SELECTION FOR HIGH AND LOW BRISTLE NUMBER IN THE MUTANT STRAIN "REDUCED"¹

FERNANDUS PAYNE

Zoological Laboratory, Indiana University, Bloomington, Indiana

[Received April 29, 1920]

TABLE OF CONTENTS

INTRODUCTION.....	501
The experiment.....	503
The analysis of the differences.....	515
DISCUSSION.....	537
SUMMARY.....	540
LITERATURE CITED.....	541

INTRODUCTION

In a former paper (PAYNE 1918) I gave the results of an experiment to increase bristle number on the scutellum of *Drosophila melanogaster*. While plus and minus variations in bristle number may occur in cultures of wild stock, it may be said that four bristles is the normal number in wild flies. In the strain selected for extra bristles, a female with five and a male with four bristles were the starting point. Throughout the experiment which covered 38 generations, flies with the greatest number of extra bristles were used as parents. The mean bristle number was increased to 9.123 in the thirty-fourth generation. The analysis of this line showed that there are at least two and probably more factors concerned in the production of extra-bristle number on the scutellum.

The mutant "reduced" appeared in the sixth generation of the line selected for extra bristles (April 15, 1916). A single male occurred with one bristle on the scutellum. This male was mated to a wild female. In F₁ there were 328 flies, 327 with four bristles and one with five. Five pairs of F₁ flies were mated and gave offspring as follows: 1594 females with four or more bristles, 834 males with four or more bristles, and 804 males with less than four bristles. Of those having less than four bristles, 255 had no bristles, 296, one bristle, 219, two bristles, and 34

¹ Contribution from the Zoölogical Laboratory of INDIANA UNIVERSITY, No. 173.

three bristles. From these numbers it was evident that the reduced bristle condition behaved as a sex-linked recessive. The character was transferred to the female by mating F_2 reduced males to F_2 females. Of 49 F_2 females tested, 27 were heterozygous for the reduced factor. The offspring of 18 of these heterozygous matings are given in table 1.

TABLE 1
Male "reduced" (1 bristle) $\times$ female, wild type (4 bristles).

A. Bristle number of offspring

4 bristles	5 bristles
327	1

B. $F_1 \times F_1$; bristle number of offspring

Females				Males						
4	5	6	7	0	1	2	3	4	5	6
1546	40	6	2	255	296	219	34	826	7	1

C. F_2 reduced male $\times$ F_2 heterozygous female; bristle number of offspring

Female						Male						
0	1	2	3	4	5	0	1	2	3	4	5	6
117	166	291	192	1044	3	392	293	181	30	850	2	1

Table 1 gives the results of the cross of the mutant male "reduced" to a wild female; $F_1 \times F_1$; and F_2 heterozygous females $\times$ F_2 reduced males. In F_1 there were 327 flies with four bristles and one with five. In F_2 there were 1594 females with four or more bristles, 834 males with four or more bristles, and 804 males with less than four bristles. Practically half of the males then showed the "reduced" character. Note the extra-bristled males and females here, indicating that the reduced male mutant carried factors for extra bristles as well as a factor for the reduced condition. The character reduced is transferred to the female by mating F_2 reduced males to F_2 heterozygous females.

Of the reduced offspring 509 had no bristles, 459 had one, 472 two, and 222 had three bristles.

Here was a mutant character which varied, the number of bristles ranging from zero to three and as we shall see later to four and five. I was working at that time on selection for extra-bristle number and when this variable mutant appeared I planned a second experiment.

THE EXPERIMENT

From the F_3 offspring two lines were started, a minus line by mating parents with no bristles, and a plus line by mating parents with two and three bristles. After the first few generations all parents in the plus line had four bristles. These flies with four bristles were not genetically wild type as they threw the characteristic sex-linked ratios when crossed to the wild. The reduced flies with four bristles could be distinguished readily, however, without the breeding test, as all reduced flies have one or more of the ocellar bristles absent. These two lines, the plus and the minus, have been bred by mating brother and sister for 60 and 65 generations respectively. In the plus line 111,640 flies have been examined and in the minus line 104,362.

The mean bristle number of the flies in the first generation of the plus line was 1.95 (table 2) and the percentage with four bristles was 7.20. For the next two generations these figures were not increased. In fact, they were slightly less in the second and third generations. In the fourth generation, however, the mean rose to 2.08 and the percentage of flies with four bristles to 8.39. The mean in the fifth generation was the same as in the fourth, but from the fifth on it rose gradually, without a break in the increase, to 3.25 in the eighteenth generation. From the eighteenth to the forty-sixth generation the mean fluctuated somewhat, never going below 3.04 nor above 3.35. From the forty-sixth generation there was a rise in the mean to 3.52 in the fifty-fifth generation. From the fifty-fifth to the sixtieth there was a decrease to 3.11 (table 2 and figure 1). If we follow the percentage of flies with four bristles we find a curve similar to that of the mean (tables 2 and 4 and figure 2). From the fifth generation, the percentage rises gradually to 46.90 in the eighteenth generation. From the eighteenth to the forty-sixth generation there is considerable variation, the percentage going as low as 36.20 in the forty-first and as high as 52.94 in the thirty-first. From the forty-sixth to the fifty-fifth generation the percentage rises from 51.06 to 64.25. From the fifty-fifth to the sixtieth there is a decrease to 40.56. The sudden rise in both curves from the forty-sixth to the fifty-fifth generation is somewhat difficult to explain. I was ready to drop the experiment when this sudden rise appeared. The line had remained more or less stationary, except for fluctuations which might be considered environmental, for 28 generations. During this time, more than a year, the line had been subjected to about all the varying environmental conditions which one meets in a laboratory. When the rise came there was

TABLE 2
Summary of plus-selected line.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 or more bristles	Percent of all offspring with no bristle	Bristle number of offspring												Total
				Female						Male						
				0	1	2	3	4	5	0	1	2	3	4	5	
1	1.95	7.20	8.19	14	36	139	108	48		44	124	156	36	3		708
2	1.88	5.33	11.14	49	231	1081	746	239		469	704	907	214	9		4649
3	1.81	5.57	12.01	54	142	533	326	131		239	417	502	90	5		2439
4	2.08	8.39	4.30	10	47	305	216	105		47	213	323	51	6		1323
5	2.08	7.77	6.32	5	86	469	420	167		135	334	470	121	5		2212
6	2.28	12.60	4.15	2	64	548	616	372		128	409	750	215	22		3126
7	2.36	13.34	2.28	1	30	498	562	361		65	348	759	243	25		2892
8	2.41	15.17	2.42		27	229	302	202		36	153	393	118	23		1483
9	2.54	17.52	1.79		5	117	194	142	1	16	68	254	79	14		890
10	2.83	27.62	0.55		3	70	210	252		6	66	253	170	45		1075
11	2.90	29.57	0.30	1	7	118	355	465	2	5	78	465	357	118		1971
12	2.88	27.51	0.15		3	176	513	603		4	95	662	470	127		2653
13	2.96	30.74	0.43		2	100	361	423		8	62	379	374	148		1857
14	3.01	34.58	0.15		2	46	246	344	3	2	40	297	224	104	2	1310
15	3.03	35.60	0.14		3	43	124	181		1	19	138	126	70		705
16	3.14	41.60	0.31		2	70	169	379	2	4	23	224	254	156		1283
17	3.17	42.00	0.05		3	109	331	580		1	27	298	330	216		1895
18	3.25	46.90	0.00		3	79	257	553			14	259	323	272	1	1761
19	3.21	45.47	0.07		8	152	401	883		2	37	436	513	409		2841
20	3.24	45.67	0.10			37	142	279		1	11	134	171	138		913
21	3.29	47.77	0.09		1	56	176	351	1	1	7	129	204	173		1099
22	3.29	48.23	0.00		2	31	121	247			6	94	141	121		763
23	3.24	47.40	0.00			24	65	153			8	80	97	94		521
24	3.19	42.20	0.00		3	32	105	192			4	101	137	87		661
25	3.12	39.05	0.14		4	76	230	394		2	18	250	275	154		1403
26	3.29	49.19	0.05		5	82	227	587		1	20	219	359	297		1797
27	3.20	43.81	0.06		1	70	206	462	1	1	15	228	288	168		1440
28	3.18	42.72	0.41		1	82	229	393	1	6	23	209	281	226		1451
29	3.30	50.67	0.05		2	78	236	566		1	17	235	309	336		1780
30	3.25	50.64	0.25		3	27	54	112		1	7	48	50	83		385
31	3.35	52.94	0.18		1	22	87	181		1	5	56	76	98		527
32	3.28	52.60	0.00			17	44	119			12	42	49	63		346
33	3.22	46.90	0.54			16	84	185		3	12	83	94	73		550
34	3.05	41.31	0.56		3	20	42	104		2	19	55	65	41		351
35	3.12	42.21	0.32	1	2	47	142	266		2	17	169	158	127		931
36	3.12	40.66	0.00		5	74	192	325			39	191	233	178		1237
37	3.19	43.71	0.19		3	69	234	440		3	33	217	305	231		1535
38	3.20	45.76	0.15		4	31	93	209		1	12	100	111	88		649
39	3.31	50.74	0.19		2	55	214	504		3	21	193	270	277		1539
40	3.23	47.31	0.26		7	53	221	494		4	36	220	263	228		1526
41	3.04	36.20	0.25		2	91	248	411		4	39	306	302	152		1555
42	3.08	37.76	0.07		6	87	196	367		1	28	255	292	158		1390
43	3.35	52.06	0.00		2	33	194	594			12	201	335	250		1621

TABLE 2 (continued)

