THE RUBY EYE-COLOR MUTANT IN THE HOUSE FLY, *MUSCA DOMESTICA* L., A CASE OF DUPLICATE GENES

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THE ruby eye-color mutant (*rb*) in the house fly, *Musca domestica* L., was originally found by HIROYOSHI (1960) in the progeny of an "orange-eyed" stock collected in the field by L. D. ZINGRONE (Illinois State Natural History Survey, Urbana, Illinois, 1955). HIROYOSHI reported that the *rb* and *ct* (cut-wing tips) mutants were linked though the "results of all F_2 tests deviated significantly from expected segregation ratios, probably due to poor viability of the *rb* mutant, or the eyes of some prospective *rb* individuals might be normal when the larvae are reared in a mixed culture." Thus he implied that a reduction in the *rb* class was responsible for the deviant ratios and that *rb* was the result of a single recessive gene.

Evidence accumulated in this laboratory shows that the *rb* mutant phenotype results from the simultaneous expression of two recessive loci of two nonhomologous chromosomes (duplicate genes). Also, the ruby stock has been shown to contain the factor(s) giving holandric inheritance of one member of the third pair of autosomes (segregation of the third pair of autosomes is sex-determining). The phenomenon of holandric inheritance of chromosome III was reported to arise spontaneously in native populations of house flies (MILANI and FRANCO 1959; SULLIVAN 1958, 1961; HIROYOSHI 1964; TSUKAMOTO, BABA, and HIRAGA 1961; KERR 1960). Normally, segregation of the X and Y chromosomes in the male is sex determining (females are XX).

MATERIALS AND METHODS

Adult house flies were maintained in metal-frame, gauze-covered cages at room temperature and humidity and were supplied with both water and a diet of 3 parts powdered milk, 3 parts sugar, and 1 part powdered egg yolk. Eggng was achieved by introducing a small plastic cup containing a black cloth saturated with fermented CSMA medium and water. After approximately 4 hr, the cups were removed and an appropriate amount of eggs (up to 1,000) was placed on top of 2 quarts of CSMA (Chemical Specialties Manufacturing Association) medium mixed with 375 ml of water in 1-gallon plastic buckets with a plastic bag liner. The buckets were held in constant temperature ($26.5 \pm 1^\circ\text{C}$) and humidity ($55 \pm 5\%$ RH) cabinets while the larvae grew and pupated. Shortly before adult eclosion (after about 8-9 days), the buckets were removed from the cabinets, and the pupae were scraped into 24-oz wax-coated drinking cups. Then the cups were put into the 1-gallon buckets (with the plastic liner and the spent medium removed) and the buckets were returned to the constant temperature and humidity cabinets. Once adult emergence began, the adults were collected and scored daily until emergence was complete. All crosses represent mass matings.

TABLE 1
Results of $rb \times ro$ crosses (disregarding ro); $rb \overset{v}{\bar{Q}} \times ac; ar \delta$ cross, and $car, tw \overset{v}{\bar{Q}} \times rb \delta$ cross

Cross	No. of progeny	F_2 segregation		
		Percent observed	Percent expected (2 loci)	
$rb \overset{v}{\bar{Q}} \times ro \delta$				
$F_1 \bar{Q} \times F_1 \delta$	233	$92.7 \pm 3.4^* (+)$	7.3 $\pm$ 3.4 (rb)	93.75 (+) 6.25 (rb)
$ro \overset{v}{\bar{Q}} \times rb \delta$				
$F_1 \bar{Q} \times F_1 \delta$	1,088	$93.2 \pm 1.5 (+)$	6.8 $\pm$ 1.5 (rb)	93.75 (+) 6.25 (rb)
$rb \overset{v}{\bar{Q}} \times ac; ar \delta$				
$F_1 \bar{Q} \times F_1 \delta$	426	$96.2 \pm 1.9 (+)$	3.8 $\pm$ 1.9 (rb)	93.75 (+) 6.25 (rb)
(classes treated independently)		$52.8 \pm 4.8 (+)$	$24.9 \pm 4.2 (ac)$	22.3 $\pm$ 4.0 (ar) 50.0 (+) 25.0 (ac) 25.0 (ar)
$car, tw \overset{v}{\bar{Q}} \times rb \delta$				
$F_1 \bar{Q} \times F_1 \delta$	987	$71.5 \pm 2.8 (+)$	$28.5 \pm 2.8 (car, rb \text{ and } car; rb)$	70.3 (+) 29.7 (car, rb and car; rb)

