THE GENETIC BASIS OF NATURAL VARIATION.

X. RECURRENCE OF *cve* POLYGENES^{1,2}

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ALLELES with simple phenotypic correlates, notably electrophoretic mobility variants of enzymes, have now been shown in many cases (e.g., LEWONTIN and HUBBY 1966; PRAKASH and LEWONTIN 1968; O'BRIEN and MACINTYRE 1969) to participate in recurrent polymorphism: the occurrence of the same two or more alleles in each of many, widely separated natural populations. Similarly, recurrent polymorphism at the gross phenotypic level is well known (FORD 1965), and it can sometimes be related to polymorphism at a single gene locus.

On the other hand, less is known about the great middle ground of natural variation, where phenotypic variation is associated most directly with combinations of alleles rather than with individual alleles. Here, phenotypic variation assumes many common forms, but the genetic basis of each variant may not be constant at all. Some traits, like size and fertility, are sensitive to allelic variation at a vast number of loci; but others, including crossvein defects in *Drosophila*, seem to monitor a workably small number of genes (MILKMAN 1960a,b). Posterior crossvein defects are rare but ubiquitous in *D. melanogaster*: they have been seen in the progeny of every natural population in which they have been sought (MILKMAN 1964). Other *Drosophila* species are similar in this regard (TIMOFÉEFF-RESSOVSKY 1934; DUBININ 1948; PREVOSTI 1951). The crossvein defects observed in the progeny of wild *D. melanogaster* have a polygenic basis, as demonstrated by the analysis of true-breeding crossveinless strains derived from them (MILKMAN 1962, 1964). Furthermore, three true-breeding strains, each derived from a wild inseminated female caught in a fruit market, all turned out to contain the same *cve* genes (MILKMAN 1965b).

There is, however, abundant evidence for the existence of numerous sets of *cve* polygenes (MILKMAN 1962, 1964, 1965b). Do these sets coexist? What is the duration and area over which a given set can be extracted?

The present paper describes the comparative study of 12 true-breeding crossveinless strains, each selected from a progeny of a single female in one of numerous samples taken over a 5-month period from two wild populations. Evidence was sought for a repeated occurrence of *cve* alleles at the same loci.

¹ This paper is dedicated to Professor THEODOSIUS DOBZHANSKY on the occasion of his 70th birthday.

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MATERIALS AND METHODS

Beginning in June, 1966, DR. PHILIP T. IVES sampled two Amherst, Mass., populations of *D. melanogaster* and sent me 12 females each week until the end of the season (September in one population, November in the other). These populations are named "Sam," for Sammy's Fruit Stand, and "Mark," for Markert's Apple Storage. The two sites are about 3 miles apart. DR. IVES reports that there was no obvious breeding site at or near the fruit stand; the apple dump is, of course, one vast and highly productive breeding site. Each female was isolated and allowed to produce progeny; in general, almost all wild-caught females have been inseminated, and most are presumably carrying sperms from one male only.

The first generation progeny flies were allowed to develop at 18°C, where penetrance and expressivity of crossvein defects are generally greater, and examined where possible. In each line 200 second generation flies were scored for crossvein defects. Lines with 1% or more cve flies at 18° are likely to respond to direct selection and eventually produce true-breeding, polygenic strains with extreme crossvein defects even at 25°, while those with fewer than 1% cve flies at 18° rarely do. Accordingly, lines with 2 or more cve flies per 200 were saved for selection, those with 1 were saved for reexamination, and those with none were discarded.

More than 400 lines were examined, and the first 12 true-breeding crossveinless strains developed were used in the study. Observations were made at 25°C unless otherwise noted.

The preliminary experiments followed the original program of the identity test (MILKMAN 1965b): two cve strains were mass mated, passed through several generations without selection, and then selected back in the direction of the normal phenotype. Failure to respond to selection indicates genetic identity at the relevant loci. After this procedure revealed that two lines, one derived from a June collection (M29) and one from November (M237), were identical, a modified procedure was evolved which produced more detailed information about similarities among other strains. Pair matings were now made between strains, and the F_1 flies were selected for *high* expressivity. Thus flies might be obtained that were homozygous for alleles which were present in both parental strains but fixed only in one or in neither. Downward selection began in the F_2 and continued until the appearance of the F_{10} , or until at least half the flies had normal crossveins at 18°C.

