Reports

Spontaneous-Mutation Rates at Specific Loci in Drosophila Males and Females

In spite of the extensive work carried out since 1927 on the frequencies of radiation-induced mutation in *Drosophila melanogaster,* there is a dearth of information about the frequencies of spontaneous mutation at specific loci in this genetically best-known organism. Timoféeff-Ressovsky (*1*) reported a rate of about 3×10^{-6} for the occurrence of white eye color ($w^+ \rightarrow w$) but a far higher rate for the reverse mutation from bobbed-lethal to nonbobbed ($bb^{lx} \rightarrow bb^+$)—namely, 8.3×10^{-5}. The information was not given to indicate whether these mutations were the sum of occurrences in both the male and female parental germ lines or in only one.

Muller, Valencia, and Valencia (*2*) published preliminary data on mutation frequencies in the female germ line occurring at nine selected sex-linked loci. Sixty thousand individuals were examined. A very high rate of mutation, apparently characteristic of the strain used in the study, was obtained (3×10^{-5} per locus as the average for the nine loci).

Muller *et al.* divided this rate by 4, in order to bring it to a rate comparable to that of other strains, and concluded that "the frequency of gene mutations at the nine loci would *ordinarily* average between 10^{-5} and 7×10^{-6} per locus in females." (They also referred to a failure to detect any mutations of sex-linked eye colors in some 50,000 individuals examined by L. and E. Altenburg, which would indicate a rate significantly below 3×10^{-5} but not below 10^{-5} per locus.)

Besides the nine loci, the cut locus yielded nine mutations in 60,000 individuals, or a rate of 1.5×10^{-4}. In addition, there were 18 to 21 mosaic mutations involving one of the nine selected loci, four involving miniature, and four involving cut. None of these was transmissible. No gross rearrangements were found, but three mutants, all lethal white eye-color mutants, were considered to be presumptive deficiencies; of two that were examined cytologically, one was definitely found to be such.

These data, valuable as they are, suffer from the atypical character of the strain used, and even more from the fact that, insofar as useful comparisons are concerned, the spontaneous-mutation frequency was examined in the female germ line, whereas the induced-mutation studies have been almost wholly limited to studies of the male germ line. There is now ample evidence (*3*) to show that induced-mutation frequencies in the two sexes are not alike in many respects. It is therefore necessary to have a comparison of spontaneous-mutation rates at specific loci in both male and female germ lines.

In the course of an extensive study undertaken to determine the frequency of spontaneous dominant minute-bristle mutations in both sexes of *Drosophila melanogaster,* it was possible to detect mutation at certain marker loci as a by-product of the study (*4*). In each of the two crosses made, one for the determination of the mutation frequencies in the female germ line and the other in the male germ line, four marked loci were observed; but unfortunately, because of the high infertility found when yellow males are crossed with nonyellow females, the tester stocks used in the two crosses had to be different, and only one of the four loci was therefore checked in both crosses. The crosses were as follows:

Oregon-R ♀ X *pr cn; by; ci* ey^R ♂ (1)
Oregon-R ♂ X *y; bw; e; ci* ey^R ♀ (2)

Thus the mutants in the tester stocks to which the Oregon-R wild-type flies were crossed included, in the test of the female germ line, the genetic markers purple (*pr*) and cinnabar (*cn*) eye colors, blistery wings (*by*), and eyeless (ey^R); and in the test of the male germ line, yellow body color (*y*), brown eye color (*bw*), ebony body color (*e*), and eyeless (ey^R). No examination of individuals for the phenotype of the *ci* marker was undertaken. The results are set forth in Table 1.

The average mutation rate observed in the female germ line for the four marked loci was 2.5×10^{-6}. In the male germ line, excluding mosaic individuals, it was 5.75×10^{-5}—that is, 20-fold greater. Excluding all sterile mutant individuals, which could not be retested for transmissibility or for the genetic identity of the mutant, the rate in the male germ line is still 3.25×10^{-5}. Much of this high rate is evidently due to the high mutability of the *y* locus. If this locus is excluded in the comparison of the frequencies of spontaneous mutation in the male and female germ lines, the rate in the male germ line still amounts to 2.3×10^{-5}, a full order of magnitude greater than the rate in the female germ line.

