THE GENETIC BASIS OF NATURAL VARIATION. V. SELECTION FOR CROSSVEINLESS POLYGENES IN NEW WILD STRAINS OF *DROSOPHILA MELANOGASTER*¹

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THE genetic basis of natural variation in posterior crossvein morphology in *Drosophila* has been studied for over 30 years. As part of a program to catalog the various alleles in natural populations which influence this vein, the present work includes the analysis of F_2 's of wild *D. melanogaster* pairs, the selection of crossveinless (cve) strains from them, the comparison of the strains *inter se* and with other cve strains, and preliminary attempts to localize the relevant alleles.

In 1923, TIMOFEEFF-RESSOVSKY discovered a gene, *venae transversae incompletae* (*vti*), which causes defects in the posterior crossvein of *D. funebris*. He subsequently (TIMOFEEFF-RESSOVSKY 1934) published an extensive study of a broad array of genetic modifiers which influence the penetrance, expressivity, and specificity (area affected) of *vti*. Although these genes were studied in the context of their modification of the action of another gene, TIMOFEEFF-RESSOVSKY's work documented the tremendous degree of genetic variation in natural populations. Later, GORDON, SPURWAY and STREET (1939) analyzed the occurrence of posterior crossvein defects in *D. subobscura*, and PREVOSTI (1951, 1952) extended the analysis both in detail and geographically. DUBININ (1948) analyzed the genetic basis of natural variation in wing venation in several species of *Drosophila*.

The results of all this work lead to the conclusion, in modern terms, that the posterior crossvein is affected by a great number of common alleles; that these alleles have individually small effects; and that various combinations of alleles (the combinations being rarer than the alleles themselves, of course) cause modifications of the posterior crossvein. It is reasonable to call these genes "polygenes," using the word as a synonym for "multiple factors" in MATHER's original (1941) sense, which does not imply close linkage (although the general likelihood of linkage is stated later in the same paper).

WADDINGTON's (1953) extension of the investigation to include the sensitivity of posterior crossvein formation to high temperature made possible the practical investigation of the vein's formation as a sensitive developmental process by BATEMAN (1959), MILKMAN (1961, 1962a, 1962b, 1963), MOHLER (1962 and unpublished data), and others. The direct observation of stages in posterior crossvein formation was also made by WADDINGTON (1941) and is being continued,

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using various wild-type and crossveinless strains, by MOHLER (1962, 1964). In short, this structure can be studied effectively from a number of points of view, and it is a good specific example with which to try out some general questions. The working assumption is, of course, that what is true for the natural variation of the posterior crossveins of *D. melanogaster* applies to natural variation in general; and that the degree and variety of response of all phenes to genetic variation is dependent on their developmental stability or canalization.

Now evidence will be presented to close the question of the high natural frequency of *cve* polygenes in *D. melanogaster*. The question as to whether these polygenes are common individually or as a class will be further defined and discussed in terms of new data.

MATERIALS AND METHODS

Individual wild inseminated females were allowed to produce progeny under standard conditions. 1000 flies in each F_2 , raised at 18°C to maximize the frequency of crossvein defects, were examined. Selection for such defects was carried on where possible, first at 18°C, and later at 25°C, where they are normally less frequent. Other standard techniques were employed, together with the use of certain simple recessives as "boosters" (MILKMAN 1960) to enhance the effect of individual *cve* alleles. Crossvein defects are rated from 0 (normal) to 12 (posterior crossveins completely absent) for each fly. These ratings are averaged for classes of flies (MILKMAN 1962a).

RESULTS

Analysis of the F_2 's of wild inseminated females: The F_2 's were examined for posterior crossvein defects, and the results are given in Table 1. The sporadic occurrence of crossveinless flies is similar to that reported in comparable lines from Ann Arbor, Michigan (MILKMAN 1962a). The distribution implies strain differences once more, and the average incidences of *cve* flies in the strains from the three locales are quite similar.

TABLE 1
Number of cve flies (N) out of 1000 F_2 flies counted

Strain	N	Strain	N	Strain	N	Strain	N	Strain	N
A	5	K	2	U	4	P-2	6	SL-1	1
B	1	L	1	V	55	P-5	1	SL-2	1
C	16	M	14	W	0	P-6	46	SL-3	0
D	1	N	3	X	0	P-7	1	SL-4	5
E	19	O	2	Y	4	P-8	0	SL-5	18
F	1	P	1	Z	1	P-9	1	SL-6	7
G	6	Q	2	AA	5	P-10	11	SL-7	0
H	3	R	3	BB	63	P-12	0	SL-8	20
I	7	S	2	CC	5	P-13	2	SL-9	3
J	1	T	18	P-1	4	Ht	2		

Percent *cve* flies, Syracuse=0.8; percent *cve* flies, South Lancaster=0.6; percent *cve* flies, Ann Arbor (MILKMAN 1962a)=0.6.