In this way, 8 strains were crossed in duplicate in each direction. Six were from the Amherst populations (3 each from "Mark" and "Sam," strain numbers prefixed M and S, respectively). To these were added strains derived from more distant populations, one from Syracuse (E'; MILKMAN 1964, 1965b) and one from South Lancaster, Mass., about 50 miles east of Amherst (SL-8; MILKMAN 1965b). Beginning with the F_2 's, two vials were made up with 4 males and 4 females, not necessarily virgin, and selection for the normal phenotype was pursued until the F_{10} was obtained, or until at least half the flies were normal at 18°.

Later, crosses were made between all remaining pairs of all 12 Amherst crossveinless strains. Initially up to 10 crosses were made between each pair; selection lines were discarded as soon as a substantial response was obvious (3 of 20 flies having normal crossveins at 25°C, or an average rating below 7).

Standard culture methods and manipulative procedures were employed. Defects in the posterior crossveins were quantified in terms of imaginary 6ths, irrespective of position. Thus a fly would be rated from 0 (normal) to 12 (crossveins completely absent on both wings). Ten flies of each sex were rated routinely, and the average rating for all flies with defects (r') and for all flies (r) was computed for each sex. In the present paper, some tables display r averaged for both sexes. At 18°, ratings average about 6 units higher than ratings of similar flies at 25° (within the limits of the range). For this reason, as an approximation 6 can be subtracted from the observed ratings of flies at 18°, increasing the range to 18 units, running from -6 to 12.

The *Cy/Pm;D/Sb* stock, obtained from J. D. MOHLER, contains the following inversions: *In(2LR)SM1* (with *Cy*), *In(2LR)bw^{V1}* (with *Pm*), *In(3LR)Dcx^F* (with *D*), and *In(3R)Mo* (with *Sb*). The last-named inversion is not effective in reducing crossing over on the left arm of the third chromosome. Further information on the inversions may be found in LINDSLEY and GRELL (1967).

Additional, more specific procedures will be described along with the results of certain experiments.

RESULTS

The initial observations on the progenies of the wild females reveal the same sporadic occurrence of posterior crossvein defects previously observed (MILKMAN 1962, 1964), although frequencies of *cve* flies are lower than in previous studies, where they averaged about 0.7%. Table 1 summarizes some aspects of these

TABLE 1
Occurrence of cve flies in the progeny of wild inseminated females

Population	Generation	Strains (N)	Total flies (N)	cve flies (N)	% cve
Mark	F ₁	182*	9,091	16	0.18
Sam	F ₁	116*	6,537	26	0.40
Mark	F ₂	268	49,332	106	0.21
Sam	F ₂	187	34,611	112	0.32

* Not all the F₁ strains were examined for cve.

observations. After roughly 10 to 20 generations of selection, beginning at 18°C and moving to 25°C, the true-breeding strains were obtained and eventually crossed in all combinations.

In the preliminary experiments, crosses between Mark 29 and Mark 237 invariably responded very little to selection. In about 20 crosses, followed by selection for wild type for up to 12 generations, the average rating never dropped below 6.5, and it often stayed above 10. Many individual crossveinless strains studied in the past never achieved an average rating above 10. In contrast, Mark 29 and Sam 10 invariably produced progeny with low ratings—or nearly normal crossveins—and quickly lost most of their remaining expression. Other crosses were not so clear cut. In some cases, there was little or no response for several generations, followed by a gradual or abrupt loss of expression.

The modified procedure, involving upward selection in the F₁, was now employed, and the results are shown in Table 2. Since this table summarizes a rather large body of information, it requires some explanation. First, it is seen that within strains (diagonal) there is essentially no response to selection. The ratings remain high. In other lines, too, high ratings indicate the absence of response to selection and therefore the similarity of parents with respect to a set of *cve* alleles. The Roman numerals, in contrast, indicate dissimilarity. They represent the generation after which selection was discontinued because it had gone quite far: specifically, at least half the flies (including at least 40% of each sex) were wild type, even when raised at 18°C. A third category of lines is characterized by ratings between 6 and —4. These lines may have some *cve* alleles in common, but not a complete set.

