

ISO-ALLELES AT THE BOBBED LOCUS IN *DROSOPHILA HYDEI* POPULATIONS

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THE mutant, bobbed, is among those which have been recorded frequently in the genus *Drosophila*. It was first found by BRIDGES in *Drosophila melanogaster* in 1915, and 40 mutations at this locus have been listed for the species. Of these, half were found by BRIDGES (BRIDGES-BREHME 1944). Parallel mutations have been recorded in *D. simulans* (STURTEVANT 1929); in *pseudoobscura* (STURTEVANT and TAN 1937); in *affinis* (STURTEVANT 1940); in *ananassae* (MORIWAKI 1935; KIKKAWA 1938); in *subobscura* (JERMYN, PHILIP, RENDEL, and SPURWAY 1943); in *hydei* (CLAUSEN 1923; SPENCER 1927); in *funebri*s (TIMOFÉEFF-RESSOVSKY 1931; SPENCER 1934); and in *virilis* (CHINO 1936). In all of these species except *ananassae* the locus of bobbed occupies a position at the centromere end of element A. This places it in a terminal position on the genetic map of the X chromosome when this contains only one euchromatic arm or in a central position on the map where there are two euchromatic arms in the X as in *pseudoobscura* and *affinis*. In *D. ananassae* KIKKAWA (1938) has shown that the locus is in the small fourth chromosome. In general the mutant is strongly sex-limited in phenotypic expression. Through studies on non-disjunction and gynandromorphs STERN (1926a, 1927) first showed that the Y chromosome carried a bobbed locus, and thus accounted for phenotypic differences in male and female when X and Y chromosomes carried different alleles. Later, by combining cytological and genetic techniques, STERN (1929a) demonstrated crossing over between the X and Y chromosomes. By using females varying cytologically from XX to XXYY and males varying from X to XYY in constitution, and carrying different combinations of bobbed and non-bobbed alleles, including a bobbed-lethal, he was able to demonstrate the additive effects of two or more genes in conditioning bristle size (STERN 1929b).

The bobbed phenotype includes a reduction in the size of all bristles, abnormal abdominal sclerites, lengthening of larval-pupal period, lowered fecundity, fertility and viability, and lethal effects in homozygotes in extreme alleles. In species other than *D. melanogaster* the most extensive analysis and account of the bobbed phenotype has been given by LÜERS (1937) for a dominant bobbed allele in *D. funebri*s. In *D. simulans*, STURTEVANT (1929) has shown that Y chromosomes derived from stocks collected from widely separated geographical areas carried bobbed alleles more extreme than the three mutations to bobbed in the X found in different laboratory stocks. Consequently males showed a more extreme bobbed phenotype than females. One wild Y chromosome derived from a Woods Hole stock, however, when combined

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with a mutant X gave males no more extreme than the females of the mutant stock. In *D. pseudoobscura*, STURTEVANT and TAN (1937) have found a condition similar to that in *D. simulans*.

Bobbed was first discovered and reported in *D. hydei* by CLAUSEN (1923). The allele found by him produced a moderate reduction in bristle size and some abnormality in abdominal sclerites. In November, 1925, the author collected a few rotting tomatoes, in which *Drosophila* were breeding, from a fruit cellar in Wooster, Ohio. *D. hydei* derived from this collection was bred in mass culture until March, 1926, when 50 pair matings were made up. Three of these gave some females with reduced bristles, and from one of them by further inbreeding a pure bobbed stock was isolated. Compared to CLAUSEN's bobbed this stock was more extreme in bristle reduction but without abnormal abdominal sclerites. During subsequent work on this species mutations at other loci were found and laboratory stocks built up from collections made at different times. It was noticed from time to time that some laboratory strains contained bobbed and it was assumed that these had been contaminated either accidentally or in experimental crosses from CLAUSEN's bobbed, of which a stock had been secured, and from the original Wooster bobbed stock. The facts to be presented in this paper, however, would indicate that bobbed was probably present in the original wild strains from which the laboratory stocks were derived. A brief summary of the data on bobbed alleles in *D. hydei* has been reported elsewhere (SPENCER 1938).

THE AZUSA POPULATION OF DROSOPHILA HYDEI

In the winter of 1936-1937 the author, then at the CALIFORNIA INSTITUTE OF TECHNOLOGY, received from DR. J. SCHULTZ a small sample of *D. hydei*, collected at a citrus dump near Azusa, Southern California. These flies were mated in pairs, and from them several new strains of bobbed were isolated, differing among themselves and from the original alleles. Certain other mutants appeared in the material, and it was decided to make an extensive study of this population.

The citrus dump was located about a mile from Azusa and thirteen miles from Pasadena in a tract of waste land near the San Gabriel River. Here were many tons of rotting citrus fruit, mostly oranges, and casual inspection showed that the citrus dump supported a huge population of *D. hydei*. The adults were there by the million, and the material contained great masses of larvae. Pupae were collected by scooping them up with a trowel. During the spring when the population was at its peak *Drosophila* could be trapped in almost incredible numbers at a considerable distance from the breeding ground. One half-pint bottle containing fermenting banana mash was hung in a bush about fifty yards from the citrus dump on April 21 and taken in at 4:30 P. M. on April 23, temperature 23°C. It contained 1403 *Drosophila*, of which 846 were *D. hydei* males and 357 *D. hydei* females. On March 20 a collection was made by holding a cellophane sack over crevices in the mass of citrus fruit. In a few minutes 933 males and 321 females of *D. hydei* were taken along with 14 *D. immigrans*, three *D. pseudoobscura*, two *D. melanogaster*, and one *D. busckii*. Both of these

collections were made in cool weather, and the high preponderance of males was due to the fact that in this species the males are more active than the females at low temperature and not to any such abnormal sex ratio as the collection figures alone would indicate.

