SPOTTING INHERITANCE IN *DROSOPHILA BUSCKII* COQ.¹

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INTRODUCTION

Few groups of animals or plants have received so much study by the geneticist as the fruit fly of the genus *Drosophila*. This work, carried on largely by MORGAN and his co-workers, has consisted almost entirely of an analysis of the inter-relations of variations found in the laboratory. Large numbers of flies have been minutely examined for germinal variations and this critical study has resulted in the finding of a large number of mutations of various sorts. These mutations were usually observed soon after their appearance and further work with them has resulted in adding much new and valuable light to our knowledge of heredity.

Although many mutations have been found in the laboratory, very few have been recorded in flies taken from nature. This fact has led some writers to question whether these variations found in the laboratory are really potent factors in the evolutionary process. The difference may lie in the fact that flies in nature have not been given the exhaustive study which has revealed the mutations appearing in the laboratory. However, when one examines any large number of flies in nature he is struck, not by the large number of variations which may be of evolutionary value, but rather by the lack of these. There is certainly very little evidence of any variations similar to those found in the laboratory, which seem to be taking the place of any existing contrasted characters.

THE PROBLEM

The problem of this paper has been to analyze variations which were found in nature. In studies of this sort it is not possible to know anything concerning the origin of the variations and hence the analysis is more difficult. The variation studied was the spotting of the abdomen. The study of the variations in the male, together with a variation in female spotting, which later appeared in the writer's stocks, composes the work of this paper. In its final form the problem became an attempt to analyze critically the factors which govern or influence the spotting character or characters in the fly. To narrow the field still further the writer has limited himself to the spots on a single segment of the abdomen. Whether the inheritance of the spots on the other segments of the abdomen are dependent upon the same factors remains in question.

DESCRIPTION OF THE SPECIES

The fruit fly *Drosophila busckii* is slightly smaller than the well known

Drosophila melanogaster and is readily distinguished from it by conspicuous spots on the abdominal segments. Each segment, excluding the last two, bears six spots. These spots are arranged on each segment as follows. There is a pair of rather large distinct spots on the dorsal surface of the segment, one on each side of a mid-dorsal line. A second pair appears more laterally, one on each side just below the last-mentioned pair. The third pair of spots are small ones, latero-ventral in position. The spots are arranged so as to make six rows running lengthwise of the abdomen. There is seldom any variation in the spotting of the above-described segments.

The spotting of the fifth segment differs from these, however, and the variations found on that segment furnish material for this study. In the female it is like that of the other more anterior abdominal segments,² but in the male there are variations. The large dorsal pair of spots is always present and the presence of this pair alone seems to be the more common type of spotting among males found in nature. Either one or both of the other two pairs may be present or absent or any one spot of a pair may be combined with any other spots, thus giving all the possible combinations of the constant dorsal pair and the other four variable spots. These various combinations are shown in figure 1. The spots show a tendency to be present or absent in pairs, that is, if the outside spot on one side is missing the corresponding spot on the other side is also likely to be missing. This tendency is especially true of the outside pair of spots and one outside spot occurs without the other so infrequently that no record of this type of spotting has been made in the tables which follow.

MATERIAL AND METHODS OF BREEDING

The flies used in these experiments were collected in the vicinity of Bloomington, Indiana, during the autumns of 1915 and 1916. In nature they breed in decaying fruit and vegetable matter but most frequently in rotting tomatoes. At first it was attempted to breed them on banana in the laboratory. It was found that they could be reared on this food, but with some difficulty. Later, moistened bran was tried and used very successfully, being a food which is both convenient to handle and one upon which the *Drosophila busckii* breeds very well. This species is very prolific and requires about fourteen to twenty-five days for a generation, depending upon the temperature. It does not well withstand high temperatures and the extreme summer heat encountered in central Indiana sometimes killed stocks of flies.

² One exception was found which will be discussed later in the paper.

FIGURE 1.—Diagram of the various types of spotting found on the fifth abdominal segment of the male. Each type represents a flattened segment showing the spots upon it. The large dorsal pair was always present and was found in every possible combination with the other four smaller spots. The spots tended to be present or absent in pairs and are referred to throughout the paper as the dorsal pair, the middle pair and outside pair. Type 1 was most common in nature.

MALE SPOTTING

Selection lines

This study was suggested by the observation that there were variations in the spotting of the males. Preliminary work upon these variations indicated that they were heritable to a certain degree. The earlier work consisted largely of crosses between the more extreme variations to see how they would behave, genetically. Further than establishing to the writer's satisfaction that these variations were heritable, very little was accomplished, for no pure strains of any of the variations had been acquired. So it was decided that the best method of procedure would be to try to establish by selection, strains which were pure for some of these variations. Selection was made for the two extremes of the various types of spotting in the males. These two extremes were the normal or two-spotted condition (fig. 1, type 1, and plate 1, no. 1) and the six-spotted condition (see fig. 1, type 16, and plate 1, no. 3). As well as

LEGEND FOR PLATE I

Some of the types of spotting found in *Drosophila busckii*. The work of this paper is confined to the fifth abdominal segment (the last segment showing spots).

No. 1.—Normal male with only the dorsal spots.

No. 2.—Male with dorsal spots and small outside one.

No. 3.—Male having full set of three pairs of spots.

No. 4.—Normal female with full set of three pairs of spots.

No. 5.—Abnormal female with middle pair of spots missing.

No. 6.—501 type of male with very large outside spots and also small middle spot.

No. 7.—Male with dorsal spots and small middle spot.

No. 8.—501 type of male with large outside spot.

being an attempt to isolate two desired types of males the problem was outlined as a selection problem. From flies taken in nature three stocks were started which were not known to be related. To begin with quite a number were examined in each stock to learn what was the ratio of the various types of male spotting in each. Then from among the offspring of a single pair in each stock a "high" and a "low" strain were started. The high strain was one in which the paternal parent in each succeeding generation was to be a male with a full set of six spots, while the low strain was one in which the father of each generation was to be a male of the normal or two-spotted type. At the same time that these two strains were started a control strain was also started in each stock. In the control the parents were selected at random with no knowledge of the nature of the spotting of the fathers. The three stocks in which the selection was started were known as 500, 502 and 504 and after the selection was started there was a low strain, a high strain and a control strain in each.

Selection line 500

Three pairs of flies were taken in nature and placed in a bottle which was numbered 500. This was the origin of the strain 500. A record was made of the spotting of the offspring from this bottle and six pairs of the offspring were mated *inter se*.³ Of these six matings, one was used as the starting point of the high and low strains of line 500. This mating was a single-pair mating and the female was virgin, so all of the offspring were necessarily full brothers and sister. This gave the two strains as nearly uniform starting conditions as possible. The only difference in conditions at the starting of the high and low strains was that two-spotted males were used in the low strain and six-spotted males were used in the high strain. In as far as possible uniformity of all conditions except the one mentioned was maintained during succeeding generations of selection. Tables 1, 2, and 3 show the selection in the three strains of line 500.

In this line a control was not started at the beginning of selection but was originated by mating the high and low strains at generation 3. All succeeding generations of the control were mated at random with no knowledge of the type of male used.

³ It will be noted that the counts obtained from the earlier matings were rather small as compared with the later generations of selection. This was due to the fact that the flies were at first reared on banana upon which they do not thrive well. Later wet bran was used and found much more satisfactory.

TABLE I
Selection in strain 500 low.

Generation	Percent selected type	Types of male spotting										Total males
		Type I	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection }	56	152	14	11	7	7	22		9	3	48	273
1	28	91	9	13	5	3	145	7	6	11	36	326
2	85	430	4		2		66		2		1	505
3	92	1166	6	11	12	1	46	1	8		17	1268
4	96	606	7	6	6	1	8	1	2		5	642
5	99	717	4		4							725
6	94	239	5		5	2		1	1		1	254
7	97	477	3		10	1			1		1	493
8	97	433	3		10							446
9	99	347	2		1							350
10	94	546	5	2	6	8	9				3	579
11	93	435	7		5	2						469
12	99	372	1		1							374
13	99	350	1		1		1				1	354
15	89	181	1		1	1	17	1			2	204

Table I shows the selection for the two-spotted type of male (type 1) in strain 500 low. The column showing the percent of selected type gives a fair idea of what was accomplished by selection. The column headings, type 1, type 2, etc., refer to the types of male spotting found. These types are shown in figure 1. Some of the types given in figure 1 are omitted in the tables which follow. This is due to the fact that there were so few individuals in some of the types that space was not given them in the tables.

In generation one of the low strain eight matings were made but in practically all of the succeeding generations two stock matings were made in each generation of each strain. The graph shown in figure 2 gives a fair idea of the results obtained by selection in this line. Selection was much more effective in the direction of the two-spotted males. The graph representing the low strain in generation 4 drops to near one-hundred percent two-spotted males and remains so during the rest of the selection. Selection in the opposite direction was less progressive. The graph representing the high strain is very irregular and a high percentage of six-spotted males was not reached till generation 9. During the first eight generations of selection the high strain frequently threw more two-spotted males than six-spotted ones. This seemed to be characteristic of line 500. There was a tendency in all strains to produce a high number of two-spotted males and the percentage of six-spotted was difficult to increase. The control in which there was no selection, al-

TABLE 2
Selection in strain 500 high.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	18	152	14	11	7	7	22		9	3	48	273
1	21	10		1			17	1		2	8	39
2	43	29	1	4	3	1	22	4	2	2	50	118
3	24	179	8	24	7	4	47	4	24	7	98	402
4	43	122	6	27	7	4	51	5	22	6	186	436
5	7	348	19	15	25	22			17		35	481
6	12	360	20	22	26	22	5	4	12	3	62	536
7	17	335	19	28	20	18	27	4	26	6	100	583
8	34	146	2	18	14	7	90	19	18	15	171	500
9	79	1		2		1	13	10	2	8	138	175
10	61	99	10	12	23	28	49	23	6	24	422	696
11	56	22	1	3	2		134	21	2	18	261	464
12	86	9	3	2	6	4	13	4		2	268	311
14	66	8					42	5		3	111	169

Table 2 shows the selection for the six-spotted type of male (type 16) in strain 500 high. The types referred to in the column headings are found in figure 1. The increase in percent observed as one reads down the second column shows what was accomplished by selection.

TABLE 3
Counts for control strain of line 500.