Syracuse strains A through CC were collected from a fruit market; P-1 through P-13, from a peach orchard; and Ht from a kitchen. The SL strains are from South Lancaster, Massachusetts.

To establish the genetic basis of this phenotypic variation, selection for crossvein defects was tried on all Syracuse strains having over 10 *cve* flies per 1000 in the F_2 . The F_2 *cve* flies were not used; those from later generations were. Selection failed in the C and P-10 strains and was successful in E, M, T, V, BB and P-6. From E, two lines were selected, the second (E') beginning with an 8th-generation vial in which the trait was expressed particularly strongly. The course of selection is outlined in Tables 2 and 3.

The ultimate comparison among the *cve* strains lies in genetic analysis of the highest resolution, but beginnings may be made by the consideration of a number of ascertainable attributes. The strains under consideration are similar in that they seem to contain numerous alleles whose effects on the posterior crossvein are individually small, additive, and greater at 18° than at 25°C. Moreover, the defects begin at the junction with the fourth longitudinal vein, and any remnants of the crossvein are at (or, rarely, near) the fifth longitudinal vein. The degree of expression varies within strains and between strains. The latter variation seems reasonably attributable to the number of *cve* genes fixed in the strain, but there is no direct evidence on this point. The search for distinctions among the strains led to the experiments which follow.

As each line appeared to reach a plateau, it was crossed to other *cve* lines derived from the present experiment, to other *cve* lines derived elsewhere, to relevant simple recessives, and to inbred Oregon-R. The percentages of *cve* flies of each sex in the F_1 's of such crosses are shown in Table 4. In considering these data, it is well to note that not all crossveinless polygenes interact positively (see the P-6 crosses). On the other hand, positive interactions are too common to be of much value in finding allelic relationships. As has been pointed out before (MILKMAN 1962a), the selection of the *cve* strains entails that the various *cve* alleles interact positively with one another. Their respective interactions with a

TABLE 2
Percent cve flies in strains plateauing early

Generation	Strain								
	M			BB			P-6		
	Male	Female	T(°C)	Male	Female	T(°C)	Male	Female	T(°C)
0	0.2	3	18	1	12	18	4	5	18
1	0.5	8	18	7	34	18	16	28	18
2	10	38	18	22	54	18	75	84	18
3	8	40	18	21	62	18	90	95	18
4	6	41	18	24	55	18	96	96	24
5	7	26	18	23	53	18	98	98	24
6	5	34	18	32	54	18	..	..	..
7	..	..	..	..	..	..	97	100	25
8	..	..	..	4	6	25	..	..	..
September 1963	3	23	18	25	62	18	82	90	25

The *cve* alleles in these strains seem to be largely fixed. The September 1963 count was made after about ten generations without selection.

TABLE 3
Percent cve flies in strains plateauing later

Generation	Strain											
	E			E'			T			V		
	Male	Female	T(°C)	Male	Female	T(°C)	Male	Female	T(°C)	Male	Female	T(°C)
0	1	3	18	..	..	..	1	3	18	5	6	18
1	5	11	18	..	..	..	17	19	18	15	27	18
2	11	22	18	..	..	..	17	38	18	33	53	18
3	21	37	18	..	..	..	35	46	18	43	54	18
4	35	51	18	..	..	..	45	58	18	40	58	18
5	16	26	24	..	..	..	39	50	18	41	48	18
6	42	68	18	..	..	..	55	50	18	55	58	18
7	23	36	24	..	..	..	74	80	18	61	75	18
8	56	70	24	..	..	..	88	88	18	78	90	18
9	57	52	25	..	..	..	10	11	25	8	9	25
10	42	60	25	83	84	25	35	48	25	23	44	25
11	54	52	25	79	94	25	51	54	25	39	59	25
12	40	49	25	..	..	..	57	56	25	41	50	25
13	49	48	25	75	79	25	37	41	25	26	36	25
14	..	..	..	82	84	25	..	..	..	6	20	25
15	..	..	..	83	82	25	54	63	25	48	53	25
16	72	68	25	44	56	25	38	38	25	41	45	25
17	81	87	25	85	84	25	72	77	25	..	..	..
18	42	66	25	62	73	25	68	53	25	57	53	25
19	91	92	25	73	84	25	..	..	..	..	..	..
20	80	71	25	76	88	25	..	..	..	..	..	..
21	92	97	25	68	96	25	..	..	..	..	..	..
22	..	..	..	95	100	25	..	..	..	..	..	..
April 1964	91	96	25	87	89	25	83	82	25	60	66	25

The *cve* alleles in these strains seem to be largely fixed. The April 1964 count was made after several generations without selection. Generations from which these last flies are derived are as follows: E, 20; E', 21; T, 18; V, 17. Comparison of the values indicates sensitivity to uncontrolled environmental variable(s).

variety of other strains is presented, although it must be pointed out that the data are little better than semiquantitative. The crosses were made when selection had covered most of its course, but in many cases, it was still proceeding slowly. On the other hand, some clear cut differences can be seen. The overall pattern points to similarities between T and V, and, more generally, among E, E', M, T and V. Needless to say, a broader range of comparisons is necessary.