It was therefore decided that a truer sample of the population could be secured by making collections of mature pupae and rearing them out. The pupae were collected in large open cans which were placed in paper sacks. These sacks were pinned shut, and daily collections of the adult flies emerging were made from them. These adults were then taken to the laboratory and examined under the binocular microscope for mutant characters.

BOBBED PHENOTYPES IN THE AZUSA POPULATION

Examination of these flies showed that a considerable number of the females were phenotypically bobbed; these ranged from extreme types in which the bristles were reduced to the size of large hairs to slightly reduced bristle forms. It soon became apparent that the less extreme bristle types graded up close to or into the normal bristle form. The normal type could easily be checked, since all males belonged to this class. The flies were quite uniform in size, indicating optimum food conditions; no difficulty in classification due to undersize flies was encountered.

Of the 27,805 flies examined there were *D. hydei* males, 12,031, females, 15,575; *D. melanogaster* males, 45, females, 62; *D. immigrans* males, 32, females, 40; *D. pseudoobscura* males, one, females, nine; *D. busckii* males, five, females, five. Of the 15,575 *D. hydei* females 495 or 3.1 percent were classified as phenotypically bobbed. Only those flies showing distinct bristle reduction were so classified, and it was apparent that many slight bobbed individuals were being placed in the normal class. The significantly lower number of males was probably due to the presence of some sex-linked lethals in the wild population, and more particularly to sex-linked recessive semi-lethal and lowered viability mutants. Large scale laboratory experiments on *Drosophila* have shown similar sex-ratios.

One vermilion, one extreme scute, and one light male, the first two sex-linked recessives and the latter a sex-linked semi-dominant, were found. These were of interest in connection with the report of DUBININ and collaborators (1937) that sex-linked recessives were practically absent from very large collections of *D. melanogaster*. Among 129,582 flies taken they found only one sex-linked mutant, a yellow male. However, more recently BERG (1942a, 1942b) has collected large numbers of sex-linked mutant males, mostly yellow, in certain populations of *D. melanogaster*. STURTEVANT (1915) has reported a considerable number of the mutant, light, a sex-linked semi-dominant, in collections of *D. repleta*. METZ found a yellow male in a collection of *D. simulans* (STURTEVANT 1929). The author has found yellow in wild specimens of both *D. melanogaster* and *D. immigrans* and has recorded vermilion in large numbers in the Wooster population of *D. hydei* (SPENCER 1932). While selection undoubtedly tends to reduce the numbers of sex-linked mutant individuals

the supposition that they are almost non-existent in wild populations would seem to be erroneous, at least under certain breeding conditions.

BOBBED GENOTYPES IN THE AZUSA POPULATION

The presence of a complex series of bobbed phenotypes in the Azusa population could have been due either to one bobbed allele acted on by modifiers or to multiple alleles or to a combination of these causes. Tests were set up as follows. Since the females reared from pupae were virgin when collected (minimum age of sexual maturity after eclosion in this species is two days for the females and five days for the males), phenotypically bobbed females of different grades were mated singly to standard bobbed males. Some tests were run using the Wooster bobbed stock, others using the bobbed stock of CLAUSEN, hereafter designated as Berkeley bobbed. The females chosen for these tests constituted a representative sample taken from the 495 bobbed females collected from the wild population, grading from slight to extreme types. In all, 54 such females were tested. When their offspring emerged, the daughters fell into either one or two classes with relatively little variation among the members of a class. Five members of each class were measured with an ocular micrometer for length of posterior scutellar bristles.

These data are shown graphically in figure 1, with full explanation in the legend to this figure. It will be noted that all but 15 of the females tested contained in their two X chromosomes separable bobbed alleles which in heterozygous combination with the tester bobbed allele gave two distinct phenotypes in the daughter offspring. The distribution of the daughter phenotypes, either one or two grades in each test, but many grades when all the tests were considered, indicated that the phenotypic variations were not due to recessive genetic modifiers acting on one bobbed allele, for in heterozygous condition the effect of such modifiers would be lost. There was, of course, some variation in bristle length in individuals owing to size differences in the flies, and it is likely that separable alleles could have been demonstrated in some of the 15 females referred to above by extensive tests and statistical analysis. The heterozygous daughter classes ranged from bristle grade 12, posterior scutellar bristle length 252 microns, to grade 33, posterior scutellar bristle length 693 microns, the standard bristle grade of males.