Generation	Types of male spotting										Total males
	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	152	14	11	7	7	22		9	3	48	273
1	195	6	1	6		11	2	1	1	11	234
2	134	1		1	1						137
3	184	5	5	12	2		1	2		6	217
4	209	6	2	5	3			1			226
5	199	8	8	4	8	45	3	3	6	23	307
6	179	2		2	1						184
7	123	1		4	1						129
8	109	3	1	4	3	9				10	139
9	148	13	2	10	6	6	1		1	1	188

Table 3 shows the counts for each generation of the unselected strain 500 control. These counts should be compared with those in tables 7 and 2. The behavior of this strain in relation to the two selected strains in line 500 is best shown in figure 2.

FIGURE 2.—Results of selection in line 500. The dash line represents the high strain, the dotted line the control, the solid line the low strain. The abscissae represent the generations of selection while the ordinates represent the difference between the percentage of two-spotted and six-spotted males in any generation. Differences in favor of the six-spotted males are plotted above the zero line while those in favor of the two-spotted males are plotted below the line; *e.g.*, in generation 2 of the high strain, 43 percent of the males were six-spotted and 24 percent were two-spotted, so the difference was 19 in favor of the six-spotted males and was plotted at 19 above the zero line. The data for this graph are found in tables 1, 2 and 3.

though somewhat more irregular, did not range far from the low strain. The extra spots of the six-spotted males of the high strain were all rather indistinct as compared with those in line 502.

Results

There can be no doubt but that the high and low strains have been made different by selection. Although a much lower percentage of males of the desired type were obtained in the high strain than in the low strain, it is evident that more was really accomplished by selection

in the high strain. This is true because the line 500 at the beginning had a tendency to produce two-spotted males and by selection a greater difference was brought about between the high strain and the control than between the control and the low strain.

Selection line 502

This selection experiment (tables 4, 5 and 6) was started and carried out in the same manner as on line 500. The initial stock was taken from nature, bred in the laboratory for two generations and then from among the offspring of a single pair the selection strains and control were started. This line soon showed itself to be characteristically different from line 500. Line 502 showed a tendency to produce a high percentage of six-spotted males. The graph (figure 3) representing the behavior of the low strain in this line was much more irregular than the

TABLE 4
Selection in strain 502 low.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection }	31	78	8	14	4	10	30	1	10		98	253
1	22	26		1	1		73	2		3	11	117
2	24	96		9	2	3	123	6	12	8	135	394
3	82	173	1	2	2		26		2		4	210
4	94	739	5	14	7	1	12		8		2	788
5	85	622	24	3	26	9	4	4	7		28	727
6	84	722	19	7	23	15	14	3	11	2	39	855
7	93	480	7	7	13	4	2		1	1	3	518
8	94	410	4	1	4	2		1			2	424
9	79	586	18	13	25	23	33	5	6	8	27	744
10	32	92		1	4	1	166	7	1	4	8	284
11	54	230	3	2	6	5	139	8	5	15	12	425
12	81	370	8	3	10	1	52	4		3	7	458
13	50	78	3	1		1	48	13		2	9	155
15	47	98	2		1		94	1		2	11	209

The counts in table 4 show the results of selection for the two-spotted type of male (type 1) in strain 502. The types referred to in the column headings are shown in figure 1. The second column shows the effect of selection and the decrease in the percent of selected type in the latter part of the experiment will be seen to be due to an increase in the type 11 males. The type 11 male (see figure 1) is one with only the outside spots and these spots are affected by temperature variations. Temperature is probably the cause of the decreased percent in the last few generations.

TABLE 5
Selection in strain 502 high.

Generation	Percent selected type	Types of male spotting										Total males
		Type I	Type 2	Type 4	Type 5	Type 6	Type II	Type 12	Type 13	Type 15	Type 16	
Counts before selection	38	78	8	14	4	10	30	1	10		98	253
1	11	8					44	3		2	7	64
2	62	48	1	7	3		108	4	7	8	305	491
3	70	33		8	2	1	40	5	8	10	246	353
4	60	26	1	9			127	28	6	18	326	541
5	75	33	2	7	3	2	62	13	5	9	416	552
6	70	2	1	4			104	20	2	20	351	504
7	89			1			38	20	1	16	585	661
8	55			1			141	31		7	217	397
9	86						10	2		4	96	112
10	100									1	502	503
11	93						24	1			351	376
12	100						1	1			458	460
14	98						1			2	147	150

Table 5 gives the results of selection in strain 502 high. Selection was for the six-spotted or type-16 male. Figure 1 gives a diagram of the types referred to in this table. It will be seen from column two that it was possible by selection to obtain almost one hundred percent of the desired type.

TABLE 6
Counts for control strain of line 502.

Generation	Types of male spotting										Total males
	Type I	Type 2	Type 4	Type 5	Type 6	Type II	Type 12	Type 13	Type 15	Type 16	
Counts before selection	78	8	14	4	10	30	1	10		98	253
3	374	3	7	6	1	83	4	9	3	16	506
4	166	5	2	10	9	6	2		1	21	222
5	89	3			2			1		1	96
6	135	5	3	5	5	13	3	3	3	19	194
7	92	2	7	5	4	35	9	5	5	88	252
8	38		4	2	3	33	7	1	5	35	128
9	30	2	2	1		26		1	1	17	80
10	7		1			98	10		1	66	183
11	62		1	8	6	33	19		2	44	175

The counts in table 6 represent the behavior of strain 502 control. This was the unselected strain of line 502. The best comparison of the behavior of this strain with the high and low strains of 502 is shown in the graph in figure 3.

FIGURE 3.—Results of selection in line 502. See figure 2 for explanation of graph. The dash line represents the high strain, the dotted line the control and the solid line the low strain. Tables 4, 5 and 6 give the data for this graph.

corresponding one in line 500. Also the percentage of two-spotted males in the corresponding generations was lower than in strain 500 low. The percentage of six-spotted males in the high strain of 502 gradually increases and in the last few generations nearly approaches one hundred. The apparent loss of effect of selection in the last few generations in the low strain was probably due to temperature and will be discussed later. The extra spots on the males of the high strain of 502 were very distinct.

Results

Here again there is clear-cut evidence of results having been accomplished by selection. The graphs representing the two strains show a wide divergence and the control, although rather irregular, occupies an approximately midway position. There was a greater difference brought about by selection between both strains and the control than in line 500.

Selection line 504

The selection strains in this experiment were started as in the other two experiments. The data for this line are found in tables 7, 8 and 9. This line behaved somewhat similarly to line 500 and perhaps a little more extremely. There was a tendency to produce two-spotted males. Very early in selection (generation 2) the low strain produced nearly one-hundred percent two-spotted males as is shown by the graph and data for this strain. The graph (figure 4) then runs very uniformly near one-hundred percent except in the last two generations when there was a rather sudden rise. This irregularity was due to temperature and will be discussed under the topic of temperature. In the high strain the results were rather variable for the first few generations of selection. The percentage of six-spotted males was hard to increase and not until the last few generations of selection did the graph representing the behavior of the high strain rise above the zero line, thus showing a preponderance

TABLE 7
Selection in strain 504 low.

Generation	Percent selected type	Types of male spotting										Total males
		Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	31	117	15	14	7	14	89	7	12	1	97	373
1	93	332	1				25					358
2	98	1287	4	1	4		9	1	2		1	1309
3	99	632	1		1		1					635
4	100	235										235
5	98	437	2		2	4						445
6	99	547	5		2							554
7	99	458	2		1				1			462
8	98	452	4	1	4	1						462
9	92	462	3	1	3	1	29		1	1		501
10	97	302	2		5	1						310
11	99	389	1									390
12	98	406		1	3	1	3					414
14	62	133	1		1		78	2				215

Table 7 gives the selection for the two-spotted male (type 1) in strain 504 low. The types referred to in this table are shown in figure 1. It is seen that selection was very effective in this strain. All of the strains of line 504 showed a tendency to produce males of the two-spotted type. In this respect this strain is quite different from the low strain of line 502. By comparing the results of this table with those in table 4 this difference is readily seen.

TABLE 8
Selection in strain 504 high.

Generation	Percent selected type	Types of male spotting										Total males
		Type I	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	26	117	15	14	7	14	89	7	12	1	97	373
1	2	106	4		2		81	2			4	199
2	3	384	6	3	6		45	2	5	1	14	466
3	4	728	27	13	42	33	67	3	16	3	34	966
4	3	521	17	5	16	12	50	4	6	3	21	655
5	10	429	14	13	17	15	193	23	8	13	80	805
6	6	429	18	19	24	26	117	21	8	9	46	717
7	6	219	12	6	20	12	89	8	7	3	22	398
8	14	238	19	14	17	15	170	19	5	20	82	599
9	4	39	2		1		199	6	2	13	10	272
10	32	2		1		1	221	25		18	127	395
11	51	62	8	6	19	14	86	16	5	8	230	454

In table 8 are shown the results of selection in strain 504 high. It was difficult by selection to increase the percent of six-spotted males (type 16) due to the tendency of all of the strains of this line to produce two-spotted males. However by examining the graph in figure 4 it will be seen that selection made the two selected strains act quite differently.

TABLE 9
Counts for control strain of line 504.

Generation	Types of male spotting										Total males
	Type I	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	
Counts before selection	117	15	14	7	14	89	7	12	1	97	373
1	290	7	1	15	1	33	1	3	1	8	360
2	131	1		4							136
3	138	3		1	2	8	1	1	1	2	157
4	193	5	1	7	5			1			212
5	242		4	7	3	18					282
6	105	1			1						107
8	147	7		7	1					5	167
9	114	2		7	1			1			125
11	110	1	1	4	2	11					129

Table 9 shows the counts for each generation of the unselected strain 504 control. The behavior of this strain in its relation to the two selected strains of line 504 is shown in figure 4.

FIGURE 4.—Results of selection in line 504. See figure 2 for explanation of graph. The dash line represents the high strain, the dotted line the control, and the solid line the low strain. Tables 7, 8 and 9 give the data for this graph.

of six-spotted males. The percentage of six-spotted males never rose as high as in the high strains of lines 500 and 502. The extra spots on the six-spotted males in the high strain were much less distinct than in line 502. The control was not started from the original stock of line 504 but was originated as a cross between the high and low strains after two selections. The control threw a rather high percentage of two-spotted males but is shown by the graph as running more irregularly and slightly above the low strain.

Results

Although in line 504 it was impossible by selection to produce a very high percentage of six-spotted males, there can be no question about the significance of the results. The divergence of the two lines repre-

senting the high and low strains clearly shows what has been accomplished.

