THE GENETIC BASIS OF NATURAL VARIATION.
I. CROSSVEINS IN *DROSOPHILA MELANOGASTER*¹

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Received July 2, 1959

AN understanding of natural variation is basic to the understanding of evolution and, more particularly, to the evolutionary potential of a group of individuals. In this regard, it is essential to understand the genetic basis of natural variation. One question on this subject stands out as important and approachable: what proportion of natural variation has a single-gene basis and what proportion has a polygenic basis?

In wild populations, it is easy to find examples of both. For example, SPENCER (1957) has isolated rare flies bearing any one of a number of genes, well known to the student of *Drosophila* genetics, which cause striking deviant phenotypes. On the other hand, LERNER (1954) describes many cases of what he calls "phenodeviants"—rare but widespread deviant phenotypes resulting from certain polygenic combinations. Each example leads to a different outlook as to the localization of potential variation. If one individual out of a thousand differs phenotypically because of a rare gene, then a small sample of the population would probably not have that gene, and the progeny would be bereft of the phenotype in question until a new mutation came along. On the other hand, if the one individual out of a thousand differed because of a rare combination of common genes, then even a small number of individuals, when allowed to produce a large number of progeny, would probably produce one with a deviant phenotype. The application of these alternative genetic mechanisms to human populations and all populations is clear; and since polygenic systems certainly have more than trivial importance, it is of interest to study some. Evaluation of the role of polygenic systems can come only after the thorough investigation of some examples.

Few genetic analyses indeed have ever been made on polygenic systems to the extent of localizing the component genes and characterizing them beyond their participation in the group effect. BARIGOZZI (1956) and WADDINGTON (1954) have attempted such analyses, on genes affecting tumors and crossveins respectively in *Drosophila melanogaster*. The work to be discussed here is also such an attempt (MILKMAN 1956).

¹ Most of this paper is taken from a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the subject of Biology at Harvard University.

The major part of the research described was supported by a predoctoral fellowship of the National Science Foundation for the academic year 1955-56.

Current work referred to is supported by grants from the Horace H. Rackham Fund and the Michigan-Memorial-Phoenix Project, both of The University of Michigan.

This polygenic system affects the ability of *Drosophila melanogaster* to form posterior crossveins during adult development. It is similar to the crossveinless polygenes studies by WADDINGTON; in fact, use of these genes (here called *cve*) for study stems from the successful repetition of WADDINGTON's phenocopy-selection experiment. In such an experiment, one selects for reduced crossvein-making ability, as manifested by low resistance to disturbance of crossvein formation by heat shock. When one selects far enough, one begins to get a small percentage of flies that can't even make perfect crossveins in the absence of heat shock. These flies, selected further, may ultimately produce a true-breeding, polygenic, crossveinless strain. Figure 1 illustrates the character "crossvein-making ability", amount of heat shock, and resultant phenotype as a nomogram.

MATERIALS AND METHODS

In addition to the crossveinless strain isolated as described above and various marker strains, an inbred Oregon R, propagated by virgin pair matings was valuable in this work. Flies were raised under usual conditions, temperature being maintained at $25^{\circ}\text{C} \pm 1$ unless otherwise noted. Special techniques will be discussed as they become relevant.

The heat shock used to induce crossvein defects was administered by immersing shell vials with pupae on their walls in a Precision constant temperature water bath at $40.5^{\circ} \pm 0.03^{\circ}\text{C}$. The vials were stoppered, and an air tube rose above the water surface. Moist cotton was kept in the vials to keep the pupae from desiccating during and after the treatment. Thermocouple records and comparison of twin treatments with single treatments both showed the warm up time to be exactly five minutes. Experiments on the temperature coefficient of the heat phenocopying (MILKMAN, in press) showed that time spent warming up produced no significant effects; it was therefore ignored. The animals were collected as white prepupae at the time of puparium formation. Successive collections shortened the age variation to about half an hour, generally. Treatment came 21 hours later.

FIGURE 1.—The relationship of crossvein-making ability, heat shock during the sensitive period, and the completeness of the posterior crossveins is shown as a nomogram. For example, a fly with intermediate ability ("A") might make normal crossveins under normal conditions but defective ones in response to even slight heat shock.