In all, 40 backcross tests of heterozygous daughter classes carrying one Wooster bobbed tester gene and one wild Azusa gene to Wooster bobbed males were set up. These included daughters from 16 of the original tested females and of the following grades: 12, 13, 14, 17, 18, 19, 22, 24, 25, 27, 28, and 29. However, none of the tests for grades 12, 13, or 14 were successful, although 18 cultures were started. The extreme bristle grades are generally highly infertile. At least some cultures of all the other grades tested came through, and the results were uniformly consistent with the interpretation that the bobbed variations were due to alleles at that locus rather than to dominant modifiers. In table 1 data are presented of the micrometer measurements made on posterior scutellar bristles of males and females of Wooster

bobbed stock and from several of these backcross experiments. The data show the usual range of variability in bristle length within the members of each class in the backcross tests. It will be noted that the variability in the females of a class is no greater than the variability in the males from the same culture, or in males and females from the inbred tester stock. Minor variations in the

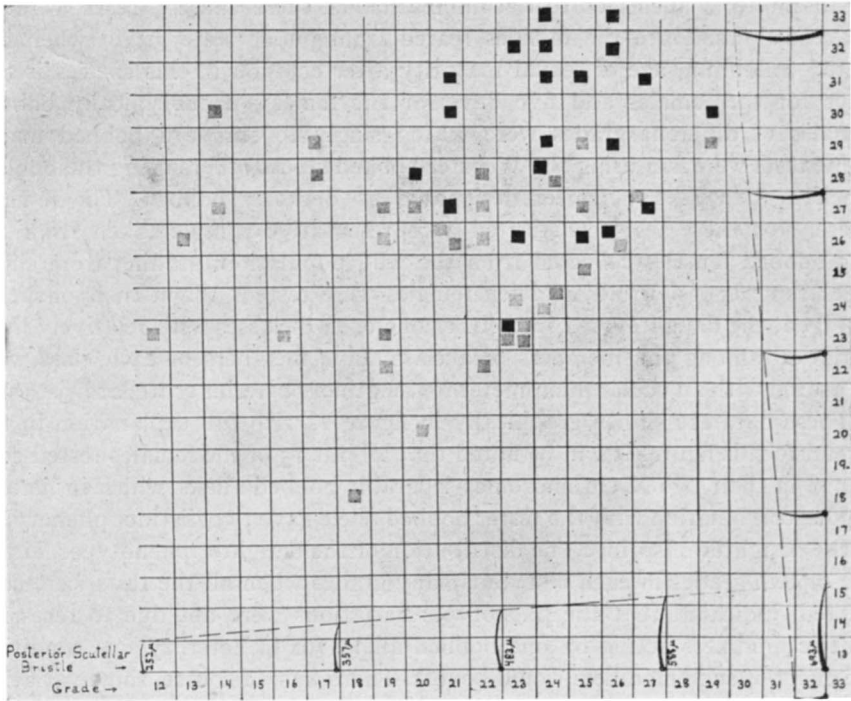


FIG. 1.—Tests of 54 phenotypically bobbed females reared from pupae collected from the Azusa population of *Drosophila hydei*. The position of each square designates the bristle length or grade in the heterozygous daughters from the mating of an Azusa bobbed female to a male from a standard bobbed stock. A black square indicates mating to a Berkeley male, the X chromosome of which carried a bobbed gene which in XX females of the stock gave a bristle grade of 28, slight bobbed. A gray square indicates mating to a Wooster male carrying a grade 20, medium bobbed, gene in the X chromosome. For example the square at the extreme left of the figure indicates a mating of a female from the Azusa population to a Wooster male carrying grade 20 bobbed in his X chromosome. The female carried in one X a gene which in heterozygous combination with the grade 20 tester gave daughters of grade 12 and in the other X a gene which in heterozygous combination with the grade 20 tester gave daughters of grade 23. It will be noted that all but 15 of the females tested carried two alleles distinguishable in the heterozygous combinations with the tester gene used.

mean bristle length of males in different cultures was due to environmental variables difficult to control. These environmental factors would likewise be expected to produce such variation within a class of females as was observed. By referring to figure 1, it will be noted that in two cases where the Berkeley tester was used in analysing bobbed females one class of daughters was pheno-

typically non-bobbed. In general it will be noted that the heterozygous combinations with Berkeley grade 28 gave an average bristle length greater than the combinations with the more extreme Wooster grade 20 tester. It was obvious that the complex series of bobbed alleles in heterozygous combinations showed no dominance within the series, but rather gave phenotypic classes conditioned by the strength of the two alleles present in the heterozygote.

The test of the female represented by the square at the extreme left of figure 1 was of particular interest. One class of her daughters was of grade 23, the other of grade 12. She was the most extreme bobbed type recovered from the wild population. It seemed likely that if these grade 12 daughters, carrying the Wooster bobbed 20 gene, showed an extreme phenotype of greatly reduced bristles and abnormal abdomen, that the wild gene recovered from their mother would be lethal in homozygous form. To test this a series of pair matings of these daughters to their brothers, all phenotypically alike but half carrying the extreme, presumably lethal, wild gene, and the other half the less extreme wild gene, was made up. Some of these matings gave two classes of daughters, one of grade 23 and the other of less extreme grade. The other matings produced only one type of female, grade 12, and the sex-ratio indicated the presence of a bobbed-lethal, killing off half of the females.

TABLE 1

Frequency distributions of males and females from Wooster bobbed stock and of the classes of males and females from four of the backcross tests of heterozygous wild Azusa/Wooster bobbed females to Wooster bobbed males.

