

Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

2010: A Century of *Drosophila* Genetics Through the Prism of the *white* Gene

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ABSTRACT

In January 1910, a century ago, Thomas Hunt Morgan discovered his first *Drosophila* mutant, a white-eyed male (MORGAN 1910). Morgan named the mutant gene *white* and soon demonstrated that it resided on the X chromosome. This was the first localization of a specific gene to a particular chromosome. Thus began *Drosophila* experimental genetics. The story of the initial work on *white* is well known but what is less well appreciated is the multiplicity of ways in which this gene has been used to explore fundamental questions in genetics. Here, I review some of the highlights of a century's productive use of *white* in *Drosophila* genetics.

There is nothing new in the world except the history you do not know.

HARRY S. TRUMAN

IN a pedigree culture of *Drosophila*, which had been running for a considerable number of generations, a male appeared with white eyes. The normal flies have brilliant red eyes." So began a short 1910 Science article by T. H. Morgan on the inheritance of the white eye phenotype. Thus began the transformation of Mendel's rather abstract hereditary "factors" to Johanssen's more concrete, if still mysterious, "genes."

The result of breeding the white male to red females and additional crosses in subsequent generations led Morgan to the conclusion that the inheritance of white eye color parallels the normal meiotic segregation of the sex chromosomes in this fly. This represented the first concrete evidence that chromosomes have a role in inheritance as had been suggested nearly a decade earlier by Sutton and Boveri. Interestingly, neither the discoverer nor the exact date of discovery of the white-eyed fly are stated in Morgan's article. Unsurprisingly, as a result of those omissions, the exact "who" and "when" became controversial. Elsewhere, this has been discussed *in extenso* (GREEN 1996). Morgan later asserted that he alone made the discovery. The date of his discovery was determined to be January 1910.

Why did Morgan, trained in embryology and comparative anatomy, an experimenter par excellence and a prolific writer of scientific articles and books on biological development, who was at the peak of his creativity, turn fairly abruptly to do research on Mendelism? A fair surmise is that he was uncomfortable with what he saw as the misuse of Mendelism. This is illustrated by a statement he made to a convention of animal breeders in 1909: "In the modern interpretation of Mendelism, facts are being transformed into factors at a rapid rate. If one factor will not explain the facts, then two are invoked; if two prove insufficient, three will sometimes work out. The superior jugglery sometimes necessary to account for results may blind us, if taken too naively, to the common-place that results are so excellently 'explained' because the explanation was invented to explain them etc" (MORGAN 1909). As STURTEVANT (2001), in a reminiscence of Morgan given in 1967, confirms, "this didn't suit Morgan. So by about 1910, he could fairly be described as anti-Mendelian." Morgan's skepticism moved him to see for himself the plausibility of Mendelism by searching for heritable phenotypic changes in the vinegar fly, *Drosophila melanogaster*. The rest is history summed up in the narrative which follows.

By 1913, Morgan had found an array of *Drosophila* mutations that altered the eye color and wing morphology phenotypes. Five new mutations were sex linked. One new mutant, eosin eye color, was inseparable from *white*; all the others in pairwise test were separable by recom-

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bination (crossing over). Using recombination frequency as a measure of genetic distance, A. H. STURTEVANT (1913) as an undergraduate student, (later Morgan's graduate student), in a tour de force constructed a linear genetic map of the six sex-linked mutations. The verity of the gene order was proved cytologically when the polytene chromosomes were rediscovered.

Volume 1 of the new 1916 journal GENETICS opened auspiciously with an article by another Morgan student, C. B. Bridges, entitled "Non-disjunction as proof of the chromosome theory of heredity" (BRIDGES 1916). The cytogenetic data he presented proved that in a *Drosophila* cross of white-eyed females to red-eyed males, linkage of *white* to the X chromosome explains not only the regular inheritance of red females and white males but also the infrequent exceptions, namely, white females and red males. These exceptions were attributed to the infrequent failure of the two X chromosomes in *white* females to disjoin (nondisjunction) during meiosis, producing an ovum with two X chromosomes, which if fertilized by a Y-bearing sperm, produced a matroclinous *white* XXY female. Alternatively, if the X chromosomes were directed to the polar body, a nullo-X ovum was produced, which when fertilized by an X-bearing sperm, produced a red-eyed patroclinous XO male. This hypothesis was proved cytologically for both classes of exception. Somewhat later, BRIDGES (1921) demonstrated that nondisjunction was not unique to the X chromosomes but also occurred with the small fourth chromosome. The foregoing investigations by Morgan, Sturtevant, and Bridges established the foundations of classical genetics. Subsequently the *white* locus was crucially associated with additional genetic principles. These will be summarily described in the narrative that follows.

