In summary it can be stated that in Bx^r the sy^+, Bx^+, fu^+ loci as well as x, a locus to the right of fu^+, are duplicated.

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*THE PSEUDOALLELISM OF WHITE AND APRICOT IN DROSOPHILA MELANOGASTER**

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The classical example of multiple allelism is the series of eye-color mutants at the white (*w*) locus in *Drosophila melanogaster*. The alternative interpretation of this series, namely, that it is made up of "pseudoalleles," or closely linked genes with similar effects, has usually been considered ruled out by two kinds of evidence. In the first place early attempts to resolve the series by crossing over failed in spite of numerous tests involving most of the mutants available at the time.[1-4] Secondly, a heterozygote for two different mutant genes of the series does not have the pheno type expected for non-allelic genes, namely, wild-type (or red) eye color, but instead has a mutant eye color which is usually intermediate between the colors of the two respective homozygotes. In recent years, however, several cases have been found in which non-allelic genes give a positive phenotypic test for allelism by virtue of a position effect.[5-7] In such cases, which have been termed "position pseudoalleles,"[7] mutant genes at the different loci (say, *a* and *b*) give a mutant phenotype in the $a\ +/+\ b$ heterozygote, but a wild-type, or more nearly wild-type, phenotype in the $a\ b/+\ +$ heterozygote.

With the above considerations in mind and with the aid of more adequate techniques for studying crossing over than were available in the early studies, the white gene and its so-called "allele," apricot, have been reinvestigated. This paper presents the evidence that these two genes occupy separate loci and that they constitute another example of position pseudoallelism. In what follows, the apricot gene, formerly symbolized as w^a will be designated by a new symbol, namely, *apr*.

In order to investigate the possibility of crossing over between *w* and *apr*, females with attached-X chromosomes were employed so that the two complementary products from any such crossing over would sometimes be recoverable simultaneously in a single individual. The first step was the

construction of attached-X's heterozygous for *w*, *apr* and suitable marker genes on either side of the *w* locus (at 1.5 in the X-chromosome). The markers chosen were yellow (*y*, at 0.0; body and bristle color); yellow-2 (y^2, allelic and dominant to *y;* with black instead of yellow bristles); scute (*sc*, at 0.0+; missing bristles); split (*spl*, at 3.0±; abnormal bristles and rough eyes); and echinus (*ec*, at 5.5; enlarged facets).[8,9] By the standard method[10] of using attached-X triploid females, an attached-X diploid female of the following or "Type-A" constitution was obtained (see Fig. 1): y^2 *apr ec/y sc w spl*. Such a female is phenotypically yellow-2 and "dilute-apricot"; the latter is a pinkish yellow eye color which is often distinguishable from the "apricot" or yellowish pink color of homozygous *apr*. A Type-A female produces predominantly three phenotypic

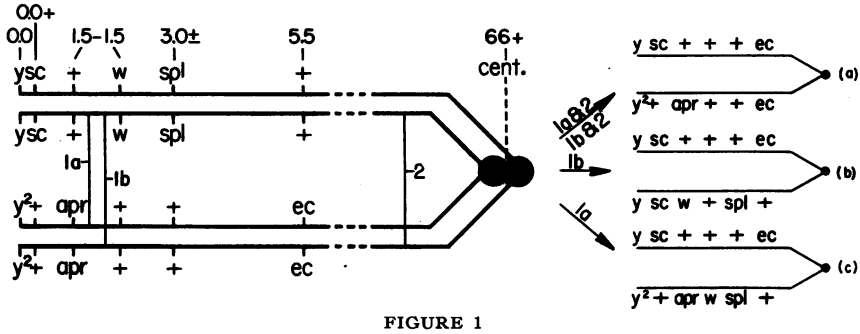

FIGURE 1

The diagram on the left is the attached-*X* constitution of Type A from which the majority of crossovers between *apr* and *w* were detected. The types of exchange which give rise to such crossovers are labeled: 1*a* (reciprocal) and 1*b* (non-reciprocal). On the right are shown the resultant three classes of crossover-containing attached-*X*'s. An additional exchange (exchange 2) between the locus of *ec* and the centromere (cent.) is required for the production of class (*a*).