These results should be contrasted with the observation that, with the exception of Mark 29 and Mark 237, all other combinations when mass mated pro-

TABLE 2

Ratings of tenth generation flies (arabic) or generations of discontinuance (roman) due to essential return to wild type

Strain of female parent	Strain of male parent							
	M29	M119	M237	S10	S118	S177	SL-8	E'
M29	11.0	11.5	10.2	V	11.5	VI	IX	V
	10.9	11.4	11.6	V	11.4	VI	VI	IX
M119	VII	10.0	9.4	VIII	11.4	3.2	2.6	X
	11.6	9.7	10.6	VII	3.6	-1.3	1.6	IX
M237	10.8	VIII	11.4	VII	V	VII	V	VI
	10.4	X	11.5	VI	V	V	VIII	V
S10	V	VI	V	12.0	0	10.4	V	-1
	V	VI	V	12.0	X	VIII	X	VII
S118	VII	11.4	11.0	-3	10.0	2.6	VI	X
	11.5	11.2	1.8	X	8.9	10.9	VIII	-4
S177	V	-2.4	V	VII	-0.9	8.6	X	0
	VI	1.5	X	VIII	2.0	11.5	X	-3.7
SL-8	-2	IX	-2	VI	11.0	VII	8.9	0
	V	11.2	-4	V	6.5	1.1	8.8	0
E'	VI	X	X	VII	0.3	-0.4	5.4	11.0
	VI	-3	VI	VII	X	VIII	VII	11.4

Each member represents a selected line on which ten flies of each sex were rated.

duced progeny capable of responding to selection for wild type and yielding a largely wild-type population, at 25° at least, after a few generations of selection. Thus the results shown in Table 2 suggest a good deal of relevant variation within at least some of these fixed strains, namely, unfixed *cve* alleles additional to a complete set of fixed *cve* alleles, since some individual hybrid lines did not respond to back selection. Table 3 lists the F₁ ratings of the crosses in Table 2. The very low F₁ ratings are associated with rapid response in Table 2. High F₁ ratings guarantee nothing, however; response to selection may be great or small.

The data in Table 2 suggest that strains M29, M119, M237, and S118 have *cve* alleles in common. Of the 24 crosses involving pairs of these strains, 16 resulted in F₁₀'s with average ratings above 9, showing essentially no response to selection. The other 8 F₁₀'s are quite low, however, resembling the progeny of mass matings selected downward from the start.

These results are consistent with the hypothesis that (aside from the common homozygous *cve* set shared by M29 and M237) unfixed *cve* alleles are contributed by each parent to produce some homozygous offspring in the F₁. If the *cve* alleles are unfixed, it follows that some parents will be missing some altogether and thus not be capable of contributing the particular necessary *cve* alleles. Thus not all crosses produced nonresponding lines.

By the same token, some parents might be homozygous for the relevant *cve* alleles, and some few pairs might, therefore, produce an F₁ uniformly homozygous for a set of *cve* alleles. (The probability of this occurrence would be greater if the variability were limited largely or entirely to one parental strain). To test

