R-LOCUS DELETERIOUS FACTORS IN MORMONIELLA¹

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IN a recent article (WHITING 1961) the four eye-color factors, *O*, *S*, *M* and *P*, found in the *R* series of allelic genes of the wasp *Mormoniella* were discussed. The term "factor" was used in an inclusive sense to denote anything accompanying the eye-color markers in meiosis which has an effect on development or which can in any other way be detected. The genes in this *R* series were described as "pleiotropic, unifactorial, bifactorial, trifactorial, etc., according to the number of their factors in which they differ from wild type." In addition, it was stated that "Any factor may have one or more mutant states which are alternatives to each other. The *alternative states* of the *factors* form subseries within the series of allelic *genes*, so that, theoretically at least, the total number of possible alleles would be equal to the product of the various states of each of the factors."

The present paper deals with several deleterious mutant conditions at the *R* locus, the symbols for which are placed after the four symbols for the eye-color factors. With a single exception, scarlet-474, the mutant genes bearing deleterious factors have been irradiation induced. Female sterility factors are either *fsa*, *fsb* or *fsx*, the letters *a* vs. *b* indicating nonhomology. The letter *x* in female-sterile-*x*, *fsx*, lethals, *lx*, semisteriles, *slx*, near-steriles, *nsx*, and male-steriles, *msx*, indicates homology not tested. Tests of these "*x*" factor states have not as yet been made with *fsb* except for mahogany-835, *mh*-835. Like the eye-color factor states, the states of the deleterious factors exist as pairs or series of alternatives differing in the different mutant genes.

Table 1 gives the formulae of the mutant genes mentioned in this paper in terms of their factor states. Each gene is designated by eye color and a three-digit number or the initials of the finder. In general, the mutant factor states act as recessives to the wild-type state so that homologies may be determined by complementation. Because of lack of crossing-over within the genes, the order of their factors must be arbitrarily assigned, the four color factors *O*, *S*, *M* and *P*, followed by the "deleterious" factors, *A*, *B* and *X*. The latter factors have normal wild-type alternatives so that the meaning of the word factor is somewhat ambiguous, referring either to a mutant condition, as lethal factor, or to that which may be either in the mutant or the normal alternative condition. This ambiguity of "factor" standing alone is cleared by the context. Factors *X* are numerous with homologies as yet undetermined. Gene *mh*-819 and possibly dahlia-845 and

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TABLE 1

Designations of R-locus genes with their formulae in terms of factor states

Designations	Factors*							Designations	Factors						
	O	S	M	P	A	B	X		O	S	M	P	A	B	X
wild type	+	+	+	+	+	+	+	scarlet-DR	+	<i>st</i>	+	+	+	+	+
apricot-837	<i>ap</i>	+	+	+	+	+	+	<i>st</i> -474	+	<i>st</i>	+	+	+	?	<i>lx</i>
dahlia-GF	<i>da</i>	+	+	+	+	+	+	<i>st</i> -689	+	<i>st</i>	+	+	<i>fsa</i>	+	+
<i>da</i> -442	+	+	+	<i>da</i>	+	<i>fsb</i>	+	<i>st</i> -808, 810	+	<i>st</i>	+	+	+	?	<i>slx</i>
<i>da</i> -817	+	<i>da</i>	+	+	<i>fsa</i>	?	<i>lx</i>	<i>st</i> -811	+	<i>st</i>	+	+	+	?	<i>lx</i>
<i>da</i> -838	<i>rdh</i>	+	<i>da</i>	+	+	+	+	<i>st</i> -812	+	<i>st</i>	+	+	<i>fsa</i>	?	<i>lx</i>
<i>da</i> -845, 846	+	+	<i>da</i>	+	+	+	+	<i>st</i> -813	+	<i>st</i>	+	+	+	?	<i>lx</i>
mahogany-441	<i>mh</i>	+	+	+	+	+	+	<i>st</i> -814, 815	+	<i>st</i>	+	+	+	?	<i>msx</i>
<i>mh</i> -819	<i>mh</i>	+	+	+	+	+	+	<i>st</i> -821	+	<i>st</i>	+	+	+	?	<i>lx</i>
<i>mh</i> -835	+	<i>mh</i>	+	+	<i>fsa</i>	+	<i>lx</i>	<i>st</i> -823	+	<i>st</i>	+	+	<i>fsa</i>	?	<i>lx</i>
orange-336	<i>or</i>	<i>st</i>	+	+	+	+	+	<i>st</i> -824	+	<i>st</i>	+	+	+	?	<i>lx</i>
oyster-DR	<i>oy</i>	+	+	+	+	+	+	<i>st</i> -829	+	<i>st</i>	+	+	<i>fsa</i>	+	+
<i>oy</i> -250	<i>oy</i>	<i>st</i>	+	+	+	+	+	<i>st</i> -830	+	<i>st</i>	+	+	+	?	<i>lx</i>
<i>oy</i> -423	<i>oy</i>	<i>st</i>	+	+	<i>fsa</i>	+	+	<i>st</i> -831	+	<i>st</i>	+	+	<i>fsa</i>	?	<i>lx</i>
<i>oy</i> -800-804	<i>oy</i>	+	+	+	+	?	<i>lx</i>	<i>st</i> -834	+	<i>st</i>	+	+	+	?	<i>slx</i>
<i>oy</i> -805	<i>oy</i>	<i>st</i>	+	+	+	?	<i>lx</i>	<i>st</i> -841	+	<i>st</i>	+	+	+	?	<i>nsx</i>
<i>oy</i> -816	<i>oy</i>	+	+	+	+	?	<i>msx</i>	<i>st</i> -842	+	<i>st</i>	+	+	+	?	<i>fsx</i>
<i>oy</i> -828	<i>oy</i>	+	+	+	+	?	<i>lx</i>	<i>st</i> -855, 856	+	<i>st</i>	+	+	+	?	<i>lx</i>
<i>oy</i> -840	<i>oy</i>	+	+	+	+	?	<i>nsx</i>	<i>st</i> -858	+	<i>st</i>	+	+	<i>fsa</i>	+	+
peach-333	<i>pe</i>	<i>st</i>	+	+	+	+	+	vermilion-MK	+	<i>vm</i>	+	+	+	+	+
								<i>vm</i> -809	+	<i>vm</i>	+	+	+	?	<i>lx</i>

