings that the *cve* genes are on all three major chromosomes and that they act additively in lowering posterior crossvein making ability, it was desirable to push the investigation towards a more detailed characterization of individual genes. Two approaches were used: one involved the relationship of known mutant genes to the *cve* system; the other made use of recombination experiments to localize the genes as precisely as possible.

EXPERIMENTAL RESULTS

Interaction with known genes that cause defective crossveins and with other suspect genes: There are a number of single mutant genes which interfere with posterior crossvein formation, some of which have characteristic effects on the posterior crossvein which distinguish them from others. With regard to these genes, three possibilities present themselves: 1. That the *cve* complex is a balanced system, heterozygous for some of these factors. 2. That alleles of these genes are the components of the complex, alleles which have no visible effects by themselves but which do in combination with other genes. 3. That these genes are not a part of the complex.

The first possibility may be discarded on the basis of evidence against the *cve* complex being a balanced system, namely, that crossover data and cytological examination fail to indicate the presence of heterozygous inversions.

The other possibilities may be tested by a combination of experiments designed to measure similarity between any gene and the components of the *cve* system. Table 1 lists the various genes tested.

The first step was to measure the interaction between a given gene and the *cve* complex. Crossvein defects were assessed in terms of the rating system already described, a rating from 0 (normal crossveins) to 12 (crossveins entirely absent). *cve* males were mated to females carrying a given gene, and the average rating of the progeny was compared to the average rating found in the F_1 of an Orgeon R $\times$ *cve* cross. The validity of this procedure depends on the similarity to the wild Oregon R genotype of the backgrounds of the stocks being tested. In extreme cases this was no problem, but intermediate results were questionable. Table 2 shows the results of these tests. It will be seen that the *cve* complex interacts strongly with a number of the genes tested.

The significance of the interaction is not easy to determine, however. In the first place, the *cve* complex interacts with *cv-c*. (This and succeeding symbols are explained in Table 1.) Does this mean that all *cve* flies are heterozygous for *cv-c*? Apparently not, for there is no 1:1 segregation for the easily recognizable *cv-c* phenotype (Table 1), nor do half the eggs die, as would be expected if the *cv-c* homozygotes were inviable with a *cve* background.

Moreover, previous crosses have shown that *cv-c* interacts with the *cve* complex in the absence of any third chromosome *cve* factor. Again (MILKMAN

TABLE 1

Genes known to affect posterior crossvein (p.c.) formation

Mutation	Locus symbol	Main effect	Other effects	Crossvein defects	
				Freq. percent	Degree
crossveinless	1-13.7 <i>cv</i>	anterior and posterior crossveins absent		100	12
crossveinless-c	3-57.9 <i>cv-c</i>	p.c. defective: crossvein fragment parallels longitudinal veins		100	10
crossveinless-d	3-65.0 <i>cv-d</i>	p.c. defective indistinguishable from <i>cve</i>		100	10
detached	3-72.5 <i>det</i>	p.c. defective indistinguishable from <i>cve</i>		†	0-8
Abruptex	1-3.0 <i>Ax</i>	longitudinal veins incomplete terminally	p.c. defective	*	0-6
dachsous	2-0.3 <i>ds</i>	wings smaller crossveins closer	p.c. defects body thicker	‡	0-?
Gull	2-12.0 <i>G</i>	wings held out and curved down	crossveins broken bristle duplications Vein L ₁ thickened	*	0-?
dachs	2-31.0 <i>d</i>	wings smaller, crossveins closer tarsi four-jointed	p.c. defects body thicker	†	0-?
elbow	2-50.0 <i>el</i>	wings bent; alulae, halteres small; p.c. defective		100	6-12
divergent	3-20.0 <i>dv</i>	wings diverge	p.c. defects	†	0-4

* - variable.

† - rare unless selected for

‡ - apparently noted only in some alleles

1960c), *cv-c* does not increase the susceptibility to phenocopy production of crossvein defects. We are thus faced with the concept that mere positive interaction does not establish that the gene in question is part of the *cve* complex.