* Standard error ($2 \times \Sigma$) is used where

$$\Sigma = \sqrt{\frac{pq}{n}}$$

and p = number of progeny of a defined type expressed as a percentage

$$q = 1 - p$$

n = total number of progeny recovered.

RESULTS AND DISCUSSION

Flies from a stock homozygous for *rb* (originally reported on linkage group IV, HIROYOSHI (1961)) were crossed reciprocally with another stock of house flies carrying the mutant rough eyes (*ro*—chromosome III). Linkage group designations are based on PERJE's (1948) numbering of the house fly karyotype in which *X* and *Y* represent the sex chromosomes and the Roman numerals I to V represent the five pairs of autosomes starting with the longest pair and ending with the shortest pair, respectively, and WAGONER's (1967, 1969) correlation of the linkage groups with particular chromosomes. Normally F_1 sibs from such a cross, when mated, would produce + and *rb* offspring in a 3:1 ratio, respectively, disregarding the *ro* marker. However, if the F_1 sibs were heterozygous for two loci on two nonhomologous chromosomes that controlled the same recessive phenotype (*rb*), one would expect a + to *rb* ratio of 15:1 in the F_2 progeny. That was the result obtained (Table 1).

Another experiment was made in which *rb* flies were crossed with a stock carrying the mutants *ac;ar* (alicurve — chromosome I and aristapedia — chromosome II). No linkage was involved in this cross because all four mutants, *ac*; *ar*; *rb_1* and *rb_2* were on nonhomologous chromosomes (I, II, III, and IV, respectively). The results of this experiment also fitted nicely with the duplicate gene hypothesis (Table 1).

The percentages of *ac* and *ar* flies recovered in the F_2 generation are given in Table 1 (without listing all the possible combinations) to show that simple recessives do segregate in the expected 3:1 ratio. Also, the *rb* loci are phenotypically listed as *rb* and + to denote the percentages of these classes disregarding the *ac* and the *ar* phenotypes. For example, some *rb* flies are *ac*, *ar*, or *ac;ar*, and the same is true in the *rb*⁺ class (the *ac* and *ar* mutants segregated normally); therefore, all eight possible phenotypic combinations were represented by ratios close to expectation.

Table 1 also shows the results of one further cross involving the *rb* mutant and a simple recessive gene, a similar unlinked eye-color mutant, carnation (*car*—chromosome II). A *car,tw* stock was used (*tw* represents twisted front legs) in which the *tw* mutant is not fully penetrant and could be disregarded in scoring. In the progeny of F_1 sib matings of such a cross in which the *car* and *rb* eye colors cannot be separated by superficial examination, one would expect the *car*⁺ to *car* ratio to be 3:1 since it is a simple recessive; then, with the two-gene hypothesis for *rb*, one would expect 1/16 of the *car*⁺ and 1/16 of the *car* flies to be *rb*—i.e., an expected ratio of 11/16 +-eyed flies to 5/16 *rb*, *car*, and *car;rb*-eyed flies (Table 1). Once again, an excellent fit with the expected results was obtained. (If the *car;rb* combination was viable, it could not be distinguished superficially from either *car* or *rb* alone since no new phenotypes were observed.)

The three experiments with *rb* flies (Table 1) were actually conducted for other purposes. After they were completed, the duplicate gene hypothesis for the inheritance of the *rb* phenotype was formulated. The earlier results were then reanalyzed and found, as indicated, to provide supporting evidence.

