[13] Goldstein, S., ed., *Modern Developments in Fluid Dynamics*, Vol. I (Oxford at the Clarendon Press, London, England, 1943).
[14] Lamb, Sir Horace, *Hydrodynamics*, 6th edition (New York: Dover Publications, Inc. 1945),
[15] Schlichting, Hermann, *Boundary Layer Theory*, translated by J. Kestin (New York: McGraw-Hill Book Company, Inc., 1955).
[16] Sneddon, I. N., *Fourier Transforms* (New York: McGraw-Hill Book Company, Inc., 1951).
[17] Jahnke, Eugene, and Emde, Fritz, *Tables of Functions* (New York: Dover Publications, Inc.).
[18] Lees, Sidney, *Integration by the Viscous Shear Process*, Report No. 6398-T10, Instrumentation Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts, 1950.

THE DISCRIMINATION OF WILD-TYPE ISOALLELES AT THE WHITE LOCUS OF DROSOPHILA MELANOGASTER

BY M. M. GREEN

DEPARTMENT OF GENETICS, UNIVERSITY OF CALIFORNIA, DAVIS

Communicated by Curt Stern, February 17, 1959

Introduction.—It is generally conceded by students of evolution that fitness of individuals in a natural population is conferred through phenotypic differences controlled by contrasting allelic genes, termed isoalleles. These phenotypic differences are slight in contrast to those usually associated with wild-type genes and their common laboratory mutant alleles. Consequently, the convincing demonstration of isoallelic differences and their genotypic basis has been difficult. Perhaps the best such analysis has been made of wild-type isoalleles associated with the *c.i.* locus of the fourth chromosome of *Drosophila melanogaster* by Stern and Schaeffer.[1] While this demonstration is convincing, the near absence of crossing over in the fourth chromosome precludes the unqualified conclusion that the differences uncovered are inherent exclusively to the *c.i.* isoalleles and not, for example, in part or wholly to closely linked loci.

A number of facts suggested that the white eye color (*w*) locus of *D. melanogaster* was particularly favorable for both uncovering distinctive wild-type isoalleles and for demonstrating their genotypic basis. In 1932 Timoféeff-Ressovsky[2] reported that two wild-type stocks of dissimilar origin, designated American and Russian, differed significantly in their x-ray induced mutability at the *w* locus. Utilizing Timoféeff's stocks, Muller[3] showed that when each w^+ X-chromosome was separately compounded with two *w* mutant alleles in triploid ♀ ♀ and compared phenotypically, a clear-cut eye color difference could be seen thereby further demonstrating the distinctiveness of the w^+ alleles.

The finding that the *w* locus is pseudoallelic[4, 5] composed of four recombinationally separate loci[6] motivated this reëxamination of the wild-type isoalleles. It appeared theoretically possible through the use of mutant pseudoalleles to pinpoint the isoallelic differences to one or more recombinational sites of the *w* locus and hence to establish unequivocally that contiguous, nonallelic loci are not involved.

Phenotypic Separation of the Isoalleles.—Since the wild-type stocks used by Timoféeff are no longer extant a search for similar or identical ones among existing wild-type *Drosophila* strains was undertaken. Identification of distinctive wild-

types was predicated on Muller's observations. For this purpose a triploid (3N) stock was synthesized in which the 3N ♀ ♀ had attached-X chromosomes homozygous for the mutant *w* plus a free X chromosome balancer carrying a complex of inversions and the mutant Bar eye (*B*). A comparable 3N stock in which the allele w^{x16} was substituted for *w* was also synthesized. Wild-type stocks tested were Canton-special ($+^c$) and Oregon-R ($+^o$). Triploid ♀ ♀ $w/w/+^c$ or $w/w/+^o$ were obtained by crossing stock 3N ♀ ♀ to appropriate wild ♂ ♂ and selecting the F_1 non-*B* ♀ ♀. Care was taken to raise F_1 flies in uncrowded cultures, at a temperature of 23–24°C and on uniform media to assure phenotypic uniformity. Phenotypes were scored after F_1 3N ♀ ♀ had been aged a minimum of five days.