classes of attached-X progeny; namely, (1) yellow-2 dilute-apricot, (2) yellow-2 apricot echinus and (3) yellow scute white split. Females belonging to Class 1 prove to be primarily of Type-A constitution like the mother. Classes 2 and 3 are the diagnostic ones for a Type-A constitution since they correspond to the two kinds of homozygotes with respect to the original linked sets of mutant genes in the mother, namely, y^2 *apr ec* and *y sc w spl*, respectively. Such homozygous classes result from certain types of exchange(s) in the region between the *ec* locus and the centromere (see e.g., exchange numbered 2 in Fig. 1). Since the latter region is over 50 map units in length, a single Type-A attached-X female usually produces numerous daughters belonging to each of the above homozygous classes; conversely, the constitution of an individual female with respect to the above group of genes is usually readily determined from inspection of the

two principal classes of homozygous daughters. Although certain types of exchange within the $y - ec$ region lead to constitutions and phenotypic classes other than those discussed above, the relatively short genetic length of this region makes such exceptions to the above rules either infrequent or absent in the progeny of a single female.

Type-A attached-X females were next made heterozygous for chromosomal rearrangements in the second and third chromosomes, since such an autosomal constitution is known[11] to be very effective in causing an increase in crossing over in the X-chromosome. For the second chromosome the two Curly (*Cy*) inversions[9] were chosen (marked by the dominant wing-mutant, *Cy*).[12] For the third chromosome a new complex rearrangement of x-ray origin was chosen. This rearrangement involves five breakage points[13] distributed throughout the third chromosome and is inseparably associated with a dominant bithorax-like change, termed Ultrabithorax-130 (Ubx^{130}). The Ubx^{130} heterozygote is readily classified on the basis of an enlarged distal segment of the haltere (while the homozygote is lethal).

The first indication of crossing over between *w* and *apr* came in the offspring of a preliminary mating, Mating 1, in which the parental yellow-2 dilute-apricot females were heterozygous for only the Ubx^{130} rearrangement. All of such females were descendants of the original Type-A female described above. The parental males in Mating 1 were heterozygous for a *Cy* chromosome which was known to carry both of the *Cy* inversions. In 19 cultures of this mating the individually mated parental females proved to have had a Type-A constitution. One of these cultures produced a single yellow-2 red echinus female. This female in turn produced 23 daughters, of which 12 were phenotypically like the mother, while 6 were yellow scute red echinus and 5 were yellow-2 apricot echinus. This is the result to be expected if the original red-eyed fly had the following constitution: $y\ sc\ ec/y^2\ apr\ ec$. As the result of detachment of the attached-X's in one of the above six homozygous *y sc ec* females, *y sc ec* males were obtained and these also proved to have red eyes. The *y sc ec* chromosome was then tested against a known deficiency for the white gene (Notch-8)[9] and the resultant females also had the wild-type eye color. Thus, the *y sc ec* chromosome appears to be completely wild-type with respect to the white region. The simplest interpretation of the origin of this chromosome is that it represents a wild-type crossover chromatid which resulted from an exchange between the *w* locus and an *apr* locus lying to the left of *w* (see exchange la or lb of Fig 1). The presence of the $y^2\ apr\ ec$ chromosome in the original red-eyed female is readily accounted for by assuming a non-reciprocal exchange (see exchange 2 of Fig. 1) between the *ec* locus and the centromere.

A search for additional red-eyed flies was continued among the progeny

of the second and final type of mating, Mating 2, in which the parental yellow-2 dilute-apricot females were heterozygous for *Cy* as well as Ubx^{130}. There were four groups of such parental females. The first group was selected from among the daughters of the 19 Type-A cultures of Mating 1, described above; the second group was selected from among the daughters of only those females belonging to the first group which proved to be of Type-A constitution; while the third and fourth groups were similarly selected from the daughters of the second and third groups, respectively. The parental females of Mating 2 were in every case individually mated to males carrying the sex-linked mutant genes, y^{31d}, sc^{8}, *apr*, *B* (Bar) and lz^{s} (lozenge-spectacled).[9] The *B* mutant served here to identify any free-X daughters, which result rarely from detachment of the attached-X's in the mother. The nearly white (*apr* lz^{s}) eye color of this type of male facilitated the search for red-eyed flies among the progeny as a whole. (The yellow

TABLE 1

THE NUMBER AND CLASSES OF RED-EYED DAUGHTERS OF y^{2} *apr ec*/*y sc w spl*, OR "TYPE-A," ATTACHED-X FEMALES (HETEROZYGOUS FOR AUTOSOMAL REARRANGEMENTS) FROM MATING 2. THE CLASSES CORRESPOND AS LETTERED TO THOSE SHOWN IN FIGURE 1