EXPERIMENTAL RESULTS

The crossveinless strain: The true-breeding crossveinless strain under discussion was selected in the manner described in the introduction. It differed from wild strains in several ways. First and foremost, the posterior crossveins were, on the average, about three fourths missing in each fly. This character showed little variability from fly to fly. In addition, viability was low, judging from observations of general activity, mating behavior, life span, and sensitivity to ether and carbon monoxide. Male genitalia were often rotated, sterility was frequent among members of both sexes, and egg survival was always below 50 percent. Unless humidity was kept close to 100 percent, *cve* larvae pupated in the food instead of on the side of the vial or bottle. Finally, the body and wings were a bit stockier than those of wild type, resembling more the dachs phenotype. These other phenotypic traits may stem from the presence of the *cve* genes or other genes fixed during selection. Nothing further was done with these observations.

In order to carry out a genetic analysis of the strain, the phenotype had to be characterized in quantitative and objective terms. Accordingly, a system of rating each wing as to its posterior crossvein was developed in which the highest reliable degree of visual resolution was taken into account. It was found practical to use a scale from 0 to 6, 6 being total absence of crossvein and 0 being a perfect crossvein. The rating of the wing, then, gave the fraction (in sixths) of crossvein absent. To rate the fly, its two wing ratings were added, making possible a range from 0 (normal) to 12 (complete absence of posterior crossveins). Similarly, a population could be rated by taking an average.

The crossveinless strain averages 9. Although the location of the missing fraction was not taken into account, this strain almost without exception displayed a single small fragment of crossvein on each wing, close to or connected with the fifth longitudinal vein. The expression and penetrance of the *cve* phenotype are temperature sensitive. This can best be demonstrated in *cve*/+ flies, which exhibit more frequent and greater defects at 18°C than at 25°C. The sensitive period is coextensive with the period during which pupae are sensitive to disturbance of crossvein formation by heat shock at 40.5°. In animals raised at 25°, this period was roughly from 13 to 25 hours after puparium formation.

The genetic analysis of this strain began with two preliminary questions:

1. Is a balanced lethal system present? Crossover tests with multiple marker stocks on each of the major chromosomes showed no evidence of inversions in the *cve* stock. Salivary chromosome smears substantiated this conclusion. Therefore we can conclude that the stock was homozygous for all its *cve* genes.

2. Is there a polygenic basis for the *cve* phenotype? The rarity of crossveinless flies in the wild population might be construed as evidence of low frequency of a single gene, of a common gene sensitive to environmental effects on penetrance or viability, or of a polygenic complex.

Early attempts to obtain a true-breeding stock by pair mating the rare cross-

veinless individuals indicate that the first possibility may be discarded, since such matings produced on the average not much greater percentage of crossveinless offspring than was to be found in the wild stock itself (see Table 1).

The response to selection, on the other hand, is clear evidence for the existence of numerous genetic factors. This response manifested itself in the three ways already discussed: susceptibility to phenocopy induction rose, frequency of crossveinless flies increased, and expression also increased.

To further demonstrate the polygenic nature of this system, and to ascertain the degree of environmentally dependent variation, a series of crosses was made, beginning with reciprocal outcrosses to inbred "isogenic" Oregon R flies. The use of Oregon R was determined by the desire to introduce as little variation into the system as possible. Although the Oregon R stock undoubtedly differs more from the *cve* stock than does the ancestral wild stock, the obvious genetic variability of the latter seemed a difficult thing to cope with.