In addition, the reciprocal relationship applies. A negative result should not eliminate a locus from consideration. Interaction differences among the alleles at the *ds* locus illustrate this point (Table 2). Moreover, until we know how powerful each *cve* gene's effect is, judgment should be reserved since a weak gene's interaction could be masked by an unfavorable background.

This procedure of testing interaction is, however, of value. Genes that give a positive interaction may well be in the *cve* complex, and they can be subjected to further investigation. Five criteria can be applied: 1. Would the gene be included in selection for the phenotype? 2. Does the gene increase phenocopy susceptibility? 3. Does the expression of the gene's crossvein effect increase at lower temperatures? 4. Does the gene lie in a region shown to have *cve* activity by

TABLE 2
Ratings of female offspring of mutant females $\times$ cve males

Mutant or strain	Experiment	No.	Avg. rating
Oregon R (wild type)	1	816	0.36
	2	186	0.33
crossveinless-c		126	7.33
crossveinless	1	117	5.19
	2	97	3.52
ancestral wild strain		20	3.60
net black cinnabar	1	107	3.20
brown	2	51	3.18
divergent	1	48	3.02
	2	39	2.80
dachsous-wide	1	72	2.89
	2	63	2.86
crossveinless-d	1	60	3.18
	2	102	2.89
	3	71	2.16
white	1	94	3.33
	2	60	1.93
detached		22	2.41
curved		8	2.38
Abruptex		24	2.38
elbow	1	35	2.09
	2	45	1.96
	3	37	1.78
roughoid hairy thread	1	36	2.22
scarlet ebony claret	2	41	1.27
dachs black	1	39	2.26
	2	62	1.50
	3	48	1.27
dachsous ^{38k}		65	0.98
cv-c allele	1	58	0.71
	2	105	0.64
	3	128	0.52
vestigial		20	0.45
rudimentary		77	0.42
Notch ^s		31	0.40
veinlet	1	124	0.69
	2	61	0.25
	3	50	0.24
yellow cut	1	57	0.47
raspberry forked	2	41	0.30
ebony		80	0.36
clot		41	0.27
dachsous		14	0.14
<i>Ratings of female offspring of cve females $\times$ mutant males</i>			
Oregon R (wild type)	1	219	0.23
	2	99	0.23
detached		24	5.0
crossveinless-c		295	4.7
divergent		30	4.3
Mohler's crossveinless polygenic strain		34	3.0

In comparison with the Oregon R strain, some of the other strains seem to have genes which interact with the *cve* complex to produce crossvein defects. These interacting genes are not necessarily the mutants for which the strains are named. An average rating over 1.0 is taken as good evidence for such interaction.

crossover tests? 5. Is the gene's phenotype exactly like the *cve* phenotype, or is it, like that of *cv-c*, similar but distinct?

Several of the genes under consideration may be eliminated immediately by application of the first criterion. None of the genes which in homozygous state independently cause defects of the posterior crossvein—or any other clear-cut phenotype for that matter—could have been involved in the selection of the *cve* complex, for they would have been noticed. Thus *cv*, *cv-c*, *cv-d*, *el*, and *Ax* are ruled out but not necessarily possible alleles of these genes.

The results of the following experiment imply that no allele of *cv-c* is involved. The F_1 of a *cv-c* $\times$ *cve* cross was inbred in several cultures, propagated for a number of generations by random mass matings, and then selected for wild type. This method got rid of the *cv-c* and fixed the *cv-c* allele present in the *cve* stock. After several generations the stock was completely wild type, and it was carried on by mass mating for several months without further selection. It is probable that the *cve* genes were now back near the level at which they had been in the wild stock except for any one that might be close to the *cv-c* locus. When flies from this stock were mated back to *cve* males, no interaction was shown (Table 2, "*cv-c* allele"), so that this locus may be removed from further consideration.

On the other hand, various known mutant genes with less pronounced effects (such as *det*, *dv*, *d*, and *ds*) could easily fit into the picture. All have effects on the crossvein even though they are incompletely penetrant.