Scoring was done in two ways: (1) by the subjective method of visual examination under the dissection microscope and (2) by the more objective method of extraction and spectrophotometric estimation of the red eye pigments. Procedures adopted for the determination of the red eye pigments were, with minor modifications, those described by Ephrussi and Herold.[7] After extraction, quantitative estimates of the pigments were made with a Beckman model B spectrophotometer at a wave length of 480 mμ. Where estimates of the brown eye pigments were made, they were extracted subsequent to the extraction of the red eye pigments. After extraction, the brown pigment was reduced with a minute crystal of sodium borohydride and measured at a wave length of 444 mμ. Quantities are expressed as the extinction (E) per number heads extracted per volume solvent. Ultraviolet and visible spectra of both pigment types were made with a Beckman recording spectrophotometer.[8]

A visual comparison of ♀ ♀ $w/w/+^s$ and $w/w/+^o$ after five days aging showed their eye phenotypes to be readily separable. The eye color of ♀ ♀ carrying the $+^c$ chromosome had a distinct reddish cast while that of ♀ ♀ carrying the $+^o$ chromosome was definitely maroon in color. Identical results were obtained after comparing 3N ♀ ♀ $w^{x1f}/w^{x16}/+^c$ and $w^{x16}/w^{x16}/+^o$. Comparable differences were noted by Muller.[3] On the basis of the eye colors, it seemed reasonable to guess that the difference seen stemmed primarily from differences in the amounts of red eye pigments in the 3N ♀ ♀. Accordingly the red eye pigments of ♀ ♀ of all 3N genotypes were extracted and their quantity spectrophotometrically estimated. The results are tabulated in Table 1 and bear out this supposition. In each case the amount of pigment, equatable to the mean E value, was significantly greater in 3N ♀ ♀ with $+^c$ than in those with $+^o$. This is supported by the fact that the ranges of E values for each comparison do not overlap. Taken together these results indicate that Canton and Oregon stocks differ at the *w* locus or at loci closely linked to *w*. The red pigment spectra proved to be identical.

TABLE 1

ESTIMATES OF RED EYE PIGMENTS OF TRIPLOID ♀ ♀ OF SEVERAL GENOTYPES

Genotype	No. Detns.	E/2.5 Heads/ml. Solvent — Mean	E/2.5 Heads/ml. Solvent — Range
$w/w/+^c$	7	0.140	0.128–0.146
$w/w/+^o$	4	0.109	0.102–0.113
$w^{x16}/w^{x16}/+^c$	2	0.135	0.134–0.137
$w^{x16}/w^{x16}/+^o$	6	0.099	0.086–0.113
y v f car/y v f car/$+^c$	5	0.224	0.218–0.233
y v f car/y v f car/$+^o$	5	0.219	0.218–0.233

Additional support for this conclusion came from comparisons of the red eye pigments of 3N ♀ ♀ whose *w* loci were unmutated. For this purpose a 3N stock whose attached-Xs were homozygous for the recessive mutants yellow body (*y*), vermilion eye color (*v*), forked bristles (*f*) and carnation eye color (*car*) was used. Triploid ♀ ♀ *y v f car*/*y v f car*/+^{c} or +^{o} were obtained and red eye pigment analyses made. The results, listed in Table 1, show no difference between +^{c} and +^{o} carrying ♀ ♀. This means that the difference between +^{c} and +^{o} is a function of genes affecting eye pigmentation at or near the *w* locus and is not related to genes in the two stocks which might affect eye size.

Because the collection of 3N ♀ ♀ in large numbers is rather laborious, a number of experiments were carried out to see whether differences between +^{c} and +^{o} could be demonstrated in diploid (2N) ♀ ♀. For this purpose ♀ ♀ heterozygous for +^{c} and +^{o} and three different white eye mutants (*w* plus w^{s4} and w^{561} both pseudo-alleles of *w*), or a deficiency of the *w* locus (w^{258-11}) were obtained and the quantities of red eye pigments determined. In addition, estimates of the red eye pigments of ♀ ♀ homozygous +^{c} and +^{o} were made. The results of these analyses are listed in Table 2 and merit the following conclusions. First, there is no difference between homozygous 2N +^{c} and +^{o} ♀ ♀. Second, an objective discrimination between +^{c} and +^{o} can be made in 2N ♀ ♀ heterozygous for a *w* mutant. It will be noted that for each comparison the +^{c} ♀ ♀ possess significantly more red eye pigment than comparable +^{o} ♀ ♀ and that the ranges of determinations do not overlap. A comparison between effects with different mutants is not warranted since these analyses were made with flies raised at different times, on different lots of media and at slightly different temperatures. These conditions all influence the quantities of pigment laid down by affecting growth and therefore eye size.