The results of these and subsequent crosses are given in Table 2 and are shown in graphic form in Figure 2. By the use of the rating system, it was seen that the

TABLE 1

*Incidence of untreated crossveinless flies in a wild Massachusetts strain from which the *cve* strain was ultimately derived*

Generations	Female	+	Male	Female <i>cve</i>	Male <i>cve</i>	Female	Percent <i>cve</i> Male	Combined
	544		561	1	0	0.2	0.0	0.1
				Inbreeding				
1	...		...	...	...	...	...	...
2	277		284	0	0	0	0	0
3	742		769	5	1	0.7	0.1	0.4
				Pair matings of untreated crossveinless flies				
F ₁	378		329	2	1	0.7	0.3	0.5
F ₂	1010		1025	2	6	0.2	0.6	0.4

TABLE 2

*Results of crosses involving *cve* and Ore R.*

Parent	Designation of	N	Females	Percent	r	r'	N	Males	Percent	r	r'
Female	Male		<i>cve</i>	<i>cve</i>				<i>cve</i>	<i>cve</i>		
Ore R × <i>cve</i>	F ₁ a	816	150	18.4	0.355	1.93	769	5	6.4	0.013	2.00
<i>cve</i> × Ore R	F ₁ b	219	27	12.3	0.228	1.85	162	18	11.1	0.172	1.55
F ₁ a × <i>cve</i>	B ₁ a	160	110	68.7	3.44	5.01	205	75	36.8	2.15	5.88
<i>cve</i> × F ₁ a	B ₁ b	200	126	63.0	3.25	5.16	231	134	58.0	3.87	4.57
<i>cve</i> × B ₁ a or B ₁ b	B ₂	361	306	84.8	6.03	7.12	301	232	77.1	5.76	7.46
F ₁ a × F ₁ a	F ₂	293	66	22.5	0.88	3.90	309	36	11.6	.55	4.75
	(<i>cve</i>)	89	89	100	9.11	9.11	95	95	100	9.17	9.17

r = Average rating, all flies.

r' = Average rating, crossveinless flies.

FIGURE 2.—Results of the crosses referred to in Table 2 are plotted as frequency distributions of crossvein defect ratings. Since the normal ("0") flies are usually by far the most numerous, they are plotted on a scale running from 0 to 100 percent. The other classes, representing various degrees of crossvein defects, are on a scale from 0 to 10 percent or 0 to 20 percent. Further explanation in text.

results were characteristic of a polygenic complex, in that repeated backcrosses brought the average crossvein defect up toward the level of the *cve* parents. The distinction between accumulation of more *cve* genes per fly and higher incidence of a single gene in the class is clearly made by the increased average expression of the crossveinless individuals as the backcrosses are made.

It will be seen in Table 2 that the more *cve* genes there are, the higher the expression and penetrance of the *cve* phenotype. Furthermore, within the scope of the phenotype, the genes seem to be acting superadditively. For example, the F_{1a} and F_{1b} females should have half as many *cve* genes as the *cve* females, yet their average ratings are well below one tenth that of the *cve* females.

On the other hand, a glance at some of the distributions in Figure 2, notably those of the F_1 's, gives the impression that they are truncated—artificially cut off by the limits of the rating scale. Moreover, there is a basis for believing that many of the "0" flies should be rated on the minus side of the scale; this would be rating them on the basis of their crossvein-making ability, whose units would be taken from the scale already set up for the range of visible variation, the crossvein defects (see Figure 3).

It would be well to pause long enough to set forth in some detail the implications both of the term "crossvein-making ability", and of its relation to the rating system. It is here postulated, and supporting evidence will soon be given, that

FIGURE 3.—The frequency distributions of ratings of females from *cve* $\times$ Ore R and from Ore R $\times$ *cve* crosses look like truncated portions of a normal distribution. This fact, suggests that these flies may be normally distributed with respect to a broader variable, crossvein-making ability. Conformity to such a truncated portion is demonstrated by the use of probits, as shown in Table 3 and Figure 4.

all the *cve* genes act on the same prerequisite of posterior crossvein formation. It may be the synthesis of a substance, the mobilization or alteration of cells, or any one of a number of processes; what is important to us is that the degree to which it is carried out has relevance to the ultimate form of the posterior crossvein. It is this process which is the physiological counterpart of the term "crossvein-making ability" used here.

As STERN (1943) and HOUSE (1953) have shown so clearly in their respective studies on gene interaction relating to wing venation, the structural reflection of developmental change may be a threshold phenomenon. Thus, no visible manifestation of various levels of a hypothetical substance "P" was seen until a threshold was crossed. Then vein defects appeared. The three genes, recessive and superadditive with respect to the vein defects, were shown to be semi-dominant and additive with respect to the underlying process which HOUSE chose to call the synthesis of "P".