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 or more bristles	Percent of all offspring with no bristle	Bristle number of offspring												Total
				Female						Male						
				0	1	2	3	4	5	0	1	2	3	4	5	
44	3.16	41.16	0.11		4	85	299	539		2	26	291	355	204		1805
45	3.10	39.73	0.08		11	184	388	631		2	45	368	402	292		2323
46	3.29	51.06	0.00		2	30	85	274			11	118	145	134		799
47	3.42	57.03	0.00			23	91	291			4	86	150	179		824
48	3.35	53.60	0.21		2	31	112	344		2	12	106	172	161		942
49	3.41	55.26	0.00			16	87	228			7	66	130	150		684
50	3.40	56.33	0.20		2	48	192	550		3	15	139	252	290		1491
51	3.47	59.89	0.00		4	57	220	573			11	120	251	417		1653
52	3.51	62.87	0.11			43	259	1029		3	12	233	419	612		2610
53	3.45	60.13	0.25	1		57	328	1277		7	38	307	515	643		3173
54	3.49	63.65	0.17		5	74	376	1585		7	56	334	606	969		4012
55	3.52	64.25	0.23	3	3	112	605	2801		13	65	531	1106	1581		6820
56	3.46	61.31	0.20	1	8	89	448	1882		9	70	468	809	1132		4916
57	3.51	63.23	0.09		5	129	658	2812		7	53	589	1183	1701		7137
58	3.36	54.69	0.07		9	173	589	1971	5	4	98	634	1042	1101		5626
59	3.35	51.68	0.09	1	2	125	302	720		1	19	160	362	320		2012
60	3.11	40.56	0.56	2	6	103	271	489		8	48	281	333	229		1770
Total.....																111,640

Table 2 gives a summary of the data of the plus-selected line generation by generation. The distribution of the bristles of the males and females is tabulated separately. The mean bristle number, the percentage of all offspring with four or more bristles, and the percentage of all offspring with no bristles are given in each generation. The total number of flies in each generation is also given. A grand total of 111,640 flies were examined during the sixty generations of selection. Note the rise in the mean and in the percentage of flies with four bristles; also the shift in the distribution toward three and four bristles.

nothing unusual about food or room temperature. The rise also lasted for a considerable period, nine generations. The sudden fall in the curve from the fifty-fifth to the sixtieth generation seemed to be due to lack of vitality within the strain. It was impossible to keep the strain going except in mass cultures.²

² Since writing the preceding paragraph I have started selection in the plus line again after several months of mass culture. In the first generation the mean rose to 3.51 and the percentage of four-bristled flies to 62.08. In the second generation the mean reached 3.53 and the percentage of four-bristled flies 64.19, practically what they were at the peak of the curve in the fifty-fifth generation. In the third generation there was a slight recession (see table 27). The immediate return of the line to the high point reached in the fifty-fifth generation lends support to the view that the decline in the mean and percentage of four-bristled flies in the last few generations of selection was due to environmental conditions. It further indicates that the rise in the curves from the forty-sixth to the fifty-fifth generation might have been due to a genetic change within the line.

Even if we grant that the rise in the curves from the forty-sixth to the fifty-fifth generation is of no special significance, we must admit that a considerable change within the line has been produced by selection. The mean was increased from 1.95 to 3.01 in the fourteenth generation and it never goes below this in any of the succeeding generations, but does go as high as 3.52 in the fifty-fifth generation. The percentage of flies with four bristles was increased from 7.20 to 35.60 in the fifteenth generation. It never goes below this in any of the succeeding generations, but does go as high as 64.25 in the fifty-fifth generation.

In the minus line the mean bristle number in the first generation was 0.504. There is a marked difference between this mean and that of the first generation in the plus-selected line (1.95). This difference is no doubt due in part to the fact that when F_2 males were mated to F_2 heterozygous females, nearly all the male parents had no bristles. So selection began really in a minus direction when the F_2 parents were mated. The mean in this line was gradually reduced until it reached 0.004 in the seventeenth generation. From this time on to the end of the experiment or to the sixty-fifth generation it remained fairly constant, but fluctuated somewhat, going as high as 0.034 in the twenty-sixth generation and as low as zero in the fortieth (table 3). The percentage of flies with no bristles was gradually increased from 64.61 in the first to 99.52 in the seventeenth generation (table 3 and figure 3). From the seventeenth generation to the end of the experiment this percentage fluctuated slightly, going as low as 96.60 in the twenty-sixth generation and as high as 100 in the fortieth. A glance at table 3 shows that at the beginning of selection in the minus line some of the flies had no bristles, some had one, some two, some three, and a few had four bristles. After the third generation no flies had four bristles; after the seventh generation none had three; after the thirtieth generation only seven flies had two bristles; and after the fifty-sixth no flies had two. It seemed impossible, however, to produce a strain of flies with no bristles. An occasional fly with one bristle appeared in every generation except the fortieth. This generation gave 100 percent zeros.

In the plus-selected line the distribution ranges from zero to four at the beginning of the experiment (table 2). In the first generation 8.19 percent of the flies have no bristles, 22.59 percent have one bristle, 41.66 percent have two bristles, 20.33 percent have three bristles, and 7.20 percent have four bristles. Most of the flies then have one, two, and three bristles with the emphasis on the twos. During the course of selection this distribution is gradually shifted in the direction of four

FIGURE 1.—The curves for the mean bristle number in the plus line and the back selection line. The data from which the curves are plotted are given in tables 4 and 7.

FIGURE 2.—The curve for the percentage of flies with four bristles in the plus line. See table 4 for the data.

FIGURE 3.—The curve for the percentage of flies with no bristles in the minus line. See table 5 for the data.

TABLE 3
Summary of minus-selected line.

Generation	Mean bristle number of offspring	Percent of offspring with no bristle	Bristle number of offspring									
			Females					Male				
			0	1	2	3	4	0	1	2	3	4
1	0.504	64.61	346	208	134	26	2	581	106	20	4	
2	0.421	70.03	1562	755	368	65	20	2184	311	77	3	4
3	0.293	77.39	888	297	112	10	3	1060	119	28		
4	0.145	88.10	857	146	56			980	46			
5	0.127	89.07	1634	298	60	2		1735	46	7		
6	0.087	92.28	1637	202	37			1724	41	1		
7	0.094	91.85	3050	397	79	3		3379	87	4		
8	0.069	93.78	2533	273	42			2747	35			
9	0.051	95.20	2018	171	17			2113	20			
10	0.044	95.95	1213	76	7			1135	14	2		
11	0.029	97.29	919	41	4			806	3			
12	0.025	97.57	1069	47	3			1064	3			
13	0.024	97.90	759	24	6			737	2			
14	0.023	97.72	820	36	2			857	1			
15	0.006	99.35	612	8				613				
16	0.005	99.45	935	8				894	2			
17	0.004	99.52	1079	9				1024	1			
18	0.009	99.14	1186	14	2			1263	5			
19	0.009	99.06	993	17				1018	2			
20	0.021	97.92	1259	49	1			1336	1			
21	0.014	98.55	1416	39	1			1386	1			
22	0.019	98.01	990	37				1039	4			
23	0.023	97.82	705	26	2			689	3			
24	0.033	96.81	1176	71	3			1252	6			
25	0.032	96.43	837	55	4			920	6			
26	0.034	96.60	666	43	1			757	6			
27	0.017	98.20	324	8				332	4			
28	0.009	99.02	798	15				821	1			
29	0.011	98.92	1050	18	2			1149	4			
30	0.014	98.56	1047	25	1			1013	4			
31	0.007	99.24	665	10				658				
32	0.003	99.69	608	2				695	2			
33	0.011	98.86	466	8				494	3			
34	0.024	97.53	201	10				195				
35	0.011	98.80	40	1				43				
36	0.037	96.69	101	4				104	2	1		
37	0.013	98.64	140	4				152				
38	0.001	99.85	681	2				733				
39	0.009	99.06	497	7				563	3			
40	0.000	100.00	186					194				
41	0.001	99.86	360					366	1			
42	0.014	98.51	118	4				148				
43	0.015	98.45	193	5				190	1			
44	0.009	99.17	274	4	1			327				

TABLE 3 (continued)

Generation	Mean bristle number of offspring	Percent of offspring with no bristle	Bristle number of offspring									
			Females					Male				
			0	1	2	3	4	0	1	2	3	4
45	0.006	99.38	445	6				528				
46	0.006	99.39	956	9				1002	3			
47	0.008	99.16	405	5				484	3			
48	0.010	98.95	930	17				965	3			
49	0.007	99.21	393	3				370	3			
50	0.010	99.10	421	7	1			468				
51	0.019	98.10	366	10				358	4			
52	0.003	99.69	335	2				309				
53	0.005	99.44	279	2				262	1			
54	0.005	99.46	288	3				275				
55	0.005	99.06	496	7	2			462				
56	0.015	98.63	453	10	2			417				
57	0.002	99.73	345	1				417	1			
58	0.004	99.58	442	3				515	1			
59	0.009	99.03	293	6				325				
60	0.008	99.30	266	2				303	2			
61	0.003	99.68	623	4				654				
62	0.004	99.54	566	3				532	2			
63	0.007	99.25	479	6				458	1			
64	0.003	99.66	144					158	1			
65	0.002	99.79	230	1				262				

Table 3 gives a summary of the minus-selected line generation by generation. The bristle counts for the males and females are tabulated separately. The mean bristle number and the percentage of all flies with no bristles are given for each generation. Note the gradual lowering of the mean toward zero and the rise toward 100 in the percentage of flies with no bristles. Note also the shift in the distribution. Toward the end nearly all flies have no bristles. A few have one bristle. In only one generation, the fortieth, was there 100 percent with no bristles.

bristles until in the fifty-fifth generation 64.25 percent of the flies have four bristles, 25.08 percent have three bristles, 9.42 percent have two bristles, 0.99 percent have one and 0.23 percent have no bristles. In the later generations then, most of the flies have three and four bristles.

A comparison of tables 2 and 3 and figures 1 and 2 shows clearly that the minus and plus lines are widely separated at the end of the experiment. They came originally from the same pair of parents. This difference has been produced by selecting parents with few or no bristles on the one hand and parents with the highest number of bristles on the other. The two lines are not only different somatically but they are different germinally. Plus selection has been attempted several

times in the minus line without results. Three attempts at minus selection have been made in the plus line without marked results. The data for one of these are given in table 6 and figure 1. The mean drops slightly for the first generation and then remains practically the same

TABLE 4
Summary of the plus-selected line grouped two generations in a group.