The clearest case is *det*, which resembles the *cve* complex genes in many ways. It has essentially the same phenotype; it responds to selection; and it has low fertility. Moreover, its expression and penetrance are greater at 18°C than at 25°C.

dv resembles wild type under some conditions. The various *ds* alleles are associated with a body and wing structure just like that of the *cve* stock. Therefore these genes could have been selected for without being recognized as *dv* or *ds*. Dachs, however, causes four-jointed tarsi, and this phenotype has not been observed in *d* $\times$ *cve* crosses. With the possibility that other alleles occur and that four-jointed tarsi may not be irrevocably tied to *d*, the locus should not be ignored.

These criteria merely tell us whether a certain gene could have been selected for in assembling the *cve* complex. To determine whether a gene is actually present in the *cve* complex, crossover tests must be tried.

Crossover tests: The use of *cv-c* as a "booster" simplified matters a great deal. Interaction with *cv-c* enables one to detect fewer *cve* genes than would otherwise have been possible (MILKMAN 1960b).

Two marked stocks were used: for the first chromosome, yellow (γ , body color), cut (*ct*, wing form), raspberry (*ras*, eye color), forked (*f*, bristle form); and for the third chromosome, Roughened (*R*, eye texture), Dichaete³ (*D*³, wing form), bithorax-Dominant (*bx*^D, haltere form), Prickly (*Pr*, bristle form). All combinations for each chromosome are distinguishable.

One test of the X chromosome proved surprisingly simple. A single *cve* male was mated to a γ *ct* *ras* *f* female. All the daughters had the same X chromosome

from the father, and they were mated to *cv-c* males. The experiment measured interaction, then, between factor(s) on one X chromosome and *cv-c*.

Although one might say that these factors are not necessarily identical with the *cve* factors, two facts argue in favor of the validity of this procedure. One is that the *cve* flies, in contrast to other stocks, contain factors on the three major chromosomes which interact with *cv-c*, and so interaction with *cv-c* seems to be one of the most general properties of the *cve* complex. The other is that the same result was obtained by MOHLER (1955) who looked for *cve*-type factors on the X of a similar stock without using *cv-c*.

Results, using the rating system previously described, are listed in Table 3. It may be seen readily that only one region is involved, the region very near or beyond the forked locus. Because of possible effects from other chromosomes even in the absence of the *cve* X, it is impossible to say definitely whether the region is near or beyond *f*. The best way of attacking this problem further would be to use markers in the region of *f*. One may disregard the possibility that *f* suppresses the *cve* effect. Were this so, one should expect some definite variation in the data for the various crossover types, due to the segregation of the hypothetical *cve* gene(s) at regions other than near *f*. That no such variation was encountered is taken as proof that the results may be taken at face value.

The third chromosome presents a more complex picture. At least two loci are involved, and the conclusions arrived at after the crossover tests are not to be taken as proven. The third chromosome dominant marker stock previously described, *R D^sbx^oPr*, was used, with *cv-c* being used as a booster.

The rating system as described was used. Three methods for using the data in analysis were available: each crossover class might be graded merely on the percentage of crossveinless flies; the average rating; or the probit average rating which compensates for minus values.

TABLE 3
First chromosome crossover data

Crossover class	Total no.	No. <i>cve</i>	Percent	Avg. rating*
<i>γ ct ras f</i>	264	31	12	0.52
<i>+++ +</i>	418	216	52	3.18
<i>γ +++ +</i>	100	45	45	2.86
<i>+ ct ras f</i>	69	12	18	0.61
<i>γ ct ++</i>	67	35	52	3.25
<i>+ + ras f</i>	103	17	17	0.40
<i>γ ct ras +</i>	110	62	56	3.44
<i>+++ + f</i>	133	34	26	0.97
<i>γ +++ f</i>	16	2	12	0.81
<i>+ ct ras +</i>	22	9	41	2.82
All males	1,324	466	35	2.00
All females	1,591	651	41	1.81

* Only males showed crossover types. For method of determining average rating, see text.