GRADE OF POSTERIOR SCUTELLAR BRISTLES	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
Wooster bobbed ²⁰ males													1	1	4	19	2	1	
Wooster bobbed ²⁰ females					5	4	4												
Sons of (Wb ²⁰ /Az) ¹⁸ female × Wb ²⁰ male																2	5	6	7
daughters of (Wb ²⁰ /Az) ¹⁸ female × Wb ²⁰ male			2	11	10	2	4	2	2										
Sons of (Wb ²⁰ /Az) ²⁵ female × Wb ²⁰ male																	1	7	2
Wb ²⁰ /Wb ²⁰ daughters of (Wb ²⁰ /Az) ²⁵ female × Wb ²⁰ male			1	2	3	2													
Wb ²⁰ /Az daughters of (Wb ²⁰ /Az) ²⁵ female × Wb ²⁰ male								1	5	5	2								
Sons of (Wb ²⁰ /Az) ²⁸ female × Wb ²⁰ male																2	2	6	6
Wb ²⁰ /Wb ²⁰ daughters of (Wb ²⁰ /Az) ²⁸ female × Wb ²⁰ male	1			1	3														
Wb ²⁰ /Az daughters of (Wb ²⁰ /Az) ²⁸ female × Wb ²⁰ male									1	3	2	8	3						
Sons of (Wb ²⁰ /Az) ²⁹ female × Wb ²⁰ male																2	5	1	
Wb ²⁰ /Wb ²⁰ daughters of (Wb ²⁰ /Az) ²⁹ female × Wb ²⁰ male			3	3	2		1												
Wb ²⁰ /Az daughters of (Wb ²⁰ /Az) ²⁹ female × Wb ²⁰ male												3		2	2	1			

While the above analysis has demonstrated the presence of a large series of multiple alleles in the Azusa population, through the testing of phenotypically bobbed wild females, it also showed that the mere tabulation of wild females as bobbed or non-bobbed could not give an accurate quantitative picture of the frequencies of the many bobbed grades present in the genotypes. Since all males seen in the population were phenotypically alike, grade 33, there was no chance of error through selection of a weighted sample in the following test (Note: the male recorded as Y-bobbed in *Drosophila* Information Service No.

11, page 17, was later shown by linkage tests not to be bobbed). One hundred and six Azusa wild males were pair-mated to standard grade 20 Wooster bobbed females. The results of this test are shown in figure 2. While the sample may be too small to be entirely representative, it should furnish an approximate analysis. It will be seen that of the 106 genes tested, 27, or 25.5 percent, gave phenotypically bobbed daughters. Each test gave daughters of a single grade. These ranged from an extreme of grade 17 to very slight of grade 32. Further tests of representatives from the heterozygote grades 27 to 32 showed that when these genes were homozygous, they had no visible bobbed effects. They had been distinguished from one another only by the use of the fairly

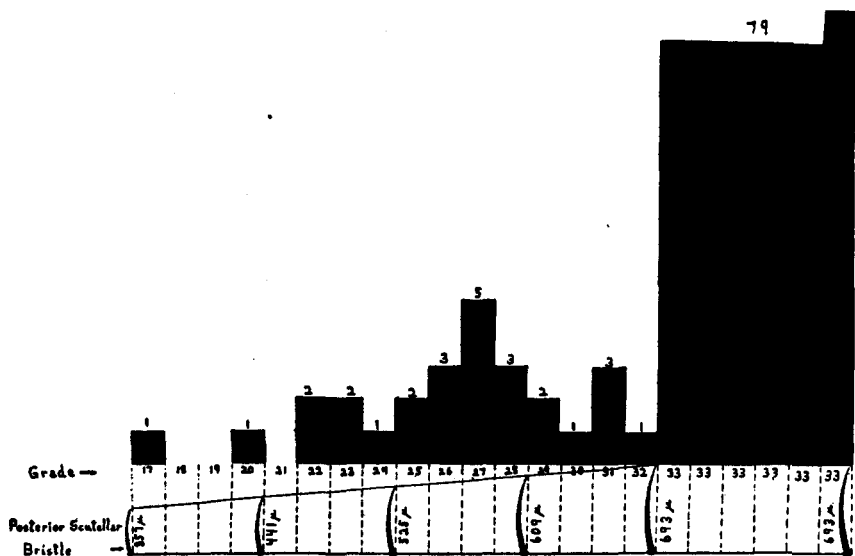


FIG. 2.—Bristle length in the heterozygous daughters of 106 Azusa males, all phenotypically identical, mated to Wooster grade 20 females. Twenty-seven or 25.5 percent of males carried alleles in their X chromosomes forming phenotypically bobbed compounds with grade 20 tester genes.

strong Wooster bobbed 20 as a tool. Following STERN and SCHAEFFER (1943), we shall refer to these and other genes in the series, requiring testers to distinguish them and to seriate them, as iso-alleles. Such iso-alleles are indistinguishable from one another in homozygous form.

Since Wooster bobbed 20 had given more extreme heterozygous combinations than Berkeley bobbed 28, it was assumed that the bobbed-lethal extracted from the Azusa population would prove a still more effective tool. It was therefore made up in heterozygous combination with a grade 22 bobbed also extracted from the Azusa population. This combination was somewhat more viable and fertile than Wooster 20/Azusa lethal. The Azusa 22/Azusa lethal females were pair-mated to 12 wild Azusa males collected from the population and all phenotypically alike. The results of these matings are shown in figure 3. It will be noted that of the 12 wild genes present in the 12 Azusa males picked at random three of them, designated b^a , b^b , and b^c , gave phenotypically

bobbed heterozygotes with Azusa 22 and much more extreme heterozygous combinations with Azusa lethal. In fact b^a gave a lethal combination with the latter and probably was itself a homozygous lethal, although the test was not made. Furthermore, of the nine genes which gave phenotypically non-bobbed heterozygotes with Azusa 22, three designated as b^d , b^e , and b^f , gave phenotypically bobbed combinations but of visibly different grades with Azusa lethal.