Gener- ation	Mean bristle number of all offspring	Percent of all off- spring with 4 or more bristles	Percent of all offspring with no bristles	Mean bristle number of females	Percent of females with 4 or more bristles	Mean bristle number of all males	Percent of males with 4 or more bristles	Totals
1, 2	1.89	5.58	10.75	2.38	10.66	1.40	0.45	5357
3, 4	1.90	6.56	9.30	2.37	12.62	1.45	0.58	3762
5, 6	2.20	10.60	5.05	2.70	19.60	1.66	1.04	5338
7, 8	2.38	13.96	2.33	2.86	25.36	1.89	2.22	4375
9, 10	2.70	23.10	1.11	3.19	39.63	2.19	6.07	1965
11, 12	2.89	28.39	0.21	3.33	47.61	2.47	10.26	4624
13, 14	2.98	32.33		3.40	50.42	2.60	15.48	3167
15, 16	3.10	39.63		3.45	57.75	2.73	22.26	1988
17, 18	3.21	44.36		3.48	59.18	2.91	28.08	3656
19, 20	3.30	46.77		3.50	61.09	2.93	29.53	3754
21, 22	3.29	47.95		3.51	60.75	3.04	33.56	1862
23, 24	3.21	44.50		3.49	60.10	2.96	29.76	1182
25, 26	3.21	44.75		3.50	61.12	2.93	28.27	3200
27, 28	3.19	43.27		3.48	59.26	2.90	27.24	2891
29, 30	3.29	50.66		3.52	62.89	3.07	38.54	2165
31, 32	3.32	52.80		3.54	63.69	3.06	40.04	873
33, 34	3.16	44.72		3.54	63.65	2.77	25.50	901
35, 36	3.12	41.32		3.42	56.07	2.84	27.37	2168
37, 38	3.19	44.32		3.49	59.92	2.90	28.97	2184
39, 40	3.27	49.03		3.56	64.38	2.97	33.33	3065
41, 42	3.06	36.94		3.41	55.25	2.73	20.16	2945
43, 44	3.25	46.32		3.57	64.74	2.92	27.08	3426
45, 46	3.15	42.63		3.41	56.38	2.88	28.08	3122
47, 48	3.38	55.20		3.64	71.02	3.12	38.99	1766
49, 50	3.41	56.00		3.63	69.27	3.17	41.82	2175
51, 52	3.49	61.71		3.68	73.31	3.29	49.51	4263
53, 54	3.48	62.26		3.73	77.28	3.21	46.29	7185
55, 56	3.49	63.01		3.74	78.67	3.23	46.90	11736
57, 58	3.44	59.46		3.70	75.38	3.19	43.69	12763
59, 60	3.23	46.48		3.47	59.42	2.96	31.17	3782
Total								111,640

Table 4 gives a summary of the plus-selected line grouped two generations in a group. From the data given in this table the curves (except the one for back selection) in figures 1 and 2 have been made.

TABLE 5

Summary of minus-selected line grouped two generations in a group.

Generation	Mean bristle number of all offspring	Percent of flies with no bristles	Total number of flies
1, 2	0.43	68.96	6776
3, 4	0.22	82.24	4602
5, 6	0.10	90.65	7424
7, 8	0.08	92.71	12629
9, 10	0.04	95.47	6786
11, 12	0.02	97.44	3959
13, 14	0.02	97.81	3244
15, 16	0.005	99.41	3072
17, 18	0.006	99.32	4583
19, 20	0.016	98.41	4680
21, 22	0.016	98.33	4913
23, 24	0.029	98.17	3933
25, 26	0.033	96.50	3295
27, 28	0.012	98.78	2303
29, 30	0.013	98.74	4313
31, 32		99.46	2640
33, 34		98.44	1377
35, 36		97.29	296
37, 38		99.64	1712
39, 40		99.31	1450
41, 42		99.49	997
43, 44		98.89	995
45, 46		99.38	2949
47, 48		99.02	2872
49, 50		99.15	1666
51, 52		98.84	1384
53, 54		99.45	1110
55, 56		98.86	1849
57, 58		99.65	1725
59, 60		99.16	1197
61, 62		99.62	2384
63, 64		99.35	1247
Grand total.....			104,362

Table 5 gives a summary of the minus-selected line grouped two generations in a group. From these data the curve in figure 3 has been plotted.

for the rest of the thirteen generations. The mean in the first generation was 3.17 and in the thirteenth 3.09. In mass cultures the two lines remain distinct. Each line has been continued in mass culture since the end of the experiment. Small counts were made eight months after the cultures were started. In the minus line 1409 flies were examined. Of these 1401 had no bristles and 8 had one bristle. In the plus line

TABLE 6
Summary of back-selected line.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 bristles	Bristle number of offspring										Total
			Female					Male					
			0	1	2	3	4	0	1	2	3	4	
I	3.17	41.20		3	26	103	178		7	99	123	75	614
2	2.82	23.95		4	88	189	178	I	24	231	152	39	906
3	2.91	30.40		5	68	137	187		18	193	132	55	796
4	2.98	28.73		1	45	128	151		9	146	167	49	696
5	3.04	35.49		2	40	141	213	2	19	153	150	66	786
6	3.00	32.15		1	65	172	245	I	17	210	205	73	989
7	2.91	26.88		2	86	179	169		13	171	139	48	807
8	2.83	25.44		3	97	153	164		14	227	139	52	849
9	2.82	24.16		I	67	114	126	I	22	160	134	33	658
10	3.02	30.64			36	144	165	I	7	130	137	36	656
11	2.85	25.77			65	144	134	I	18	181	115	48	706
12	2.83	24.20		I	43	98	89		24	113	100	32	500
13	3.09	36.65			11	64	103	2	7	62	70	22	341

Table 6 gives a summary of the back-selected line generation by generation. It gives the distribution of the bristles in the males and females, the mean bristle number, and the percentage of all offspring with four bristles in each generation. Note that the mean was 3.17 in the first generation and 3.09 in the thirteenth.

TABLE 7
Back-selected line summarized two generations in a group.

Generation	Mean bristle number of all offspring	Percent of all offspring with 4 bristles	Total
1, 2	2.96	30.92	1520
3, 4	2.94	29.62	1492
5, 6	3.02	33.63	1775
7, 8	2.87	26.14	1656
9, 10	2.92	30.64	656
11, 12	2.84	25.12	500

Table 7 gives the data for the back-selected line grouped two generations in a group. From these data the curve for the back-selection line in figure 1 is plotted.

544 flies were examined. Of these 277 had four bristles, 156 three bristles, 96 two bristles, 14 one bristle, and one no bristles. The mean of these is 3.27. The mean of the sixtieth generation at the end of selection was 3.11. Both of these counts, then, show that the two lines have remained for eight months practically the same as they were at the end of selection. They have been not only separated by selection but they have become fixed by selection.

THE ANALYSIS OF THE DIFFERENCES

What is this germinal difference and how has selection brought it about? In an attempt to answer these questions I have made use of the phenomena of linkage and crossing over and have also made other crosses to bring out the genetic differences.

Before taking up the analysis of the germinal differences between the two lines a few words should be said about the effect of the environment upon bristle number. My own observations during selection had led me to think that temperature was one factor which caused considerable variation. Following up this notion one of my students, Mr. ALBERT FROEMMING, made some experiments in which he demonstrated that low temperature increases bristle number in both the plus and minus lines. His experiments were made after about one year of selection.

In the experiments with the plus line he mated females with three bristles to males with two bristles. First he placed the parents in an incubator with the temperature varying from 23° to 26°C. Here the bottles were allowed to remain until the flies had completed their life cycle, which in this case was about ten days. He examined 7668 offspring. They showed a mean bristle number of 2.03. A second experiment was made in a cave room where the temperature varied from 10 to 13°C. At this temperature the adults would not deposit their eggs. So they were kept at room temperature until larvae appeared in the bottles. The bottles were then transferred to the cold room. They were kept here until the larvae pupated. Pupation would occur in the cold but the flies would not emerge. Hence after pupation the bottles were again transferred to room temperature. Three thousand two hundred twenty-five flies gave a mean bristle number of 2.40. The life cycle in this case was about thirty days. The flies bred in the cold room have a higher mean bristle number than those bred in the incubator.

Another experiment and even more convincing than the first two, was carried out by using the same parents under the different temperatures. He permitted the bottles to remain at room temperature until larvae appeared. The parents were transferred to new bottles and the bottles with larvae transferred to the cave room. The new bottles with the parents were put into the incubator. The mean bristle number of the 2047 flies reared at 10° to 13°C was 2.34; that of the 2929 flies reared in the incubator at 23° to 26°C was 2.14. These experiments leave no doubt, I think, that low temperature increases bristle number in the plus-selected line. They also demonstrate that the effect is produced during larval life.

In the minus-selected line similar experiments were made. One will suffice to demonstrate the effect of the lower temperature in increasing bristle number. Flies with no bristles were used as parents. They were placed in bottles and permitted to remain until they had deposited eggs. The parents were removed to bottles with fresh food and placed in the incubator. The bottles with eggs and larvae were placed in the cold room. Those reared in the incubator (804) gave 96.64 percent with no bristles; those reared in the cave room (542) gave only 79.52 percent with no bristles. In the minus-selected line, then, also, the lower temperature increases bristle number.

These conclusions reached about three years ago agree with the recent work of SEYSTER (1919) on the effect of temperature on facet number in the bar-eyed mutant.

A study of tables 2 and 3 shows clearly that at the end of selection the plus line is much more variable than the minus line. In the minus line only a very few flies have one bristle. The rest have none. In the plus line most of the flies have three and four bristles but flies also appear with two, one, and no bristles. The figures and tables also show that the females have more bristles than the males. This is brought out in figure 1 and table 4 for the plus line. In the early generations the difference is greater than later when the line has become somewhat stable. It approaches and even reaches one in the ninth and tenth generations. After the sixteenth the difference is about one-half and remains fairly constant. In the line selected for extra bristles (PAYNE 1918) a similar difference between males and females was present. The analysis showed that a sex-linked factor was responsible for the difference. The plus line in the present experiment has been tested for such a factor. The case here is slightly different, for the reduced condition itself is due to a sex-linked factor. Could there be another sex-linked factor, which modifies bristle number, in addition to the primary one? The analysis shows the presence of such a factor. The test was made by crossing the plus line to eosin miniature and then mating the F_1 female to an eosin miniature male. Table 8 gives the results. Only males are included in the table as all females in this type of cross are wild type with respect to bristle number. Reduced and eosin miniature are the non-crossover classes. Of the crossover classes miniature reduced and eosin are the largest. Reduced and eosin are found together in only a small percentage of the crossovers. These facts demonstrate that eosin and reduced lie very close together. If there are factors in the X chromosome, other than reduced, which modify bristle number, a comparison of the crossover

class, miniature reduced, with the non-crossover class, reduced, ought to bring this out. If there are no other factors, the miniature reduced flies should show approximately the same bristle number and distribution as the reduced. On the other hand, if there are additional factors, and if they cross over in the F_1 female, then the two classes should differ with respect to bristle number and distribution. A comparison of the reduced and the miniature reduced flies show very clearly a difference.