TABLE 3
F₁ ratings of lines described in Table 2

Strain of female parent	Strain of male parent							E'
	M29	M119	M237	S10	S118	S177	SL-8	
M29	11.8	9.4	10.6	1.8	6.8	7.3	2.1	2.8
	11.2	8.3	10.9	1.0	11.6	7.6	9.6	1.7
M119	8.7	9.8	7.4	8.4	10.4	10.4	9.7	6.2
	9.4	10.4	6.7	n.c.	9.2	11.6	5.1	10.8
M237	11.2	11.1	11.4	0.6	0.2	6.4	0.7	1.0
	11.3	4.2	11.4	0.7	6.0	3.0	0.8	0.8
S10	5.4	10.3	5.4	11.9	9.4	11.6	9.8	9.2
	3.0	10.8	5.6	11.8	9.4	12.0	10.6	8.8
S118	1.6	11.0	5.7	9.8	11.6	9.2	5.8	5.7
	5.8	10.4	5.6	9.6	9.6	11.3	2.0	7.4
S177	6.2	11.4	2.5	10.8	9.2	11.6	6.4	6.6
	5.5	10.8	6.3	12.0	9.0	12.0	5.7	8.1
SL-8*	0.4	7.9	3.6	8.0	11.0	9.1	9.2	7.1
	0.7	8.0	8.0	8.4	10.2	10.6	n.c.	8.3
E'*	3.2	5.5	1.2	5.7	9.6	10.9	9.3	11.0
	7.0	5.6	0.6	9.9	9.6	9.3	7.8	11.3

n.c.—not counted, but used to make F_2 .

* SL-8 has a strong sex-linked *cve* allele and in these crosses, E' also shows a strong X-linked contribution, so ratings on these lines are for female progeny only. The male averages were all between 9.4 and 12.0.

this possibility, 37 pair matings between M29 and M119 (about half in each direction) were analyzed. The F_1 results are listed in Table 4. As usual, 10 flies of each sex were rated, but a combined average is given because sex differences were minor.

Several lines were now chosen for further analysis. First, two high pairs and two low pairs were taken from each of the chosen F_1 , so that four F_2 's resulted. The combined average ratings are reported in Table 5, from which the following conclusions may be drawn: First, since some upward selected F_2 's have rather low ratings and contain flies with normal crossveins, true-breeding *cve* lines are not to be expected in every case. Second, the difference between high and low F_2 's from a single F_1 confirms the existence of genetic variation within some F_1 's. And third, cases of very high ratings in both high and low F_2 's suggest that some of the original pairs were homozygous in common for a complete set of *cve* genes.

The array of modified identity tests was now completed by crossing the 12 *cve* strains in all remaining combinations. The preparation of up to 10 pair matings per cross (as opposed to 2 or 3 in the previous large experiment) permitted a better choice of F_1 's for upward selection. Nevertheless, only a few pairs of strains shared *cve* alleles at enough loci to produce a true-breeding strain (Table 6). At most, three promising lines were chosen for selection, except S48, M225 (four) and M119, M225, where a second trial was thought useful. Sometimes the F_1 flies were all wild type or nearly so; in other cases, the F_2 flies had rather low ratings.

TABLE 4

Average ratings of F_1 flies from pair matings

Cross	No.	Rating	Cross	No.	Rating
29×119	1	5.2	119×29	1	7.6
	2	6.4		2	7.6
	3	9.4		3	6.9
	6	7.3		4	7.4
	7	10.4*		6	8.9
	8	7.4		7	6.1
	9	7.6		8	7.6
	11	7.6		9	9.0
	12	6.9		10	6.9
	13	8.2		11	7.6
	14	6.0		13	6.9
	16	8.2		14	7.0
	18	7.4		15	6.0
	19	7.0		16	9.5
	21	11.3*		17	8.8
	22	10.6*		18	6.9
	24	7.6		19	8.6
				20	5.6
				21	8.8
				23	10.0

* Apparent high strains.

TABLE 5

Ratings of high and low selected F_2 's from F_1 's in Table 4

Cross	Ratings of F_1 pair	F_2 rating	Cross	Ratings of F_1 pair	F_2 rating
29×119 (1)	10-12	8.2	29×119 (11)	11-11	9.0
	11-10	8.8		10-11	9.2
5†	2-0	5.4	1†	2-2	9.6
	0-0	3.8		0-2	6.6
29×119 (2)	11-12	12.0*	29×119 (21)	12-12	11.2*
	11-10	10.5*		11-12	11.6*
17†	5-3	6.2	2†	10-10	10.6*
	3-1	5.0		10-10	10.4*
29×119 (3)	12-12	6.4	119×29 (4)	10-11	10.6*
	12-12	6.6		10-10	10.4*
1†	5-3	6.0	3†	2-0	3.4
	6-0	6.8		0-0	9.5
29×119 (6)	9-11	7.1	119×29 (19)	11-11	9.8*
	9-10	10.6*		10-10	10.3*
6†	3-4	5.7	1†	8-8	8.4
	3-0	6.7		8-3	10.6*

* No "0" flies in sample.