A similar problem is faced with the *cve* genes. Here an attempt will first be made to delineate the norm of reaction mathematically, in order to have a way of better understanding the *cve* genes. Later, an attempt will be made to corroborate the physiological reality of this norm of reaction by an attack from a different direction.

Now, finally, one more thing must be said about the rating system before the data are presented. The rating units are set up in the simplest empirical way; they correspond to fractions of missing crossvein. But there is no reason to assume

that the amount of crossvein made is linearly proportional—threshold or no—to any underlying developmental process. That remains to be demonstrated. What is to be hoped is that in the quantitative terms of the underlying process the difference between a “1” fly and a “2” fly is the same as between a “6” and a “7”.

One way of demonstrating that this is the case is to show that the variation in rating is normally distributed. This normal distribution would best be sought in the absence of genetic variation, since, if the number of genes involved were small, genetic variation would introduce several modes into the distribution.

If the rating system is internally consistent, it may be extrapolated beyond the “0” threshold. If the genes can now be shown to behave additively (with respect both to other genes and to their homologs, i.e. without dominance), then one can infer that these *cve* genes determine the norm of reaction relating to one aspect of crossvein formation.

Since the evidence is strong that the *cve* genes are homozygous, and since the Oregon R stock is highly inbred, the F_1a should have no relevant genetic variation. The normality of its rating distribution may be tested by converting frequency into probits, as is done in Table 3. These transformed data should fall on a straight line if the distribution is normal, and they do, as is seen in Figure 4. The line is extrapolated to the probit value of five, where the mean may now be measured. It is -1.75 , a value for the norm of reaction of this genotype in rating units. Standard deviation is graphically estimated at 2.75 units.

If the heterozygous *cve* genes of the F_1 now segregate in a backcross or F_2 , a multimodal distribution should be seen. That this happens is shown in Figure 2. For the F_1 's, however, even where the data show very few crossveinless individuals, it seems reasonable to assume that the hypothetical ratings are distributed normally on the other side of the threshold, so that the norm of reaction can be calculated from the few positive ratings available.

Since the backcross data were unsuitable for probit transformation, visual extrapolations were made instead, to compensate for the truncation of the distribution. The average of the norms of reaction corresponding to the various genotypes in the backcross progeny could then be roughly estimated.

TABLE 3
Conversion of F_1a female rating frequencies into probits

Rating class	Frequency	Cumulative frequency	Percent	100—%*	Probits
7	1	1	0.12	99.88	8.04
6	2	3	0.37	99.63	7.68
5	1	4	0.49	99.51	7.58
4	9	13	1.59	98.41	7.15
3	30	43	5.26	94.74	6.62
2	34	77	9.45	90.55	6.32
1	74	151	18.50	81.50	5.90
0	665	816	100.	0	...

* This step is unnecessary but was done because the probit table used has an extra decimal place above 98 percent.

FIGURE 4.—Transformation of a normal curve into a straight line is done by converting variate units into units of standard deviation plus 5. Points obtained from similar treatment of data from F_1 females are fairly close to a straight line. The mean is where the line intersects the probit value of 5, and the standard deviation in rating units is graphically demonstrated as the horizontal deflection of the line as it moves one probit unit.

The rating of the parental *cve* stock could be computed merely by averaging individual ratings, since none of these flies were wild type. The assumption is made in doing this that here, too, the rating units are equivalent to the units lower down on the scale.

Additive action of the cve genes: As may be seen from Table 2, differences in the average ratings of F_1 a and F_1 b males suggest that a sex-linked *cve* factor is present. This fact has a bearing on the choice of data to examine in order to find out whether or not the *cve* genes are acting additively on a process underlying crossvein development. If the genes are acting additively, there should be a quantitative correlation between corrected ratings and number of *cve* genes.

From the F_1 and backcrosses, progeny classes can be chosen for comparison so that one is genetically intermediate between the other and the *cve* parents of one sex. If the corrected ratings follow the same pattern, the *cve* genes may be assumed to act additively. If this is the case, and since the F_1 females are genetically intermediate between the Oregon R females and the *cve* females, the norm of reaction for Oregon R with respect to this aspect of crossvein formation can be calculated.