CLASS	PHENOTYPE	CORRESPONDING GENOTYPE	NUMBER OF FLIES
(a)	yellow-2 echinus	$\frac{y\ sc\ +\ +\ +\ ec}{y^{2}\ +\ apr\ +\ +\ ec}$	2
(b)	yellow scute	$\frac{y\ sc\ +\ +\ +\ ec}{y\ sc\ +\ w\ spl\ +}$	4
(c)	yellow-2	$\frac{y\ sc\ +\ +\ +\ ec}{y^{2}\ +\ apr\ w\ spl\ +}$	4

body color of this type of male served a similar purpose with respect to any non-yellow progeny, which might result from crossing over between *y* and y^{2}, but none of these was found.)

Among 897 fertile cultures[14] of Mating 2, a total of 794 cultures proved to have had parental females which were either of Type-A constitution or were heterozygous for at least *y*, *sc* and *spl*, as well as *apr* and *w;* while the remaining 103 cultures had to be discarded either because of too few progeny or because of an insufficiently marked maternal constitution. From the above 794 adequately constituted cultures, there was a total of 12 independent occurrences of red-eyed females among an estimated 40,100 attached-X offspring. Ten of these 12 red-eyed females came from Type-A mothers. As shown in table 1, these ten were distributed among three phenotypic classes with respect to the sex-linked marker genes. Each of the two yellow-2 red echinus flies of Class (a) proved on progeny testing to have had attached-X's made up of a *y sc ec* or wild-type crossover chromosome and a y^{2} *apr ec* chromosome, as in the first case of a red-eyed fly from Mating 1, above. Of the four yellow scute red females of Class (b), one died and each of the others proved to have had a *y sc ec* chromosome

associated with a *y sc w spl* chromosome. Class (b) individuals are those expected following a single non-reciprocal exchange (see exchange 1b of Fig. 1) between *apr* and *w*. The four yellow-2 females of Class (c) each produced the following principal classes of homozygous attached-X daughters: yellow scute red echinus and yellow-2 white split. Each of the four females of Class (c) must therefore have represented a case of simultaneous recovery of a *y sc ec* wild-type crossover chromosome and the complementary double mutant, or y^2 *apr w spl*, crossover chromosome. Of the two remaining red-eyed flies among the above group of 12, one was yellow-2 in phenotype and arose from a y^2 *apr*/*y sc w spl* mother (identical in constitution with Type A except for the loss of *ec*). The principal two classes of homozygous daughters of this red-eyed female were phenotypically yellow scute red and yellow-2 white split. The other red-eyed fly, also of yellow-2 phenotype, came from a y^2 *w spl*/*y sc apr ec* mother (identical with Type A except for an interchanging of the markers distal to *apr*). The principal two classes of homozygous daughters of this latter red-eyed female were yellow-2 red echinus and yellow scute white split in phenotype. Thus, each of the above two red-eyed flies must have represented a case in which both crossover chromatids from an exchange between *apr* and *w* had been recovered simultaneously.

The above analysis of the 12 red-eyed attached-X females from Mating 2 actually provided a total of 18 crossovers between *apr* and *w;* that is, each of the 12 carried a wild-type crossover while six of them carried the complementary double mutant crossover, as well. The observed amount of crossing over was 0.03% (12/40,100 ±). This value probably overestimates the standard map distance between *apr* and *w*, since the observed total amount of crossing over in the whole region from *y* to *spl* in these experiments was calculated to be 11.5% (based on a complete phenotypic analysis of 11,985 attached-X progeny from a large sample of Type-A cultures of Mating 2), or nearly four times the standard value of 3.0±%.[15] This increase, however, was not distributed uniformly over the *y* − *spl* region. Thus, the *y* − *apr* region was increased from 1.5 to 8.9% or nearly sixfold;[16] while the *w* − *spl* region increased from 1.5± to 2.6% or about 1.7 times. By assuming that crossing over in the *apr*−*w* region was increased by a factor lying within these limits, the standard map distance between *apr* and *w* can be calculated as being within the range of 1/6 to 1/1.7 of the observed value of 0.03 unit, or roughly 0.005 to 0.02.