Discussion of the selection experiments

Selection has been carried out upon three distinct stocks of flies. In each case two strains have been produced which behave quite differently with respect to the character upon which selection acts. As to how selection acted is a more difficult problem. There are two conflicting views: first, the pure-line view that selection can only isolate existing pure lines, or the elimination and accumulation of multiple factors; second, the opposing view until recently held by CASTLE that selection modifies the gene.

This experiment offers no demonstration of the correctness of either view. In the experiment under consideration the object of selection was not to increase and decrease the intensity of a given character but rather to vary the percentage of individuals showing the character. The character was the presence or absence of four spots on a given abdominal segment. It was found that each line selected from nature seemed to have a more or less characteristic behavior with respect to the presence or absence of these spots. The ease with which a strain could be varied by selection in a given direction and the range of variation seemed to be a characteristic of the strain itself. The easiest explanation of this behavior is that this is a case of isolating pure lines. This in a way would be comparable to the classic experiments of JOHANNSEN upon beans. The difference in range of variation of the different strains is hardly sufficiently great to make a good case of this sort. However the rather striking difference in amenability to selection in a given direction does lend support to the pure-line theory and causes the writer to believe that the lines 500, 502, and 504 were all different genetically when taken from nature. It should be added, however, that at the end of the selection experiment the two high strains of lines 502 and 500 (see discussion of results of crosses between various male strains, which follow later in the paper) were crossed and the F_1 results indicated that at least some of the factors for spotting were common to both high strains. So although the two high strains reacted differently to selection and even remained somewhat different at the end of selection, they appeared to have the same genetic constitution at the end. Selection may have changed the genetic constitution of one of them. The writer is unable to see how the assumption of multiple factors would assist in explanation of this case and therefore merely presents the observed facts allowing the reader to give his own interpretation.

Permanency of the strains established by selection

An experiment was carried out to test whether the difference brought about by selection in the high and low strains was permanent. Line 502 was used in this test and at generation 5 of selection, a strain was isolated from each of the high and low strains. These strains were to be carried along with the selection strains but the male parents of each generation were to be taken at random with no knowledge of the type of spotting which they possessed. Random selection was carried on for seven generations and then counts were taken of the offspring. The results (table 10) show that there had been no retrogression in either strain in spite of the fact that selection had been stopped for several generations. In the high strain the count showed that seventy-six percent of the males were of the six-spotted type and at generation 5 when this strain was isolated to be carried without selection, the strain showed seventy-five percent of the males to be of the six-spotted type. So the high strain had not changed. At the time that selection was stopped, the low strain showed eighty-five percent of the males to be two-spotted and after seven generations of mass culture without selection it still showed eighty-nine percent of the males to be two-spotted. These two strains were carried for twenty-three generations without selection and the intention was to make another count after a long period. Pressure of other work prevented these counts from being made and finally the two strains were killed by the extreme summer heat of Alabama where the writer was then located. However, in transferring the flies from generation to generation it was observed that there always remained a striking difference in the spotting of the two strains and it is believed that the effects of selection remained permanent.

Discussion of behavior of male line 501

Before discussing the genetic behavior of the various selection strains it will be well to consider an unusual type of male spotting which was found in the stocks carried. A number of stocks of flies were taken from nature at the time that the male selection lines were started. One of these stocks (line 501) was observed to produce males which had unusually large outside spots on the fifth abdominal segment (see plate I, no. 8, page 64). The spots of this outside pair were frequently almost as large as those of the dorsal pair. This was unusual, for these spots were ordinarily much smaller than the dorsal ones. Selection was started using the most extreme males in an attempt to establish a pure

TABLE 10
Permanency of effect of selection.

A. After five generations of selection

Strain	Percent selected type	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
502 low	85	622	24	3	26	9	4	4	7		28	727
502 high	75	33	2	7	3	2	62	13	5	9	416	552

B. Same strain after seven additional generations without selection

502 low	89	281	2		1		32	1				317
502 high	76	4			1		45	13		4	217	284

Table 10 shows the permanency of the effect of selection in line 502. Strain 502 low and 502 high originally came from the same stock. In strain 502 low selection was made for type 1 males while in strain 502 high selection was for type 16 males. (These types are shown in figure 1.) After 5 generations of selection in which considerable difference was brought about, stocks were taken from the two strains and carried unselected for seven generations. At the end it is seen that the difference in the two strains remained permanent.

stock of this type of male. Immediately an almost pure stock was obtained. Practically all of the males possessed the outside pair of spots and these spots were unusually large in most cases. Some of the males also possessed the middle pair of spots (plate 1, no. 6) making a male of the six-spotted type. Selection was carried on for several generations until practically all of the males possessed the unusual outside spots.

Genetic behavior of line 501 when crossed to strain 504 low

The 501 line was crossed with the low strain of 504. It will be remembered that the low strain of 504 was practically pure for the two-spotted condition or the absence of the outside pair of spots which were characteristic of line 501. The 501 males and females were each crossed with the opposite sex from 504 low. The F_1 results are shown in table 11. Eleven matings were made by crossing the 504 females, by the 501 males, and ten matings of the reciprocal cross. The data for these crosses show a rather striking difference in the results where the 504 female was crossed with a 501 male, as compared with the cross in the opposite direction. When the female was from 501 there was a much higher percentage of the males in the F_1 showing one or both spots of the middle pair than when the male of the same line was used. This is not a chance fluctuation but holds true for practically every mating. The

middle pair of spots was not the one which was of primary interest in this set of matings for only a small percentage of the males of 501 possess the middle spots. From figure 1 it can be seen that all of the types listed in table 11, except types 1 and 11, possess one or both of the middle spots. When the 501 female was used in the cross, about thirty percent of the males in the F_1 have one or both of the middle spots, but when the 501 male was used, less than three percent of the males possessed one or both of the middle spots. The only explanation that can be offered for this peculiarity is that one or more of the genes affecting the presence of the middle spots are located in the sex chromosome. Not all

TABLE 11
Strain 501 × strain 504 low.

F_1 generation											
Type of mating	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
504 ♀ × 501 ♂	373		1			1539	18	2	13	18	1964
501 ♀ × 504 ♂	244	4	8	2	1	953	90	8	105	311	1726

F_2 generation												
Type of mating	Type 1	Type 2	Type 4	Type 5	Type 6	Type faint 11	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
Faint type 11 ♂ from cross 504 ♀ × 501 ♂	314	14	10	24	19	613	398	42	2	27	149	1612
Type 16 ♂ from cross 501 ♀ × 504 ♂	357	19	1	17	15	694	433	38	1	20	148	1743
Good type 11 ♂ from cross 501 ♀ × 504 ♂	376	13	3	9	11	667	416	26	2	14	95	1632
Total for F_2 generation	1047	46	14	50	45	1974	1247	106	5	61	392	4987

Table 11 shows the results of the cross between strain 501 and strain 504 low. Strain 501 possessed very large outside spots while strain 504 lacked them. In the F_1 results note the difference in percent of type 16 males depending on whether the male or female from 501 was used in the cross. The F_2 results indicate that it makes no difference in the results of that generation what sort of an F_1 male is used as father of the F_2 generation.

of the genes for the middle spots can be in the sex chromosome, for in that case a female from 501 should transmit the character to all of her sons in the F_1 generation. After observing this tendency toward sex-linked inheritance of the middle pair of spots in 501 the writer examined some of the other data in which the middle pair of spots was involved to

see if the same behavior had been overlooked there. A cross had been made between a strain known as the abnormal strain⁴ and 502 high. In the abnormal strain the males were practically all two-spotted or lacking both in the middle and outside pair of spots, while the males of the 502 high possessed all three pairs. In the crosses between these two stocks, there was no difference in the F_1 results whether the male or female from 502 high was used. There is no indication of sex-linked inheritance with respect to the middle pair of spots, though such linkage should have been manifest in this cross since the abnormal strain was practically pure for the absence of the middle pair of spots while the 502 high was practically pure for their presence. Thus it seems necessary to conclude that there is a gene in the sex chromosome in line 501 which influences the presence of the middle pair of spots while in strain 502 high none of the genes influencing the inheritance of the middle pair of spots are in the sex-chromosome.

Turning now to the genetic behavior of the outside pair of spots for which the cross between 501 and 504 low was made, it will be seen from table 11 that in the F_1 there is a more or less incomplete dominance of the presence of the outside spots in 501 over their absence in 504 low. The count shown in the first column (type 1) of the table represents the males with neither outside spot while practically all of the other columns were for males which possessed one or both of the outside spots. As to the percentage of the males in the F_1 showing the outside spots, it seems to have made no difference whether the male or female from 501 was used in the cross. However, one difference was evident in the F_1 generation between the crosses where the 501 male or female was used in the mating. When the female of the cross was from 501 the male offspring showing the outside spots in the F_1 showed them in greater intensity than when the 501 male was used in the cross. In the cross of 501 male by 504 female, the outside spots which appeared on the males of the F_1 generation were rather faint. When the cross was made in the opposite direction many of the male offspring were almost typical 501 males with respect to the intensity of the spots. The F_2 results are also given in table 11. Three types of matings were made in the F_1 generation. From the F_1 of the cross 501 female by 504 male, both the six-spotted males and the almost typical 501 males were mated to sisters while in the F_1 of the cross 504 female by 501 male, the males showing the outside spots faintly were mated to sisters. The F_2 results show no difference between these three sorts of matings. In the table of the F_2 results the

⁴ The behavior of this strain is discussed later in this paper.

column headed type 11 represents the count of males which were typical 501 while the column just preceding it contains males with the outside spots showing faintly. The males which show the outside spots faintly are probably heterozygous while the apparently typical 501 males are probably pure, segregated males of that type. The males in the first column are typical 504 males with only the dorsal pair of spots. Some of these typical 501 and 504 males were taken from the F_2 generation and mated back to females from their respective lines. The resulting offspring were typical of the line to which the males were back-crossed showing that the two types of males used in the cross had segregated after the Mendelian manner.

These crosses indicate that the outside pair of spots which are characteristic of line 501 is inherited in the usual Mendelian manner except that there is a lack of dominance of its presence or absence. There is a difference in the intensity of the outside spots on males of the F_1 generation depending upon whether the 501 male or female is used in the cross. The more intense spots appear when the 501 female is used. This might be taken to indicate that a sex-linked factor is involved in the inheritance of the outside spots. There is fairly definite evidence from the crosses made that sex-linked inheritance is involved in the transmission of the middle pair of spots in 501 although there is no evidence that the same spots in other stocks are inherited in that way.