† Total number of similar examples, including the one presented.

Either of these results led to the discarding of the lines before the F_3 , and this is referred to in Table 6 as "0" generations of selection. As a result of this experiment, two additional lines, M225 and S48, were seen to have some *cve* alleles in common with the four already noted, M29, M119, M237, and S118.

In order to determine which strains were carrying the unfixed *cve* alleles, numerous crosses were next set up involving 5 females and 1 male. The females were subsequently separated and their progenies counted. Two sorts of differences were possible: within sets of 5 females, and between sets of 5 females. The former would point to variation within the maternal strain and the latter to variation within the paternal strain. A typical set of data is illustrated in Table 7. Here strain S118 proves to be variable. In other cases, both parental strains can be seen to vary in their contributions to an F_1 , even though each breeds true for the *cve* phenotype. From similar sets of data, the relationships listed in Table 8 were concluded. Note that variation relevant to one strain may not be relevant to another.

The true-breeding strains derived from crosses between M29 and M119 were now intercrossed, and they proved to be indistinguishable: 10 pairs of 29/119 (or 119/29) strains were mass mated and propagated without selection for 4 generations. There was essentially no change in any line.

To learn more about the genetic composition of the various nonresponding strains derived from crosses between two strains, some 10th generation strains were mass mated back to both parental strains and to some other *cve* strains as well, for comparison. Downward selection was begun *at once* and carried on for a total of 5 generations of selection, or until a large response was observed. The mixed strains might be identical in fixed *cve* alleles to one parent, to both, or to neither. The results are summarized in Table 9. Crosses of this type also help rule out certain types of procedural errors as the possible basis of the results reported in Table 2.

Before discussing the observations recorded in Table 9, one event should be mentioned. Strain S118 became fixed for the *cve* allele(s) that completed its "M29" set: now, when crossed to M29 or to M237 it invariably produces true-breeding progeny. (In crosses to other strains, M29 and S118 remain clearly different, however). The exact time of this fixation, with respect to the data now to be reported, is uncertain. Thus similarities in the results of crosses to M29 and S118 cannot be interpreted unambiguously. One feature of the results, however, is quite clear: any true-breeding hybrid line, both of whose parents were drawn from the following set of strains, M29, M119, M225, M237, S48, and S118, breeds true with M29.

In Table 9, "no response to selection" indicates the maintenance of r above 10. "Slight response" refers to a drop to between 8.0 and 9.9 with very few individual ratings below 6. "Yes" refers to average ratings below 6, or to higher values where a considerable number of "0" flies were present.

The data suggest that M119, M225, and S48 have unfixed alleles that correspond to fixed alleles in M29. The results of all the experiments taken as a whole are consistent with the sharing of a set of fixed *cve* alleles by M29, M237,

TABLE 6

Identity tests of additional strains

Strains crossed	Crosses(N)	Lines selected(N)	Nonresponding lines(N)	Generations of downward selection
S10, S18	10	2	0	3
S10, S34	10	3	0	3
S10, S48	10	2	0	3
S10, M187	11	2	0	0
S10, M189	10	3	0	3
S10, M225	10	2	0	4
S18, M29	10	0	0	0
S18, S34	10	2	0	0
S18, S48	10	3	0	3
S18, S118	5	2	0	6
S18, M119	5	2	0	1
S18, S177	5	2	0	5
S18, M187	9	0	0	0
S18, M189	10	3	0	1
S18, M225	10	2	0	2
S18, SL-8	5	1	0	1
M29, S34	9	2	0	0
M29, S48	9	3	3	8
M29, M187	10	0	0	0
M29, M189	8	0	0	0
M29, M225	9	3	3	8
M34, S48	12	2	0	3
S34, S118	5	2	0	8
S34, M119	5	2	0	2
S34, S177	5	2	0	1
S34, M187	10	0	0	0
S34, M189	10	2	0	5
S34, M225	6	2	0	3
S34, SL-8	5	1	0	1
S48, S118	5	2	2	7
S48, M119	5	2	2	8
S48, S177	5	2	0	2
S48, M187	10	0	0	0
S48, M189	11	3	0	5
S48, M225	8	4	2	7
S48, SL-8	5	1	0	4
S118, M187	1	0	0	0
S118, M189	5	2	0	3
S118, M225	5	3	3	8
M119, M187	1	0	0	0
M119, M189	5	2	0	3
M119, M225	16	8	3	10
S177, M187	1	0	0	0
S177, M189	5	2	0	10
S177, M225	5	1	0	1