In Table 5, average ratings are shown for various marker classes in the progenies of crosses involving a balanced strain, Curly/Plum Dichaete/Stubble. The data are pooled for individual marker classes so that progeny carrying either second-chromosome marker (Curly or Plum) are grouped together, as are progeny carrying either third-chromosome marker (Dichaete or Stubble). The proportions of the various markers are not constant, but the analysis of the data before pooling indicates that no major distortion has resulted from this procedure. Again, one is best advised to restrict his consideration to the major differences,

TABLE 4

Percent cve flies in progeny at 18°C

		E	E'	T	V	M	BB	P-6	det	per	rec	cv	cv-c	Ore-R
E	♂	96	100	99	84	27	33	0	46	42	27	12	96	0
	♀	96	100	97	88	13	58	4	44	54	26	47	92	1
E'	♂	96	99	88	94	61	57	9	52	35	54	17	65	13
	♀	94	96	92	93	64	69	9	61	41	58	54	63	20
T	♂	88	98	97	91	23	41	1	69	40	51	4	79	6
	♀	95	96	98	94	28	66	0	66	16	66	35	71	12
V	♂	99	100	99	91	32	38	2	43	51	21	2	87	2
	♀	94	100	100	91	39	65	6	41	23	31	38	72	3
M	♂	14	40	22	16	3	2	0	2	2	0	0	5	0
	♀	33	57	50	55	23	54	0	7	4	2	0	8	0
BB	♂	54	62	21	22	16	25	0	38	3	1	0	0	2
	♀	62	82	57	58	62	62	0	50	3	1	3	1	5
P-6	♂	3	10	2	2	0	0	84	0	0	0	0	0	0
	♀	3	12	2	7	0	0	98	1	1	0	0	0	0
det	♂	56	63	39	27	4	0	0	99	2	3	2	6	0
	♀	73	67	77	71	21	28	2	99	15	37	30	33	1
per	♂	20	41	8	14	1	0	0	0	100	100	0	0	0
	♀	40	61	23	22	8	8	0	5	100	100	0	0	0
rec	♂	27	52	19	15	2	0	0	7	100	100	0	0	10
	♀	46	80	38	47	3	5	0	20	100	100	0	0	14
cv	♂	100	100	100	100	100	100	100	100	100	100	100	100	100
	♀	57	44	43	75	0	6	0	26	0	9	100	0	0
cv-c	♂	87	35	90	84	15	0	0	6	4	0	0	100	0
	♀	92	63	80	92	9	2	0	23	4	8	0	100	0
Ore-R	♂	0	1	0	0	0	0	0	0	0	0	0	0	0
	♀	4	7	1	3	0	1	0	0	0	0	0	0	0

Female parents at the left; male parents at the top.

200 flies counted in each cross (generally 100 for each sex).

per, a polygenic crossveinless strain derived from a wild inseminated Ann Arbor female (MILKMAN 1962a); rec, a polygenic crossveinless strain derived from the progeny of a per × Ore-R cross; det, a polygenic crossveinless strain with a major component (detached) at 3-72.5. The simple recessives cv (crossveinless, 1-13.7) and cv-c (crossveinless-c, 3-57.9) are well known.

such as the absence of crossveinless flies in P-6 where the P-6 second chromosome is not homozygous; the differences in apparent contributions of the second chromosome of E and E' from that of T and V; and the fact that, in contrast to the other crosses, M shows substantially higher ratings in the cv-c crosses than in the backcrosses.