TABLE 8

F_1 ♀ (from plus line ♀ × eosin miniature ♂) × eosin miniature ♂.

Type	Number of bristles	Number of male offspring	Percentage	Mean
Wild.....	4	55		
Reduced.....	3 2 1 0	78 371 402 496	5.78 27.54 29.84 36.82	1.022
Miniature.....	4	11		
Miniature reduced.....	3 2 1 0	20 147 211 377	2.64 19.47 27.94 49.93	0.748
Eosin miniature.....	4	1324		
Eosin miniature reduced.....	3 2 1 0	1 5 10 13		
Eosin.....	4	682		
Eosin reduced.....	3 2 1 0	2 2 0 2		

Table 8 gives a summary of the results when an F_1 female produced by mating the plus line to eosin miniature, is back-crossed to an eosin miniature male. The data are for the males only as none of the females show the character "reduced." It gives the number of flies and their bristle number in each class. Reduced and eosin miniature are the non-crossover classes. Of the crossover classes, miniature reduced and eosin are by far the largest. This table affords a comparison of the non-crossover class, reduced, with the crossover class, miniature reduced. The two are different, demonstrating the presence of a plus-modifying factor in the X chromosome near miniature.

TABLE 9
F₁ ♀ (from plus line × eosin ruby forked) × eosin ruby forked ♂.

Type	Number of bristles	Number of male off- spring	Percentage	Mean
Wild.....	4	44		
Reduced.....	3 2 1 0	63 135 146 121	13.54 29.03 31.39 26.02	1.301
Forked.....	4	23		
Forked reduced.....	3 2 1 0	25 82 107 129	7.28 23.90 31.19 37.60	1.088
Eosin ruby forked.....	4	499		
Eosin ruby forked reduced.....	3 2 1 0	3 4 1 4		
Eosin ruby.....	4	279		
Eosin.....	4	33		
Eosin forked.....	4	4		
Ruby forked.....	4	1		
Ruby forked reduced.....	3 2 1 0	2 5 11 9		
Ruby.....	2 1 0	6 1 3		
Eosin ruby reduced.....	2 0	1 2		

Table 9 shows the results of back-crossing an F_1 female, produced by crossing the plus line to eosin ruby forked, to an eosin ruby forked male. The reduced and eosin ruby forked flies are the non-crossovers. The forked reduced and eosin ruby are the principal crossover classes. A comparison of the non-crossover class, reduced, with the crossover class, forked reduced, shows the reduced flies to have a higher mean bristle number, 1.301 as compared to 1.008 for the forked reduced. Of the reduced flies only 26.02 percent have no bristles while 37.60 percent of the forked reduced have no bristles.

The mean of the reduced flies is 1.022 while in the miniature reduced flies it is 0.748. The distribution of the bristles differs also. Of the reduced flies 36.82 percent have no bristles; of the miniature reduced flies 49.93 percent have no bristles. It would seem then that in addition to the factor for reduced we have an additional modifying factor near miniature which does not pass into the miniature reduced crossover class. Hence this class has fewer bristles than the non-crossover class, reduced.

The same fact is shown, but less clearly, by mating the plus line to eosin ruby forked and back-crossing the F_1 females to eosin ruby forked males (table 9). A comparison of the non-crossover class, reduced, with the crossover class, forked reduced, shows that the reduced flies have a higher mean bristle number (1.301) than the forked reduced (1.008). There are also fewer flies with no bristles, 26.02 percent as compared with 37.60 percent in the forked reduced. The fact that the differences here are not so marked as in the eosin miniature cross is no doubt due to the fact that the second factor lies near miniature and in many of the crossovers both bristle factors cross over. This would raise the bristle number of the forked reduced flies above that of the miniature reduced.

Attempts were also made to locate factors outside the X chromosome. First the plus line was mated to black pink bent stock and the F_1 females back-crossed to black pink bent males. The data for the males which show the reduced character are given in table 10. So far as I can see, though, it gives no certain clew. One fact stands out very clearly, however, and that is that the bent reduced have practically the same mean bristle number and the same distribution as the reduced flies. The mean of the bent reduced is 1.20 and of the reduced flies it is 1.19. In contrast with this the black reduced, and pink reduced flies have a much lower mean and a much higher percentage of the flies have no bristles. The mean for the black reduced flies is 0.35 and for the pink reduced 0.72. This indicates the possibility of plus modifying factors in the second and third chromosomes. The plus line was also crossed to black, to spineless, to sooty and to eyeless. In each case the F_1 female was back-crossed to black, to spineless, to sooty, and to eyeless males, respectively (table 11). These crosses brought out the same thing as the cross to black pink bent. The black reduced, spineless reduced, and sooty reduced flies had a lower mean bristle number than the reduced. The eyeless reduced flies, as in the case of the bent reduced, had practically the same mean and distribution as the reduced flies.

TABLE 10
F₁ ♀ (from plus line ♀ × black pink bent ♂) × black pink bent ♂.

Type	Number of bristles	Number of male off-spring	Percentage	Mean
Reduced.....	3	28	11.81	1.19
	2	71	29.95	
	1	57	24.05	
	0	81	34.17	
Black reduced.....	3	3	1.57	0.35
	2	13	6.80	
	1	33	17.27	
	0	142	74.34	
Pink reduced.....	3	15	7.10	0.72
	2	28	13.27	
	1	51	24.17	
	0	117	55.45	
Bent reduced.....	3	9	9.78	1.20
	2	28	30.43	
	1	28	30.43	
	0	27	29.34	
Black pink reduced.....	3	0	0.00	
	2	7	4.45	
	1	12	7.64	
	0	138	87.89	
Black bent reduced.....	3	0	00.00	
	2	13	13.54	
	1	26	27.08	
	0	57	59.37	
Black pink bent reduced.....	3	0	00.00	
	2	3	4.91	
	1	11	18.03	
	0	47	77.04	
Pink bent reduced.....	3	8	8.78	
	2	21	23.07	
	1	18	19.77	
	0	44	48.35	

Table 10 gives the results of back-crossing an F_1 female (produced by crossing a plus line female to black pink bent male) to a black pink bent male. Only the males showing the character reduced are given in the table as all others are wild type with respect to bristle number. The factors for black, pink and bent are located in the second, third and fourth chromosomes, respectively. A comparison of the reduced flies with black reduced, pink reduced and bent reduced, shows that the mean and distribution of the bristles in reduced and bent reduced are very similar. On the other hand the mean in the black and pink flies is very much less and the distribution is shifted toward the zeros.

TABLE II

A. F_1 ♀ (from *plus line* × *black*) × *black* ♂.

Reduced			Black reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	68	15.17	3	11	2.55
2	117	26.11	2	45	10.44
1	131	29.24	1	93	21.57
0	132	29.46	0	282	65.42

B. F_1 ♀ (from *plus line* × *spineless*) × *spineless* ♂.

Reduced			Spineless reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	17	3.60	3	4	0.94
2	43	9.12	2	4	0.94
1	68	14.43	1	16	3.78
0	343	72.82	0	399	94.32

C. F_1 ♀ (from *plus line* × *sooty*) × *sooty* ♂.

Reduced			Sooty reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	20	5.31	3	8	2.65
2	54	14.36	2	11	3.65
1	94	25.00	1	35	11.62
0	208	55.31	0	247	82.05

D. F_1 ♀ (from *plus line* × *eyeless*) × *eyeless* ♂.

Reduced			Eyeless reduced		
Bristle number	Number of flies	Percent	Bristle number	Number of flies	Percent
3	56	11.02	3	49	12.37
2	98	19.29	2	118	27.79
1	140	27.55	1	107	27.02
0	214	42.12	0	122	30.80

TABLE 11 (continued)
E. F₁ ♀ (from plus line × wild) × wild ♂.

Reduced		
Bristle number	Number of flies	Percent
4	18	3.73
3	49	10.16
2	105	21.78
1	116	24.06
0	194	40.24

Table 11 gives a series of crosses of the plus line to black, to spineless, to sooty, to eyeless and to the wild, and then in each case back-crossing the F_1 female to the black male, the spineless male, the sooty male, the eyeless male and the wild-type male, respectively. The table gives only the distribution of the bristles in the males which show the character reduced. The factor for black is in the second chromosome, those for spineless and sooty in the third, and that for eyeless in the fourth. These crosses are similar to the one made to black pink bent, and the results are also very similar, i.e., the eyeless reduced flies are similar in mean and distribution of bristles to the reduced, while the black, spineless and sooty have a lower mean and the distribution is shifted toward the zeros.

In an attempt to determine the presence or absence of factors in the third chromosome, the plus line was crossed to sepia spineless kidney sooty rough, the factors of which lie in the third chromosome. The F_1 females were back-crossed to sepia spineless kidney sooty rough males. Four groups of the males, because only the males show the reduced character, have been recognized. These are: First, reduced; second, sepia spineless kidney sooty rough reduced; third, sepia reduced and sepia reduced in combination with any other factor or factors except rough; and fourth, rough reduced and rough reduced in combination with any other factor or factors except sepia (table 12). A study of this table shows that the means and the distributions of the bristles of the reduced and the rough reduced flies are very similar. The mean of the reduced is 0.995; that of the rough reduced 0.946. Of the reduced flies 38.28 percent have no bristles; of the rough reduced 38.39 percent have no bristles. On the other hand the sepia reduced and the sepia spineless kidney sooty rough reduced males have a similar mean and a similar distribution of bristles. The mean of the former is 0.680; that of the latter 0.642. The percentage of flies with no bristles is 58.82 in the sepia reduced and 58.89 in the sepia spineless kidney sooty rough reduced. Table 12 is a compilation of two separate crosses made at different times. Each by itself showed practically the same result as the sum of the two.