Thus it was shown that certain bobbed genes, which not only in homozygous condition but even when acting in heterozygous combination with the rather strong Azusa 22 gave no phenotypically bobbed effects, could be seriated as to their bobbed potency by the use of the strong bobbed-lethal tester. How-

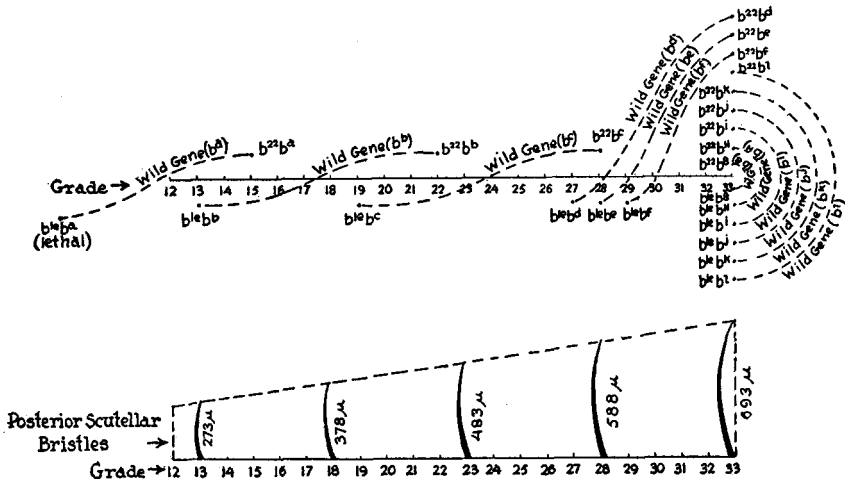


FIG. 3.—Tests of 12 Azusa males to heterozygous tester females of composition Azusa bobbed 22/Azusa bobbed-lethal. Genotypes of each daughter class placed directly above their corresponding phenotypes, represented in the reference scale of bristle lengths. Grade 33 (693 microns) is maximum bristle size in the species. The bobbed-lethal tester distinguishes and seriates six of the genes tested; bobbed 22 distinguishes and seriates three of them.

ever, other genes were so strong in their bristle forming potency as to pull the heterozygotes with the extreme bobbed-lethal into the class of maximum bristle size.

In this presentation of data we have purposely avoided the use of the term wild type because it connotes a homogeneous class of genes in the wild population of which the mutant form is the rare exception. Actually the situation at the bobbed locus in the Azusa population has been shown by this analysis to be quite different. There is no one wild type allele mutating rarely to bobbed. Rather there is a complex series of bobbed alleles grading from extreme lethal types at one end and through genes which in homozygous condition give bristles of many different grades to those at the other end, which in homozygous condition are non-bobbed phenotypes but in heterozygous combination with moderate bobbed genes show visible effects. Finally at this end of the series there are some genes so strong in bristle forming potency as to override

the effect of bobbed-lethals in heterozygous combinations. This, however, does not prove that these form a homogeneous class. By implication they do not. It would be of interest to test for iso-alleles in the more extreme members of the series by using such a gene as b^s (see fig. 3) as a tool in searching for more extreme bobbed-lethals, and using such a lethal in turn in locating and seriating extremes at the other end.

While bristle grade 33 represents the end point in a morphological series, the evidence indicates that minimum grade 33 is far from the end point in what may be designated as a potency series. Using the morphological guide of bristle length in that range of the series where phenotypic effects are secured in heterozygous combinations, we might assign to gene b^c a potency of about 34, b^d a potency of 50, b^e of 52, and b^f of 54. At the other end Azusa lethal would have a potency of about four, and b^a of about eight, itself a homozygous lethal but less extreme than the Azusa lethal tester gene. Heterozygous or homozygous females in which the sum of the potencies of the two alleles present is 66 or more are of maximum bristle grade 33. A potency series was set up by STERN (1929b) to represent the additive effects of bobbed alleles in *D. melanogaster* in haplo, diplo, triplo, and even more complex combinations. A series of this nature was postulated by MOHR (1932) to explain the action of different alleles at the vestigial locus in *D. melanogaster*. It should be mentioned that the effects of some bobbed alleles found in *D. melanogaster* cannot be interpreted in terms of a simple potency series such as proposed here. Such exceptions can better be explained by the substrate-gene interaction theory recently developed by STERN (1943). It seems probable that an extended study of the moderate *D. hydei* bobbed alleles in primary non-disjunction males and in heterozygous combinations with bobbed-lethals would show similar exceptions best interpreted by the substrate-gene interaction theory.

BOBBED PHENOTYPES IN THE WOOSTER POPULATION

In order to determine whether the presence of multiple alleles at the bobbed locus was a characteristic of the species, *D. hydei*, or peculiar to certain populations such as that at Azusa, an investigation of a population of *D. hydei* in the environs of Wooster, Ohio, was made in the fall of 1937 and the summer of 1938. This population was breeding on a large refuse dump maintained back of a wholesale fruit and grocery concern. Here were thrown banana stalks, over-ripe bananas, citrus fruit, melons, tomatoes, and other vegetables. At the peak reached in this latitude in October and November the adult population was estimated at not less than 500,000, much smaller than the Azusa population, estimated at 100,000,000.