TABLE 2

ESTIMATES OF RED EYE PIGMENTS OF DIPLOID ♀ ♀ OF VARIOUS GENOTYPES

Genotype	No. Detns.	E/5 Heads/ml. Solvent — Mean	E/5 Heads/ml. Solvent — Range
+^{c}/+^{c}	8	0.410	0.398–0.420
+^{o}/+^{o}	8	0.418	0.403–0.426
w/+^{c}	4	0.381	0.366–0.387
w/+^{o}	4	0.323	0.319–0.328
w^{561}/+^{c}	7	0.285	0.270–0.294
w^{561}/+^{o}	3	0.234	0.299–0.237
w^{s4}/+^{c}	4	0.375	0.356–0.387
w^{s4}/+^{o}	5	0.260	0.252–0.276
w^{258-11}/+^{c}	6	0.334	0.329–0.366
w^{258-11}/+^{o}	7	0.301	0.284–0.310

Genotypic Delineation of the Isoalleles.—Since the *w* locus is pseudoallelic, the following questions may be asked. Do +^{c} and +^{o} differ at all or a selected number of *w* loci? An answer to this question can be provided by the synthesis of wild-type stocks whose *w* loci are derived in part from +^{c}, in part from +^{o}. Before such a synthesis is outlined the following information must be considered. The crossing-over frequency between any pair of contiguous *w* mutants differs rather strikingly. Using the mutants w^{col}, w^a, w^{ch} and *sp-w* to represent each of the four loci as they occur sequentially from the distal end toward the centromere along the X chromosome, these recombination fractions have been observed for each interval: w^{col}-w^a $^1/_{50,000}$; w^a-w^{ch} $^1/_{10,000}$; w^{ch}-*sp-w* $^1/_{50,000}$.[6, 9, 10] (It should

be noted that in each instance the crossover frequency was enhanced with the heterozygous autosomal inversions *Cy* and *Ubx*^{130}.) These frequencies suggested that synthesis of wild-types derived in part from both $+^c$ and $+^o$ could be accomplished best by using w^a and w^{ch} as markers and taking advantage of the comparatively high frequency of crossing over between them. Thus, for purposes of discussion, the *w* loci have been arbitrarily divided into two groups, each with two loci and marked by the mutants w^a and w^{ch}. The Canton and Oregon wild-types may now be described by the genotypic notations $+^c+^c$ and $+^o+^o$, respectively.

Synthesis of the derived wild-types was carried out as follows. A tester stock of genotype *y su-w*^{a} w^a w^{ch} *spl; Cy; Ubx*^{130}*/Xa* was made up and crossed separately to $+^c$ and $+^o$ (*y* = yellow body, *su-w*^{a} = suppressor of w^a, *spl* = split bristles). From heterozygous ♀ ♀ *y su-w*^{a} w^a w^{ch} *spl*/+ + $+^c+^c$+ or + + $+^o+^o$ + and carrying the *Cy* and *Ubx* inversions recombinants between w^a and w^{ch} were sought. These recombinants are: *y su-w*^{a} w^a $+^c$ + and + + $+^c$ w^{ch} *spl* or *y su-w*^{a} w^a $+^o$+ and + + $+^o$ w^{ch} *spl*. By combining the appropriate recombinant chromosomes, ♀ ♀ of the following genotypes were obtained: (1) *y su-w*^{a} w^a $+^c$ +/+ + $+^o$ w^{ch} *spl* and (2) *y su-w*^{a} w^a $+^o$ +/+ + $+^c$ w^{ch} *spl*. Crossing over between w^a and w^{ch} in ♀ ♀ of genotype (1) will produce wild-type recombinants with the *w* locus genotype of $+^o+^c$ and in ♀ ♀ of genotype (2) wild-type with the *w* locus of $+^c+^o$. Each type was successfully recovered.

A phenotypic comparison among the Canton, Oregon and derived wild-types followed and was based on these considerations. If the difference between the Canton and Oregon wild-types resides primarily in the left section of the locus, the $+^o+^c$ wild-type should be phenotypically equivalent to the $+^o+^o$ wild-type when compounded to a *w* mutant. Similarly the $+^c+^o$ wild-type should be equivalent to $+^c+^c$ when made heterozygous with a *w* mutant. The converse should hold if the difference between the wild-types lies in the right section of the *w* locus. Finally if the difference between the wild-types is cumulative, confined in part to each segment, the $+^c+^o$ and $+^o+^c$ wild-types would be expected to produce essentially inseparable phenotypes when compounded with a *w* mutant, phenotypes intermediate between those of $+^c+^c$ and $+^o+^o$ compounds.

Concurrently homozygous *w* ♀ ♀ were separately crossed to the four types of wild ♂ ♂ and the red eye pigments analyzed. The results of these analyses are listed in Table 3 and are essentially self-explanatory. The mean *E* values for ♀ ♀ heterozygous for $+^c+^c$ and $+^o+^c$ are alike and differ significantly from the mean *E* values for ♀ ♀ heterozygous for $+^o+^o$ and $+^c+^o$ which in turn are alike. Hence the primary difference between the Canton and Oregon wild stocks resides in their right member loci marked by the mutants w^{ch} and *sp-w* and little difference exists in their left loci marked by the mutants w^{col} and w^a.