This shows very clearly, I think, the presence of a factor in the third chromosome near the sepia end. Otherwise why the difference in these two types of crossovers? No further study was made of the second chromosome because of lack of suitable material for analysis.

TABLE 12

F_1 ♀ (from plus line $\times$ *sepia spineless kidney sooty rough*) $\times$ *sepia spineless kidney sooty rough* ♂.

Type	Number of bristles	Number of male offspring	Percentage	Mean
Reduced.....	3	16	7.20	0.995
	2	52	23.42	
	1	69	31.08	
	0	85	38.28	
Sepia reduced and sepia reduced in combination with any other factor or factors except rough.....	3	8	6.72	0.680
	2	16	13.45	
	1	25	21.00	
	0	70	58.82	
Rough reduced and rough reduced in combination with any other factor or factors except sepia.....	3	9	8.03	0.946
	2	19	16.96	
	1	41	36.60	
	0	43	38.39	
Sepia spineless kidney sooty rough reduced.....	3	8	4.39	0.642
	2	28	15.38	
	1	37	20.32	
	0	109	59.89	

Table 12 gives the results of back-crossing F_1 females (produced by crossing the plus line to *sepia spineless kidney sooty rough* stock) to *sepia spineless kidney sooty rough* males. The factors for these characters lie in the third chromosome. Only males showing the character reduced are given in the table. The reduced and the *sepia spineless kidney sooty rough* reduced are the non-crossover classes. Only two classes of crossovers are recognized. These are, first, *sepia* reduced and *sepia* reduced in combination with any other factor or factors except rough; and second, rough reduced and rough reduced in combination with any other factor or factors except *sepia*. The mean and bristle distribution in the reduced and rough reduced class of crossovers are practically the same. Also the mean and distribution of the bristles in the *sepia* spineless kidney sooty rough reduced and in the *sepia* reduced class of crossovers are about the same, but they are different from the other two.

We are forced then to conclude that in the plus line, at least three factors are present which modify bristle number, one primary factor which we have called "reduced," and which keeps the bristle number below that of wild flies, and two modifying factors which increase bristle number up to the same as or near that of wild flies.

In the analysis of the minus-selected line, the line was crossed to eosin miniature and the F_1 females back-crossed to eosin miniature males. A comparison of the reduced and the miniature reduced classes shows them practically the same with respect to bristle number. The mean of the reduced flies is 0.071 and of the miniature reduced flies 0.061. The percentage of flies with no bristles among the reduced is 93.04 and among the miniature reduced it is 94.56 (table 13). Hence we are forced to

TABLE 13
 F_1 ♀ (from minus line ♀ × eosin miniature ♂) × eosin miniature ♂.

Type	Number of bristles	Number of male offspring	Percentage
Reduced.....	2	2	0.24
	1	55	6.70
	0	763	93.04
Miniature reduced.....	2	3	0.74
	1	19	4.69
	0	383	94.56
Miniature.....	4	2	
Eosin reduced.....	0	3	
Eosin.....	4	359	
Eosin miniature.....	4	767	
Eosin miniature reduced.....	1	1	
	0	23	
Wild.....	4	27	

Table 13 gives the results of back-crossing an F_1 female (produced by crossing the minus-selected line to eosin miniature) to an eosin miniature male. Reduced and eosin miniature are the non-crossover classes. Miniature reduced and eosin are the principal crossover classes. A comparison of the non-crossover class, reduced, with the crossover class, miniature reduced, shows the two very similar.

conclude that in the minus line the modifying factor found in the X chromosome of the plus line is not present.

The minus line was also crossed to black pink bent and the F_1 females back-crossed to black pink bent males (table 14). The mean and the distribution of the bristles were very similar in the reduced, black, reduced, pink reduced, and bent reduced males. Hence we see no indication of factors outside the X chromosome.

TABLE 14
*F*₁ ♀ (from minus line ♀ × black pink bent ♂) × black pink bent ♂.

Type	Bristle number	Number of male offspring	Percentage
Reduced.....	3	0	00.00
	2	3	1.33
	1	14	6.22
	0	208	92.44
Black.....	3	0	00.00
	2	0	00.00
	1	4	2.11
	0	185	97.88
Pink.....	3	0	00.00
	2	0	00.00
	1	33	15.06
	0	186	84.93
Bent.....	3	0	00.00
	2	0	00.00
	1	2	1.68
	0	117	98.31
Black pink.....	3	0	00.00
	2	0	00.00
	1	2	1.45
	0	135	98.54
Black bent.....	3	0	00.00
	2	0	00.00
	1	3	4.00
	0	72	96.00
Black pink bent.....	3	0	00.00
	2	2	2.89
	1	2	2.89
	0	65	94.20
Pink bent.....	3	0	00.00
	2	0	00.00
	1	11	10.09
	0	98	89.90

Table 14 gives the results of crossing *F*₁ females (produced by mating the minus line to black pink bent) to black pink bent males. Only the males showing the character reduced are tabulated as all other flies are wild type with respect to bristle number. The percentage of zero flies and the distribution of the bristles is practically the same in each class.

We must conclude then that in the minus line only a single factor is present and that this acts as an inhibitor which prevents the appearance of the normal bristle number.

In a further attempt to test the genetic differences between the plus and minus lines, the two lines were crossed out to the wild, with each other, and also to the line selected for extra bristles. Can the results of these crosses be explained on the basis of the conclusion that in the minus line there is a single inhibiting factor present and in the plus line this same inhibitor and two or more plus factors? In fact if the conclusions are correct they must explain the results.

Let us consider the plus line first. When the plus-line male is crossed to a wild female and the F_1 flies inbred, one-half of the F_2 males are reduced. Of these extracted F_2 males 15.57 percent have four bristles, 24.84 percent have three bristles, 32.21 percent have two bristles, 20.42

TABLE 15
 $F_1 \times F_1$ (from plus line male $\times$ wild female).

	Female	Male, wild type	Male, reduced				
			0	1	2	3	4
Bristle number.....	4	4	0	1	2	3	4
Frequency.....	983	482	33	97	153	118	74
Percent.....	100.00	100.0	6.94	20.42	32.21	24.84	15.57

Table 15 gives the results of inbreeding the F_1 flies produced by crossing the plus-line male to a wild female. About one-half of the males show the character reduced. Note the distribution of the bristles in the reduced males.

percent have one bristle, and 6.94 percent have no bristles (table 15). The males of the parental generations showed 46.71 percent with four bristles, 32.34 percent with three bristles, 18.35 percent with two bristles, 2.22 percent with one bristle, and 0.35 percent with no bristles. The mean of the males in the parental generations was 3.22 while in the extracted F_2 males it was 2.21. This is a considerable regression toward the unselected line. The ninth and tenth generations of selection showed a mean of 2.19 for the males and the thirteenth and fourteenth generations showed 15.48 percent with four bristles (table 4). A certain amount of regression should be expected, however, if there are three factors for bristle number in the plus line, and if they become separated, as they would, in such an out-cross.

The plus-line female was crossed to the wild and the F_1 males compared with the F_2 extracted males. Table 16 gives the results. Of these males 17.65 percent have four bristles, 26.23 percent have three bristles, 40.10 percent have two bristles, 12.50 percent have one bristle, and 3.50 percent have no bristles. The mean is 2.42. Here also is a considerable regression. Perhaps more than we should expect. The difference between these males and the F_2 extracted males is not very great. The F_1 males get their X chromosome from the plus-line female and hence they have the inhibitor and one plus modifier. The second plus modifier is also present in a heterozygous condition. The F_2 extracted males get their X chromosome from the plus-line male. Some of these males would be homozygous for the factor in the third chromosome, some would be heterozygous and some would not have it at all. The inhibitor and one plus modifier are sex-linked. They may become separated by cross-

TABLE 16
Plus-line female $\times$ wild male.

	Females	Males, reduced				
Bristle number.....	All wild type	0	1	2	3	4
Frequency.....		51	182	584	382	257
Percent.....	100.00	3.50	12.50	40.10	26.23	17.65

Table 16 gives the results of crossing plus-line females to wild males. All the males are reduced as they get their X chromosomes from the plus-line female. Note the distribution of the bristles.

ing over in the F_1 female. If separated, the bristle number would be decreased, as a fly without the inhibitor is a wild-type fly. Hence, upon analysis the difference between these groups of males should not be so great.

The one puzzling result is the cross of the plus-line female to the minus-line male. If there is a single factor in the X chromosome of the minus line, the F_1 males from this cross might be expected to have as many bristles as the F_1 males when the plus-line female is crossed to a wild male. They do not, however. Table 17 shows only 3.32 percent of them have four bristles, 9.20 percent have three bristles, and 29.71 percent have two bristles, while 33.03 percent have one bristle and 24.72 percent have no bristles. Compare these percentages with those in table 16 where 17.65 percent of the males have four bristles. Why this difference when in both cases the F_1 male gets his X chromosome from the

plus-line female? The difference must be due to factors outside the X chromosome. While at first glance these results seem difficult to understand, yet perhaps they should not be counted as opposing our conclusions when we recall that we know nothing of the factor or factors which keep the number of bristles in wild flies practically constant. Certainly the normal number cannot be due to a single factor. In fact, I am convinced that several bristle factors are present in wild flies.

Wild flies differ genetically with respect to extra-bristle factors. During my selection experiments several strains have been tested. In one strain (PAYNE 1918) the bristle number was increased until the mean was nine plus. All wild strains which have been examined have a few flies with one extra bristle. Selection has been tried in two strains without results. At the present time I have a strain which has been selected for extra bristles for six generations. The mean in the fifth

TABLE 17
Plus-line female × minus-line male.