The events that led to the two-locus hypothesis were as follows: A check cross was set up between *ac;ar;bwb;ct;ocra* virgin females (*bwb*—brown-body—chromosome III and *ocra*—ocra-eye color—chromosome V) and *rb* males. This cross was used to test all mutant stocks (1) for the presence of autosomally mediated holandric inheritance, (2) for the proper assignment of mutant markers to linkage groups, and (3) for the presence of any other genetic phenomena.

The *rb* stock fitted into two categories. It contained a holandrically inherited (male-determining) third chromosome (absence of *bwb*⁺ females and *bwb* males in the backcross to F₁ males), and *rb* not only segregated against the *ct* mutant as expected (*rb* and *ct* were known to be linked previously), but it was also associated with the holandrically inherited chromosome III (III^M) (only *rb* males were present, i.e., no ruby females were present in the progeny of the cross of F₁ sibs), a phenomenon not previously reported (Table 2). This information linked *rb* with chromosome III (*rb*₁) and chromosome IV (*rb*₂) and was the first indication that *rb* was indeed controlled by two genes on two nonhomologous chromosomes.

The progeny of this backcross would give both males and females of the six classes listed in Table 2 if no holandrically inherited autosome were present in the tested mutant stock (treating each mutant independently). Since the *rb* stock contained a holandrically inherited autosome III (III^M-*bwb*⁺), and since *rb* males were used in the P₁ cross, all F₁ males carried the male-determining autosome-III^M-*bwb*⁺ from their male parent and the normal homologue of chromosome III (III-*bwb*) from their female parent. Thus, no *bwb* males were found in the F₂ progeny of either the sib mating or the backcross because all flies receiving the III^M chromosome from the P₁ male (via the F₁ male) were necessarily F₂ III^M *bwb*⁺ males; also, no *bwb*⁺ females were recovered in the F₂ progeny of the backcross because they all obtained the normal chromosome III (with respect to sex determination) from the F₁ male, the contribution of the P₁ female (i.e., III-*bwb*). The backcross females contributed only III-*bwb* chromosomes.

In all stocks examined cytologically by the author in which holandric inheri-

TABLE 2

Results of *ac;ar;bwb;ct;ocra* ^V♀ × *rb* ♂ cross
(where ++ = class present and — = class absent)

Cross		F ₂ segregation classes and backcross					
		+	<i>ac</i> (I)	<i>ar</i> (II)	<i>bwb</i> (III)	<i>ct</i> (IV)	<i>ocra</i> (V)
<i>ac;ar;bwb;ct;ocra</i> ^V ♀ × <i>rb</i> ♂							
	♂ <i>rb</i> ⁺	++	++	++	—	++	++
	♂ <i>rb</i>	++	++	++	—	—	—
	♀ <i>rb</i> ⁺	++	++	++	++	++	++
	♀ <i>rb</i>	—	—	—	—	—	—
<i>ac;ar;bwb;ct;ocra</i> ^V ♀ × F ₁ ♂							
	♂	++	++	++	—	++	++
	♀	—	++	++	++ (all)	++	++

FIGURE 1.—Chromosomes of the *rb* house fly strain showing the XX sex chromosome complement in the male (from testes squashes—gonial mitosis).

tance of chromosome III occurs, male and female flies show an XX sex chromosome complement instead of the normal XX female and XY male situation (Figure 1). HIROYOSHI (1964) found a few exceptional strains giving holandric inheritance of one of the members of the third pair of autosomes which showed cytologically that they were made up of XX males and females but also contained X0 males.

As noted, the segregation patterns of the sib matings listed in Table 2 were made to check linkage relationships. Normally in such matings, a simple recessive of a type not represented in the multiply-marked stock will be found in combination with 4 of the 5 markers of the multiply-marked stock in the F_2 progeny and will not be found in combination with the remaining marker, the marker representing the same linkage group (there is no crossing over in male house flies). The first male and female rows (Table 2) represent flies that are rb^+ when each of the five markers in the multiply-marked stock are considered independently (columns). The second male and female rows are *rb* flies in combination with the five markers. Note the dashes in the *rb,ct* class (Table 2) after the male heading. This class is expected to be absent in such an F_1 sib-mating test cross since *rb* and *ct* have already been reported to be linked (further evidence that rb_2 is linked to other mutants on chromosome IV will be given later). The *ocra* column opposite the *rb* rows contains dashes by necessity since an *ocra*-eyed fly cannot show the ruby phenotype.