TABLE 3

COMPARATIVE RED EYE PIGMENTATION IN CANTON, OREGON AND DERIVED WILD-TYPE HETEROZYGOTES

Genotype ♀ ♀	No. Detns.	E/5 Heads/ml. Solvent — Mean	E/5 Heads/ml. Solvent — Range
$w/+^c+^c$	15	0.352	0.329–0.366
$w/+^o+^o$	11	0.298	0.276–0.310
$w/+^o+^c$	15	0.335	0.323–0.347
$w/+^c+^o$	13	0.310	0.301–0.319

Added support for this conclusion came from another source. In synthesizing the derived wild-types, ♂♂ of the genotypes *y su-w^a w^a +^{c}* and *y su-w^a w^a +^{o}* were recovered. Visual comparison showed that although the *su-w^a* effective in both in shifting the *w^a* phenotype toward wild-type, the phenotypes were not identical. The +^{c} carrying ♂♂ were distinctly darker than the +^{o} ♂♂. After homozygous stocks were established analyses of both red and brown eye pigments of both ♂♂ and ♀♀ of these genotypes were undertaken.

The results of these analyses, listed in Table 4, demonstrate that the phenotype associated with the *su-w^a w^a* genotype is clearly dependent on whether a +^{c} or +^{o} component is present in the right half of the *w* locus. A significantly greater amount of both eye pigments occurs in both ♂♂ and ♀♀ of the *su-w^a w^a +^{c}* genotype than in ♂♂ and ♀♀ *su-w^a w^a +^{o}*. These results parallel exactly the phenotypic effects noted for the wild-types heterozygous for the *w* mutant and reinforce the conclusion that the primary difference between the Canton and Oregon wild-types resides in the right section of their *w* loci.

TABLE 4

EFFECT OF CANTON AND OREGON ON EYE PIGMENTATION OF *su-w^a w^a* ♂♂ AND ♀♀

Genotype	No. Detns.	Red Pigment, E/40 Heads/ml. Solvent Mean	Red Pigment, E/40 Heads/ml. Solvent Range	Brown Pigment, E/40 Heads/ml. Solvent Mean	Brown Pigment, E/40 Heads/ml. Solvent Range
♀♀ *y su-w^a w^a +^{c}*	5	0.142	0.137–0.165	0.122	0.108–0.143
♀♀ *y su-w^a w^a +^{o}*	9	0.088	0.081–0.096	0.073	0.051–0.086
♂♂ *y su-w^a w^a +^{c}*	7	0.134	0.124–0.137	0.113	0.102–0.142
♂♂ *y su-w^a w^a +^{o}*	8	0.082	0.076–0.091	0.065	0.051–0.081

Because of the low crossing-over frequency between *w^{oh}* and *sp-w*, no further attempt was made to localize the wild-type isoalleles.

Discussion and Summary.—In the foregoing study it has been shown that the phenotypically inseparable Canton and Oregon wild-type stocks of *Drosophila melanogaster* differ genetically at the white eye locus. The analysis has demonstrated that Canton and Oregon differ through isoalleles localized to the *w^{ch}* and *sp-w* loci which make up the right segment of the pseudoallelic *w* locus. Thus the genetic attributes of wild-type isoalleles are not different from those of mutated genes with more drastic phenotypic effects. The difficulties of genetic analysis stem from problems of phenotypic identification.

It is quite conceivable, therefore, that for a particular gene locus there may exist an array of wild-type alleles differing through phenotypic effects which are difficult to demonstrate. While these differences are slight they may assume a more pronounced role where natural selection operates. Such a model demonstration has been made with the *c.i.* isoalleles by Hochman.[11]

[1] Stern, C., and E. W. Schaeffer, these PROCEEDINGS, **29**, 361–367 (1943).

[2] Timoféeff-Ressovsky, N. W., *Biol. Zbl.*, **52**, 568–476 (1932).

[3] Muller, H. J., *J. Genetics*, **30**, 407–414 (1935).

[4] Lewis, E. B., these PROCEEDINGS, **38**, 953–961 (1952).

[5] Mackendrick, E. M., and G. Pontecorvo, *Experientia*, **8**, 398 (1952).

[6] Green, M. M., *Heredity* (in press).

[7] Ephrussi, B., and J. L. Herold, *Genetics*, **29**, 148–175 (1944).

[8] The pigment analyses were carried out by Dr. R. A. Kroman to whom sincere thanks are due.

[9] Lewis, E. B., *Rec. Genetics Soc. America*, **25**, 651 (1956).

[10] Green, M. M., unpublished observations.

[11] Hochman, B., *Genetics*, **43**, 101–121 (1958).