Female bristle number					Male bristle number				
0	1	2	3	4	0	1	2	3	4
93	207	385	236	153	223	298	268	83	30
Percent.....					24.72	33.03	29.71	9.20	3.32

Table 17 gives the results of crossing the plus-line female to the minus-line male. Note drop in the percentage of flies with four bristles and the increase in the other groups, when compared with table 16.

generation is 5.17 and 75.60 percent of the flies have extra bristles (table 18). Yet in this strain no fly has had more than seven bristles and only a few have that number. Nearly all of the extra-bristled flies have one or two extra bristles. As yet I am not absolutely sure, but I feel convinced that, unless some further genetic change occurs in the strain, I shall never be able to increase the mean much beyond what it is at the present time. I may be able to produce a strain in which 100 percent of the flies have extra bristles, but the number of bristles will not go beyond seven. It seems to me then that in the wild fly there may be plus modifiers which have been eliminated in the minus line by selection.

We meet with no difficulties when similar crosses are made with the minus line. When the minus-line female is crossed to a wild male 78.56 percent of the F_1 males have no bristles, 18.58 percent have one bristle, and 2.85 percent have two bristles (table 19). Here as in the plus line

TABLE 18

Summary of selection for extra bristles in a second wild strain.

Genera- tion	Mean bristle number of parents	Bristle number of offspring							Total	Mean bristle number of offspring	Percent with extra bristles	Percent with seven bristles
		Female				Male						
		4	5	6	7	4	5	6				
1	4.50	268	31	3		268	3		573	4.06	6.45	—
2	4.70	622	138	70	1	660	48	9	1548	4.22	17.82	—
3	5.61	283	149	80	5	372	62	6	957	4.41	31.55	—
4	5.80	50	76	62	5	146	52	13	405	4.72	51.48	1.23
5	6.00	31	68	143	6	80	92	35	455	5.17	75.60	1.31
6	6.00	50	107	159	7	126	112	64	625	5.09	71.84	1.12

Table 18 gives a summary of a second line selected for extra bristles. The starting point was three females, each with one extra bristle, and three wild-type males. The three pairs were from the same stock culture which had been inbred in the laboratory for two or three months. The mean bristle number of the offspring gradually rises from 4.06 in the first generation to 5.17 in the fifth. In the sixth there is a slight decrease to 5.09. No flies have appeared with more than seven bristles, and only a few with that number. It is doubtful whether selection will carry the change much further.

TABLE 19

Minus-line female $\times$ wild male.

Females	Male bristle number				
4	0	1	2	3	4
All wild type	689	163	25		
Percent.....	78.56	18.58	2.85	0.00	0.00

Table 19 gives the results of crossing minus-line females to wild males. All the males are reduced as they get their X chromosomes from the minus-line female. Note the distribution of bristles.

TABLE 20

F₁ $\times$ F₁ (from wild female $\times$ minus-line male).

Female	Male, wild type	Male, reduced				
4	4	0	1	2	3	4
557	849	632	160	57	6	2
Percent.....		73.74	18.66	6.65	0.70	0.23

Table 20 gives the results of inbreeding the F₁ flies produced by crossing the minus-line males to wild females. One-half of the F₂ males show the character reduced. Note the distribution of the bristles in these reduced males.

is a regression toward the unselected line. It indicates, as suggested, that the wild fly has more than one bristle factor. In this case it would seem that plus modifiers in the wild fly cause the change. If the minus-line male is crossed to the wild and the F_1 individuals inbred, the F_2

TABLE 21
Minus-line female $\times$ plus-line male.

Female					Male				
0	1	2	3	4	0	1	2	3	4
90	185	283	174	68	416	226	136	23	4
Percent.....					51.67	28.07	16.89	2.85	0.49

Table 21 gives the results of crossing the minus-line female to the plus-line male. Note particularly the drop in the percentage of males with no bristles. The drop is much greater than when the minus-line female is crossed to the wild.

TABLE 22
Wild $\varnothing \times$ plus line σ^7 .

Generation	Mean bristle number of parents	Bristle number of offspring														Totals	Number with extra bristles	Mean bristle number of offspring	Percentage of flies with extra bristles
		Females					Males			Males, reduced									
		4	5	6	7	8	4	5	6	0	1	2	3	4					
F ₁	4.00	221	5				218	4							448	9	4.02	2.00	
F ₁ × F ₁	4.37	352	51	17			190	10	3	17	30	56	46	41	623	81	4.16	13.00	
F ₂ × F ₂	5.06	441	102	20	1		566	28	1						1159	152	4.15	13.11	
F ₃ × F ₃	5.43	152	98	34	3		231	45	4						567	184	4.40	32.45	
F ₄ × F ₄	5.68	195	159	61	12	7	351	58	4						847	301	4.48	35.53	
F ₅ × F ₅	6.50	109	94	76	2		180	78	34						573	284	4.69	49.56	
F ₆ × F ₆	5.92	37	66	62	7		88	41	13						315	190	4.89	60.31	

Table 22 gives the results of crossing the plus-line male to a wild female and inbreeding for six generations the flies with extra bristles. The mean in F_1 is 4.02 and the percent of flies with extra bristles is 2. These figures are gradually increased in later generations of inbreeding until in F_6 the mean reaches 4.89 and the percentage of extra bristles is 60.31. Do the extra-bristle factors come from the plus line?

reduced males show about the same regression as the F_1 males show when the minus-line female is crossed to the wild (table 20). The cross of the minus-line female to the plus-line male brings out an interesting fact. Table 21 shows that only 51.67 percent of the F_1 males have no bristles.

Why a greater regression here than when crossed to the wild unless the plus line carries more plus modifiers than the wild fly? That the plus line does carry more plus modifiers than the wild is indicated by the fact that when the plus line is crossed to the wild some of the F_1 flies have extra bristles and that when these extra-bristled flies are inbred a strain of extra-bristled flies can be produced (table 22).

TABLE 23
A. Plus line $\times$ line selected for extra bristles.

Bristle number of parents	Bristle number of offspring											
	Female						Male					
	4	5	6	7	8	9	4	5	6	7	8	9
$\varnothing, 9 \times \sigma^7, 4$	14	11	5		1		16	5	14	3	1	
$\varnothing, 9 \times \sigma^7, 4$	18	9	3				5	11	14	13	4	
$\varnothing, 9 \times \sigma^7, 4$	37	8	7	6		1	8	13	9	6	2	1
Totals.....	69	28	15	6	1	1	29	29	37	22	7	1

B. $F_1 \times F_1$.

Bristle number of parents	Bristle number of offspring																	
	Female									Male								
	4	5	6	7	8	9	10	11		4	5	6	7	8	9	10	11	Male, reduced 1 2 3
$\varnothing, 4 \times \sigma^7, 4$...	22	7	5	4	1					11	3	1	1	2				11 2
$\varnothing, 6 \times \sigma^7, 6$...	15	7	6	2	12	6	1	1		1	3	4	3	4		1		3 17 7
$\varnothing, 4 \times \sigma^7, 4$...	21	13	6	5	9	5	2			2	4	2	7	6	1	1	1	4 21 12
Totals.....	58	27	17	9	22	11	3	1		14	10	7	11	12	1	2	1	7 49 21

Table 23 gives the results of crossing the plus line to the line selected for extra bristles. Also the results of inbreeding the F_1 flies. Note the appearance of extra-bristled flies in F_1 and F_2 . Note particularly that more of the F_1 males than females have extra bristles.

The cross of the plus and minus lines to the line selected for extra bristles gives interesting results. It should be recalled that the mutant "reduced" appeared in the sixth generation of the extra-bristle line. When the plus line is crossed to the extra-bristle line from which the mutant arose, some of the F_1 males and females have extra bristles (table 23). This might have been expected since extra bristles is partially dominant to the wild (table 24). When the minus line is crossed to the extra-bristle line we might expect the same result, but instead very few

of the F_1 females, 5 out of 229, have extra bristles while many of the males, 40 out of 102, have extra bristles. In each of the five females only one extra bristle was present. Of the 40 males, 29 have one extra

TABLE 24
High-selection-line female $\times$ wild male.

Bristle number of offspring										
Female					Male					
4	5	6	7	8	4	5	6	7	8	9
135	135	89	32	5	124	102	58	20	10	1

Table 24 gives the results of crossing the high-selection line of my previous paper (PAYNE 1918) to the wild. Extra bristles is partially dominant.

TABLE 25
Minus line $\times$ line selected for extra bristles.

Bristle number of parents	Bristle number of offspring								
	Female		Male				Reduced male		
	4	5	4	5	6	7	0	1	2
♀, 0 $\times$ ♂, 8.....	78	3					44	36	10
♀, 10 $\times$ ♂, 0.....	50		35	12	4				
♀, 0 $\times$ ♂, 9.....	42	1					25	7	
♀, 9 $\times$ ♂, 0.....	54	1	27	17	5	2			
Totals.....	224	5	62	29	9	2	79	43	10

B. $F_1 \times F_1$.

Bristle number of parents	Bristle number of offspring														
	Female							Male				Male, reduced			
	4	5	6	7	8	9	10	4	5	6	7	0	1	2	3
♀, 4 × ♂, 5.....	55	8	6	3	3	3	1	22	4		1	33	12	1	1
♀, 4 × ♂, 4.....	67	16	10	6	3		1	27	6		1	22	13	7	4
♀, 4 × ♂, 6.....	19	4	3	1	7			9	6	2	4	7	2		
Totals.....	141	28	19	10	13	3	2	58	16	2	6	62	27	8	5

Table 25 gives the results of crossing the minus line to the line selected for extra bristles and the inbreeding of the F_1 flies. Note that very few of the F_1 females have extra bristles (5 out of 229). Forty out of 102 males have extra bristles. The ratio is in favor of the females in F_1 .

bristle, 9 have two, and 2 have three extra bristles (table 25). In the case of the plus-line cross, 51 out of 120 F_1 females have extra bristles while 96 out of 125 males have extra bristles. Here then also more F_1 males than females have extra bristles. Why this difference in the F_1 males and females, and especially why the difference in favor of the males when in the extra-bristle line the females have on the average about one more bristle than the males? Can these results be explained on the basis of the conclusion that there is present in the minus line a single factor which inhibits or keeps the number of bristles below four, while in the plus line this same inhibitor is present, and in addition two modifying factors, one in the X chromosome and one in the third chromosome, which increase the bristle number to as high as four and in exceptional cases to five? I think they can.