Indirect proof was obtained above for the occurrence of six double mutant, or *apr w*, crossovers from Mating 2. In none of these cases, however, has it been possible to distinguish, phenotypically, *apr w* from *w*. Thus, the heterozygote, *apr w*/+ +, invariably has red eyes in striking contrast to the pinkish yellow eye color of *apr* +/+ *w* heterozygotes; while the *apr w* homozygote is like that of *w* in having white eyes. In

addition, two *apr w* detachment males were obtained from one of the females belonging to Class (c) of table 1. Such males also have white eyes. Similarly, *apr w*/+ *w* females have white eyes, and *apr w*/*apr* + females have pinkish yellow eyes like those of +*w*/*apr* +.

An attempt was next made to obtain direct proof for the presence of *apr* in the double mutant combination by searching among the progeny of *apr w*/+ + females for a dilute-apricot phenotype. For this purpose a third type of mating, Mating 3, was employed. The parental females in this case were phenotypically yellow-2 red (and were in some cases heterozygous for *Cy* and/or Ubx^{130}). Each was derived as either a second or third generation daughter of one of the original four yellow-2 red females from Mating 2 (the same one, in fact, from which the above *apr w* detachments were ultimately derived). The parental females of Mating 3 were individually mated to males which carried wild-type X-chromosomes (and were heterozygous for Ubx^{130}). To forestall, in so far as possible, any contamination of this mating with dilute-apricot attached-X females, all cultures containing such females had been purposely destroyed before Mating 3 was initiated, except for one Type-A culture in which, however, all of the flies were homozygous for the Gowen gene (*c3G*).[9] Since this gene results in complete suppression of crossing over in the female, Type-A females from this latter type of culture are readily recognizable because they breed true for the Type-A constitution and are nearly sterile. From one culture of Mating 3 in which the parental female proved to have had the constitution, *y sc ec*/y^2 *apr w spl*, a single dilute-apricot (yellow-2) female (which also happened to carry *Cy* and Ubx^{130}) arose among the otherwise red- or white-eyed progeny. The principal classes of homozygous daughters of this latter female were yellow-2 apricot echinus and yellow scute white split in phenotype; thus, the maternal constitution in this case must have been y^2 *apr ec*/*y sc w spl*, or Type A. In this case there must have been simultaneous recovery of the *w*+ and *apr* + crossover chromatids that are to be expected following an exchange between the *apr* and *w* loci in the parental *apr w*/+ + female.

Discussion.—The above crossing-over studies have shown that at least two loci are at the basis of the so-called "multiple allelic" series of white mutants. Only brief consideration can be given here to the relation of this finding to results obtained from several other types of studies that have already been made of this series. Firstly, phenotypic studies have shown that this series is comprised of two qualitatively distinct groups of mutants. Thus, the eye color of mutants belonging to the so-called "apricot" group is darker in the male than in the female; while the converse is true for mutants of the "eosin" group. Similarly, Bridges' specific modifier, Pale, acts to darken the eye color of mutants of the apricot group, and to lighten it for those of the eosin group.[17] Moreover, it has been

pointed out[17] that "*w*" (presumably the same *w* as used in the present studies) belongs to this latter group since the associated "white" phenotype is made even lighter in color in the presence of Pale.

The above difference in properties between the apricot and eosin group of mutants will more than likely turn out to reflect a functional difference between the apr^+ and w^+ genes. Superimposed on such a difference, however, would be the position effect associated with these genes; namely, the striking phenotypic difference that exists between *apr*+/+ *w* (with pinkish yellow eye color) and *apr w*/+ + (with red eye color). This result suggests that a mutant gene at one of the loci blocks, or impairs the functioning of, the normal allele of the gene at the other locus, when both are present in the same chromosome, as in the *apr* +/+ *w* heterozygote; while no impairment of the functioning of the two different wild-type alleles is phenotypically detectable when both of these are present in the same chromosome. The simplest assumption is that the effect is one-way; that is, that the mutant gene *apr* impairs the functioning of w^+, or that *w* impairs that of apr^+. This leads to a simple model in which one of the genes controls a step A→B, and the other a step, B → C, in a biochemical reaction chain of the type: A → B → C. The position effect can then be assumed to result from a failure of substance B to diffuse readily from one chromosome to the other so that the chain of reactions in one of the chromosomes of the heterozygote is carried out more or less independently of the chain in the homologous chromosome. Further details of the application of this type of model to position pseudoallelism have been given elsewhere.[7]