The backcross showed that III^M was present in the ruby stock, and no *rb* females were found in the F_1 sib cross, which confirmed both the presence of III^M and the fact that *rb* must be located on chromosome III as well as on chromosome IV since otherwise one would expect to find both *rb* males and females in the F_2 generation. All of the F_2 *rb* flies (males) must receive a $III^M bwb^+, rb_i$ from the F_1 male and a $III-bwb^+, rb_i$ chromosome from the F_1 female (crossing over would not affect the outcome). Thus, all the *rb* flies are male and are at the same time *bwb* $^+$. F_2 females would receive either a $III-bwb,rb_i^+$ or a $III-bwb^+,rb_i$ chromosome from the F_1 female and a $III-bwb,rb_i^+$ chromosome from the F_1 male. Thus F_2 females may be *bwb* or *bwb* $^+$, but, because of the contribution from the F_1 male (rb_i^+), they cannot show the *rb* phenotype (crossing over does not occur

TABLE 3

Results of $rb \overset{v}{Q} \times Iv \delta$ cross, $rb \overset{v}{Q} \times Aw \delta$ cross, $ye \overset{v}{Q} \times Iv \delta$ cross, $Aw \overset{v}{Q} \times ye \delta$ cross, and $rb, ye \overset{v}{Q} \times Aw \delta$ cross

Cross	No. of progeny	F ₂ segregation				Percent expected
		Percent observed				
$rb \overset{v}{Q} \times Iv \delta$						
$F_1(Iv) \overset{v}{Q} \times F_1(Iv) \delta$	692	23.7 $\pm$ 3.2 (+)	70.7 $\pm$ 3.4 (Iv)	2.7 $\pm$ 1.2 (rb)	2.9 $\pm$ 1.3 (Iv;rb)	 (crov.)*
$rb \overset{v}{Q} \times F_1(Iv) \delta$	651	26.0 $\pm$ 3.4 (+)	53.1 $\pm$ 4.0 (Iv)	20.9 $\pm$ 3.2 (rb)	0.0 (Iv;rb)	25.0 (+) 50.0 (Iv) 25.0 (rb) 0.0 (Iv;rb)
$rb \overset{v}{Q} \times Aw \delta$						
$F_1(Aw) \overset{v}{Q} \times rb \delta$	1,188	20.8 $\pm$ 2.4 (+)	61.9 $\pm$ 2.8 (Aw)	17.1 $\pm$ 2.2 (rb)	0.3 $\pm$ 0.3 (Aw,rb)	 (crov.)
$rb \overset{v}{Q} \times F_1(Aw) \delta$	809	23.9 $\pm$ 3.0 (+)	51.3 $\pm$ 3.6 (Aw)	24.8 $\pm$ 3.0 (rb)	0.0 (Aw,rb)	25.0 (+) 50.0 (Aw) 25.0 (rb) 0.0 (Aw,rb)
$ye \overset{v}{Q} \times Iv \delta$						
$ye \overset{v}{Q} \times F_1(Iv) \delta$	696	25.7 $\pm$ 3.4 (+)	25.7 $\pm$ 3.4 (Iv)	23.9 $\pm$ 3.2 (ye)	24.7 $\pm$ 3.2 (Iv;ye)	25.0 (+) 25.0 (Iv) 25.0 (ye) 25.0 (Iv;ye)
$Aw \overset{v}{Q} \times ye \delta$						
$ye \overset{v}{Q} \times F_1(Aw) \delta$	450	4.4 $\pm$ 1.9 (+)	48.4 $\pm$ 4.8 (Aw)	47.1 $\pm$ 4.8 (ye)	0.0 (Aw,ye)	0.0 (+) 50.0 (Aw) 50.0 (ye) 0.0 (Aw,ye)
$rb, ye \overset{v}{Q} \times Aw \delta$						
$rb, ye \overset{v}{Q} \times F_1(Aw) \delta$	895	6.3 $\pm$ 1.6 (+)	44.5 $\pm$ 3.4 (Aw)	49.3 $\pm$ 3.4 (ye)	0.0 (Aw,ye)	0.0 (+) 50.0 (Aw) 50.0 (ye) 0.0 (Aw,ye)