It should be recalled that in the extra-bristle line there are at least two factors for extra bristles. One is in the X chromosome and the other in the third chromosome. Let us take the minus-line cross first. Our conclusion here was that there is a single inhibiting factor which keeps the number of bristles below four and we might even say below two as in the later generations of selection no flies had two bristles and only a very few had one bristle. The rest had none. If we use an extra-bristle-line female in the cross, all eggs will be alike, assuming of course that all factors for extra bristles are homozygous. They will have a factor for extra bristles in the X chromosome and one in the third. There will be two kinds of sperm in the minus-line male, the female-producing with the X chromosome carrying an inhibitor, the male-producing with no factors, either inhibitors or plus modifiers. The egg then with its two extra-bristle factors, when it meets the male-producing sperm of the minus-line male with no modifiers, produces males with extra bristles. On the other hand the X chromosome of the female-producing sperm carries an inhibitor. When it meets the egg, the zygote will have one X chromosome carrying a factor for extra bristles, the other carrying a factor for bristle reduction. Judging from the results, the factor for bristle reduction must neutralize the effect of the factor for extra bristles. The presence of an extra-bristle factor in one of the third chromosomes probably accounts for the few extra bristles which do appear. When the F_1 flies are inbred, there are as many extra-bristled females as males in F_2 (table 25).

When the plus line is crossed to the extra-bristle line both F_1 males and females have extra bristles. Here also, as previously mentioned, more males than females have extra bristles. We have concluded that

in the plus line there are three factors present, one an inhibitor the same as in the minus line, and two other factors which modify bristle number in a plus direction. Reciprocal crosses gave similar results in F_1 . For analysis let us use the extra-bristle female and the plus-line male. The eggs of the female parent will be the same as in the cross to the minus line. There will be one factor for extra bristles in the X and one in the third chromosome. In the plus-line male there will be two kinds of sperm, the female-producing with the X chromosome carrying the inhibitor and a plus modifier, and the third chromosome carrying a plus modifier. The male-producing sperm will carry only the plus modifier in the third chromosome. When the female-producing sperm meets the egg the zygote will have two X chromosomes, one from the extra-bristle line carrying a factor for extra bristles and one from the plus line carrying an inhibitor and also a plus modifier. This plus modifier was not present in the F_1 female of the minus-line cross. Of the third chromosomes the one from the extra-bristle line will carry a factor for extra bristles and the one from the plus line will carry a plus modifier. This plus modifier was not present in the F_1 female of the minus-line cross. Here then are two plus modifying factors present which were not present in the F_1 female of the minus-line cross and hence we should expect more extra bristles and more flies to show extra bristles. When the same egg with its two extra-bristle factors is fertilized by the male-producing sperm with one plus modifier in the third chromosome we have a zygote with one plus modifier in the X chromosome from the extra-bristle line. Of the third chromosomes one will carry an extra-bristle factor from the extra-bristle line and a plus modifier from the plus line. This zygote then has three plus modifying factors and no inhibitor. Hence more of the males than females have extra bristles. This F_1 male has one more plus modifier than the F_1 male from the minus-line cross and hence should have more bristles.

That the two plus modifiers in the plus-selected line may cause the appearance of extra bristles is indicated by crossing the plus line to a wild strain. This cross has been made to two separate wild strains and the result is the same in each. Table 22 gives the result of one such cross in which the plus-line male was used as one parent and the wild female as the other. Only a few extra-bristled flies appeared in F_1 (9 out of 448, or 2 percent). In F_2 however, 13 percent of extra-bristled flies is obtained. Inbreeding was continued for six generations using flies with extra bristles as parents. The percentage of flies with extra bristles was gradually increased from 2 percent in F_1 to 60.31 percent in F_7 . The mean bristle number was increased from 4.02 in F_1 to 4.89 in F_7 .

The minus-line male was also crossed to the same wild strain, in part to determine whether our conclusion that a single inhibiting factor is present would explain the results. If there are no plus modifiers here, we should expect no more extra bristles in F_1 and in later generations of inbreeding than we find in the wild strain. In fact we might expect to find fewer extra bristles in F_1 and F_2 if we have an inhibiting factor and no plus modifiers in the minus line. It should be recalled that when the minus line was crossed to the extra-bristle line only a very few of the F_1 females had extra bristles while more of the F_1 males had extra bristles. In the F_1 female the inhibiting factor in the X chromosome of the minus line apparently neutralized the effect of the extra-bristle modifier in the X chromosome of the extra-bristle line. In the cross to the wild strain we should likewise expect more of the F_1 males than females to have extra bristles. Such is actually the result shown in table 26. Of 240 F_1 females none had extra bristles. Three out of 237 F_1 males had extra bristles. This ratio, 3 to 474 is less than in the wild strain to which the minus line was crossed. Counts in the wild strain about the time the cross was made gave 327 flies with four bristles and 19 with five bristles. This is a percentage of 5.81 with one extra bristle. I may state that this wild strain had been bred in the laboratory about four years. When it was first brought into the laboratory a count of 613 flies gave 612 with four bristles and only one with one extra bristle. The continued inbreeding in the laboratory probably accounts for the presence of so many flies with extra bristles. In F_2 the percentage with extra bristles was increased to 2.35; in F_3 it dropped to 1.67; and in F_4 it rose to 6.30. Contrast this with 32.45, the percentage of extra-bristled flies in F_4 of the plus line crossed to the wild. Even the percentage of 6.30 is only slightly in excess of that found in the wild strain. I think we may safely conclude that the minus line has brought in no plus, modifiers.

Again it should be recalled that the mutant "reduced" from which both the plus- and minus-selected lines have come, appeared in the sixth generation of the line selected for extra bristles. In this sixth generation of the extra-bristle line 52.06 percent of the flies had extra bristles. The mean bristle number of the parents was 6.482; that of the offspring 4.88. The highest number of bristles on any one fly was nine. The extra-bristle line at this time must have carried factors for extra bristles. Since the reduced male appeared as a mutant from this line, it seems to me it would be possible and even probable that the mutant would carry these plus or extra-bristle modifiers. In the plus-selected line we have

demonstrated the presence of two plus modifiers which push the number of bristles up to four and sometimes to five. Can these plus modifiers be the same as the plus modifiers in the extra-bristle line? I am inclined

TABLE 26
Wild ♀ × minus line ♂.

Generation	Bristle number of offspring												Totals	Number of flies with extra bristles	Percentage of flies with extra bristles
	Female			Male			Male reduced								
	4	5	6	4	5	6	0	1	2						
F ₁	240			234	3							477	3	0.62	
F ₁ × F ₁	586	13	2	283	5	1	281	26	2			890	21	2.35	
F ₂ × F ₂	599	11		518	8							1136	19	1.67	
F ₃ × F ₃	130	18	1	97	3							349	22	6.30	

Table 26 gives the results of crossing the minus-line male to a wild female and then inbreeding for three generations the flies with extra bristles. The percentage of extra-bristled flies was increased to 6.30 in F₄. This is about the same percentage found in the wild strain to which the minus line was crossed.

TABLE 27
Selection in the plus line after seven months in mass culture.

Generation	Bristle number of offspring										Mean	Percent with four bristles
	Female					Male						
	0	1	2	3	4	0	1	2	3	4		
1			2	39	119	1		28	43	66	3.51	62.08
2			5	53	237		7	48	104	152	3.53	64.19
3			7	29	176	1	4	39	67	86	3.50	54.05

Table 27 gives the results of three generations of renewed selection in the plus line after seven months of mass culture. The mean and percentage of four-bristled flies are brought back to what they were at the peak of the curve in the fifty-fifth generation.

to think that such may be the case, especially since the cross of the plus line to the wild gives extra-bristled flies in F₁ and an increasing number of extra-bristled flies in later generations of inbreeding (table 22). The extra-bristle factors must have been brought in by the plus line.

DISCUSSION

The present paper adds another link in the chain of accumulating evidence in favor of the multiple-factor hypothesis as an explanation of the effects of selection in bisexual forms. The conclusions reached agree with those of my former paper (PAYNE 1918) on selection for extra-bristle number on the scutellum. They also agree with the earlier conclusions of MACDOWELL (1915, 1917) on bristle inheritance in *Drosophila*; and finally with those of STURTEVANT (1918) on selection for high and low bristle number in the mutant race, *dichaete*. A review of the literature and a full discussion of all work bearing on selection need not be repeated here as this has been done by several writers recently. For a thorough and impartial discussion of the disputed points I refer the reader to the paper of STURTEVANT (1918).

A recent short article by CASTLE (1919) should be mentioned. His work on selection with the hooded rats has long stood as a stumbling block in the way of the multiple-factor hypothesis. With some new facts at hand he now interprets those results in favor of this hypothesis.

This change of CASTLE leaves JENNINGS and his students as the principal opponents of the multiple-factor hypothesis. In the unicellular forms they have been accumulating evidence of changes by minute variations and have interpreted their results in favor of the Darwinian theory of natural selection. In addition to these small variations they find occasionally larger ones which they call mutations.