Table 4 shows two series of three progeny classes each and their ratings. The middle member of each series is exactly intermediate genetically between the other two. It will be seen that the ratings follow this quantitative relationship

TABLE 4

Relation of cve chromosomes to corrected rating

Sex	Sets of <i>cve</i> autosomes	Sets of <i>cve</i> X chromosomes	Class	Corrected rating
f	2	2	<i>cve</i>	+ 9.11
	1½ (avg)	1½ (avg)	B ₁ a	+ 3.25
	1	1	F ₁ a, F ₁ b	- 2.
m	2	1	<i>cve</i>	+ 9.17
	1½ (avg)	½ (avg)	B ₁ a	- 1.
	1	0	F ₁ a	- 8.

Calculation of rating of parental Ore R flies

Sex	Sets of <i>cve</i> autosomes	Sets of <i>cve</i> X chromosomes	Class	Corrected rating
f	2	2	<i>cve</i>	+ 9.11
	1	1	F ₁ a, F ₁ b	- 2.
	0	0	Ore R	-13. (calc)
m	2	2	<i>cve</i>	+ 9.17
	1	½ (avg)	½ (F ₁ a+b)	- 5.
	0	0	Ore R	-19. (calc)

fairly well also. We may say, then, that the *cve* genes do act additively, and the crossvein-making ability of the Oregon R flies may now be calculated. Table 4 shows the series that are used. The F₁ females, -2, are intermediate between the *cve* females, +9, and the Oregon R females. Since $+9 - (-2) = 11$, then $-2 - 11 = -13$, the value for Oregon R females. The Oregon R females, then, have 13 units of crossvein-making ability (c.m.a.) in excess of their needs.

For the males, the values of the F₁ males are average at -5. The *cve* males are +9. Since $+9 - (-5) = 14$, then $-5 - 14 = -19$, the value for the Oregon R males. The farther a c.m.a. value is from 0, the greater the reserve ability to carry on this aspect of crossvein formation. This rating difference between Oregon R males and females reflects a fact we already know, and indeed the calculated value results from it: that many fewer F₁ males than females are crossveinless.

Corroboration of the values calculated for the norms of reaction of Oregon R males and females: In order to give substance to these numbers, -13 and -19, brief mention may be made of an experiment which corroborates these estimates from an entirely different angle. Fuller discussion will come in a later paper (MILKMAN, in press).

The norms of reaction of the Oregon R flies given above were measured with respect to a certain process underlying crossvein development. If this same process could be altered by nongenetic means, a study of the relationship of treatment dose to response should enable us to calculate the same norm of reaction.

When 23-hour pupae are treated at 40.5°C, no change in adult morphology is

noted unless the treatment lasts longer than about 25 minutes. Above this threshold, response (crossvein defects) increases with duration, apparently linearly. Each sex has its own rate. Extrapolation of these curves back to zero time gives the following values for the norm of reaction. Oregon R males, -22 ; females, $-12\frac{1}{2}$. These values compare well with -19 and -13 , respectively.

Roughly, then, we can quantify a physiological state, whose nature we know nothing of, except that it is one measure of a fly's ability to make posterior crossveins. The further below zero it is, the more reserves the fly had in the face of outside interference. This state is quantitatively controlled by the genes which make up the *cve* complex, and a diagram of the relationships involved is given in Figure 5.

Evidence for the multichromosomal nature of the cve complex: The F_1 data have already shown that *cve* factors are present both on the X chromosome and on one or more autosomes. A simple demonstration of the involvement of all three major chromosomes comes from the following experiment: *cve* females were mated to *Cy/Pm D/Sb* males. *Cy/Pm D/Sb* is a multiple inversion stock containing Curly and Plum in repulsion on the second chromosomes and Dichaeete and Stubble in repulsion on the third chromosomes. These dominant markers affect wing shape, eye color, wing position, and bristles, respectively. They are all lethal when homozygous. From the above cross, as is illustrated in Table 5, several crossveinless males were taken and mated back to *Cy/Pm D/Sb* females. The crosses gave rise to progeny whose unmarked second and third chromosomes were uniform and contained whatever *cve* factors were present in the

FIGURE 5.—Under normal conditions, crossvein-making ability is directly translated into crossveins. One may choose a degree of c.m.a., which is a norm of reaction, see the crossvein rating the fly would have, and see this expressed visually as the fraction of crossvein absent.