Effect of temperature on the male spotting

During the course of the selection experiment it was fairly evident that some environmental factor was influencing the male spotting. Observations indicated that temperature was such a factor and to test this out a series of experiments were planned. These experiments were carried out at the tenth generation of selection when the divergence of the high and low strain was about as extreme as at any time during the selection experiment. The strains 500 low and high and 502 low and high were used in the tests. It had been noted that low rather than high temperature seemed to affect the results ordinarily obtained under room temperatures, so attention was directed chiefly to the effects of low temperatures as compared with room temperature.

A set of matings was made from each strain, some of them being kept under room temperatures (17° — 23°C), and others being placed in a basement room where the temperatures ranged between eleven and

TABLE 12
Effects of temperature on spotting of males.

A. Strain 500 low

Temperature condition	Type 1	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
Room (17-23° C)	546	5	2	6	8	9				3	579
Basement (11-15° C)	69					418	6		3	4	520

B. Strain 500 high

Room (17-23° C)	99	10	12	23	28	49	23	6	24	422	696
Basement (11-15° C)						54	10		15	73	152

C. Strain 502 low

Room (17-23° C)	92		1	4	1	166	7	1	4	8	284
Basement (11-15° C)	4					249			1		254

D. Strain 502 high

Room (17-23° C)									1	502	503
Basement (11-15° C)						15	8		5	97	125
Incubator (25° C)									1	61	62

In table 12 are shown the results of a series of experiments to test the effect of temperature on the spotting of males. Under room temperatures strain 500 low was almost pure for type 1 males, while strain 502 high was almost pure for type 16 males. Strain 500 high gave a fairly high percent of type 16 males under room temperatures while strain 502 low gave a good percent of type 1 males. From the tables it will be seen that the lower temperatures caused an increase in the type 11 males or in other words they brought out the outside pair of spots in all strains in which it did not already appear.

fifteen degrees centigrade. The species will not breed under temperatures much lower than those in the basement. The results are shown in table 12. No effects of high temperature had been observed in the laboratory, but to test the effect of high temperatures one culture of 502 high was reared in an incubator where the temperature was kept at 25°C. Strain 500 low which at room temperature produced almost exclusively males of the two-spotted type, reacted quite differently at lower temperatures. When 500 low was reared at the basement temperatures a large percentage of the males possessed very distinct outside spots. The middle pair of spots was not affected by the low temperatures. Strain 500 high which at room temperatures ordinarily produced a large

majority of six-spotted males, at basement temperature produced no two-spotted males at all, but only males having the outside pair of spots. Strain 502 low never produced as high a percentage of two-spotted males as the other low strains. This strain proved to be always more susceptible to the effect of low temperatures. The count for room temperature in table 12 is hardly characteristic of the strain, for at that time the percentage of two-spotted males was somewhat low. In the stocks of 502 low reared at the basement temperatures practically all of the males were those possessing the outside pair of spots. The stock of 502 high which at the time of the test was practically pure for six-spotted males when reared at room temperatures was also reared in the basement room. Since this stock was, at room temperatures, already pure for six-spotted males, low temperatures could not increase the percentage of males possessing the outside pair of spots. The results show that the low temperatures had no effect on the outside pair of spots but did seem to reduce somewhat the number of males possessing the middle spots. Instead of being pure for six-spotted males as was the condition under room temperatures a few of the males had one or both of the middle spots missing. Strain 502 high was also subjected to high temperatures. It was reared in an incubator where the temperature was kept at 25°C. Table 12 shows that results obtained at this temperature were not different from those obtained under room conditions.

These tests show that the outside pair of spots could be brought out by low temperatures regardless of the spotting condition of the strain induced by selection. Even in lines where none of the males under room temperatures possessed the outside spots, these spots could be made very distinct in a large majority of the males under lower temperatures. In strains where the males did not possess the middle pair of spots under room temperatures, the lower temperatures did not bring out these spots as it did the outside pair. Strains which under room temperatures possessed the middle pair of spots showed a tendency to lack them at low temperatures. In fact the lower temperatures showed a slight tendency to reduce the distinctness of this pair of spots. The lower temperatures also had a tendency to intensify the general pigmentation of the fly. Temperatures higher than room temperatures seemed to have no effect upon either the middle or outside pair of spots. It is true that the data on the effects of higher temperatures are rather meager and more matings would have given more conclusive results. It was the intention to test this out further at a later date but pressure of other work has made

this impossible. However, judging from observations made during the course of the selection experiment, I feel confident that more extensive data would only substantiate the results already recorded.

The facts brought out by this experiment have a definite bearing upon the selection experiments. Low temperatures occasionally encountered in the laboratory would have considerable influence upon the results of the selection experiment. This effect, especially upon the low strains, was noted several times. The effect of low temperatures is responsible for some of the irregularities in the graphs representing the behavior of the strains (see figures 2, 3 and 4). The rather sudden upward divergence of the graphs for the low strains near the end of the selection experiments can be correlated with a period of low temperatures which decreased the percentage of two-spotted males.

Crosses between the various male strains

At the end of the selection experiment when the various male strains had become fairly well established, crosses were made between some of them. A large series of experiments were planned but only a limited number were carried out because of the interference of other duties. Two types of matings were made, one set in which the high and low strains of the same line were crossed and the other in which the high strains of two different lines were used.

For the set of matings where the high and low strains of the same line were crossed, line 500 was used. Generation fifteen of the 500 low was crossed with generation fourteen of strain 500 high. The cross, 500 low male by 500 high female, and its reciprocal were both made and the results from the two types of crosses were the same (table 13). As to dominance or recessiveness, it would hardly be expected that either strain would act as a complete dominant for neither was absolutely pure for its type. The F_1 results show a high percentage of the males to be of the two-spotted type, but there are also considerably more six-spotted males than were to be found in the low strain. So the results show spotting in the males which is intermediate between the two strains. The lack of Mendelian ratios is probably due to the lack of dominance of either type. This would affect not only the F_1 generation but also the ratios obtained in the F_2 generation.

For the cross between two high strains, lines 500 and 502 were used. The fourteenth generation of the 500 high was crossed with the same generation of the strain 502 high. At the time of the cross the 502 high

TABLE 13
*F*₁ generation of crosses between various male strains.

A. Strain 500 high × strain 500 low

Type of cross	Type I	Type 2	Type 4	Type 5	Type 6	Type 11	Type 12	Type 13	Type 15	Type 16	Total males
500 high ♀ × 500 low ♂	245	17	1	20	12	34	10		1	59	399
500 low ♀ × 500 high ♂	465	17	1	18	11	47	8		5	40	612
Total	710	34	2	38	23	81	18		6	99	1011

B. Strain 502 high × strain 500 high

502 high ♀ × 500 high ♂	5	2			1	14	5			425	452
500 high ♀ × 502 high ♂	4			1		3	1		1	136	146
Total	9	2		1	1	17	6		1	561	598

The results in table 13 are for the crosses between the high and low strains of line 500 and between the high strains of lines 500 and 502. The condition of the two strains of line 500 at the time of this cross is shown in the last generation of selection of these strains (tables 1 and 2). The cross gave a result which was intermediate between the two strains. The cross between the two high strains gave a result which might be considered like either strain since the two strains were very similar at the time of the cross.

strain was practically true for the six-spotted type of males while strain 500 high still threw a few two-spotted males in each generation. The cross was made in both directions with no difference resulting between the two types of crosses. The results found in table 13 show a very high percentage of six-spotted males. The results which are about characteristic of either strain show that the same factors are responsible for the spotting character in each strain.

Summary

1. Selection was effective in changing the percentage of two-spotted or six-spotted males in a strain. There was a difference in amenability to selection of the different lines taken from nature.

2. The effects of selection remained permanent, at least for several generations.

3. The crosses with the 501 type of male indicate that there is sex-linked inheritance involved in the case of both the middle and outside pairs of spots.

4. Low temperatures tend to bring out the outside pair of spots on the male and also seem to have a slight tendency to cause the middle pair of spots to disappear.

5. Neither type is dominant in the F_1 generation when strains with two-spotted males are crossed to strains with six-spotted ones.

6. The results of crosses between the high strains of different lines indicate that their spottedness is due to the same factors.

FEMALE SPOTTING

Abnormal female

While examining flies on March 16, 1916, in one bottle, six females were found which had the middle pair of spots missing from the fourth abdominal segment. The other two pairs of spots were normal in size and position but there was no indication of the middle pair (plate I, no. 5, page 64). These females were mated to brothers and from this mating originated a strain of flies which was called the "abnormal female" strain or for the sake of brevity the "ab" strain. This strain is characterized by the absence of the middle pair of spots from the fifth abdominal segment.

Since there is sexual dimorphism in the spotting of the fifth abdominal segment, the problem of analyzing the spotting inheritance became rather difficult. In mating the original ab females to brothers, the males had to be selected blindly, as there was no way of knowing what relation if any, the male spotting bore to the female spotting. So in selection for purity with respect to the ab condition, the type of males used was kept constant, only those being used which possessed but one pair of spots, the large dorsal ones (plate I, no. 1).

Selection of ab strain

As already mentioned the six original ab females were mated to two-spotted brothers in stock culture and the total of their offspring showed 576 normal females and 105 ab females. (It might be said here that these early counts of ab females are not absolutely accurate as there are always a few flies which have the ab spotting on one side and normal on the other. These intermediates at first were called ab or normals depending upon the intensity of the spots. Later they were recorded in the counts.) It was hoped that by mating ab females to their brothers a pure strain, with respect to the ab female, might be established. At first the results seemed rather puzzling for in each cross ab females were

TABLE 14
Selection from the original abnormal females to establish a pure abnormal strain.

ration	<u>226 N-6 ab</u>						
I	<u>6</u>						
	<u>576 N-105 ab</u>						
2	<u>2</u>						
	<u>245 N-74 ab</u>						
3	6	3					
	<u>53 N-115 ab</u>	<u>63 N-62 ab</u>					
4	1	4	7	28	29	32	33
	2 N-30 ab	2 N-20 ab	10 N-121 ab	1 N-66 ab	1 N-53 ab	10 N-80 ab	33 N-95 ab
4	3	5	17	18	19	21	
	<u>6 N-131 ab</u>	<u>0 N-79 ab</u>	<u>1 N-64 ab</u>	<u>0 N-44 ab</u>	<u>20 N-91 ab</u>	<u>12 N-72 ab</u>	
5	11	12	13	26	22	23	
	18 N-44 ab	0 N-33 ab	0 N-66 ab	13 N-7 ab	0 N-7 ab	1 N-5 ab	

The letter "N" in table 14 indicates normal females and "ab" stands for abnormal females. The figures above the line indicate the mating number in the generation. This selection line was lost at the end of the fifth generation.

obtained but the relative number of ab and normal females was quite variable. Table 14 shows the selection which was carried on for five generations. It will be seen that in the fourth generation of selection there were two matings (matings 5 and 18) which gave only ab females. Similar results were obtained in the fifth generation. Unfortunately at this time the selected strain was lost, due to the extreme summer heat. Previously, however, the ab strain had been crossed to a wild strain which did not show the ab condition in the females. From the F_1 of this cross a few ab females were saved and mated to brothers and in this way the selection was renewed.