Each method has its drawbacks. The percentage method does not utilize all available information. In both the percentage method and the average rating method all wild type flies are classified as zero, which results in inaccuracies when comparisons are made. The probit plot in this case is difficult to use because the distribution is not perfectly normal. Nevertheless, all three methods result in approximately the same answer, and it seems reasonable to assume that this picture is correct.

The probit method which was used to determine the particular localizations is described as follows. The offspring of 16 crosses were classed and rated, as shown in Table 4. These data were then put on a probit plot, and a "true" average rating for each class was estimated. Table 5 contains these data. In order to facilitate

TABLE 4
Third chromosome crossover data

Class	Total	0	1	2	3	Rating 4	5	6	7	8	9	10	11	12
<i>RDBP</i>	356	320	14	9	5	5	1	1	1	0	0	0	0	0
++++	598	118	32	47	44	42	51	61	48	64	36	44	11	0
<i>R</i> +++	203	83	16	24	18	16	18	11	7	3	2	3	2	0
+ <i>DBP</i>	263	188	19	14	12	13	4	5	0	4	3	1	0	0
<i>RD</i> ++	55	28	8	7	3	2	4	1	1	0	0	1	0	0
++ <i>BP</i>	73	45	7	3	2	1	3	3	2	5	2	0	0	0
<i>RDB</i> +	182	138	21	12	4	2	2	2	0	0	0	1	0	0
+++ <i>P</i>	290	110	17	19	18	21	22	30	17	15	10	5	5	1
<i>R</i> ++ <i>BP</i>	24	23	1	0	0	0	0	0	0	0	0	0	0	0
+ <i>D</i> ++	22	10	2	1	0	2	4	1	1	1	0	0	0	0
<i>RD</i> + <i>P</i>	12	10	0	2	0	0	0	0	0	0	0	0	0	0
++ <i>B</i> +	16	5	2	1	3	1	1	1	0	2	0	0	0	0
<i>R</i> ++ <i>P</i>	117	64	13	11	9	6	4	3	2	0	1	4	0	0
+ <i>DB</i> +	125	63	14	8	10	3	4	6	4	6	3	3	1	0
<i>R</i> + <i>B</i> +	2	1	0	1	0	0	0	0	0	0	0	0	0	0
+ <i>D</i> + <i>P</i>	8	6	0	1	1	0	0	0	0	0	0	0	0	0

Degree of crossvein defect is rated from zero (wild type) to 12 (posterior crossveins completely absent). Intermediate numbers represent intermediate degrees. *R*=Roughened (eyes). *D*=Dichaete (spread wings). *B* stands for *bx^D*=bithorax-dominant. *P*=Prickly (bristles). See text.

TABLE 5
Transformation of rating data to percentages of total third chromosome cve effect

Marker class	Avg. rating	Percent of difference between ++++ and <i>RDBP</i>	Marker class	Avg. rating	Percent of difference between ++++ and <i>RDBP</i>
<i>RDBP</i>	-4.3*	0	++ <i>BP</i>	-0.2*	44
++++	+5.1	100	<i>RDB</i> +	-2.0*	24
<i>R</i> +++	+2.0	68	+++ <i>P</i>	+3.0	78
+ <i>DBP</i>	-1.5*	30	<i>R</i> ++ <i>P</i>	+0.6	51
<i>RD</i> ++	+0.7	53	+ <i>DB</i> +	+0.7	52

* Estimated from distribution. See text.

calculations, the ratings were converted to comparable values from zero to 100 as follows: the lowest rating (parental marked class) was -4.3 , and the highest (parental unmarked class) was 5.1 , making a range of 9.4 . By setting -4.3 at zero and $+5.1$ at 100 , it was possible to convert the other ratings by use of the formula:

$$\frac{\text{rating} - (-4.3)}{5.1 - (-4.3)} = \frac{\text{rating} + 4.3}{9.4}$$

The resulting fraction was multiplied by 100 . Table 5 also lists the converted values for each crossover class.