TABLE 5
The BL crosses

Original cross:	+/+	Cy/Pm	D/Sb	×	cve/	cve/cve	cve/cve
Backcross:	+/cve	M/cve	M/cve	×	+/	Cy/Pm	D/Sb
Final cross:	Back cross progeny			×	+/	+/+	cv-c/cv-c
Possible genotypes							
Chromosome X	Backcross progeny II	III		X	BL progeny II	III	
+/	M+/cve	M+/cve		+/	M/+	M/cv-c	
+/+	M+/+M	M+/+M		+/+	cve/+	cve/cv-c	
cve/				cve/			
cve/+				cve/+			

M stands for Cy, Pm, D, or Sb.

parental second and third chromosomes. Also, the females had an X chromosome from the *cve* grandmother.

It was desired to test the contribution of each of the major chromosomes by mating these flies in such a way as to permit measurement of the *cve* effect of each unmarked chromosome. The partial dominance of the *cve* genes made crossing back to the *cve* flies undesirable.

At this point, advantage was taken of the interaction of the *cve* complex with *crossveinless-c*, a completely recessive third chromosome mutant which produces a crossveinless phenotype. Evidence presented in the second paper in this series shows that the *cv-c* locus is probably not involved in the *cve* complex. The *cv-c* allele used here is certainly not. This interaction proved valuable in providing a uniform booster which enabled small numbers of *cve* genes to have a morphological effect. An illustration is given by the crosses under discussion.

When a group of Marker/*cve* sisters or a group of brothers was mated to *cv-c* flies, the markers and *cve* genes segregated in such a way as to reveal the contribution of each chromosome to the *cve* complex. These crosses were designated BL. Table 6 lists the average ratings of the progeny of these crosses, which are not compensated for sub-zero values. It may be seen from Table 6 that each *cve* chromosome has an effect both individually and in combination with others. The contribution of the X chromosome may be seen by comparing the data for males of crosses 1, 2, and 3, whose mothers were heterozygous for the *cve* X chromosome, with the data for males of cross 4, whose mothers did not supply their *cve* factors.

The frequency distributions were not all normal, so that the probit method of estimating the true means could not be used, but the averages give some idea of the relative contributions of each chromosome. The second and X chromosomes seem equally important, the third about twice as important as either.

Surely two of the outstanding properties of the crossveinless complex are the interaction of the components with one another and the similarity of their inter-

TABLE 6
Results of BL crosses

Crosses 1, 2, 3									
$\frac{M +}{+ cve}$		$\frac{+ M +}{++ cve}$	$\frac{++}{+ cve}$	$\times$	$\frac{++}{++}$	$\frac{cve ++}{cv-c ++}$	$\frac{+}{+}$	50% of males get <i>cve</i> X	
								50% of females get <i>cve</i> X	
Cross 4									
$\frac{++}{++}$		$\frac{cv-c ++}{cv-c ++}$	$\frac{+}{+}$	$\times$	$\frac{M +}{+ cve}$	$\frac{+ M +}{++ cve}$	$\frac{cv-c +}{+}$	no males get <i>cve</i> X	
								50% of females get <i>cve</i> X	
Average ratings of BL progeny									
Marker Class	Cross	50 percent with <i>cve</i> X chromosomes		None with <i>cve</i> X chromosomes	<i>cve</i> Autosomes	Pooled average ratings			
		Female	Male	Male		Female 1,2,3	4	Male 1,2,3	4*
++	1	8.36	9.00						
	2	8.19	8.57						
	3	7.88	8.21		II, III	8.19	8.17	8.62	5.50
	4	8.17		5.50					
Cy +	1	5.17	6.53						
	2	6.65	7.65						
	3	4.00	5.60		III	5.84	5.60	7.00	1.33
	4	5.60		1.33					
Pm +	1	5.61	6.72						
	2	6.28	6.54						
	3	5.64	7.05		III	5.84	5.61	6.72	1.73
	4	5.61		1.73					
+ D	1	...	...						
	2	0.11	0.89						
	3	1.50	3.00		II	0.42	1.00	1.00	0.40
	4	1.00		0.40					
+ Sb	1	2.36	6.15						
	2	0.94	2.40						
	3	3.32	7.23		II	2.06	...	4.32	...
	4	...	...	...					
Cy D	1	0	0.65						
	2	0.08	0.48						
	3	0	0.67		..	0.03	0.57	0.58	0
	4	0.57	...	0					
Pm D	1	0.04	0.39						
	2	0.24	0.24						
	3	0.00	0.75		..	0.10	0.13	0.39	0
	4	0.13	...	0					
Cy Sb	1	0.13	0.52						
	2	0.42	1.81						
	3	0.13	1.36		..	0.25	...	1.16	...
	4	...	...	...					
Pm Sb	1	0.26	0.94						
	2	0.20	1.14						
	3	0.19	1.66		..	0.22	0.50	1.18	0
	4	0.50		0					