The year round structure of these two populations differed greatly as reported by the author (SPENCER 1938, 1941). Since *D. hydei* is native to the tropics, the Wooster population was greatly reduced in the winter, where all survivors overwinter in buildings. In a collection of flies reared from pupae taken from this population in July 1938 of a total of 2,251 there were 813 *D. hydei* males and 1,422 *D. hydei* females. Of these latter 51 or about 3.6 percent

were classified as distinctly bobbed, of which the most extreme was grade 16. Another collection yielded a total of 2,083 flies, 11 *D. melanogaster*, three *D. busckii*, 891 *D. hydei* males, and 1,178 *D. hydei* females. A careful check of the latter showed 57 or 4.8 percent visibly bobbed. As in the Azusa population, several wild males showed other sex-linked recessives, seven vermilion and one singed. In other collections, in which bobbed was not recorded, of 1,220 males 12 were vermilion, one extreme scute and eight light, a sex-linked semi-dominant body color.

BOBBED GENOTYPES IN THE WOOSTER POPULATION

In November, 1937, a test was made to determine the distribution of bobbed alleles in the Wooster population in comparison to that in the Azusa population. In order to secure maximum information for the work involved,

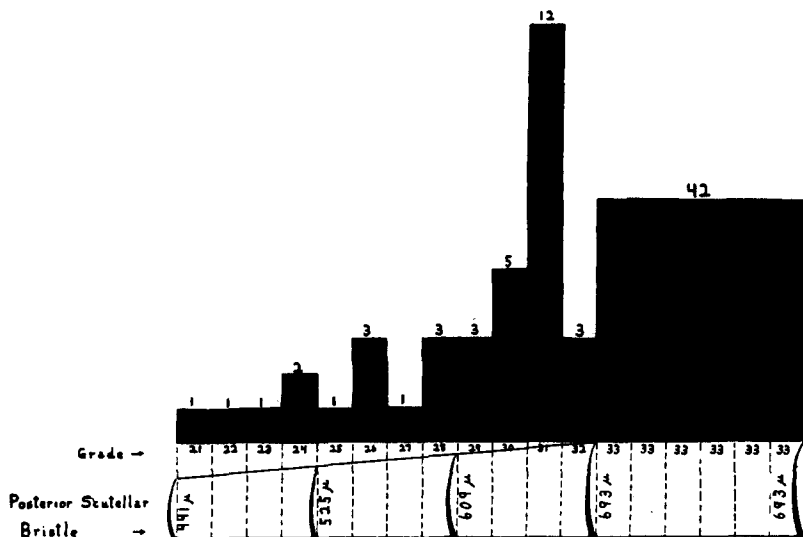


FIG. 4.—Tests of 78 genes carried in 39 females chosen at random from a Wooster, Ohio, population of *Drosophila hydei*. The graph shows bristle lengths in the heterozygous daughter classes from matings of the 39 females to males carrying Wooster grade 20 tester gene in their X chromosomes. Thirty-six or 46.2 percent of genes tested formed phenotypically bobbed compounds with the grade 20 tester.

virgin wild females were pair-mated to Wooster bobbed 20 males. Two genes were tested by each pair-mating. Care was taken that this sample should not be weighted either by selecting or excluding phenotypically bobbed females for the test. The results are shown in figure 4. Of the 78 genes tested 36, or 46.2 percent, formed phenotypically bobbed compounds with standard bobbed 20. This is in contrast to the 25.5 percent of genes of these grades in the Azusa sample. However, the sample shows relatively more of the heterozygotes from the Wooster population in the high grade ranges, 30–32. This might well account for the smaller difference in the percentages of visibly bobbed females in the two populations (3.1 percent for Azusa versus 3.6 to 4.8 per-

cent for Wooster) than might be expected if only the quantity and not the quality of the alleles demonstrated in the two populations were considered. The frequencies of the bobbed alleles in the two populations falls into line with the analysis of gene frequencies at other loci which the author has reported on briefly (SPENCER 1938, 1941). The Wooster population has shown a higher concentration of a few mutant genes, gray body color, vermilion eye, nicked wing, but a lower number of mutant loci per 100 flies tested than were present in the Azusa population. The year-round pattern of the Wooster population gives a sharper bottle-neck effect due to winter killing. In like manner the Wooster population shows a higher concentration of a few bobbed alleles and less spread in the grade range.

OTHER *DROSOPHILA HYDEI* POPULATIONS

While no quantitative data have been secured from other *D. hydei* populations, it should be noted that small collections from Coffeyville, Kansas, and Long Island have yielded bobbed. In analysing about 100 males from Gatlinburg, Tennessee, for the presence of mutants at other loci and with no intention of checking for bobbed, a lethal at the bobbed locus was discovered. It seems safe to conclude that this species generally carries in its populations, where these have a moderately large effective breeding size, a complex series of alleles at the bobbed locus, similar to those demonstrated.

PLEIOTROPIC EFFECTS OF BOBBED

It is not our intention here to discuss in detail the question of whether so-called pleiotropic effects (see GRÜNEBERG 1943) represent actual multiple or unit gene action. However, in those morphological and physiological effects conditioned by mutant changes there is an actual difference in complexity from locus to locus and among the alleles at one locus. Often the characters conditioned by a single mutant change are not only multiple in the descriptive sense but manifold in terms of their fundamental physiological and even adaptive potentialities. The tracing of the pathways from such characters back to or toward the gene constitutes an important field of research. Until these developmental paths are mapped and probably after it would seem convenient to retain a term for demonstrable multiple effects in contrast to single effects. However, the more careful investigation of many genes now known for one phenotypic effect should demonstrate others, often more important in terms of the physiology of the organism (DOBZHANSKY and HOLZ 1943).