Now the contribution of the *cve* chromosome region opposite each marker could be calculated merely by subtracting the value for a class with the marker from the value for a class identical except for not having that marker. Table 6 lists these calculations. It will be noted that four calculations may be made for each of the end regions, two for each of the inner ones. More are possible in each case, but these would involve values for very small multiple crossover classes and would be meaningless.

It should be noted that although no effort was made to adjust the data, values for reciprocal crossover classes equal close to 100 in each case. Moreover, the four values obtained for the four regions added up to about 97 before the figures were rounded off. This degree of internal consistency seems quite satisfactory.

In the analysis of these values, one assumption must be made; namely, there is no *cve* factor in region 2. As will be shown later, this region is only 8.6 units long,

TABLE 6

Calculation of the contribution of the region opposite each marker gene

For $+^R$:	$++++ - R +++ = 100-68 = 32$
	$+DBP - RDBP = 30-0 = 30$
	$+DB+ - RDB+ = 52-24 = 28$
	$++++P - R+++P = 78-51 = 27$
	$+^R = 30$ (average)
For $+^D$:	$++BP - +DBP = 44-30 = 14$
	$R++++ - RD++ = 68-53 = 15$
	$+^D = 15$
For $+^{bx}$:	$++++P - ++BP = 78-44 = 34$
	$RD++ - RDB+ = 53-24 = 29$
	$+^{bx} = 32$
For $+^P$:	$++++ - +++P = 100-78 = 22$
	$RDB+ - RDBP = 24-0 = 24$
	$+DB+ - +DBP = 52-30 = 22$
	$R++++ - R+++P = 68-51 = 17$
	$+^P = 22$

These values represent the percentage of the total *cve* effect of the third chromosome assigned to each region by comparison of effects associated with various chromosome marker combinations.

due to the suppression of crossing over near the *D* locus by an inversion associated with *D^s*. This assumption simplifies matters by implying that each marker locus is next to only one region with *cve* factors in it, and this enables us to localize the *cve* factors within their regions by comparing the contributions of the parts of the chromosome near each of the markers. The *R* and *D^s* data serve for region 1, and the *bx^v* and *Pr* data for region 3. The ratio of contributions of the two marker areas is inverted to find the ratio of distances from each marker to the *cve* factor (if there is only one per region).

Region 3 is the simpler case. $+^{Pr}$ contributes 22 percent of the third chromosome *cve* effect, and $+^{bx}$ contributes 32 percent. The ratio of distances from a single *cve* gene (22/32) shows the distance to *Pr* to be greater. The distance from *bx* to *Pr* is 31.2. *Pr* is located at 90.0, so 32/54 of the *bx-Pr* distance would place the *cve* gene at 71.5. It is suggested that the gene in question is *det* (72.5), recalling the evidence previously presented. There are other possibilities; namely, two *cve* genes occur in region 3 with neither being *det*, or three, with one being *det*. These possibilities are predicated on the location of the center of the *cve* effect at 71.5. This effect contributes about 55 percent of the third chromosome's *cve* effect.

Region 1 presents difficulty. By the same method of calculation, a single gene could be at 12.1. There are no known genes between 1.4 and 19.2, however. The data are not sufficient to permit one to decide exactly where two genes would be, since their relative contributions would also be unknown. But one gene would clearly have to be very near *R*, between zero and 1.4. The other would lie between 19.2 and 50, the approximate place where crossing over is no longer suppressed by the inversion associated with *D^s*.

If there is only one gene on the left arm, it contributes 45 percent of the third chromosome's *cve* effect. If two genes are involved, one may at least calculate limits for their contributions. The gene not near *R* would have a minimum of 15 percent, the share assigned to $+^{D^s}$, if it did not recombine with *D^s*. If it were as far from *D^s* as possible, at approximately 19, its contribution would be 25 percent, according to the method described above. The *cve* gene near *R* would contribute the rest, from 20 percent to 30 percent.