* For explanation see text. Homologies of deleterious factors listed under *X* cannot, in general, be determined without the use of diploid males. CASPARI showed *fsa* of *oy*-423, *nsx* (= "*fsb*") of *st*-841 and *fsx* (= "*fsc*") of *st*-842 to be non-homologous. A mutation in factor *B* which produces female sterility, *fsb*, has been shown in *da*-442 only. Test for presence of *fsb* in other genes with deleterious factors has been made of *mh*-835 only.

da-846 carry a factor, *C*, (not indicated in the table), suppressing crossing-over with purple body color, *pu*.

Phenotypes of compounds may be readily determined from the formulae of their two genes as given in Table 1. Thus, the compound female, oyster-800/scarlet-689 (*oy*.+ .+ .+ .+ .+ .*lx*/+ .*st*.+ .+ .*fsa*.+ .+) is wild type in eye color and fertile; *st*-831/*oy*-423 (+ .*st*.+ .+ .*fsa*.+ .*lx*/*oy*.*st*.+ .+ .*fsa*.+ .+) is scarlet-eyed and sterile. Genes having deleterious factors may be maintained in compound with "normal" mutant genes, genes bearing mutant eye colors and the wild-type alternatives to the deleterious factors.

The *R*-locus genes discussed in the present paper are selected from a large number that have been obtained by irradiation. Unless otherwise indicated (as by initials of finders in parentheses—MARJORIE E. GROESBECK, GEORGE B. SAUL, DAVID T. RAY, MARION KAYHART), it is to be understood that they occurred in experiments carried out by SARAH B. CASPARI which are summarized in her publication on an X-ray sperm-dose-action curve (CASPARI 1958). MRS. CASPARI's method was to X-ray wild-type males and to cross them to females of a stock with peach eye color and purple body color, *pe*-333 *pu*. Peach and purple are linked, normally giving about 11 percent crossovers.

The formula of peach in terms of factors *O.S.M.P.A.B.X* may be written *pe.st.+.+.+.+* (or briefly *pe.st*) indicating that it is mutant in two of the four eye-color factors and is normal respecting any deleterious factors. It will be designated simply peach, *pe*, in this paper.

In practice, gene formulae are shortened by omitting symbols for wild-type alternatives, +, except that these symbols must be included to the left of mutant eye-color symbols in order to indicate factors involved—as *st-DR* = *+.st*, *da-845* = *+.+.da* and *da-442* = *+.+.+.da.fsb*.