Cytological studies of Panshin[18] and of others[9] have shown that the w^+ gene is located within the confines of the 3C2-3 doublet, or two-banded, structure of the salivary gland chromosomes (see Bridges' revised map[19]). Moreover, the evidence of Panshin was based on the critical method of synthesizing a deficiency for the gene in question by combining parts of two appropriate rearrangements, neither of which acts like a deficiency for that gene. In this case, the rearrangements were the white-mottled-5 (w^{m5}) and roughest-3 (rst^3) inversions in the X-chromosome. The right break-points of these two inversions are essentially similar and in the heterochromatic region. The left break-point of w^{m5} lies between 3C1 and 3C2 (Sutton[9]);[20] while that of rst^3 lies between 3C3 and 3C4 (Emmens and others[9]). Panshin combined, as the result of crossing over, the left end of w^{m5} with the right end of rst^3. The resultant chromosome is a deficiency for the 3C2-3 doublet and it proved to act like a deficiency for the w^+ gene; on the other hand, other synthesized deficiencies either for the 3C1 band, or for bands to the right of the 3C2-3 doublet, did not act as though they were deficiencies for this gene. From these results, it is probable that apr^+ also lies within the 3C2-3 doublet.

The doublet structure has been implicated before in certain cases of position pseudoallelism. As has been discussed more fully in connection with these other cases,[5,7] the probable significance of this cytological finding is that the pseudoallelic genes associated with a doublet represent an established duplication of a single ancestral gene. To explain the finding that the two members of the duplication now appear to differ in function, it has been presumed that one of the genes has, by mutation, diverged in function from the other, and, in such a way, that it now controls a reaction successive to (or, on Horowitz' hypothesis for the evolution of biochemical synthesis,[21] antecedent to) that of the original gene.

Summary.—The white (*w*) and apricot (*apr*, formerly w^a) genes of the so-called "multiple allelic" white-series in *Drosophila melanogaster* are found to be pseudoallelic genes whose order in the chromosome is *apr*—*w*. This result was based on a total of 21 crossovers between the *apr* and *w* loci. The total percentage of crossing over between them was 0.03%, under conditions giving two to six times the normal amount of crossing over in the surrounding regions. The standard map distance between *apr* and *w* is inferred to be in the range of 0.005 to 0.02 unit. The *apr* and *w* genes are a typical example of position pseudoallelism. Thus, a position effect is present, whereby *apr* +/+ *w* females have a pinkish yellow eye color, while *apr w*/+ + females have a wild-type or red eye color. The use of attached-X chromosomes enabled the *apr w*, or double mutant, combination to be derived simultaneously with the complementary wild-type crossover in a total of six instances. The results are discussed in relation to phenotypic and cytological studies of the white-series, and also in relation to other studies of position pseudoallelism.

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[13] The new rearrangement in the third chromosome is as follows: 3L tip to 61A-C/96A to 93B/89D-E to centromere to 74/61A-C to 74/89D-E to 93B/96A to tip of 3R.

[14] The author is indebted for technical assistance in the preparation of these matings to W. Gencarella.

[15] No definite case of a double crossover in the $y - spl$ region was observed.

[16] Two verified crossovers between y and sc were detected as y^2 *sc w spl* phenotypes.

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ON THE NUMBER OF BOUND STATES IN A CENTRAL FIELD OF FORCE

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1. The present note contains some fairly elementary remarks concerning the number of bound state solutions of the Schrödinger equation

$$\nabla^2\psi + E\psi = V(r)\psi$$

for a central field of force, more specifically, the number n_l of bound state solutions of the radial wave equation

$$\phi'' - l(l+1)r^{-2}\phi + E\phi = V(r)\phi \tag{1}$$

for angular momentum l. We assume the integral

$$I = \int_0^\infty r\,|V(r)|\,dr \tag{2}$$

to be *finite*, and we wish to estimate n_l in terms of I. (In the units chosen V has the dimension (length)$^{-2}$, so that I is dimensionless.) R. Jost and A. Pais (ref. 1, p. 844) have shown that no bound states occur if $I < 1$. Our aim is to derive the more general inequality

$$(2l+1)n_l < I \tag{3}$$

(equality excluded). The number n_l counts the distinct stationary energy values corresponding to equation (1). If the $(2l+1)$-fold degeneracy of each of them is taken into account it is seen that for a given angular momentum l there are less than I bound states, and no bound states occur if $l \geq \frac{1}{2}(I-1)$. The estimate (3) is best possible in the sense that for a given l potentials may be constructed which have a prescribed number n_l of bound states for that angular momentum and for which I approaches