* Crossing over can occur in this cross. Since the map distance between the markers is not known, expected ratios cannot be given.

in male house flies). The duplicate-gene hypothesis was therefore formulated, and the earlier data were reviewed to confirm the hypothesis.

Further tests were performed to confirm the location of the two ruby loci. Segregation of rb_1 (III) and rb_2 (IV) was tested by making two crosses with dominant mutants, *Iv* (Interrupted veins—chromosome III) and *Aw* (Airplane wing—chromosome IV) (Table 3).

The important results from these crosses were obtained from the backcross data. The F_1 males in both series produced no recombinations of the double mutant type (rb_1Iv or rb_1Aw), however, when crossing over was possible (F_1 females), double mutant recombinations were expected and found. The expected ratios in the backcross to F_1 females (rb virgin females $\times Iv$ males) and the F_1 sib cross (rb virgin females $\times Aw$ males) (Table 3) are left blank because map distances between the mutants used in these crosses have not been reported. With the $rb_1;rb_2$ hypothesis, the new combination + class would be expected to be present in the F_2 of F_1 male backcrosses because the F_1 male from the *Iv* cross would carry rb_2+ from the $P_1 Iv$ male and the F_1 male from the *Aw* cross would carry rb_1+ from the $P_1 Aw$ male.

Iv and *Aw* were then crossed with other simple recessives to be sure they segregated as expected. The simple recessive *ye* (yellow eye color—chromosome IV) was crossed with both *Iv* males (III) and *Aw* males (IV) (Table 3). The *Iv* mutant segregated normally with the *ye* marker and gave the independent assortment expected of two unlinked genes. The *Aw* virgin female $\times ye$ male cross was not clear cut, probably because of the poor penetrance of the *Aw* character. No new combinations (+ or *Aw, ye*) were expected in the backcross to F_1 males, but a few normal (+) flies did appear. These exceptional flies did not significantly skew the expected 50:50 ratio of *Aw* to *ye* flies and they probably carried the *Aw* gene but did not express it phenotypically. (The matter was not tested.)

A similar cross involving $rb;ye$ virgin females $\times Aw$ males gave similar results in an F_1 male backcross to $rb;ye$ virgin females. The results of such a cross should be like those of the previous *Aw* virgin female $\times ye$ male cross because *ye* covers the *rb* phenotype (Table 3). Once again, the results were not significantly skewed from the expected 50:50 ratio, and the class that should not be present (+) probably represents poor penetration of the *Aw* mutant. If the + flies were added to the *Aw* total, the ratio is close to 50:50. Thus, dominant genes on chromosomes III and IV were both shown to be located opposite ruby loci (rb_1 and rb_2 , respectively).

Three additional crosses were made for further testing (Table 4). The first consisted of mating *ye* virgin females $\times rb$ males and backcrossing the F_1 progeny to $rb;ye$ flies. The results of the F_1 male backcross fitted expectation well. Here, because of the III^M chromosome, all non-*ye* males should be *rb* (25%), all non-*ye* females should be + (25%), and 50% of the flies should be *ye* males and females in equal numbers. This was the result. Also, though the map distance between *ye* and rb_2 is not known, the F_1 female backcross will fit expectations if the distance is small. Reciprocal products of crossing over would increase and decrease the *ye* and + phenotypes from + and *ye*, respectively (therefore no ratio change) and

TABLE 4

Results of $ye \overset{v}{\delta} \times rb \overset{v}{\delta}$ cross, $rb \overset{v}{\delta} \times ct \overset{v}{\delta}$ cross, $rb \overset{v}{\delta} \times Orlando$ regular wild-type δ cross, and $rb \overset{v}{\delta} \times Bx \overset{v}{\delta}$ cross