Heterochromatin and phenotypic variegation or ever sporting: In his detailed report on the mutagenicity of X-rays, MULLER (1930) noted that with one exception X-ray-induced mutations were phenotypically no different from those of spontaneous origin. The one exception was those exhibiting a *white* variegated or mottled phenotype. Cytogenetic study demonstrated that the variegation was invariably associated with either a chromosome inversion or translocation. Ostensibly, the normal allele of *white* was placed proximal to the centromeric heterochromatin either by inversion of the X chromosome or by translocation to an autosome. The crucial question posed was: What is the status of the normal *white* allele? To answer this question, three different approaches were used. In one, GRIFFEN and STONE (1940) X-rayed the X-4 translocation *white*-mottled-5 (w^{m-5}) and recovered phenotypic reversions to normal. Polytene chromosome cytology showed that new translocations had been induced and the normal *white* gene was now proximal to euchromatin. In a second, heroic experiment, JUDD (1955) employed crossing over in heterozygous females, T(1;4) w^m 258-21/ w^+

to remove the *w* allele in the translocation X chromosome from its heterochromatin association into a normal sequence chromosome and restored the normal phenotype. Finally, NOVITSKI (1961) analyzing the heterochromatin-associated X chromosome inversion *roughest-3* (rst^3) succeeded in exactly reinverting the inversion by X-raying females heterozygous for the inversion and a normal X chromosome thereby restoring the normal phenotype. Taken together, these experiments demonstrated that normal gene function was altered by proximity to heterochromatin, without any change to its physical integrity.

Dosage compensation: In *Drosophila*, there is little observable difference in phenotype between females with two copies of a sex-linked mutant gene and males with only one copy. MULLER (1932) coined the term "dosage compensation" to describe this situation of equalized expression for different gene dosages between the two sexes. Dosage compensation applies to the vast majority of sex-linked mutants in *Drosophila*. One noteworthy exception, however, exists among *white* locus mutants with pigmented eyes. Whereas the eye color of homozygous white-apricot (w^a) females is inseparable from that of hemizygous w^a males, the eye color of homozygous white-eosin (w^e) females is distinctly darker than the eye color of hemizygous w^e males. This phenotype difference extends to females that have a *w* locus deletion. Thus females w^e/w^{deletion} and w^e/w^a are phenotypically the same but females w^e/w^{deletion} are clearly lighter than the eye color of w^e/w^e females. This difference extends to intragenic mapping where w^a and other compensated white mutants map to one site and w^e and all other uncompensated mutants map to a separate site (GREEN 1959a).

Mobile DNA elements: One of the most unexpected discoveries of the 20th century was made by Barbara McCLINTOCK (1950, 1951) with her description in maize of DNA elements, which she called "controlling elements" and which are now designated mobile DNA elements or transposons. Employing only cytogenetic methods, she demonstrated that these elements are unstable and move from one chromosome site to another. For almost two decades, the maize mobile elements were unique, engendering more than a little skepticism as to their origin and mobile property, not to mention the general significance of the phenomenon. In 1965, however, consistent with the Batesonian exhortation of "treasure your exceptions," Green discovered a mobile element at the *white* locus in *D. melanogaster* (GREEN 1967). The discovery was made in an experiment designed to measure the frequency of X-ray-induced reversions of the white-ivory (w^i) mutation of *D. melanogaster*. To maximize the efficiency of the search, attached X (two X chromosome with one centromere) females homozygous w^i and a linked marker gene were X-rayed and reversions sought among their female progeny. (Thus, two irradiated w^i mutants were scored and only