TABLE 6 (continued)

M187, M189	10	0	0	0
M187, M225	6	0	0	0
M189, M225	6	2	0	5
M189, M237	4	0	0	0
M189, SL-8	5	2	0	10
M225, M237	3	1	0	3
M225, SL-8	5	2	0	4

and (the recently fixed) S118, and the presence of some or all of these alleles in M119, M225, and S48. If this is true, then the same set of alleles has been demonstrated in the progenies of 6 females obtained at different times throughout the season.

Further evidence that mixed strains derived from these 6 *cve* strains all carry the same set of fixed *cve* alleles is seen in the results of intercrosses of various high F_{10} lines derived from the 6 strains. These failed to respond to downward selection, as is shown in Table 10. Additional crosses involving mixed lines ultimately derived from S48 and M225, as well as from the other 4 strains, are in progress and have not yet showed any response to selection.

Returning to the data in Table 2, several high F_{10} values are seen for crosses involving SL-8, as well as for one S10/S177 line. These lines all yielded to more rigorous selection in subsequent generations; as we have seen from their behavior in various additional experiments involving crossing and selection, the previously discussed high F_{10} 's did not. It should also be noted that S10 and S177 produce progeny with very high ratings, affording little basis for selection, though a return to the normal phenotype usually begins after several generations and proceeds rapidly after that. Perhaps the S10 *cve* genes are rather closely linked to those of S177.

False positive results, then, are possible, and it is therefore necessary to review the experiments on M29, M119, M225, M237, S48, and S118 to make certain that they have been interpreted properly. First of all, it will be recalled that mass matings selected downward involving M29 and any one of the other original strains except M237 invariably shows a prompt response. Thus, the lack of response in the M29 crosses described in Table 9 must be considered as meaningful. Most compelling of all are the two crosses between M29 and the 119/48 strains: neither M119 nor S48 is identical to M29. Furthermore, the responses shown in other columns of Table 9, and the results of the M29 cross to 118/177 show that the mixed strains are not different from other *cve* strains in a way that affects response to selection.

The minimum number of loci involved in a given strain may be estimated by determining the number of chromosomes contributing to the *cve* phenotype. The data, reported in Table 11, for crosses of the form illustrated in Figure 1, suggest that both major autosomes carry *cve* genes in each of several strains.

TABLE 7

Crosses of several females to a common male

Female parent(M237)	Male parent(S118)	+	(N)	cve(N)	r
1A	1	9		11	3.4
1B	1	9		11	4.8
1C	1	11		9	3.4
1D	1	8		12	5.7
1E	1	10		10	4.1
2A	2	0		20	10.7
2B	2	0		20	10.8
2C	2	0		20	10.6
2D	2	0		20	11.0
3A	3	0		20	10.2
3B	3	0		20	11.3
3C	3	0		20	10.2
4A	4	12		8	1.3
4B	4	12		8	1.6
4C	4	15		5	1.4
4D	4	13		7	1.4
4E	4	17		3	0.9

Female parent(S118)	Male parent(M237)	+	(N)	cve(N)	r
1A	1	0		20	11.2
1B	1	0		16	11.2
1C	1	0		20	11.2
1D	1	0		17	11.2
1E	1	0		20	11.2
2A	2	0		20	11.4
2B	2	0		20	11.4
2C	2	0		20	11.2
2D	2	0		20	11.3
2E	2	0		20	11.2
3A	3	0		20	10.0
3B	3	15		5	0.5
3C	3	0		20	10.2
3D	3	0		20	10.5
3E	3	0		13	10.2
4A	4	0		20	11.6
4B	4	0		20	11.0
4C	4	0		20	11.2
4D	4	0		20	11.3