It should be noted that the cross from which the ab strain was regained was one in which a male from the ab strain had been mated to a normal female from a wild strain (recorded as original cross in table 15).

Although the male himself did not show the ab character, he was capable of transmitting it to his offspring. In the F₂, or generation B, a large number of flies were obtained and the selection for a pure ab strain was once more started. In the third generation eighteen matings were made with the ab females as mothers, and in only one case (mating C 8) were the females obtained all ab and this was probably due to chance as the count was very small. In generation D the ab females were used in twenty-nine matings and six of them proved to be pure with respect to the ab condition. It was then thought that the strain had reached purity for the ab condition and thirteen matings were made from among the offspring of the matings which bred true. The results of this generation were again disappointing as the thirteen matings did not all breed true. Four matings were made from D27 and they were all pure, five were made from D44 and none of them were pure, four were made from D45 and two were pure. These inconsistencies could not be explained at this time but later matings added new light which made them explainable. From among the offspring of D27 more matings were made all of which bred true again. This is the origin of the ab strain which has bred true for many generations.

While the strain producing the ab females was being selected for purity, back selection was also attempted. This was to see if selection was effective in both directions. From mating B1 (table 15) back

TABLE 15
Selection in strain producing abnormal females.

Generation	Parent	Female spotting				Generation	Parent	Female spotting			
		Normal	Ab	Left	Right			Normal	Ab	Left	Right
Original cross	A2 B1 A2 B1 B1 B1 B1 B1 B1 B1 B1 B1 B1 B1 B1 B1 B1	10	9			E7	D2	1	135	3	5
A2		6	7			E8	D15		130		1
B1		200	138	34	31	E11	D15	22	171	5	5
C3		51	92	18	11	E13	D30		17		
C4		46	48	8	5	E15	D31		117		
C5		70	36	6	11	E16	D44	8	94	10	4
C6		16	65	9	10	E17	D27		69		
C7		105	30	10	6	E18	D27		124		
C8			5			E20	D27		126		
C10		59	47	10	7	E21	D27		128		
C12		1	14	2		E23	D44	13	62	2	3
C13		51	13	3	1	E24	D45	57	165	5	7
C16		8	16	2	1	E27	D15	7	349	12	11
C17		55	9	8	2	E28	D30		135		
						E29	D30		125		

TABLE 15 (continued)

Generation	Parent	Female Spotting				Generation	Parent	Female spotting			
		Normal	Ab	Left	Right			Normal	Ab	Left	Right
C18	B1	54	20	5	4	E30	D30		115		2
C19	B1	24	33	7	5	E33	D33	1	179		1
C21	B1	2	11	2	2	E34	D33	4	153	2	
C22	B1	26	33	7	4	E36	D44	24	159	6	12
C26	B1	9	69	6	6	E37	D44	31	155	9	3
C27	B1	3	14	1		E39	D44	2	251	1	
C32	B1	2	18	1	1	E40	D45	1	237		1
D2	C1	8	81	4		E41	D45		198	1	
D3	C4	10	26	5	3	E42	D45		63		
D4	C4	39	67	3	9	E47	D4	27	242	8	7
D6	C4	29	46	6	3	F1	E18		161		
D7	C3	46	16	2	4	F2	E20		176		
D8	C3	17	44	4	7	F3	E20		84		
D9	C3	26	14	2	3	F4	E20		82		
D11	C6	2	16	4		F5	E20		175		
D12	C5	14	14	2	3	F6	E28		222		
D13	C5	25	30	4	2	F7	E28		76		
D15	C6	43	50	6	11	F8	E28		254		
D16	C7	3	41	2	2	F9	E28		178		
D17	C3	3	64	1	2	F11	E41	1	316	4	2
D18	C4	7	37	4	2	F13	E41	37	192	8	7
D22	C12		81	1		F16	E25	66	251	19	13
D26	C8	25	250	7	13	F17	E37	6	133	5	5
D27	C12		98			F18	E37	2	106		1
D30	C12	1	124			F19	E37	15	83	7	3
D31	C8	7	16	3		F25	E25	10	48	4	2
D33	C12	9	23	2	3	F26	E24		240	2	
D37	C6		27		1	F29	E24	84	159	22	16
D38	C6	18	36	3	3	F30	E41	15	207	12	3
D44	C26		253	1		F31	E29		414		1
D45	C26		138	2		F32	E21	2	370		
D46	C32		93			G1	F2		195	1	
D47	C32	10	31	2	1	G2	F2		176		
D49	C32	16	37	4	6	G3	F8		401		
D50	C26	9	145	1	1	G4	F2		66		
D51	C32	5	30	1	2	G5	F8		100		
E1	D15	10	285	8	12	G6	F2		128		
E2	D15	14	288	11	13	G7	F8		59		
E3	D15		247	1		H1	G3		186		
E4	D2		57			H2	G3		198		
E5	D2	1	4		2	I1	H1		67		
E6	D2	3	59	2		I2	H2		214		

Table 15 shows the selection required to bring the abnormal strain to purity. The letters in the column to the left indicate the generation of selection and the figures which follow them indicate the mating number in the generation. "Left" indicates a female which is abnormal only on the left side and "right" one which is abnormal on the right side.

TABLE 16
Back selection of abnormal strain.

Generation	B 1	
	200 N-138 ab	
I	14	
	153 N-3 ab	
2	20	21
	29	32
	50 N-6 ab	61 N-24 ab
	64 N-8 ab	16 N-2 ab
3	25	
	133 N-130 ab	
4	14	15
	20	21
	22	
	103 N-95 ab	233 N-126 ab
	151 N-8 ab	129 N-19 ab
	111 N-137 ab	
5	12	13
	250 N-0 ab	427 N-15 ab
6	6	4
	371 N-18 ab	147 N-56 ab
7	9	8
	224 N-9 ab	40 N-17 ab
8	6	5
	313 N-4 ab	203 N-72 ab
9	2	1
	283 N-0 ab	395 N-65 ab

Reverse selection using abnormal females

Table 16 gives the results of selection for the normal female in the strain producing a high percent of abnormal females. The selection here is in a direction opposite to that in table 15 but started with mating B1 of that table. At generation 6 selection was again reversed (selecting for ab females) and is shown as the offspring of mating 4 of generation 6. The selection for normal females was still continued and is shown as the offspring of mating 6 of generation 6.

selection was started, using normal females. At the time back selection was started the strain was throwing about an equal number of ab females and normal females. Selection of this sort was carried on for nine generations and the strain became practically pure for normal females (table 16). In the fifth generation of back selection when the strain was approaching purity for the normal females, selection was started again in the reverse direction. This was done by using ab females as mothers. Selections in opposite directions were carried along for four generations, by which time one strain had become practically pure for the normal female, while the other was again throwing a large percentage of ab females. These experiments show that selection is effective in either direction before purity is reached and that purity may be reached for either type if selection is carried far enough.

The question as to how the ab strain originated,—whether as a simple mutation from the beginning, or as a result of selection—will not be discussed at present. Before taking up this question we will examine the results of the matings of this strain to normal strains. These matings were made in attempting to analyze this variation.

Matings of the ab strain with the normal strain 500 low

The strain 500 low to which the ab strain was crossed was one which had been bred in the laboratory for six generations and was in no way related to the ab strain. The males of this strain were almost all of what may be called the normal male type, having only the two large spots. The 500 low strain had been selected for three generations for the above type of male and from the beginning showed a tendency to produce males of that sort. The females of the 500 low strain were all normal, that is, having a full set of six spots. In all strains bred in the laboratory a few ab females appeared, but they seemed to be somatic variations as will be shown later (page 102). Since this strain was very similar to the ab strain, as far as the male spotting was concerned, and normal with respect to the female spotting, it was considered a good strain with which to cross in testing the genetic behavior of the ab strain.

As will be seen in table 17 twelve matings were made between abnormal females and males of the normal strain, and nine matings were made between males of the abnormal strain and females of the normal strain. For the first type of cross the totals were 977 normal females, 1645 ab females, 329 "left" females and 273 "right" females.¹ In the

¹ By "left" female is meant a female which is abnormal only on the left side and the expression "right" refers in the same manner to the right side.

TABLE 17
F₁ generation of cross abnormal strain × normal strain 500 low.

Abnormal ♀ × normal ♂					Normal ♀ × abnormal ♂				
Mating number	Female spotting				Mating number	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
150	57	158	17	17	157	164	190	42	46
151	64	81	30	19	158	204	206	56	39
153	15	142	19	15	159	35	91	21	21
154	72	124	28	21	168	66	177	27	32
155	79	187	25	29	169	123	329	51	59
162	24	103	14	13	170	75	261	43	42
163	57	77	17	11	171	24	158	13	10
164	53	92	22	24	172	52	183	30	22
165	91	26	13	8	250	154	230	60	55
166	92	139	33	26					
188	281	227	71	49					
251	92	289	40	41					
Totals	977	1645	329	273	Totals	897	1833	343	326

Grand total, 1874 normals, 3478 abnormalities, 672 lefts, 599 rights.

In table 17 are shown the F_1 results of a cross between the abnormal female strain and strain 500 low. The females in strain 500 low were all normals. Crosses in both directions are shown. Both normal and abnormal females appeared in the F_1 generation with about twice as many abnormalities as normals.

second set of matings in which the male from the ab strain was used the totals were 897 normals, 1833 abnormalities; 343 lefts and 326 rights. These matings were all single-pair matings. The total of the F_1 is 1874 normals, 3478 abnormalities, 672 lefts and 599 rights. These F_1 results threw very little light upon the nature of the abnormal variation. They do show however that either the male or the female of the ab strain will transmit the ab character even though the males do not show it. It is also seen that the ratio of abnormalities and normals is about the same regardless of which way the cross was made.