among the female progeny.) Reversions to w^+ were found as well as one female whose phenotype was scored as a partial reversion, *i.e.*, the eye color was darker than w^j but not normal eye color. The partial reversion was named white-crimson (w^c). Since the exceptional attached X female had one w^c and one w^j chromosome, homozygous w^c females via crossing over were sought and recovered among the female progeny. When the presumptive homozygous w^c females were bred and their progeny scored, it was found, however, that in addition to homozygous w^c females, a few females of unexpected phenotype were also found: those with normal eye color and those with w^j color. (The exceptions carried the marker gene and thus were not contaminants.) The attached Xs were detached and a homozygous w^c stock with free Xs produced. When tested, the free X homozygous females as well as w^c males were mutationally unstable generating w^j and normal progeny. Homozygous w^c females were also found to produce spontaneously at low frequency two unexpected classes of nonoverlapping, fixed endpoint w locus deletions: those beginning at the w locus and extending to the right and those extending to the left. The nonoverlapping deletions were analogous to the controlling element-generated deletions described by McClintock. Additionally, transpositions of w^c to the third chromosome were recovered (GREEN 1969). Taken together the conclusion was drawn that the instability of w^c is caused by a controlling-like element inserted into w^j . With the advent of molecular biology, the correctness of this conclusion was proved by COLLINS and RUBIN (1982) who demonstrated that a so-called foldback DNA sequence was inserted into w^j , this being the source of the instability. It did not take long before *D. melanogaster* was shown to contain a broad spectrum of mobile DNA elements, the causal agents of many gene mutations.

Tandem duplications and unequal crossing over:

The X chromosome *Bar* (B) mutation is probably the most ubiquitous dominant mutation in *D. melanogaster*. Found in 1912, and easily scorable, it is used as a marker for almost all X-chromosome balancer inversions. Early on, it attracted study because its phenotype varied in response to environmental change. Additionally, it was found to spontaneously revert back to normal or mutate to a more extreme phenotype called ultra *Bar* (B^u). The mechanism of B mutation was unclear until subjected to the incisive intellect of A. H. STURTEVANT (1925). He noticed that the B mutations, forward and back, occurred only in homozygous B females, not in B males. Since at the cellular level meiotic crossing over occurs only in *Drosophila* females and not males, ergo mutation of B could be associated with crossing over. In a classical experiment, cited universally in textbooks of genetics but seldom read, by using flanking marker genes to monitor crossing over in homozygous B females, Sturtevant demonstrated that the B mutations did, in fact, mutate in association with crossing over.

Since the mutations occurred in homozygous B females, he concluded that B was not a "point" mutation but a tandem duplication and B^u was a triplication and the reciprocal B^+ was the normal sequence. With the advent of polytene chromosome cytology, Sturtevant's conclusion was confirmed cytologically by BRIDGES (1936) and independently by MULLER *et al.* (1936) who demonstrated that a segment of the X chromosome including the polytene chromosome bands 16A1-7 was duplicated in tandem. What remained unresolved was the mechanism by which B arose spontaneously. To date no repeat occurrence has been found. The solution to this puzzle stems, in part, from fine structure genetics and, in part, from the discovery of mobile DNA elements. (By coincidence, the research to be described was carried out independently by the late Burke Judd and by the author with only trivial differences. We used different w alleles and different mobile elements. Because I am more familiar with my own research, I will describe my own results without prejudice to Judd's research. In fact, the findings are identical.)