The multiple marker strain used, called *Cy/Pm;D/Sb* for short, carries inversions on both homologues of each major chromosome. Furthermore, crosses using the marker strain yellow cut raspberry forked indicate the participation of the X chromosome in some strains as well. We may compare the average ratings

TABLE 8

Summary of conclusions of experiments exemplified in Table 7

Cross	Source of variation
M29 × M119	M29, M119
M29 × M237	None
M119 × M237	M119, M237
M29 × S118	M29
M119 × S118	S118
M237 × S118	S118
M237 × M237	None
M29 × M29	None
M119 × M119	(slight M119)

TABLE 9

*Response to downward selection of progeny of nonresponding
F₁₀ lines mass mated to original cve strains*

Generations of selection	F ₁₀ line	M29	M237	S118	M119	S48	M225	E' or S10 or SL-8	S177
4	29/119(1)	sl.*	...	no	yes	...	...	yes S10	...
4	29/119(2)	no	...	no	yes	...	...	yes E'	...
4	119/29(2)	no	...	no	yes	...	...	yes E'	...
4	119/237(1)	sl.	sl.	no	sl.	...	...	...	yes
6	119/237(2)	...	sl.	yes	yes	...	...	yes SL-8	...
4	29/118(1)	no	...	no	yes	...	...	yes E'	...
4	29/118(2)	no	...	no	yes	...	...	yes E'	...
5	118/29(2)	no	...	no	yes	...	...	yes S10	...
5	119/237(1)	sl.	...	no	yes	...	...	yes S10	...
5	118/119(1)	no	...	no	sl.	...	...	...	...
5	118/119(2)	...	sl.	no	sl.	...	...	yes E'	...
4	119/118(1)	no	...	no	yes	...	...	yes SL-8	...
4	119/48(2)	no	no	no	sl.	yes	...	...	...
4	119/48(4)	no	no	no	sl.	yes	...	...	...
4	118/225(1)	no	...	no	yes	yes	yes	...	...
4	118/225(2)	no	...	no	yes	yes	yes	...	...
4	118/225(4)	no	...	no	yes	...	yes	...	...
4	225/29(2)	no	...	no	yes	yes	yes	...	...
2	225/29(4)	no	...	no	yes	yes	yes	...	...
3	29/225(3)	sl.	...	...	yes	yes	yes	...	...
4	48/118(5)	no	...	no	...	yes	...	...	...
4	48/29(2)	no	...	no	...	yes	...	...	...
4	48/29(4)	no	...	...	yes	yes	...	...	...
5	118/177(2)	yes	...	no	yes	...	...	yes E'	no

* Slight response (see text)

TABLE 10

Response of crosses between mixed lines to downward selection

Cross	Generations of selection	Average ratings	Designation of strain after selection
S118/M119 (1) × M119/S118 (1)	7	11.6*	A
S118/M237 (1) × M119/M29 (2)	7	11.2	B
M119/M29 (2) × M119/M237 (2)	7	10.8	C
S118/M29 (2) × M119/M29 (2)	7	11.2	D
S118/M29 (2) × S118/S177 (2)	7	2.3	
A × D	5	11.2	
B × C	5	11.4	