It has been noted that of the two bobbed alleles first described in *D. hydei*, one, Berkeley 28, has slight bristle effect but in addition abnormal abdominal sclerites. In contrast, Wooster 20 shows more extreme bristle change but no abnormal abdominal sclerites. Observations on the many bobbed alleles in the populations studied revealed a complex situation in regard to pleiotropic effects. The characters observed may be listed as follows: reduction in bristle size, abnormal abdominal sclerites, reduction in pigment spots around thoracic bristles, lengthening of larval-pupal period of development resulting in late emergence, lengthening of time to sexual maturity after eclosion, lowered vi-

ability, fecundity, and fertility, and finally lethal effects. Seriation for one of these characters was not absolutely correlated with seriation for others. Thus bristle types of as high grade as 28 might be associated with abnormal abdomen, and types of as low grade as 20 with normal abdomen. Some slight bristle grades showed more larval-pupal retardation than other more extreme types. MOHR (1932) has described a similar lack of correlation in seriation of vestigial alleles for wing reduction and breaks in the second longitudinal vein.

In general it may be stated that the bristle effect was much more often observable than the abnormal abdomen effect and that extreme bristle types were more likely to show abnormal abdomen and other phenotypic characters than slight bristle types. It is not to be understood that all of the alleles placed in one grade in the bristle analysis were indistinguishable on other morphological and physiological grounds. However, due to the complexity of the situation, it was deemed expedient to concentrate on the one character most suitable for metrical analysis. It should also be emphasized that the seriation of a character by counts or measurement may not represent an accurate picture of physiological and developmental grades of equal values. The whole question of thresholds is involved here. Nevertheless, until better tools are at hand it would seem that such measuring of adult characters has value, as long as the method is recognized as giving at least a qualitative and at most only an approximate quantitative picture.

DISCUSSION

We may raise the question as to whether the bobbed situation in *D. hydei* is unique and therefore a detail of passing interest as far as an interpretation of genetic phenomena is concerned. Certainly too many competent observers have worked with many species of *Drosophila* not to have discovered such a marked phenotypic bobbed complex if it were present. However, the difference in the several species studied is probably not so fundamental as it might appear to be. As mentioned before, 40 mutations to bobbed have been recorded by BRIDGES and others in *D. melanogaster*. These, in general, show a similar phenotypic complex to that found in *D. hydei*. Furthermore, the additive effects of different gene doses of bobbed on the bristle character in *D. melanogaster*, so ably demonstrated by STERN (1929b), have been shown by the author (SPENCER 1930) to hold in *D. hydei* subject to the limitations imposed by chromosome behavior in this species. Using bobbed X chromosomes marked with white, it was shown that XO primary non-disjunction males in *D. hydei* were more extreme bobbed than XX bobbed females. Also XXY primary non-disjunction females carrying bobbed in their X's but the strong non-bobbed allele in the Y were non-bobbed. However, since bobbed alleles in the Y giving phenotypic bobbed combinations have not been discovered in this species, and since it is impossible to build up multiple Y stocks in this species, owing to the absence of secondary non-disjunction, the complete demonstration presented by STERN in *D. melanogaster* is not possible in *D. hydei*.

The absence of phenotypically bobbed males in populations of this species might conceivably be due to the rapid elimination of Y chromosomes carrying

strong bobbed alleles through negative selection. An alternative explanation would be the evolution of some compensatory mechanism in the Y chromosome acting as a suppressor and partially reducing this negative selective factor in a species where the center of the mutational galaxy for bobbed may recently have been brought close to the phenotypic threshold. It might be asked why evolution resulting in adaptive adjustments had partially failed in this case. The answer may well be sought in terms of relative growth rates and compromising adjustments where certain advantages override what may seem to be and in some cases actually are deleterious effects. Furthermore, those changes which might act as suppressors of bobbed in the X may not yet have become established as they apparently have for the Y. On this interpretation the present bobbed condition in *D. hydei* may be thought of as relatively recent in origin.

It seems likely that the genetic structure of a species, through developmental adjustments, may furnish a background against which certain classes of mutations at the same and different loci may be shifted toward or away from the phenotypic threshold or level of expression. It becomes of interest to examine other reduced bristle mutants in this species. By far the commonest class of mutations in *D. melanogaster* are dominant Minute bristle types, lethal in homozygous form and often highly inviable and partially sterile in heterozygous condition. The author has found many of them. But in more extended observations on *D. hydei* no dominant Minutes have been found. It is not unreasonable to suppose that when mutations at these loci occur they are either dominant lethals or are covered up through the evolution of a suppressor mechanism. It may be concluded that the two species, *D. hydei* and *D. melanogaster*, differ in the quantity if not the quality of visible bristle reducing mutations at different loci.

The first case of iso-alleles in *Drosophila* was described by STERN (1926b). He found that two genes at the ebony locus, designated wild type and e^{12} , were phenotypically identical in homozygous form but gave visibly different phenotypes in heterozygous combinations with another ebony allele. STERN and SCHAEFFER (1943) have recently described iso-alleles at the cubitus interruptus locus in *D. melanogaster*. Here the three wild genes tested showed different potencies when combined with certain cubitus interruptus alleles, particularly if raised at low temperature. Even in homozygous condition, one of the iso-alleles was shown to differ from the others when they were reared at low temperature.