As noted above, the pigmented w alleles are separable into discrete sites by crossing over. The dosage-compensated mutants are in one site, the uncompensated in the adjacent, nonoverlapping site. Further separation by crossing over failed, with one exception. When females of the genotype w^c/w^{c^4} plus flanking markers were tested, white-eyed male exceptions associated with crossing over were recovered always with the polarized identical markers. No reciprocal w^c recombinants were found. A genetic test for w deficiencies established the w exceptions to be small, cytologically irresolvable deficiencies (GREEN 1959b). Subsequently, the reciprocal crossover, a small duplication, was identified. (The details of this discovery are spelled out in GREEN 1963.) In principle, this is unequal crossing over. Despite the recovery of both duplication and deficiency, reciprocal products of crossing over, the precise mechanism remained obscure. The probable mechanism evolved during a visit I made to see Walter Gehring while I was on sabbatical in Geneva. A number of research groups including Gehring's were trying to clone w^c , which was circumstantially linked to the mobile element copia. During conversations with Gehring, the unequal crossing over in w^c/w^{c^4} females arose. Out of the conversation the following possible mechanism for w^c/w^{c^4} unequal crossing was proposed. Suppose the w^c and w^{c^4} are associated with the copia element, but in slightly separate locations: slightly to the right of w^c and slightly to the left of w^{c^4} . Suppose the copia elements occasionally pair and a crossover occurs within the paired elements. The products of such an event will be a duplication and a deficiency, the latter viable and white-eyed in phenotype. This is unequal crossing over. A w^{c^4} stock was obtained and *in situ* hybridized to localize copia chromosomally. The results, no copia at the w locus in w^{c^4} . This was a keen disappointment, needless to

say. I made a second visit to Gehring, however, and asserted that this hypothesis was too good to be discarded; there must be a different mobile element involved. Michael Goldberg was, at the time, a postdoc in Gehring's group and was a bystander to the discussion. He volunteered to look into the duplication/deficiency situation molecularly. To make a long story short, Goldberg identified another mobile element associated with w^e and w^{e4} , which Gehring named Belshazzar (*bel*). He also found that a copy of *bel* is located between the two parts of the duplication. To account for the marker exchange in the production of the duplication, *bel* must lie just to the right of w^e and just to the left of w^{e4} . If the homologous *bel* elements pair and a crossover occurs within the paired elements, a duplication and deficiency result. This was so reported (GOLDBERG *et al.* 1983). Following on the heels of this finding, DAVIS *et al.* (1987) using a different pair of *w* mutants described an identical case of unequal crossing over where the mobile element B104 is responsible.

The two examples of unequal crossing over are a model for the origin of the *B* duplication. The model predicts that if *B* arose by the mechanisms described, a mobile element should be located between the two parts of the duplication. In fact, TSUBOTA *et al.* (1989) reported that in *B*, a copy of the element B104 does in fact lie at the juncture of the duplicated chromosome segments. The *bel* elements associated with w^e and w^{e4} are separated by 60 kb. To generate *B*, pairing had to occur between a B104 element at polytene chromosome band 16A1 and on the homolog B104 at polytene chromosome band 16A7, a distance infinitely greater than 60 kb. Such a pairing event must be extraordinarily rare and that rarity explains the unique occurrence of *B*. The reciprocal deletion most likely is lethal.

The cloning of w^e : The intimate association of w^e with copia led to its being the first *D. melanogaster* gene cloned. Using *in situ* hybridization, GEHRING and PARO (1980) demonstrated that several w^e strains have copia in the *w* gene chromosome location. This was confirmed by BINGHAM and JUDD (1981) who found tight genetic linkage between w^e and copia. This was followed by the work of BINGHAM *et al.* (1981) who succeeded in molecularly cloning DNA from the w^e mutation.

No account of the formative days of *Drosophila* genetics can be deemed complete without acknowledging the contribution of Lilian V. (Mrs. T. H.) Morgan. As a wife and mother, she nurtured a son and three daughters. As a superb *Drosophila* geneticist, she discovered and characterized the first attached-X female whose genotype was later employed to maintain without selection sex-linked female sterile mutations. Attached-X females were also used in deducing that meiotic crossing over occurs between chromatids and not chromosomes. She also found the first ring-X chromosome which was used to produce genetic mosaics for studying development. Hers was a fulfill-

ing career, at home and in the lab. This is the essence of joy!

It is a truism that big things often have small beginnings. For *Drosophila* genetics, nothing illustrates this phenomenon better than the solitary white-eyed male fly that appeared one day in January, 1910 in one of the milk bottles of T. H. Morgan's *Drosophila* collection. That fly helped inaugurate a century of groundbreaking discoveries in genetics.

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