Peach occurred as a spontaneous mutation in the unstable-*O* scarlet, *unst-O.st*, pedigree (WHITING 1960a). Scarlet, *st*, remains stable in this pedigree but factor *O* mutates giving a wide range of conditions. That of peach in *O* is symbolized *pe* but it is not known what the eye color might be were the gene + (instead of *st*) in *S*. Peach is useful in irradiation tests and in *R*-locus gene analysis but care must be taken to discount the spontaneous mutations in *O*. For this reason the linked gene purple body color is helpful. The fact that none of the unstable-*O* mutants has ever had any deleterious factors suggests that these spontaneous mutations are very localized in their action.

A mutation occurring in factor *O* or *S* of the X-rayed sperm in CASPARI's experiments would show as a mutant-type female compound for the maternal peach gene. Thus a mutation to oyster-white would give a "tinged" mutant female, a peach-oyster compound, *pe.st/oy.+*, and a mutation to scarlet would show the dominant scarlet, *pe.st/+.st*.

Most of the data given in this paper are gleaned from notebook records made while stocks were being maintained and miscellaneous genetical relationships of the various traits were being studied. Because there was no preceding knowledge or plan of all that might be done, the tests are of necessity very unevenly distributed respecting the different genes. Furthermore, the present paper is, for the most part, restricted to what may be understood from tests on the haplo-diploid level. Publication of tests on the diplo-triploid level (WHITING 1960a) are planned.

NEW DATA

Crossover suppressors: Among a total of 17,570 counted offspring of females heterozygous for purple body color and compound or heterozygous for various *R* alleles, making up 63 different combinations, there were 11.1 percent crossovers (WHITING 1956, p. 229). Because these *R* alleles included no known crossover suppressors, this crossover value has been taken as standard.

Two mutations produced genes determining very similar if not identical dark red eyes by a change in factor *M*, *da-845* (GBS) and *da-846* (MK). *da-845/pe pu* females produced sons, 735 parentals 48 crossovers, 6.13 percent crossovers. *da-846/pe pu* females produced sons, 1810 parentals, 126 crossovers, 6.95 percent crossovers. A total of these two groups gives 6.40 ± 0.47 percent crossovers.

mh-819 affects factor *O*, the mutant female *pe.st.+.+.pu/mh.+.+.+* having had dark red eyes. There is no perceptible influence of *O*-factor mahogany on the males or on the homozygous females. These are wild type. The gene must be

differentiated by a recessive such as *pe* or *oy* in *O*. This mutation would not then have been detected had it occurred among the sons of irradiated wild-type females.

mh-819 reduces crossing-over with purple body color. In earlier counts, F_2 males from mahogany by peach purple were 4854 parentals, 18 crossovers and from *oy-250* by mahogany purple there were 765 parentals, one crossover, totalling 19 crossovers among 5638, 0.337 ± 0.077 percent. Recently from *mh-819/oy-423 pu* females there have been counted 1324 wild-type (*mh-819*) and 1144 oyster purple sons. Their daughters sired by *oy-423 pu* males were 69 dahlia, 71 oyster purple. These data total 2608 parentals, no crossovers. It seems certain that *mh-819* is accompanied by a crossover suppressor and it is suggested that this action is greater in recent counts. No differences have been noted between *pe*, *oy-250* and *oy-423* as crossover modifiers in compound with other *R*-locus alleles.

Among *R*-locus alleles with deleterious factors, *st-841*, discussed below, prevents crossing-over with purple.

Female sterility: Female sterility of the *A* type, *fsa*, is not complete. The stinging apparatus is not affected and the females live for many days in the incubator, feeding on the host pupae. The tester gene *oy-423* used by CASPARI in determining homology of her female-sterile-*a* mutants had been obtained (MEG) (WHITING 1956, p. 243) by X-irradiation of *st-DR* females. A single oyster-white son proved to be *oy.st.fsa*, the changes in *O* and in *A* (normal female fertility) presumably having occurred simultaneously. *oy-423* is conveniently run in a balanced stock with a scarlet lethal, the scarlet compounds being fertile, the oyster homozygotes sterile. The oyster males are of normal fertility. Two groups including 107 homozygous *oy-423* females recently set with many pupae which they stung many times, produced only 13 offspring, 0.12 per mother. In another recent check of the *oy-423* stock, 57 *oy-423* females set with 28 pupae produced 21 offspring, 0.37 per mother.

Among the female steriles listed by CASPARI (1958) were 11 scarlet that proved to be *fsa* by test with *oy-423*.