Since *dv* (at 20.0) is a logical possibility, and since no genes have been definitely localized in the region between 1.4 and 19.2, the number and location of *cve* factors on the left arm of the third chromosome are not as yet determinable. Further study with different recessive markers and using *Ax* (on chromosome 1) as the booster should bring out the answer.

Some investigators would consider the 1.4–19.2 region to be an area for polygenes. MATHER (1941) believes that polygenes are found in particular places, especially in blocks of heterochromatin (which this region, by the way, is not). GOLDSCHMIDT (1951) assigns a similar role to heterochromatin, particularly intercalary heterochromatin. DUBININ (1948) felt that the regions around net and blistered contain many polygenes. I cannot subscribe to the idea of special locations for polygenes for two reasons; one is that some polygenes have been

localized among "ordinary" genes; secondly, the intergradation between polygenes and other genes is quite smooth. There are very few genes in *Drosophila* with minor functions, as the lethality of almost all homozygous deficiencies shows (SINNOTT, DUNN and DOBZHANSKY 1958; GOLDSCHMIDT 1955); therefore, heterozygous deficiencies induced in this region should be expected to produce new recognizable phenotypes even if spontaneous mutations were generally so slight as to produce little morphological effect. We can clearly assume, then, that the *det* locus, near 72, is the site of the only *cve* gene in the right arm of chromosome 3. The left arm may contain one *cve* gene near 12 or two *cve* genes, one near locus one and the other between 19 and 50, possibly *dv*, at 20.

With respect to the second chromosome, data are available from matings made to test for crossover suppressors on this chromosome. For this purpose, net (*net*, wing veins) black (*b*, body color) cinnabar (*cn*, eye color) brown (*bw*, eye color) flies were used; only the non-*net* flies could be assessed for the *cve* phenotype. There is an indication from limited data that *cve* activity is present on both arms of the second chromosome.

The genes of the cve complex: We can say that there are at least five *cve* factors: one on the X, near *f* (56.7); one on the right arm of the third, *det*, about 72; one or two on the left arm of the third; one or more on the right arm of the second; and one or more on the left arm of the second.

It is further possible to state the relative contributions of the genes on the X and third chromosomes and of the second chromosome as a whole. From available data it is not possible to determine whether a *cve* factor involves a single gene or two or more closely linked genes. For the present, the simpler (one gene) interpretation is used.

The X chromosome, then, has one factor whose contribution can be estimated by comparing the true mean rating of classes with the X factor to that of classes without it. A probit plot of the data shows a difference of about 6 units which is the contribution, then, of the hemizygous X factor. Since the *cve* genes are semi-dominant by the criteria we use, a heterozygous X factor has a value of about 3 rating units.

The third chromosome has a value of 9 units in the heterozygous condition, with 45 percent of the value associated with the left arm and 55 percent with the right arm. Heterozygous *det* is then worth 5 units, and the left arm gene or genes worth 4. A rough estimate of the second chromosome's contribution, based on its approximate equivalence to the X in earlier crosses, would be 3 units per chromosome.

If the values for each chromosome are added together, the sum is 30. These 30 units cover the range between the +9 rating of the *cve* flies and the value -21. The latter is the value the wild stock would have if all *cve* genes were replaced by non-*cve* alleles in the laboratory stocks with which they were compared in the crossover tests. It is a measure of the reserve crossvein-making ability of the flies. The wild stock, then, could be selected to a norm of reaction measuring at least $9 - 30 = -21$ rating units. No basis exists here for distinguishing the maximum

crossvein-making abilities of the two sexes as was done with Oregon R (MILKMAN 1960b); because no *cve*-free line of the ancestral wild stock has been made.

Two indirect lines of evidence indicate that the base value of -21 should be approximately the same for both males and females. One is that in the early selection for crossvein defects, males and females appeared with equal frequency. Another is that, in contrast with Oregon R where females responded more than males, both sexes of the wild stock respond about the same to phenocopy treatment at 40.5°C to be described in a subsequent paper.