* Combined ratings, duplicate lines, 10 flies each sex

TABLE 11

Average ratings at 25°C of progeny classes from marker crosses

Chromosomes 2 3 Strain	Genotype				X chromosome genotype	
	<i>Ore</i>	<i>Ore</i>	<i>cve</i>	<i>cve</i>	<i>γ ct ras f</i>	+ + + + (<i>cve</i>)
	<i>Ore</i>	<i>cve</i>	<i>Ore</i>	<i>cve</i>		
M29 ♂	0	5.6	0	10.0	3.4 (14/33)*	3.0 (22/74)
♀	0	7.1	0	11.0		
M119 ♂	0	6.0	0	10.6	0.3 (2/26)	2.6 (38/114)
♀	0	7.0	0	9.8		
S10 ♂	0	1.0	0	9.2	3.4 (5/14)	7.7 (17/19)
♀	0	3.1	0	10.6		
S118 ♂	0	7.8	0	11.6	5.2 (5/9)	5.0 (25/51)
♀	0	8.7	0	11.1		
S177 ♂	0	2.9	0	2.9	3.2 (10/34)	6.6 (22/31)
♀	0	2.6	0	7.2		
119/118 (2) ♂	0	0	0	0.9		
♀	0	0	0	2.4		

• Number of *cve* flies/total number

for flies with *cve* second and third chromosomes in Table II with the ratings observed in the pure *cve* strains in Table 3 (where it will be recalled that the two values are for two strains, not male and female). Strains M29 and S118, which indicate no *cve* contribution from the X chromosome on the basis of the *γ ct ras f* crosses, show no great differences between pure and partially reconstructed strains. The results relating to strain M119 are ambiguous. In those strains where the participation of all three major chromosomes has been demonstrated by the use of markers, the minimum number of *cve* genes is 3. It is also noteworthy that the hybrid strain M119/S118(2), whose average rating is known to be 2, owes its defects to both major autosomes. M119/S118(2) is at least one gene short of a full S118 set, because downward selection of S118 × M119/S118(2) cross results in a rapid return to a rating of 2, with no additional progress. Thus S118 contains

FIGURE 1.—Diagram of crosses made to determine which chromosomes in the *cve* strains contribute to the *cve* phenotype.

at least 3 *cve* genes. Finally, recent mass matings of M119 with S118, followed by immediate downward selection also level off at about 2, suggesting that M119 also has at least 3 *cve* genes, two of which are also fixed in S118.

DISCUSSION

Briefly to review our understanding of the crossveinless polygenes, the following points will be recalled:

1. A vast amount of potential genetic variation relevant to the posterior crossveins exists in natural populations of *Drosophila*.
2. In *D. melanogaster*, which does not seem atypical in this respect, about 1 in 10 iso-female lines will give rise to true-breeding, polygenic crossveinless strains in response to selection.
3. These strains typically are homozygous for *cve* alleles at several loci; the strains seem to differ widely in many cases.
4. It has been possible to select "identical" strains independently, both from a single source restricted in time and space, and (as can be seen in the present paper) from sources three miles and several months apart.

Since it has been clear for some time that crossvein defects in nature generally result from the collaborative effects of alleles at several loci, the question at hand is whether these alleles are themselves rare or common. Certainly as a class, alleles that can contribute to the production of crossvein defects are common, but does the class consist of individually common alleles at a few loci or individually rare alleles at a multitude of loci? From the present observations, it is clear that at least some of the alleles are individually common.

It is perhaps useful to show that some alleles that collaborate to produce morphological effects appear to be polymorphic in the same sense as do alleles identified with individual electrophoretic mobility variants of enzymes. Further, it is of interest to see that even in one population several genetic alternatives exist for

producing a discrete morphological change rapidly in response to selection. To consider this case in terms of evolutionary potential, it must be assumed, of course, that the crossveinless phenotype is representative of natural phenotypic variation, and also, more generally, that the genetic variation utilized in evolutionary processes is really the standing "polymorphic" variation that has now become so evident.

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SUMMARY

Twelve true-breeding, polygenic crossveinless strains were selected from the progenies of single wild-inseminated females collected over a 5-month period from two populations about 3 miles apart. Three strains, originating in June, August, and November collections, respectively, are monomorphic for *cve* alleles at the same loci. Three other strains contain *cve* alleles, polymorphic or monomorphic, at all of these same loci and at other loci. The six remaining strains differ from the above six and from one another in that they do not share enough *cve* alleles to produce true-breeding progeny with the extreme *cve* phenotype.—These observations demonstrate the maintenance at high frequencies in natural populations of *cve* alleles at several loci. Genes collaborating to produce morphological variation are thus also included among the increasingly numerous examples of persistent genic polymorphism.

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