These authors have listed a number of other cases of iso-alleles in *Drosophila* and in other forms. One of the cases is of particular interest. TIMOFÉEFF-RESSOVSKY (1932) has shown by irradiation experiments that a certain gene at the white locus, extracted from an American strain of *D. melanogaster*, mutates more frequently to or toward white than does a certain gene extracted from a Russian strain. MULLER (1935), by the use of triploids containing two white genes combined respectively with the American and Russian red alleles of TIMOFÉEFF, was able to demonstrate that the American gene had a lower pigment producing potency, was a weaker allele in this respect, than the

Russian gene. He raises the question as to whether the observed difference in mutation frequency of these two genes is real or due to the shift of the whole mutation "spectrum" toward white in the case of the American allele. On this hypothesis more of the mutations of the Russian red gene would fall in the red end of the spectrum, would themselves be iso-alleles. Critical evidence on this point seems to be furnished by the extensive data of TIMOFÉEFF-RESSOVSKY (1933) on mutations of red and of the intermediate alleles at the white locus to white. From his tables we may extract the pertinent data. 48,500 red genes tested mutated 25 times to white; 87,500 genes of intermediate alleles tested mutated 22 times to white. Thus the red mutation frequency in the experiments reported was twice as high as that of the intermediate alleles. Nor can an appreciable error have been introduced by the use of very light alleles where mutations to white might have been overlooked. Only 14,000 of the intermediate genes tested were of alleles lighter than eosin. We must conclude that factors other than or in addition to position in a phenotypic potency spectrum act to determine mutation frequency in different alleles. Otherwise we would have expected higher mutation frequencies to white in the intermediate alleles than in any red, whatever its relation to other reds. However, these studies on mutation frequency and potency have both demonstrated that these two red genes are iso-alleles. It seems likely, as MULLER has pointed out, that both ends of the visible series, red and white, represent in turn a series of iso-alleles grading away from the visible spectrum.

In extensive studies on different Russian populations of *D. melanogaster*, DUBININ and collaborators (1937) found the thoracic color pattern, "trident," in percentages ranging from 2.65 to 39.89. It would seem likely that iso-alleles showing seriation effects in heterozygous combinations might be demonstrated in this complex. The high incidence and many grades of trident in *Drosophila* stocks have often been observed. In *Drosophila immigrans*, DR. A. H. STURTEVANT first called the author's attention to a series of "net" vein alleles occurring in wild populations. A report on some of these has been presented elsewhere (SPENCER 1940). The variable semi-dominant behavior of some of the stronger of these in certain crosses may be due to the presence of iso-alleles at this locus.

It may be concluded that *Drosophila* species, differing in their genetic backgrounds, show variations in observable alleles present and in observable mutation rate at certain loci. Actual differences in mutation rate may exist, but this observed difference is apparently due in part to the variable positions in reference to phenotypic thresholds of the means of developmental potencies of mutants at these loci.

Such studies as those reported indicate the complexity of the mutation process and the reservoir of genetic variability lying below the phenotypic surface. The known effects of environmental factors on the expression and suppression of many phenotypic characters and the possibilities of genic interaction in uncovering these hidden mutant galaxies in crosses between strains would seem to provide ample material for preadaptations on which selection may act in the course of evolution.

SUMMARY

Of a large sample of females from an Azusa, Southern California, citrus dump population of *D. hydei*, 3.1 percent were bobbed. Many different phenotypes appeared.

Tests showed: (a) that the variations were due to a complex multiple allelic series at the bobbed locus and not to modifiers; (b) that most of the females tested carried two different bobbed alleles.

Of 106 males from this population 27, or 25.5 percent, produced visibly bobbed daughters when mated to bobbed tester females of grade 20 bristle length. The test indicated that the males carried bobbed alleles capable of seriation when in combination with the tester. But in homozygous condition several of these seriated alleles produced identical maximum bristle types. They are designated as iso-alleles (see STERN and SCHAEFFER 1943).

Bobbed-lethal/bobbed 22 heterozygous tester females were pair-mated to 12 wild Azusa males. Three of the genes tested were recognized as distinct from one another in combinations with bobbed 22; six were so recognized in combinations with bobbed-lethal. One of them produced a lethal compound with the latter.

Using phenotypically different heterozygous combinations with these two testers as a guide, a potency series was constructed with the bobbed-lethal tester at one end and the strongest bristle forming allele seriated at the other end.

Tests of a medium sized population of *D. hydei* from Wooster, Ohio, showed 3.6 percent and 4.8 percent phenotypically bobbed females in two large samples collected.

Of 78 genes chosen at random from this population 36, or 46.2 percent, gave phenotypic bobbed compounds with the same bobbed 20 tester gene used on the Azusa population.

The Wooster sample showed less spread in the allelic series and higher concentrations of a few grades than the Azusa sample. Differences in size and year-round pattern of the two populations probably account for these facts.

Recovery of bobbed alleles from smaller samples of *D. hydei* taken elsewhere is reported. This complex multiple allelic series, with iso-alleles forming lethals at one end and maximum bristle type at the other, is characteristic of this species and not limited to one or a few populations.

Several cases of iso-alleles reported by other workers on *Drosophila* are compared with the present findings. A discussion of differences in thresholds for phenotypic effects of mutant alleles from species to species and of the possible evolutionary value of iso-allelic series is included.

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