In all these data, there is nothing to contradict the possibility that E, E', M, T, V and BB differ simply in containing different combinations of a largely common group of cve alleles. This remains to be seen in further analysis. It would appear, however, that the P-6 strain does differ from the rest of these Syracuse

TABLE 5

Average defect ratings of flies in progenies of crosses involving marked chromosomes

cve strain	Chromosome 2 3	(CPDS × cve) F ₁		CPDS × cve		cve × CPDS		CPDS × cv-c		cv-c × CPDS	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
E	MM	0.8	0.2	1.3	1.0	0.4	0.6	0.1	0.0	0.0	0.0
	+M	..	..	2.3	2.0	1.3	1.4	0.3	0.1	0.0	0.0
	M+	..	..	2.6	2.6	2.6	2.3	1.4	0.5	0.0	0.3
	++	..	..	3.2	3.6	3.3	4.0	1.8	1.0	0.3	0.8
E'	MM	1.0	0.2	1.3	0.8	0.1	0.3	0.1	0.0	0.0	0.0
	+M	..	..	2.2	1.1	1.2	1.5	0.4	0.0	0.0	0.1
	M+	..	..	4.3	5.2	3.0	4.0	0.6	0.5	0.1	0.4
	++	..	..	6.6	5.8	5.7	6.3	1.8	0.8	0.8	1.3
M	MM	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	+M	..	..	0.2	0.0	0.0	0.0	0.1	0.3	0.0	0.1
	M+	..	..	0.4	0.0	0.0	0.0	0.5	0.0	0.5	0.9
	++	..	..	0.1	0.1	0.0	0.1	1.1	0.8	1.1	1.9
T	MM	1.4	0.5	2.2	0.6	0.3	0.2	0.2	0.0	0.1	0.0
	+M	..	..	2.4	1.5	1.1	0.4	0.8	0.2	0.0	0.1
	M+	..	..	5.5	3.0	4.3	3.2	4.8	3.0	1.9	2.6
	++	..	..	4.9	3.9	4.1	3.1	5.4	3.4	3.3	3.8
V	MM	1.6	0.4	0.8	0.3	0.3	0.3	0.1	0.1	0.0	0.1
	+M	..	..	1.8	0.9	0.5	0.8	0.3	0.1	0.1	0.1
	M+	..	..	3.0	2.0	3.3	2.8	4.5	2.2	1.1	1.3
	++	..	..	3.8	3.7	3.9	3.3	4.5	2.9	2.2	3.1
BB	MM	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	+M	..	..	0.1	0.2	0.0	0.0	0.2	0.0	0.0	0.0
	M+	..	..	0.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0
	++	..	..	0.2	0.8	0.1	0.2	0.1	0.0	0.0	0.3
P-6	MM	0.0	0.0	0.0	0.0	0.0	0.0	..	..	..	..
	+M	..	..	3.6	4.3	3.5	4.6	..	..	..	..
	M+	..	..	0.0	0.0	0.0	0.0	..	..	..	..
	++	..	..	2.9	3.4	6.3	4.9	..	..	..	..

At least 200 flies were counted in each F₁. The other 26 crosses involved 500–1000 flies each. CPDS=Curly/Plum Dichaete/Stubble. CPDS/cve is (CPDS × cve) F₁. In the second column, M stands for marker chromosome.

cve strains, and that the Ann Arbor cve strain, per, differs from all the Syracuse strains. Experiments with South Lancaster cve strains await completion of selection in SL-5 and SL-8, which is proceeding rapidly.

DISCUSSION

We come now to a statement of the problem to be attacked by higher-resolution analysis. It is clear that several different combinations of alleles interfere with posterior crossvein formation. These individual alleles are common as a class, in that it is easy to select groups of mutually interacting cve genes from the progenies of individual pairs. But we have hardly approached the geographic distribution of each allele. It is hoped that localization techniques currently being employed may lead to the eventual mapping in space and time of such distributions.

Meanwhile, the question exists as to what proportion of the *cve* genes present in each strain have been detected by selection. It is possible, for example, that there are numerous alleles in each original strain which have not been selected, either for reasons of noninteraction with the selected genes (or, indeed, antagonistic effects), or for reasons of general inefficiency. The repetition of selection on the original strains might prove interesting, but it has not been tried. On the other hand, a different form of selection has been performed on a population containing the contributions of the 34 Syracuse strains which did not give rise to *cve* strains. This selection was based for a number of generations on response to crossvein defect production at 36.5°C (MILKMAN 1962b), and direct selection for nontemperature-induced defects was performed independently on lines coming from the 9th, 10th, 11th, 12th and 13th generation of phenocopy selection. The characteristics of these 100 percent penetrant *cve* strains are now under analysis and will be discussed in a subsequent paper. Suffice it to say, expressivity and penetrance show that selection has proceeded much further than in the individual strains discussed here. Probably the larger starting population contributed to this difference; perhaps the broader based selection (MILKMAN 1960) did, too. The interpolation of non-selection generations between selection generations did not have a serious effect.

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SUMMARY

Several new polygenic crossveinless strains have been produced by selection of the progenies of wild inseminated females. The body of information now at hand proves that numerous interacting alleles with small adverse individual effects on posterior crossvein formation are common in nature, at least as a class. Strain differences indicate that there is great variety of *cve* genes in nature, and that it is appropriate to try to measure their individual frequencies over space and time.

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