Are these variations which JENNINGS and his students describe and which they use in selection, the same as the fluctuating variations of DARWIN? This question is somewhat difficult to answer as DARWIN did not specify exactly what he meant by such variations. It is the general opinion, I believe, that he referred for the most part to somatic variations. The small variations of JENNINGS and his students are certainly not somatic variations. In fact they speak of them as heritable variations. In other words they are germinal in origin. DARWIN also realized that variations, to be effective in the process of evolution, must be inherited, but he erred in thinking that practically all variations are inherited. Is then the selection of germinal variations, selection in the Darwinian sense? Or does this merely mean that among the large number of variations observed within a species some will be germinal and if such variations happen to be of marked advantage to the species they will be preserved? To be sure JENNINGS and his students have produced changes within their lines by selecting small heritable varia-

tions. Is this all the selection theory asks? Apparently to JENNINGS it is. Three questions arise, however. First, are small heritable variations more numerous than larger heritable variations? Second, are these variations more apt to occur in the direction of selection than in other directions? Third, who or what determines whether a variation is large or small? It is impossible to answer the first question except in forms where actual breeding tests have been made, as a somatic variation is not distinguishable from a heritable one in any other way. In the protozoa, according to those who work with them, nearly all variations are small. This may be due in part to the fact that only the more extreme variations are called large. In other words it may be to a certain extent a matter of personal opinion whether a variation is said to be small or large. In the flies most of the heritable variations are large, at least large enough to be called mutations by those who work with them. To the second question it may be said that a mutation or a heritable variation may occur in the direction of selection or the reverse, and so far as I know is as apt to occur in one direction as in another. I think it may be said that we are more likely to see a variation in the direction of selection than in other directions because they are the ones we are looking for. This would be true particularly where the variations are small and where the eye is the judge of the differences. Also they are the ones which would be subjected to the breeding test. ZELENY (1920 b) has shown that by using the mutations which appear in the bar-eyed fly, facet number can be increased to full eye in one direction and to ultra bar in the other. As an example of a reverse mutation the reduced strain used for selection in the present paper arose as a mutant from the line selected for extra bristles. Third, as to whether a variation be large or small depends, as previously stated, to some extent upon the one who describes it. As an illustration JENNINGS (1917) describes the series of eye colors in *Drosophila* which behave as allelomorphs to white, as small heritable variations. He also describes the several modifiers of eosin eye color as small heritable variations. Those who have described this multiple-allelomorph system and the modifiers of eosin speak of them as mutations. Whatever you call them they did not occur in a graded series. What about the forms intermediate between the large and small variations? Would they be small heritable variations or would they be mutations? What I wish to emphasize is that it depends to a large extent upon the individual who describes the variation whether it be small or large, and that there is no sharp line of demarkation between the two; they intergrade. Why then make a

distinction between them? To me there is no difference so far as evolution is concerned. They are one and the same thing. Both are genotypic changes and as such are inherited.

Are then the views of JENNINGS and his students and the multiple-factor hypothesis mutually exclusive? It seems to me they are not. In the multiple-factor explanation of selection it is not necessary to assume that all modifying factors are present at the beginning of selection. Some of them may arise as mutations during the course of selection. This was indicated in my earlier experiments (PAYNE 1918). It has been demonstrated by ZELENY (1919) to occur in selecting for facet number in the bar-eyed mutant. ZELENY has also shown that the principal gene itself may change as in the reversal of bar to full eye and in the change from bar to ultra bar. Are not such mutations germinal variations, and how do they differ from the germinal variations of JENNINGS? The only difference I can see is in the size of the variation. When we can reduce the differences between two opposing theories to a question of the size of the variations, it seems to me the differences disappear entirely. Why not call all genotypic variations, mutations, or if we object to such an interpretation for the word, mutation, leave it out of our discussion and use the expression, "genotypic variation"? Certainly no one will object to saying that a genotypic variation may be large or small.

JENNINGS does not specify whether the heritable variation is due to a change in a single gene or whether through the origin of modifying factors. He means to imply that both methods may function, I think. In fact JENNINGS (1917) seems to think that it matters but little how the variations arise. The essential fact is that they do arise and this is all the selection theory asks.

Adherents of the multiple-factor hypothesis have usually assumed that selection is effective for a few generations and then ceases. This is not necessarily the case, as in the strain selected for extra bristles (PAYNE 1918) selection was effective for 29 generations. In the present paper the peak of the curve in the plus line was not reached until the fifty-fifth generation. So if genotypic changes occur within the line in the direction of selection either as changes within the principal gene or as factors which modify the character in question there is no reason why selection should not continue to be effective for long periods of time.

CONCLUSIONS

1. The mutant "reduced" appeared in the sixth generation of the line selected for extra bristles. The name applies to the number of bristles on the scutellum. Four is the wild-type number. In this mutant the number may be four, three, two, one, or none at all.

2. The strain has been separated very clearly into minus and plus lines by mating flies with no bristles on the one hand and flies with four bristles on the other.

3. In the minus line selection was effective for 17 generations. During this time the mean was reduced from 0.504 to 0.004 and the percentage of flies with no bristles was gradually increased from 64.61 to 99.52. From the seventeenth to the sixty-fourth generation the mean and the percentage of zero flies fluctuated slightly, but certainly there was no further genetic change after the seventeenth generation. While in the fortieth generation all the flies were bristleless, it was impossible to produce a strain without bristles.

4. In the plus line the mean was increased from 1.95 in the first generation to 3.25 in the eighteenth. The percentage of four-bristled flies was increased from 7.20 in the first generation to 46.90 in the eighteenth. From the eighteenth to the forty-sixth generation the mean and percentage of four-bristled flies fluctuated considerably. With the forty-sixth generation both began to rise and continued until the mean reached 3.52 and the percentage of four-bristled flies reached 64.25 in the fifty-fifth generation. From the fifty-fifth to the sixtieth there was a decline. The mean went to 3.11 and the percentage of four-bristled flies to 40.56.

5. The decline in the mean and the percentage of four-bristled flies in the last six generations of selection was probably due to weakness in the line. This conclusion is strengthened by the fact that after the strain was continued in mass culture for eight months renewed selection brought back immediately the mean and percentage of four-bristled flies to approximately what they were in the fifty-fifth generation.

6. Back selection in both lines was ineffective.

7. In mass cultures for eight months the two lines have remained what they were at the end of selection. They are not only somatically different, but they are genetically different.

8. By linkage and crossing over it has been demonstrated that in the minus line a single sex-linked factor is present. This factor seems to act as an inhibitor.

9. By the same method it has been demonstrated that in the plus line there are present at least three factors and probably more. One is the inhibitor present in the minus line. In addition there is a plus modifier in the X chromosome near miniature and a plus modifier in the third chromosome near sepia.

10. When the plus-line female is crossed to the minus-line male, there is a greater reduction in the bristle number of the F_1 males than when the plus-line female is crossed to the wild male. This can be explained on the assumption that the wild male has plus factors not present in the minus-line male. It also leads me to believe that the four-bristled condition in the wild fly is not due to a single factor.

11. When the minus-line female is crossed to the plus-line male, fewer F_1 males have no bristles than when the minus-line female is crossed to the wild. This indicates that the plus line has more plus modifiers than the wild fly. The number of bristles then in the plus line must be kept below those of wild flies by the presence of the primary inhibiting factor, reduced.

12. When the plus line is crossed to the wild some of the F_1 flies have extra bristles. By continued inbreeding of these extra-bristled flies a strain of extra-bristled flies can be produced. These extra-bristle factors must have come from the plus line. This seems all the more probable when it is recalled that the reduced strain arose from the sixth generation of the line selected for extra bristles. This also leads me to suspect that the plus modifiers of the plus line may be the extra-bristle modifiers of the extra-bristle line.

13. Selection for extra bristles in a second strain has increased the mean to 5.17 in the fifth generation. In the sixth generation it declined to 5.09. No flies have more than seven bristles and only a few have this number.

14. Low temperature increases bristle number.

LITERATURE CITED

- BRIDGES, C. B. 1919 Specific modifiers of eosin eye color in *Drosophila melanogaster*. Jour. Exp. Zool. 28: 337-384.
- CÁSTLE, W. E., and PHILLIPS, J. C., 1914 Piebald rats and selection. Carnegie Inst. Washington, Publ. 195. 56 pp.
- CÁSTLE, W. E., 1915 Some experiments in mass selection. Amer. Nat. 49: 713-726.
- 1916 Further studies of piebald rats and selection, with observations on gametic coupling. Carnegie Inst. Washington, Publ. 241 (part 3), pp. 161-192.
- 1917 The rôle of selection in evolution. Jour. Washington Acad. Sci. 7: 369-387.
- 1919 Piebald rats and selection. Amer. Nat. 53: 370-376.

- HEGNER, R. W., 1919 Heredity, variation, and the appearance of diversities during the vegetative reproduction of *Arcella dentata*. *Genetics* 4: 95-150.
- JENNINGS, H. S., 1916 Heredity, variation, and the results of selection in the uniparental reproduction of *Diffugia corona*. *Genetics* 1: 407-534.
- 1917 Observed changes in hereditary characters in relation to evolution. *Jour. Washington Acad. Sci.* 7: 281-301.
- MAY, H. G., 1917 Selection for higher and lower facet numbers in the bar-eyed race of *Drosophila* and the appearance of reverse mutations. *Biol. Bull.* 33: 361-395.
- MACDOWELL, E. C., 1915 Bristle inheritance in *Drosophila*. *Jour. Exp. Zool.* 19: 61-97.
- 1917 Bristle inheritance in *Drosophila*. II. Selection. *Jour. Exp. Zool.* 23: 109-146.
- MIDDLETON, A. R., 1915 Heritable variations and the results of selection in the fission rate of *Stylonychia pustulata*. *Jour. Exp. Zool.* 19: 451-503.
- MORGAN, T. H., 1916 A critique of the theory of evolution. 195 pp. Princeton: Princeton University Press.
- 1919 The physical basis of heredity. 305 pp. Philadelphia: J. B. Lippincott Co.
- MORGAN, T. H., STURTEVANT, A. H., MULLER, H. J., and BRIDGES, C. B., 1915 The mechanism of Mendelian heredity. 262 pp. New York: Henry Holt and Co.
- PAYNE, F., 1918 An experiment to test the nature of the variations on which selection acts. *Indiana University Studies*. Study No. 36. 45 pp.
- PEARL, R., 1917 The selection problem. *Amer. Nat.* 51: 65-91.
- ROOT, F. M., 1918 Inheritance in the asexual reproduction of *Centropyxis aculeata*. *Genetics* 3: 174-206.
- SEYSTER, E. W., 1919 Eye facet number as influenced by temperature in the bar-eyed mutant of *Drosophila melanogaster*. *Biol. Bull.* 37: 168-180.
- STOCKING, RUTH J., 1915 Variation and inheritance of abnormalities occurring after conjugation in *Paramecium caudatum*. *Jour. Exp. Zool.* 19: 387-449.
- STURTEVANT, A. H., 1918 An analysis of the effect of selection. *Carnegie Inst. Washington Publ.* No. 264. 68 pp.
- ZELENY, C., 1920 a The mutational series, full to bar to ultra bar, in *Drosophila*. *Anat. Rec.* 17: 336.
- 1920 b Forty-two generations of selection for high and low facet number in the white bar-eyed race of *Drosophila*. *Anat. Rec.* 17: 338-339.
- ZELENY, C., and MATOON, E. W., 1915 The effect of selection upon the 'bar-eyed' mutant of *Drosophila*. *Jour. Exp. Zool.* 19: 515-529.