Cross	Number of progeny	F ₂ segregation		
		Percent observed		Percent expected
$ye \overset{v}{\delta} \times rb \overset{v}{\delta}$				
$F_1 \overset{v}{\delta} \times rb; ye \overset{v}{\delta}$	996	25.6 $\pm$ 2.8 (+)	24.3 $\pm$ 2.8 (rb)	50.1 $\pm$ 3.2 (ye)
				25.0 (+)
$rb; ye \overset{v}{\delta} \times F_1 \overset{v}{\delta}$	463	0.0 (+ δ)	25.9 $\pm$ 4.0 (rb δ)	0.0 (+ δ)
		25.7 $\pm$ 4.0 (+ δ)	0.0 (rb δ)	25.0 (+ δ)
$rb \overset{v}{\delta} \times ct \overset{v}{\delta}$				
$F_1 \overset{v}{\delta} \times F_1 \overset{v}{\delta}$	396	70.5 $\pm$ 4.6 (+)	3.5 $\pm$ 1.8 (rb)	68.75 (+)
$rb \overset{v}{\delta} \times Orlando$ regular wild-type δ				
$rb \overset{v}{\delta} \times F_1 \overset{v}{\delta}$	619	53.0 $\pm$ 4.0 (+ δ)	29.4 $\pm$ 3.6 (+ δ)	50.0 (+ δ)
$rb \overset{v}{\delta} \times Bx/+ \delta$				
$rb \overset{v}{\delta} \times (Bx) F_1 \overset{v}{\delta}$	523	24.9 $\pm$ 3.8 (+)	53.0 $\pm$ 4.4 (Bx)	25.0 (+)
$rb \overset{v}{\delta} \times (Bx) F_1 \overset{v}{\delta}$	339	27.4 $\pm$ 4.8 (+)	45.1 $\pm$ 5.4 (Bx)	50.0 (Bx)
$F_1 (Bx) \overset{v}{\delta} \times rb \overset{v}{\delta}$	451	33.3 $\pm$ 4.4 (+)	43.7 $\pm$ 4.6 (Bx)	25.0 (rb)
$F_1 (Bx) \overset{v}{\delta} \times rb \overset{v}{\delta}$	1,438	32.4 $\pm$ 2.4 (+)	45.1 $\pm$ 2.6 (Bx)	0.0 (Bx;rb)

* Crossing over can occur in this cross. Since the map distance between the markers is not known, expected ratios cannot be given.

would increase the *ye* and depress the *rb* phenotypes from expectations. No significant increase in the *ye* or decrease in the *rb* phenotype was found. Thus, the map distance between *ye* and *rb* should be small.

The second of the three crosses consisted of mating *rb* virgin females $\times$ *ct* males and crossing F_1 sibs (Table 4). The results fitted the expectation reasonably well. Since the *ct* mutant is not fully penetrant, a depression of the *ct* class in the F_2 might have occurred, but this did not happen.

The third cross consisted of mating *rb* virgin females $\times$ Orlando regular wild-type males and backcrossing the F_1 males to *rb* virgin females (Table 4). The Orlando regular stock contains a holandrically inherited chromosome III (III^M). Thus, if the *rb₁* gene is located on chromosome III and *rb₂* is located on chromosome IV, all F_2 males from a backcross involving F_1 males should be + and all females should be *rb* or + in equal numbers. Further proof that the location of *rb₁* is on chromosome III was provided. If *rb₁* were not on chromosome III, one would expect *rb* and + males and females in equal numbers in such a backcross, but the normal sex-limited (holandric) inheritance (all males +, all females mutant) was not realized because the *rb₂* locus on chromosome IV was segregating independently of the III/III^M situation. All F_2 males must be + because they get a III^M-rb_1+ chromosome from the F_1 male. However, females, even though they get a $III-rb_1$ chromosome from the F_1 male and the *rb* female and $IV-rb_2$ chromosome from the *rb* female parent, may get a $IV-rb_2+$ or a $IV-rb_2$ chromosome from the F_1 male and so should be + or *rb* in equal frequencies. The F_2 results were statistically significantly different from expected though the proper phenotypes and trends were present. The difference cannot be explained at this time.