In order to carry the analysis still further F_2 matings were made (table 18). Since there were both ab and normal females in the F_1 it was necessary to make two types of matings in the F_2 , that is, ab females with brothers and normal females with brothers. The totals for the first type of matings were 945 normals, 1248 abnormalities, 138 lefts and 112 rights, and for the second type 1879 normals, 1415 abnormalities, 167 lefts and 159 rights. If the ab condition acts as a Mendelian character

there should be no difference in the genetic constitution of the two types of females in the F_1 . Then F_1 ab and normal females when crossed should behave the same with respect to the sort of offspring to which they give rise. Three types of crosses were made in which the behavior of these two sorts of females can be compared: (a) F_1 females back-crossed to the pure ab strain, (b) F_1 females back-crossed to the normal strain which was used in the cross, and (c) F_1 females by brothers. The totals for the results of these crosses are shown in table 19. Considering the individual matings the results seem quite variable in each type of mating, but the totals show evidence of a slight difference in behavior of the two types of females appearing in the F_1 . Where the two types of females were back-crossed to the pure ab strain there is a higher relative number of females, as compared with the number of normals produced when the ab female was the mother. Where the cross was a back-cross to the normal strain there was no apparent difference between the two females, but again when they were crossed to their brothers

TABLE 18

F₂ generation of cross abnormal female strain $\times$ strain 500 low.

Abnormal F_1 ♀ $\times$ normal brother					Normal F_1 ♀ $\times$ normal brother				
Mating number	Female spotting				Mating number	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
154 A1	80	125	20	13	154 A3	164	177	10	13
154 A2	73	177	5	9	157 A1	114	137	18	22
157 A3	167	157	27	20	157 A2	178	200	12	21
157 A4	126	101	15	8	157 A5	166	113	11	5
162 A1	107	179	15	16	157 A6	188	156	24	15
164 A3	63	172	14	11	164 A1	101	72	11	4
164 A4	105	213	19	16	164 A2	87	146	9	14
169 A1	83	28	5	5	169 A4	128	89	10	10
169 A2	141	96	18	14	188 A1	262	148	33	17
					250 A1	317	88	13	20
					251 A1	174	89	16	18
Totals	945	1248	138	112	Totals	1879	1415	167	159

Grand total, 2824 normals, 2663 abnormals, 305 lefts, 271 rights.

In table 18 showing the F_2 results of the cross abnormal female strain by strain 500 low, the grand total shows about an equal number of abnormal and normal females. However the ab F_1 female gave a higher percentage of abnormals in the F_2 than the normal female from the same generation.

a higher relative number of ab females appeared when the maternal parent was an ab female. With the large number of individuals examined in these crosses it hardly seems possible that these variations could be due to chance. Assuming that ab is an ordinary Mendelian character, these two types of F_1 females should react the same genetically, but when they are each crossed to males of the same type, they each tend to beget young which show a higher percentage like their mothers. Although there is a variation in their behavior the preponderance is never large enough to obliterate any class of individuals which should have

TABLE 19

A comparison of the behavior of the two types of females appearing in the F_1 generation.

Type of male used in cross	F_1 ab female				F_1 normal female			
	Female spotting				Female spotting			
	Normal	Ab	Left	Right	Normal	Ab	Left	Right
Male from pure normal strain	2706	382	142	138	2812	486	133	127
Male from pure ab strain	203	2922	110	81	223	1588	103	81
F_1 brother	945	1248	138	112	1879	1415	167	159

Table 19 shows the results of three types of crosses comparing the behavior of the two types of females found in the F_1 generation. In two of the crosses the F_1 ab female produced more abnormals than her normal sister.

segregated out. The factor which brings about the difference seems to play entirely upon the heterozygous individuals and therefore does not prevent the ab condition from still being considered as behaving in a Mendelian manner.

The F_2 normal and ab females were back-crossed to the respective strains from which they came, to test whether there was Mendelian segregation. These results are shown in table 20. Where the F_2 ab female was back-crossed to a male from the ab strain, eight of the fifteen matings were pure for the ab condition. Where the F_2 normal female was back-crossed to a male from the normal strain, nine out of the fifteen matings bred practically pure for the normal condition. The same sort of back-crosses were made in the F_1 generation (see table 19). Out of ten crosses between F_1 ab females and males from the ab strain no matings went pure for the ab condition and likewise no all-normal counts were obtained from making eight crosses between F_1 normal females and males from the normal strain.

TABLE 20

Back crosses of the two types of females appearing in the F₂ generation.

F ₂ ab ♀ × pure ab ♂					F ₂ normal ♀ × pure normal ♂				
Mating number	Female spotting				Mating number	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
205		141			210	398	10	10	14
206	19	109	2	3	211	56		4	2
207	22	94	9	7	212	321	10	7	15
208		157			213	387	5	6	4
209		39			214	220	21	9	10
220	14	222	10	4	218	208	57	18	12
221		67			225	369			
222	3	61		1	226	314		1	
223	17	157	9	7	227	227	1	1	3
224		223			228	269	49	18	15
241		86	1		229	210		1	
256	43	251	5	10	263	361	1		1
266	20	268	9	17	264	336		1	1
269		80			265	278	2	4	3
270		113			267	263		1	1

The crosses in table 20 were to test the segregation of the normal and abnormal females in the F₂ generation. Ab females from the F₂ generation were back-crossed to males of the pure ab strain while normal females of the same generation were back-crossed to males of the pure normal strain. Of the first cross, eight out of fifteen of the females acted as pure abnormals and in the latter cross, nine out of fifteen of the females acted as pure normals.

The fact that pure stocks were regained by back-crossing the two types of F₂ females to the stock from which they originated, indicates that there is Mendelian segregation. That this could not be done with apparently the same types of females from the F₁ also shows Mendelian behavior.

Some of the F₂ females having a spot on one side only (recorded as lefts or rights) were tested as to their genetic constitution. The test was made by back-crossing them to the original strains from which they came. The number of such crosses was not large but in all of them both abnormal and normal females appeared, showing that the females used were heterozygous flies. So the flies indicated in the tables as lefts and rights are probably individuals which are heterozygous for the ab and normal condition.

Summary of various types of matings in testing the behavior of ab when crossed to the strain 500 low

1. In the F_1 generation resulting from the cross between the ab female and the 500 low strain and its reciprocal, there is not a dominance of one or the other of the contrasted characters. Both types appear in F_1 with about twice as many abnormals as normals. Both males and females from the ab strain are capable of transmitting the ab character although only the females show it.

2. The two types of F_1 females when back-crossed to their respective original strains did not give pure stock, showing that these females were heterozygous and there was no segregation in this generation.

3. The two types of F_1 females gave slightly different ratios when crossed to males of the same genetic constitution. The F_1 ab female gave more abnormals than the F_1 normal female when they were crossed to the same male.

4. There was Mendelian segregation in F_2 of the two original types. This was shown by back-crosses of the two types of females in F_2 and results of the F_2 generation.

Crosses between the ab strain and strain 502 high

In an attempt to further analyze the genetic behavior of ab, the ab strain was mated to strain 502 high. The strain 502 high was like 500 low so far as the female condition was concerned, that is, all the females were normal, i.e. with a full set of six spots. The only difference between these strains was that the males in strain 502 high had been selected for a full set of six spots while the strain 500 low had been selected for the normal or two-spotted male condition.

To the writer's surprise entirely different ratios were obtained (table 21) in F_1 when the ab strain was crossed to the strain 502 high. With apparently the same environmental conditions as before, a large majority of the F_1 flies were normal. There was practically a dominance of the normal condition over the ab. Out of the 1965 F_1 females examined only 71 were abnormal.

These F_1 results are quite different from those obtained when ab was crossed to strain 500 low (table 17). The count for the F_1 generation of the cross of ab with 500 low was 1874 normals, 3478 abnormals, 672 lefts and 599 rights. For the abnormal-by-502-high cross the counts were 1756 normals, 71 abnormals, 69 lefts and 69 rights. Since there is almost complete dominance of the normal condition over the abnormal condition in crossing ab by the 502 high strain, it would be expected that

TABLE 21
Totals of the F_1 and F_2 generations of the
cross abnormal strain $\times$ strain 502 high.

F ₁ generation			
Female spotting			
Normal	Ab	Left	Right
1756	71	69	69
F ₂ generation			
3169	1117	54	47

The counts in table 21 are the results of the F_1 and F_2 generations of the cross between strain 502 high and the abnormal female strain. Very few abnormalities appeared in the F_1 generation and an approximate 3:1 Mendelian ratio was obtained in the F_2 generation. Note that the ratios in this cross are quite different from those obtained in the cross, abnormal female by strain 500 low.

the usual 3 to 1 ratio would be obtained in the F_2 . This was found to be true as will be seen in table 21. The totals for the F_2 were 3169 normals, 1117 abnormalities, 54 lefts, and 47 rights. F_2 data were obtained from ab female by 502 high male and the reciprocal cross, but the ratios were approximately the same in each.

F_3 matings were also made to see whether a pure ab strain might be extracted. Fourteen matings were made of which two bred true to the ab condition. Since there is almost complete dominance of the normal condition, probably all of the ab females obtained in the F_2 are pure abnormalities. There is no way to determine the genetic constitution of the males with respect to the ab condition except by breeding. One-fourth of the males should be pure for the ab condition, so one-fourth of the F_3 matings should have yielded only abnormalities. Only two out of fourteen matings were of this character, but this shortage was probably due to chance because of the small number of matings.

Here we have the ab condition acting very much as an ordinary Mendelian character, the normal condition being almost completely dominant in the F_1 generation. The only thing unusual about this set of matings was that there was such a difference in behavior of the ab character here as compared with its behavior when crossed to strain 500 low. The explanation for this is found in the results of the tests which follow.

Genetic behavior of the ab strain when crossed to various strains

Since such a difference was found in the F_1 behavior of the ab strain when crossed to strains 500 low and 502 high, it was decided to test its behavior with all of the strains carried. The strains used were the selection strains of the experiment upon the male spotting. The females in all these strains were normal, having a full set of six spots. The only difference was that part of the strains had been selected for males with two spots while the rest were selected for the six-spotted condition of the males. When these matings were made the high strains had attained a condition in which a large percentage of the males were of the desired type with six spots, and likewise the low strains possessed mostly two-spotted males. The matings were all made at once and subjected to as nearly identical environmental conditions as possible. When the F_1 counts were made the same difference was again found to exist between the ratios when ab was crossed to 500 low and 502 high. Some other rather striking results were obtained from this series of crosses.