A scarlet female-sterile, *st-858* (*st-311* GBS), found in an X-ray experiment similar to CASPARI's, has been recently tested. Ninety-one homozygous females supplied with 49 host pupae stung the pupae and formed feeding tubes, lived for several days, but produced only 56 progeny, scarlet females and males, 0.615 per mother. Scarlet females compound for *oy-423* (*st-858/oy-423*) are of very low fecundity indicating *fsa*. From 158 of these set with 40 pupae there were obtained 120 offspring, 0.76 per mother. *St-858* has viability close to normal, wild-type compound (*oy-DR/st-858*) females producing 599 scarlet, 639 oyster sons.

A scarlet mutant son, *st-689*, (DTR) (WHITING 1955, p. 243) of an X-rayed wild-type female was mutant also in a factor for female sterility. Settings of 65 homozygotes resulted in no offspring and no eggs or larvae were found. Scarlet females (*st-689/oy-423*) derived from heterozygous females crossed to *oy-423* males were tested. Settings of 50 showed no evidence of eggs and no adult progeny, but one diapause larva was found. Both the homozygous scarlet and the

compound females sting the host pupae and feed as do normal females. In recent tests, three groups totalling 55 homozygous *st*-689 females given 32 pupae, which they stung many times, produced only six (scarlet) offspring, 0.11 per mother.

One of the *st fsa* mutant stocks, *st*-829, obtained by CASPARI has been maintained. Homozygous females totalling 380, set with about 110 pupae, produced 601 offspring, 1.58 per mother. Thirty-five females compound for *oy*-423, given 31 pupae, produced four offspring, only 0.114 per mother. It cannot be stated that differences between the groups in number of offspring per *fsa* female are genetical. They are more likely due to uncontrolled cultural conditions. Numbers of eggs of *fsa* females found and recorded vary from none to many. An accurate count is difficult or impossible if the offspring are to be allowed to develop. Many eggs were noted from two females but only one egg developed. No eggs were found from a setting of 20 females but three offspring appeared.

A dark red-eyed X-ray mutant-type, *da*-442 (GBS), which proved to be female sterile, has been discussed (WHITING 1961, pp. 442-443). Eye color is due to a fourth *R*-locus factor, *P*, and the female sterility, *fsb*, is nonhomologous with *fsa*. Compounds both with *oy*-423 (*oy.st.+.+.fsa.+/+.+.da.+fsb*) and with *st*-689 (*+.st.+.+.fsa.+/+.+.da.+fsb*) are wild type in eye color and of normal fertility. Neither *fsa* nor *fsb* appears to have any effect on male fertility.

"*Lethal-a, la*" genes: CASPARI's recessive lethals in compound with a gene having *fsa* (*oy*-423 or *st*-689) produced females sterile or of very low fecundity. This was taken to mean that factor *A* had undergone a deleterious modification more drastic than *fsa* so that it had a lethal effect, "*la*." Furthermore, the incidence of one of the lethals, *mh*-835, is on the pupae while the other three are egg lethals. Triple mutant alternatives of factor *A* were then suspected—female sterile, pupal lethal and egg lethal. Later studies on the diplo-triploid level have shown that "*la*" is not a simple unit but rather *fsa* masked by different lethal factors, *lx*. Formulae of the five "*la*" genes are thus expressed in Table 1.

st-812, carrying an egg lethal, in compound with normal alleles, forms highly fecund females. Of 78 females compound for *oy*-423, 55 were living after being set 22 days. All host pupae, 34, were stung, feeding tubes were formed and several eggs were laid, but no larvae developed. Transfers to fresh pupae showed stinging and formation of feeding tubes but no oviposition.

st-831 is similar to *st*-812. Twenty-three scarlet compounds with *oy*-423 set with 24 pupae laid a few eggs from which ten females developed. A transfer of the 21 females living after 11 days to six pupae resulted in only three offspring. Many (number not recorded) compounds with *st*-689 set with 17 pupae produced only 97 progeny.

da-817 is an egg lethal showing as dark red in compound with alleles having *st* in *S*. With *oy*-DR it produces wild type. A "Merry-Go-Round" (WHITING and CASPARI 1957) beginning with dahlia females, *da*-817/*pe*, crossed with *oy*-DR males produced (*F*₁) wild-type, *da*-817/*oy*-DR, and tinged, *pe/oy*-DR, females and peach males. About half of the females of each subsequent generation were tinged, the discards. The others, selected for breeding after mating with their brothers, alternated wild type in the odd generations, dahlia in the even. The

brothers were respectively, peach and oyster. This Merry-Go-Round was carried through 14 generations.