In summary, two further lines of attack on the characterization of the *cve* genes have been introduced. The interaction of the *cve* complex with various mutants has been used as a lead. Crossover analyses have led to conclusions of varying precision: that there is one *cve* factor on the X chromosome, near *f*; that there are two or three on the third, of which one is *det*, and that there are at least two on the second.

It has also been concluded that the *cve* complex is homozygous for these factors and that the wild ancestral stock's base value for crossvein making is about -21 for both sexes, as opposed to -13 and -19 for Oregon R females and males, respectively.

DISCUSSION

Using the *cve* complex as a model, we may now try to relate the gene to phenotype and to evolution. It is not the purpose of this discussion to set forth any new ideas, but rather to reaffirm those of the old ones which seem best to apply to the case at hand. The basis of the discussion will be the following set of facts: 1. Several genes have been found in a wild population which affect crossvein formation. 2. These genes act additively on a process underlying crossvein formation (MILKMAN 1960b). 3. This underlying process and a corresponding norm of reaction may be quantified. They vary over a wide range, depending upon the number of *cve* genes present (MILKMAN 1960b). 4. The part played by a single gene with respect to the norm of reaction is small. 5. The *cve* genes are not rare in wild populations. They are not to be regarded as less plentiful than their alleles, in general, and they are widespread (MILKMAN 1960a). 6. The number of genetic loci affecting crossvein formation is out of all proportion to the crossvein's morphological importance.

Considering first the relationship of gene to phenotype, the outstanding consideration is that many genes bear quite directly on the production of the *cve* phenotype. The *cve* genes are related to one another by their additive participation in defining the extent to which a developmental process is carried out, upon which crossvein formation depends. In this way, crossvein formation is like completely quantitative characters, which were once thought to have a monopoly on polygenes.

Because the *cve* genes do act additively, any of several combinations may achieve the same result, at least as relates to crossvein formation. Therefore, there are two ways in which the norm of reaction under discussion could be stabi-

lized in the population. One involves a fixed combination of genes in a homozygous state. The other involves maintaining a certain combination of heterozygous genes. The latter alternative apparently applies to the *cve* genes. The ready adaptability of this method would seem to reward its use, but it must be borne in mind that the quick morphological response to selection for crossvein defects would be of little value to the population if viability were markedly reduced, as was the case in these experiments.

The explanation of this heterozygosity would have to rest on single locus heterosis, since no single inversion is found in enough of the ancestral Amherst flies (IVES and HINTON 1946) to hold together groups of *cve* genes. Besides, the genes are on at least three chromosomes. The maintenance of such heterozygosity, which LERNER (1954) has strongly emphasized, entails a good deal of potential variability. This variability would seem to have two characteristics. Much of it is deposited in each member of the population and not in just a few, so that a small number of individuals picked at random would possess a major part of the population's variability. Also, the elements which could contribute to morphological change have already been tested physiologically. These elements are already a functioning part of the animal's genome.

The above considerations have led directly into the relationship of genes to evolution, for in this relationship the distribution of genetic variability is of extreme importance. The thinking on this subject has come far since the days when the material of evolution was thought to be sudden, rare mutations which were accepted or rejected by the environment. The great genetic variability within sexually reproducing populations has been discovered and the importance of recombination has been emphasized. Evolution is recognized as a population phenomenon, rather than a series of changes each in an individual. This trend in thought has made evolution seem much less a paradox; now the acceptance of a further step would add to our understanding of evolution. For it would seem that the genetic variability present in one individual is far greater than we imagined.

To the extent that the *cve* complex can be generalized, it can be said that each phenotypic variation among individuals in natural populations is controlled by many genes present in large numbers, rather than by single genes present in small numbers. Either alternative could account for the same amount of phenotypic variation in a population. The striking implication of the first alternative, however, is that potential variation among offspring of a few individuals would be on the order of that previously expected for the whole population.