The results show that the dominance or recessiveness in the F_1 is affected by the degree of spottedness of the males in the strain in which the ab strain is crossed (see table 22). All three of the strains which are called high give almost complete dominance of the normal female condition over the ab in F_1 while the low strains crossed to the ab strain yield nearly half or more of abnormal F_1 females. The ratios obtained were as follows: In line 500 the low strain produced 307 normals to 289 abnormals, and the high strain 710 normals to 44 abnormals; in line 502 the low strain, 176 normals to 259 abnormals, and the high strain, 457 normals to 7 abnormals; in line 504 the low strain, 166 normals to 427 abnormals, and the high strain, 296 normals to 93 abnormals. Since this consistent difference appeared in the reaction of the high and

TABLE 22
Results of F_1 generation in crossing abnormal strain with male-selection strains.

Strain crossed with ab	Female spotting				Strain crossed with ab	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
500 low	370	289	60	72	500 high	710	44	28	18
502 low	176	259	23	24	502 high	457	7	12	8
504 low	166	427	37	45	504 high	296	93	20	15

Table 22 shows the difference in reaction of the abnormal female strain in the F_1 generation when crossed to the various strains of the male selection lines. The percent of ab females is much higher in crosses to the low strains than in the crosses to the high strains.

low strains in crosses to the ab strain when all environmental factors were kept constant, it seems that the difference in genetic constitution of the two strains is the responsible factor.

*Relation of temperature to genetic behavior of abnormal
female condition*

After the ab strain became once established it was quite evident that variations of temperature had no effect upon it, for although there was considerable fluctuation in the temperature of the laboratory the strain remained pure for the ab condition.

The variability of the recessiveness or dominance of the ab in the F_1 led the writer to suspect that some environmental condition might be playing a part. In addition to the influence of the "spottedness" of the males in the strain to which it was crossed there seemed to be some other factor. When the type of male to which the ab was crossed was kept constant there was still considerable variation in the F_1 results. This was most noticeable in crosses where strains possessing normal or two-spotted males were used, resulting in F_1 ratios with a high percentage of ab females. Here were obtained rather variable results. Temperature being one of the simplest environmental factors to analyze, a series of experiments were carried out to test its influence upon the behavior of ab in the F_1 generation.

In testing the effect of temperature, flies reared at room temperature were compared with some reared at high temperature in an incubator, and also with others reared at a low temperature in a basement room without heat. The effects of temperature upon the F_1 behavior of ab when crossed to strains 500 low and 502 high were tested. The results of these two experiments are given in table 23. The only place where it was possible to keep the temperature constant was in the incubator. The laboratory and basement temperatures were somewhat variable but the range of variation was sufficiently different to make a satisfactory experiment. The incubator was kept at 25°C which was just about the maximum temperature at which it was possible to rear *Drosophila busckii*. Even at that temperature the flies were somewhat undersized and reduced in number. Immediately after pairing the flies, the culture bottle was placed in the incubator and kept there continually except while the progeny was being examined. In the case of the cold, the flies were also reared at about the minimum temperature at which they would reproduce. The basement-room temperatures were so low that it was difficult to get the flies to mate when kept there from the beginning. To overcome this the flies

TABLE 23
Effect of temperature on the F_1 behavior of the abnormal strain.

F_1 results of cross ab $\times$ 500 low					F_1 results of cross ab $\times$ 502 high				
Temperature condition	Female spotting				Temperature condition	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
Room 17-23° C	370	289	60	72	Room 17-23° C	1299	64	57	61
Incubator 25° C	466	189	71	66	Incubator 25° C	142	1	2	4
Cold 11-16° C	3	625	8	4	Cold 9-15° C	265	133	23	42

Table 23 shows the effect of temperature on the F_1 behavior of the abnormal strain when crossed to strains 500 low and 502 high. It has already been shown that the ab strain reacts differently with each of these strains in the F_1 generation under normal room temperatures. From the table it is seen that lower temperatures increase the percent of ab females in the F_1 generation in each of the crosses.

were started in the laboratory and kept there till several eggs were laid. In the cross between 500 low and ab, in the low-temperature series, the culture bottles were kept at laboratory temperatures for the first week and then kept in the cold for the rest of the time. At that time the basement temperature varied between eleven and sixteen degrees centigrade. When the experiment was carried out with the strain 502 high the basement temperatures were somewhat lower (9°—15°C) and it was necessary not only to start the cultures in the laboratory but also to alternate cultures between the laboratory and cold room.

The results obtained from these matings are as follows: High temperatures tend to increase the number of normal females in the F_1 generation while low temperatures tend to increase the number of abnormals. As will be seen in table 23 giving these results, the temperatures seem to have a greater or less range of effect. Under room temperatures, strains 502 high and 500 low, due to a difference in spottedness, reacted differently in the F_1 generation when crossed with ab. Under the different temperature conditions they still retained their characteristic influence upon the F_1 behavior of ab. The lowest temperature used made the ab condition almost completely dominant when crossed to 500 low while the highest temperatures used made the ab condition almost completely recessive when crossed to 502 high. This is a very pretty demonstration of the combined influence of two factors upon the behavior of a Mendelian character.

Since temperature influences so much the ratios of the F_1 generation it is evident that its influence is upon heterozygous flies. To further demonstrate this some bottles of the F_2 generation of the cross 502 high by ab were reared in the cold. In this case a larger percentage of the females should be of the ab type than when the F_1 generation of the same cross was reared under the same condition. In the F_2 generation some pure abnormals should segregate out and not being influenced by the low temperature would increase this class. The results of the F_2 generation reared under the low temperatures were as follows: 398 normals, 398 abnormals, 18 lefts and 17 rights. The results of the F_1 generation reared under the same conditions were (table 23); 265 normals, 133 abnormals, 23 lefts and 42 rights. It is seen that the expected increase in the percentage of abnormals in the F_2 generation occurred. This is additional evidence that temperature in modifying the ratios obtained from crosses of the ab female strain, acts upon the heterozygous flies.

Linkage

Since the writer (WARREN 1917) had earlier found two eye mutations in this species it was thought that crosses between these two mutations and ab might be of interest. Earlier crosses between these two eye mutations indicated that they were probably in the same chromosome. The two eye mutations had been called "red" and "chocolate."

Ab was first crossed to the mutation red eye. In the F_1 , as had been observed in other crosses, the ab was only partially recessive to the normal spotting in the red-eye strain. In the F_2 , in case the red-eye mutation and ab were in the same chromosome we would expect to get no red ab females (the males cannot be considered since they do not show the ab character). If the two mutations should not be in the same chromosome we would expect independent segregation of the two characters in the F_2 and the chances would be that about one-half of the red-eyed flies would be abnormals. The F_2 results (table 24) were as follows: 193 red eye, normal-spotted; 110 red eye, ab-spotted; 640 normal eye, normal-spotted; 472 normal eye, ab-spotted. So it is seen that there is no linkage between red eye and ab, or in other words they are not in the same chromosome.

Ab was also crossed to chocolate eye. Since the red- and chocolate-eye mutations had been shown to be probably in the same chromosome we would expect ab to behave in the same manner when crossed to chocolate as it did with red eye. This expectation was realized, for chocolate and

TABLE 24
*Crosses testing linkage between the abnormal character
 and two eye mutations.*

A. F_2 of red-eyed strain $\times$ abnormal strain							
Red-eyed females				Normal-eyed females			
Normal	Ab	Left	Right	Normal	Ab	Left	Right
193	110	12	12	640	472	47	34

B. F_2 of chocolate-eyed strain $\times$ abnormal strain							
Chocolate-eyed females				Normal-eyed females			
Normal	Ab	Left	Right	Normal	Ab	Left	Right
102	121	14	10	319	398	37	47

Table 24 gives the results of crosses to test the linkage between the abnormal character and two eye mutations. Since there was independent segregation of the two characters in both crosses it can be assumed that the factor for the ab character is not in the same chromosome as the two eye mutations.

ab segregated independently in the F_2 generation. The results were as follows: 102 chocolate eye, normal-spotted; 121 chocolate eye, ab-spotted; 319 normal eye, normal-spotted; 398 normal eye, ab-spotted. So the indications from these crosses are that the mutation for the ab condition was not in the same chromosome in which the red and chocolate eye occurred. Since no other mutations have been found in this species it was impossible to test the linkage further.

Attempts to establish an ab strain from other stocks

In practically every strain of flies which was carried any length of time there appeared an occasional female which had the ab type of spotting. In some cases, out of a day's count of sixty females from a bottle there might be four or five females with the ab type of spotting. So high a percentage was unusual, for sometimes several hundred females could be examined without a single female of that type appearing. However the relative number of the ab type of females appearing was a factor of considerable fluctuation and seemed to be influenced by some environmental condition. The environmental condition necessary to bring them out was not determined although close observation was made with this point in mind.

During the course of this experiment, as these females of the ab type

appeared in the stocks from time to time, some of them were isolated and mated in an attempt to establish another ab strain. In some cases these strains were selected for several generations. Also some of the females of the ab type were crossed to males from the pure ab strain.

The data for these attempts are given in table 25. The strain 508 was selected for seven generations for the ab condition, but at the end of this period the strain was no nearer purity than at the beginning. In the sixth generation seven single-pair matings were made, but none of these

TABLE 25
Attempts to establish by selection a second abnormal strain.