One further cross was made with the *rb* stock, and these results also fitted the expectations reasonably well; *rb* virgin females were crossed with *Bx* males (a dominant marker on chromosome III, lethal in the homozygous form) (Table 4). Again, no double mutants were found in the F_1 male backcross as expected, and the crossover class *rb, Bx* was not present in the F_1 female backcross though the + class was larger in two separate tests. The results cannot be explained, but it has been suspected for some time that *Bx* is not a simple gene change.

The final proof of the *rb₁, rb₂* hypothesis came from translocation studies in which the karyotypes of heterozygous X-ray induced translocations between the *Rl* (Rolled wing—chromosome I) and *rb⁺* linkage groups were examined. Three *Rl-rb⁺* translocations were obtained when P_1 *Rl/+* males (treated with 2,500 r of X rays) were mated with *rb* virgin females and F_1 (*Rl*) males were backcrossed to *rb* virgin females to check for induced translocations. Two translocations gave abnormal segregation when they were held in stock form (heterozygous translocation bearing males $\times$ *rb* virgin females), and one *Rl-rb⁺* translocation gave normal segregation. In such crosses when no translocation is present, one expects to obtain four classes in equal frequencies, +, *Rl*, *rb*, and *Rl;rb*. When a translocation is induced, the + and *Rl;rb* classes should be absent; only *Rl* and *rb* flies would normally be expected to survive. Translocation number 3 gave the expected result, but translocation numbers 1 and 2 yielded a third class, +, as well as the *Rl* and *rb* classes and did not give the *Rl;rb* class.

P₁

* translocated autosomes.

FIGURE 2.—Translocation segregation in *Rl-rb⁺* translocation stocks 1 and 2.

When the *rb₁*, *rb₂* hypothesis was worked out, the explanation for translocations 1 and 2 became clear. Such a segregation would be expected when loci on three chromosomes are involved (with two of the chromosomes reciprocally translocated) (Figure 2). In normal double reciprocal translocations, lines 1 and 2 (Figure 2) are the same and lines 3 and 4 are the same (disregarding the *rb₂* chromosome (IV) because only two markers are usually involved); thus only two viable classes are produced (the translocated chromosomes must segregate together and the normal chromosomes segregate together for viable zygote production—alternate disjunction). In translocations 1 and 2, however, the *rb₂⁺* and *rb₂* chromosome had free segregation, so the class normally expected to be *rb* may receive a *rb₂⁺* chromosome and thus be changed to a + phenotype—not normally expected in regular translocation segregation. Cytological examination confirmed the interpretation. Translocation 3 contained a triple reciprocal translocation involving the *Rl* chromosome (I) and also both suspected *rb* chromosomes (III and IV); translocations 1 and 2 contained only double reciprocal translocations. This result explained the regular segregation of translocation 3. The *rb₂* and *rb₂⁺* chromosomes were not segregating freely as they were in translocations 1 and 2. In translocation 3, the *rb₂⁺* chromosome was also tied up with the *Rl* and *rb₁⁺* chromosomes in a triple reciprocal translocation.

It has been theorized that duplicate genes (most normally found in plants) arise by evolutionary changes where whole chromosome sets are added (polyploidy) and that duplicate genes are therefore extremely rare in the animal kingdom (WAGNER and MITCHELL 1955). Whether or not the *rb* mutant actually fits

the classical definition of duplicate genes or might be described more correctly as multiple loci cannot be determined from these experiments.

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SUMMARY

The *rb* mutant (ruby eye color), in the house fly, originally reported by HIROYOSHI (1960), is the phenotypic result of duplicate genes, namely, two completely recessive genes located on two nonhomologous chromosomes (III and IV). This is the first known case in the house fly of a recessive mutant controlled by two loci.

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