When a hybrid corn plant is inbred a vast array of variation is seen in the offspring. The range of ear size, for example, is great because the parent was heterozygous for many loci affecting this phenotypic character. We know from many avenues of investigation that heterozygosity is great in *Drosophila* although little specific information has been obtained beyond the existence of this heterozygosity (DOBZHANSKY 1947, 1948; WALLACE *et al.* 1953; EPLING *et al.* 1953). It is difficult to see the value to an individual (upon whom selection works) of the various rare lethals and visible mutations floating in all populations. These cannot be

considered integrated into the gene pool, although their presence is due to the necessity and randomness of the process of mutation. The directly important heterozygosity and, therefore, the important potential variation must be high in frequency in the population.

The observed high frequency of the *cve* genes, the observed additive relationship of the *cve* genes to a process underlying crossvein information, and a survey of the nature of meaningful heterozygosity fit together to imply the following interpretation. A population does not store its variability in the form of rare genes that will spring into abundance when called upon. Rather, variation is brought about by the recombination of genes, many of which are present in any one individual, and most of which are present in any few individuals.

A striking feature of the Amherst wild population with respect to crossveins is this contrast. Although 99.9 percent of the flies have perfect crossveins, and the defects in the others are hardly recognizable, yet from the progeny of any 20 a strain can be quickly selected to give rise to a majority of *cve* members. Experimental tests strongly support this viewpoint (MILKMAN 1960a). DOBZHANSKY (1959) found that the variance of viability among the progeny of small samples of a population approaches the variation of the population itself. This combination of stability and adaptability is all the more striking when it is realized that the same situation probably holds for *Drosophila melanogaster* all over the world. Surely the potential variation is integrated into the genotype of the individual.

But just how general is this phenomenon? Does it apply only to crossveins and the few other recognized phenodeviants in *Drosophila*? It is too early to tell, but the means of inquiry are open.

The situation reminds one of the electric signs in which different combinations of lights can be lit to form an almost infinite array of pictures. No light is specific for any one picture; it may be used in countless combinations, each reducible to a numerical formula. The electric light has only two choices, on or off. At any genetic locus, the number of possible alleles is much greater. How reduced this number is in any one population remains to be seen, but clearly not too many could be present in significant frequencies. In practice, the choice might be between only two alleles, but perhaps groups of fairly equivalent alleles will be found to predominate (STERN 1943).

If a few individuals do possess as large a proportion of the population's potential variability as is here postulated, then this situation has remarkable experimental implications. For it means that most of the avenues of change immediately available to a population—and perhaps a large number of those immediately available to a species—can be found if the proper selection is applied to the offspring of a few individuals. Needless to say, this is no simple task.

It is clear that recombination of the existing genetic material cannot support evolution forever. It can only be suggested that whatever new genes are introduced to a population must have mild enough effects to be integrated into the gene pool—and individual genomes—in a fairly unobtrusive way before leading a charge to a new and striking phenotype.

SUMMARY

1. A true breeding stock, with polygenically-caused posterior crossvein defects has been selected from a wild strain of *Drosophila melanogaster*.

2. There are at least five additively acting genes involved: one near *f* on the X chromosome, *det* on the third chromosome, one or two on the left arm of the third chromosome, and at least one on each arm of the second chromosome. Localizations were done by crossover tests, using *cv-c* to enhance the expression of the *cve* genes. The relative contribution of each chromosome has been estimated.

3. The *cve* genes interact with many mutants to produce crossvein defects. The number of loci affecting crossvein formation is high.

4. The *cve* genes should not be thought of as rare. The widespread nature of genes affecting crossvein formation is discussed.

5. The natural importance of genes like the *cve* genes is discussed with particular attention to the type of genetic system where a number of heterozygous genes, each contributing a small part, govern a developmental process underlying the formation of a structure. The implications of such a system upon the distribution of potential variation within a population are described: any few individuals would have a large part of the significant variation present in the whole population.

6. The present susceptibility of natural genetic variation to detailed orthodox genetic analysis is suggested.

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