A. Selection for ab in strain 508

Generation and mating	Female spotting				Generation and mating	Female spotting			
	Normal	Ab	Left	Right		Normal	Ab	Left	Right
Gen. A mating 1	124	14	3	4	Gen. F mating 2	163	11	7	12
Gen. B mating 1	219	17	13	14	Gen. F mating 3	106	9	9	10
Gen. C mating 1	217	7	6	9	Gen. F mating 4	39	9	10	4
Gen. D mating 1	252	28	24	18	Gen. F mating 5	224	24	27	23
Gen. E mating 1	160	44	22	25	Gen. F mating 6	57	6	5	10
Gen. F mating 1	61	4	6	7	Gen. F mating 7	80	10	12	14

B. Selection for ab in strain 503

Generation	Normal	Ab	Left	Right
A	289	4	3	3
B	223	11	5	3
C	147	1		1

Table 25 shows two attempts to establish a second ab strain from occasional ab females appearing in strains 508 and 503. Although strain 508 threw an unusual number of ab-appearing females, this percent could not be increased in six generations of selection. In strain 503 only a few abnormals ever appeared.

matings showed any high percentage of abnormals. Again in strain 503, selection was carried on for three generations with no effective results. In three other cases ab females from the normal strains were mated to brothers but among the offspring there were very few if any abnormals

Another type of mating was made to test the genetic constitution of the ab type of females appearing in the normal strains. This was to cross the ab-appearing females to males from the pure ab strain. If these females were of the same genetic constitution as the females in the pure ab strain, a cross to the males in the ab strain should breed true for the ab condition. The results of these crosses (table 26) were, that no more abnormals appeared in the F_1 than ordinarily appeared when the males from the ab strain were crossed to normal females from the respective strains in which the ab females were found. So it seems that

TABLE 26
Crosses of abnormal-appearing females found in wild stock to males from the pure abnormal stock.

Types of female spotting			
Normal	Ab	Left	Right
109	95	20	28
60	149	19	25
67	327	23	24
68	194	16	18

The counts in table 26 give the F_1 results of crosses of the occasionally appearing ab females in wild stocks to males from the pure abnormal stock. Had these ab-appearing females been genetically pure abnormals all the offspring of the F_1 generation should have been abnormals. No higher percent of abnormals appeared in the F_1 generation than if the mating had been made to normal-spotted females from a wild strain.

these constantly appearing females of the ab type in the various strains were not genotypically abnormals.

Discussion of results of the analysis of the abnormal type of female spotting

From the very beginning there existed two peculiarities of the abnormal strain which made the analysis of the character both difficult and confusing. These were sexual dimorphism in the spotting of the species and the variability of the dominance or recessiveness in the F_1 generation when crossed to a normal strain.

Discussing first the origin of the ab strain,—a glance at the data might seem to indicate that the purity of the ab strain had been arrived at by the cumulative effect of selection. The data shows that it required several generations of selection to bring the strain up to a state of purity and also that a somewhat gradual change took place in the direction of

selection. However it seems to the writer that the two peculiarities mentioned above offer an adequate explanation for the selection required to bring the ab strain to purity. Since the males did not show the character being selected for in the females, selection was necessarily blind as far as the paternal parent in each generation was concerned. This, together with the fact that heterozygous females might have the same appearance as pure ab females, would furnish sufficient explanation for the period of selection required to bring the ab strain to purity. The lack of success in attempting to establish pure ab strains from the occasional ab type of female appearing in normal strains indicates that the original ab individuals were probably mutations and the later-appearing were only somatic variations brought about by some environmental condition.

As to the genetic behavior of ab, early matings seemed to indicate that it had but few things in common with the behavior of a single Mendelian character. This was due largely to the lack of complete dominance or recessiveness of the ab in the F_1 generation when crossed to normal. More confusion was added when it was found that the relative number of abnormals appearing in the F_1 was influenced by the type of male spotting in the strain to which it was crossed. But after all of the data were summarized testing the effect of temperature and male spotting the following findings seem to hold true: *The ab condition in the female behaves as a Mendelian character. Its dominance or recessiveness is affected by temperature and by the type of male spotting in the normal strain to which it is crossed.* The effect of the spottedness of the males in the strain to which ab is crossed, is that the ab condition is almost entirely recessive in the F_1 generation when crossed to a normal strain in which the males are practically all of the type with a full set of six spots. The opposite holds true when ab is crossed to a normal strain where the males are of the two-spotted type. In this case ab acts more like a dominant over the normal female. High temperatures tend to reduce the number of abnormals appearing in the F_1 generation, while low temperatures increase them, but taking the effect of these factors into account the behavior of ab seemed to be that of an ordinary Mendelian character.

Summary

1. The type of female possessing the abnormal spotting probably originated as a mutation.

2. Although selection was necessary to bring the strain to purity for the abnormal type of spotting, the resulting purity was probably due only to isolation of individuals possessing the desired type of spotting, not to a cumulative effect on the strain as might be interpreted from the data. The difficulty encountered in bringing the strain to purity was due to the fact that the character was sex-limited.

3. The abnormal-spotting character acted as a Mendelian character. However the dominance or recessiveness of this character in the F_1 generation was rather variable and was found to be influenced by at least two factors.

4. One factor which influenced the behavior of the abnormal-spotting character in the F_1 generation was the strain to which the abnormal-female strain was crossed. When the strain possessing females with abnormal spotting was crossed to a strain possessing males of the six-spotted type, the former tended to act as a recessive character, but when crossed to a strain with males of the two-spotted type, it acted more like a dominant.

5. Temperature also influenced the behavior of the abnormal-spotting character in the F_1 generation. High temperatures tended to make the abnormal spotting act more like a recessive character while low temperatures cause it to act like a dominant one.

GENERAL DISCUSSION

The simplicity of a character offers no clue as to the simplicity of the manner of its inheritance. To the writer the most striking fact brought out in this study was the complexity of inheritance. A study of the spotting inheritance of a single body segment which bears only six spots might not ordinarily be expected to be very complicated. Numerous influences and interrelations of both genetic and environmental factors were found, but when carefully analyzed the inheritance was found to be Mendelian.

One of the most striking peculiarities was the almost entire independence of inheritance in the two sexes. Variations in the males of a strain did not usually influence the female spotting. The ab female and the 501 type of male were identical as far as the type of spotting was concerned. Each had the middle pair of spots missing but still there appeared to be no relation between the two when their inheritance was considered. Both males and females might possess the three pairs of spots but a different set of factors seemed to be responsible for the inheritance of the spots in each sex.

In all of the crosses between the various types of spotting studied in this paper there were no cases of complete dominance. The lack of dominance in the crossing of contrasted characters is frequently observed but its occurrence in all of the crosses in this work is worthy of note. In the case of the cross between the ab female strain and a strain of normal females, the percentage of ab females appearing in F_1 generation was dependent upon the temperature and the type of spotting of the male in the normal strain. Under certain combinations of these influencing conditions the ab condition was almost completely dominant.

Again each pair of spots seemed to be inherited independently and was acted upon differently by the same environmental factor. Low temperatures tended to intensify the outside pair of spots on the male while the same temperature tended to cause the middle pair of spots to be less distinct. Even the same pair of spots in the male was inherited differently in different strains. As simple a character as a pair of spots was dependent upon several factors.

The fact that one cannot look entirely to the germ cells for the solution of a problem in inheritance is rather strikingly brought out in this study. The importance of environmental conditions for the realization of certain characters was repeatedly seen. Temperature was shown to play an important part in making the ab condition a dominant or recessive character. The spotting in the males which seemed to be a character independent from the female spotting also influenced the dominance or recessiveness of the ab character. Again in the male spotting, certain spots which had been made to disappear by selection could be brought out again by subjection to certain temperatures. So when we consider the interrelation of different characters and the influences of environmental factors upon Mendelian characters, it is not surprising that the solution of the problem of inheritance has become more difficult than was anticipated at the time of the rediscovery of Mendel's Law.

In recent years considerable study has been devoted to the heredity of secondary sexual characters. Since the variations discussed in this paper are limited to one sex they may be considered as secondary sexual characters. It was found that the males were able to transmit variations in the female spotting which they themselves did not show. Likewise the females transmitted male variations which did not show. This ability of one sex to transmit the secondary sexual characters of the other has already been described in other insects. A similar case is that described by FOOT and STROBELL (1913) in the bug, *Eu-*

schistus. They crossed two species, one of which possessed a black spot on the male. The females of both species lacked the spot. However, the female of the species of the black-spotted male transmitted the spot although she herself did not show it. Other similar cases are GEROULD'S (1911) work on *Colias philodice* and DE MEIJERE'S (1910) work on *Papilio memnon*.

Considering the findings of this study in their relation to the evolution of the species it is difficult to say just what is happening. The spotting of the female seemed to be the more stable type. In the female the full set of six spots seemed to be the normal type while in the male one pair of dorsal spots was the commoner type in nature. The frequent variations found in the male spotting would indicate that evolution was taking place in that sex. In case an evolutionary process is in progress there would be two possibilities. The process might be one of loss or acquisition of spots to the fly. In the case of the species losing spots, all of the males would have once possessed six spots but have been the first of the two sexes to be acted upon by the process. On the other hand if the species be acquiring spots the action of the process upon the male has been retarded, the female having already changed from a two-spotted type to a six-spotted one. These are only suggestions of what might possibly be taking place, as there is no evidence from which we can draw any dependable conclusions.

GENERAL SUMMARY

1. This paper is a discussion of spotting inheritance in the fruit fly, *Drosophila busckii*. The spotting of the fifth abdominal segment is the subject of discussion.
2. There is sexual dimorphism in the spotting of this segment, the normal female having three pairs of spots on this segment and the male having one, two, or three pairs of spots on it.
3. An unusual type of female was found (probably originated as a mutant in the writer's stocks) which had one of the three pairs of spots missing. This was found to bear no relation to a similar type of spotting in the male; in fact the various types of spotting found in the two sexes were inherited independently.
4. The mutant in female spotting required considerable selection to bring the strain to purity, but the effect of selection was probably only to isolate individuals homozygous for the character.
5. Extensive selections were made for the type of male having one

pair of spots and the type having three pairs of spots. The strains in which selection was made for the two types of males were of a common parentage. By selection strains were produced which had a high percentage of the desired type but neither was ever brought to absolute purity for the type sought.

6. The behavior of the mutant in female spotting in the F_1 generation was affected by the strain to which it was crossed. If crossed to a strain possessing a high percentage of six-spotted males, it acted as a recessive character but if crossed to a strain with a high percentage of two-spotted males, it acted more like a dominant.

7. High temperatures caused the mutated type of female spotting to act as a recessive character while low temperatures made it act as a dominant.

8. In the male spotting, high temperatures intensified one pair of spots while the same temperatures seemed to reduce another pair of spots on the same segment.

9. In the male spotting, when a strain selected for two-spotted males was crossed to a strain selected for six-spotted males neither type of spotting was dominant in the F_1 generation.

10. There was a difference in amenability to selection of different strains taken from nature; but when two strains, each selected for six-spotted males but of different origin, were crossed, the results of the cross indicated that their spottedness was due to the same factors.

11. An unusual type of male spotting was found in which the spots of one pair were very large. A study of this character indicated that sex-linked inheritance was involved in its genetic behavior.

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