Most significant is the comparison of the rates for the ey^R marker, which was the only locus checked in both tests. In the female germ line no mutations were found at this locus in 100,414 flies, indicating a rate less than 10^{-5}. In the male germ line there were six in 102,759, a rate of 6×10^{-5}, or, if sterile mutants are included, 3×10^{-5}. It should be emphasized that none of the *ey* mutants found had minute bristles or interrupted cubitus wing veins (*ci*), as would be the case if nondisjunction of the fourth chromosomes had occurred and the supposed mutants had actually been haplo-4

Table 1. Spontaneous mutations at specific loci in *Drosophila.*

Mutations in female germ line (*Ore-R*)			*Mutations in male germ line* (*Ore-R*)		
pr 1/100,414 (fertile ♂)	10^{-5}	*y*	6/51,380	(3 fertile ♀) (3 sterile ♀)	12×10^{-5}
cn 0/100,414	$< 10^{-5}$	*bw*	3/102,759	(2 fertile, ♂, ♀) (1 sterile ♂)	3×10^{-5}
by 0/100,414	$< 10^{-5}$	*e*	2/102,759	(2 fertile ♀)	2×10^{-5}
ey 0/100,414	$< 10^{-5}$	*ey*	6/102,759	(3 fertile, 1 ♂, 2 ♀) (3 sterile ♀)	6×10^{-5}
Sex-linked mutations			*Mosaics*		
f 1/50,207 (fertile ♂)	2×10^{-5}	*y*	3/51,380	(1 sterile ♀; 2 gynandromorphs)	
m or *dy* 1/100,414 (sterile ♂)	10^{-5}	*bw*	1/102,759	(sterile ♀)	
y, pn, w, rb, *cm, ct, sn, lz,* *ras, v, s, g,* *r, car* 0/702,898	$\ll 10^{-5}$				
Autosomal dominant					
S (?) 1/100,414	10^{-5}				

individuals. The relative abundance of mosaic mutant flies in the male germ line and their absence in the female germ line is also striking.

In cross 1 all sex-linked loci were also exposed to detection, but it is not clear how many might have been observed. Attention was directed only to relatively obvious changes in eye color and shape, body color, wing size and shape, and bristle size and shape. It seems clear that any mutations such as yellow or sable body color, forked or singed bristles, lozenge eyes, miniature, dusky, cut, or rudimentary wings, or prune, white, ruby, carmine, raspberry, vermilion, garnet, or carnation eye colors would have been noticed. Two mutations were observed: one to forked bristles, and another (sterile) to either miniature or dusky wings. The number of individuals examined per locus was about 50,000 (half of the total of 100,414). The mutation rate for sex-linked recessives in the female germ line may, therefore, be estimated as about 2.35×10^{-6}, which is in excellent agreement with the average frequency for the four autosomal loci specifically tested.

Although the data on spontaneous mutability should certainly be extended to include additional loci, the conclusion seems warranted from the present data that in *Drosophila melanogaster* the spontaneous-mutation rate at loci where visible mutants arise is about one order of magnitude larger in males than in females. So large a sex difference in mutability in *Drosophila* makes it imperative to investigate the question of whether a similar difference exists in other species, including the human.

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References and Notes

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4. This investigation was supported by a contract [AT(30-1)-1472] with the U.S. Atomic Energy Commission.

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Goldfish Erythrocyte Antigens and Serology

Incidental to an investigation of tissue-transplantation immunity in goldfish (*Carassius auratus*) (*1*), a preliminary study was aimed at detecting individual differences in the erythrocyte antigens of goldfish. Blood samples were taken and isoimmunization was accomplished by cardiac puncture, a 22-gage needle on a 1.5-ml syringe being most suitable. About 0.5 ml of blood may be safely taken from a 4-inch specimen, while up to 1.5 ml may be withdrawn from a 12-in-fish. Goldfish red cells keep about equally well in 0.7- to 1.0-percent saline but remain intact much longer in concentrated suspensions, especially in homologous plasma. For washing cells and making dilutions, 0.85-percent phosphate-buffered sodium chloride was used. The technique of tagging specimens and the effect of temperature on antibody production in fish have been discussed in full elsewhere (*1*).