* The cross 4 males carry no *cve* X chromosomes.
The average crossvein defect rating of a class of flies shows the influence of each of the major chromosomes.

actions with *cv-c*. For, in examining the results of the BL crosses, Table 6, one sees the two lines of evidence that there are *cve* genes in each of the major chromosomes. One is that in classes with any one of these three chromosomes unmarked, one finds some crossveinless flies. And the other is that if one compares any two classes differing only by one marked chromosome, no matter which, the class with the extra marked chromosome has the lower average rating. This may also be seen in crosses not involving *cv-c*. Table 7 lists the percentages of crossveinless flies in the results of an early experiment done before ratings were used.

The above approaches to the *cve* complex have not been of the type likely to result in information about the individual genes. Having found some of the general facts—that the genes are on all three major chromosomes, that they are semidominant and act additively in the determination of a state underlying crossvein formation—it would now be well to consider two specific approaches to the more precise characterization of the genes involved. These are (1) interaction with genes known to cause crossvein defects, and (2) crossover tests. Results of these approaches will be discussed in the next paper in this series (MILKMAN, in press).

Discussion of the results presented here will be deferred to the next paper of this series.

TABLE 7

Results of inbreeding Marker/cve heterozygotes

(<i>cve</i>)	<i>Pm</i> /+	<i>Sb</i> /+	×	(<i>cve</i>)	<i>Cy</i> /+	<i>D</i> /+		
Grouped by second chromosome marker classes								
Second chromosome	1.				2.			
	+		<i>cve</i>		+		<i>cve</i>	
	F	M	F	M	F	M	F	M
<i>Cy/Pm</i>	9	8	17	13	6	4	0	1
<i>Cy</i> /+	7	10	21	18	4	1	6	7
+/ <i>Pm</i>	8	7	22	17	5	4	3	2
+/+	0	0	0	0	0	1	2	1
Grouped by third chromosome marker classes								
Third chromosome	1.				2.			
	+		<i>cve</i>		+		<i>cve</i>	
	F	M	F	M	F	M	F	M
<i>D/Sb</i>	17	17	3	3	10	4	0	0
<i>D</i> /+	2	5	20	17	1	0	3	1
+/ <i>Sb</i>	2	3	21	18	4	6	2	7
+/+	0	0	16	10	0	0	6	3

SUMMARY

1. A true-breeding stock with polygenically caused posterior crossvein defects has been selected from a wild strain of *Drosophila melanogaster*.
2. The genes which cause crossvein defects also increase susceptibility to crossveinless phenocopying by heat.
3. The polygenes are on all three major chromosomes.
4. A character underlying posterior crossvein formation and affected by the crossveinless polygenes is defined as crossvein making ability. In terms of it, the polygenes can be seen to be acting additively. The reserve crossvein making ability (in excess of the normal need) can be measured both by analysis of crosses and by analysis of phenocopy dosage-response data.
5. The use of "booster" genes to render detectible genes with quite small effects is discussed.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the effective guidance of PROFESSOR R. PAUL LEVINE in this work.

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