Recent investigations of normal hemagglutinins in fish serums and of species differences in red-cell antigens of various fishes have been summarized by Cushing and Sprague (*2*). In the study reported here, normal serums from several different goldfish were checked for natural agglutinins at 1/4 and 1/8 saline dilutions against 2-percent suspensions of washed red cells from 12 other goldfish in standard agglutination tests (*3*). The tests were checked after 30 minutes and after 2 hours at room temperature, with and without centrifugation. No agglutination whatever was found. Normal serums from a variety of other animals were similarly tested for natural antibodies against goldfish red cells. No individual differences were detectable, because the red cells of all fish were agglutinated by a given serum to within one doubling dilution when heteroagglutinins were present. No absorptions of these normal serums were performed.

Proof of the existence of individual differences in goldfish erythrocyte antigens was first achieved by isoimmunization at room temperature. A large specimen was given a course of nine injections, each injection consisting of 0.3 ml of a 20-percent suspension of washed red cells from a particular fish of about the same age (4 years). Three injections were given per week. The agglutination titer checked by trial breedings was only 1/8 6 days after the third injection, but rose to 1/1024 7 days after the ninth injection. The serum was stored frozen and subsequently was used with no further processing.

An absorption analysis of this isoimmune serum was made using red cells from 11 unrelated goldfish. About 1 ml of blood was taken from each fish, and the cells were washed twice in saline. A small aliquot of each sample was then removed to prepare a 2-percent suspension for testing, and the remainder was divided equally in two tubes for absorptions. The isoimmune serum was diluted 1/8 in saline, and 0.5 ml of the diluted serum was thoroughly mixed with the packed cells of each fish for two successive absorptions of 30 minutes each at room temperature. The absorbed serums were then diluted 1/3 in saline for standard agglutination tests. Two fish, including the control recipient, possessed no antigen that reacted with this serum. The erythrocytes of the other nine fish gave positive reactions which revealed the presence of at least five antibody subpopulations recognizing individual antigenic specificities.

Two additional isoimmune serums were prepared as before by nine injections of two unrelated goldfish, respectively, with red cells of two parents from which offspring were being reared. The unabsorbed serums agglutinated the homologous cells to a titer of 1/1024. By the time the F_1 progeny were large enough (2 years old) to provide sufficient blood for an absorption analysis the P_1 ♂ had died. Nevertheless, 2-percent suspensions of the washed red cells of 11 F_1 siblings were tested against doubling dilutions of both unabsorbed isoimmune serums. All of the F_1 cells were agglutinated within two doubling dilutions of each other by both serums; hence, no differences were demonstrable among the F_1 siblings by this method. However, it is apparent that one or more antigenic specificities shared by the parents and their progeny are absent in the individuals that developed these isoimmune antibodies. These specificities represent inherited individual differences in the antigens of goldfish red cells.

Several rabbit antigoldfish red-cell serums were prepared by giving a series of injections of washed red cells in the marginal ear veins. Cross-absorption analyses were made with one such antiserum, using red cells from ten goldfish in addition to the donor. Washed cells prepared from 1-ml blood samples taken from each fish were divided among four tubes for absorptions. To each of the first absorption tubes 0.5 ml of antiserum at 1/8 dilution was added. Each of the first three absorptions was carried out at 10°C for 15 minutes in order to minimize hemolysis. The final absorption was at room temperature for 30 minutes. After the last absorption, each serum reagent was further diluted 1/4 in saline for lytic tests.

Each test consisted of 0.2 ml of serum reagent and 0.1 ml of 2-percent red-blood-cell suspension; after shaking, 0.1 ml of guinea-pig complement at 1/4 dilution was added. Appropriate serum, complement, and saline controls were also run. In all four of the absorption analyses, the red cells of the fish that were tested completely removed antibodies for each other but left in hemolysins for the cells of the donor used in immunization. Thus, only one antigen could be distinguished among the fish tested. The reciprocal removal of antibodies for red cells other than those of the donor was not a consequence of overabsorption, be-