Twenty dahlia females, *da-817/st-689*, stung and fed on the 19 pupae supplied but laid few eggs from which there developed only two diapause larvae. Of these 20 females, 16 surviving after 15 days were fed five pupae but produced no offspring. Thirty-two dahlia females, *da-817/oy-423*, stung and fed on the 14 pupae supplied, laying several eggs from which developed four diapause larvae. The 17 females surviving after 22 days stung five pupae but laid no eggs. Total compounds with the tester genes, *st-689* and *oy-423*, were 52 from which were obtained only six offspring, 0.115 per mother.

Mahogany-835 has very dark red eyes determined by an *S* factor mutation (WHITING 1961). The gene carries a lethal with incidence at the late pupal stage or at eclosion. A few of the haploids are able to walk about feebly and even hold on to a card upside down. If puparia infected by compound unmated females are opened after emergence of the viable males and a count is made, the dark red inviables will be somewhat over 40 percent of the total sons. Total from unmated mahogany compounds for *pe*, *oy-423* or *st-DR*, were 797 dark red sons, 1060 of the other colors, 42.92 percent dark red.

A Merry-Go-Round was run through 17 generations beginning with mahogany females, *mh-835/pe*, crossed with *oy-423* males. Only a few mahogany females, *mh-835/pe*, were set in the even generations to produce an abundance of progeny, mahogany, *mh-835/oy-423*, and tinged, *pe/oy-423*, females, peach males, in the odd generations. From 452 *mh-835/oy-423* given about 214 pupae there were 508 progeny (121 mahogany, 107 tinged females, 145 oyster, 109 dark-red males, 26 diapause larvae) 1.124 progeny per mother. From 25 mahogany females, *mh-835/st-689*, supplied with 11 pupae, there were only six offspring, 0.24 per mother.

Oyster lethal-x genes: The 48 mutations to oyster white eyes listed by CASPARI (1958) appeared as females with tinged eyes, peach-oyster compounds. Eight of these were associated with a lethal factor so that no oyster males were produced. Seven of the lethals formed wild-type compounds with *st-DR* showing them to be *oy.+* in factors *O* and *S*. The eighth lethal, *oy-805*, gave scarlet compounds with *st-DR* and proved to be *oy.st*. Seven of the lethals including *oy-805* were tested with *fsa* genes such as *oy-423*, *st-689* and *st-829* and gave normally fertile compounds. At least six of the lethals were, therefore, *oy.+lx*. *oy-805* was *oy.st.lx*.

Four of the tested oyster lethals, numbered 800, 802, 804 and 828, showed incidence in the egg stage. *oy-801* permits a small but variable number of the haploid males to develop into small sized pupae. *oy-803* is a larval lethal. If puparia infected by unmated compound females are opened after the viable males have emerged, large numbers of half-grown larvae are found. After several days these die and dry without pupating. A count was made of sons of unmated wild-type, *oy-803/st-DR*, females. There emerged 282 viable scarlet. The puparia were then opened and the contents placed in alcohol to facilitate manipulation and separation. There were found 254 of the half grown larvae.

Merry-Go-Rounds may be made by combining an oyster lethal with viable oyster and scarlet. A balanced Merry-Go-Round had the three genes, *oy*-803, (*oy*.+.+.*lx*), *oy*-423 (*oy.st.fsa*+) and *st*-689 (+.st.fsa.+). The compound scarlet females, *oy*-423/*st*-689, appearing in every generation, are sterile. The compound females bearing the lethal, wild type and oyster in alternating generations, are allowed to mate with their brothers which are oyster and scarlet, respectively, to produce the next generation.

oy-828 makes a convenient balanced stock with *st*-689, the fertile females being wild type, (*oy*.+.+.*lx*/+.st.fsa.+), the males scarlet.

An oyster scarlet lethal-x gene: The triple mutant *oy*-805 (*oy.st.lx*) which produced scarlet compounds with *st*-DR (+.st.+) was maintained by crossing these compounds to *da*-GF males (*da*.+.+) to form a Merry-Go-Round. In each generation the red females are selected for breeding after they have mated with their red brothers. The wild-type females, compounds of the two genes lacking the lethal, are discarded. When the red females are scarlet, their brothers are dahlia and in the alternate generations the red females are dahlia and the males scarlet.

To maintain the stock of *oy*-805 it had been necessary to run it in a Merry-Go-Round in order to separate the red lethal-bearing females from their sisters. After *M*-factor mutant genes were obtained, however, these could be used. Scarlet compound females (*oy.st*.+.*lx*/+.st.+.+) were crossed to *da*-845 males (+.+.da.+). Daughters, all wild type, were crossed with *da*-845 males and set singly. From those producing only dahlia sons, no scarlet, a stock was derived having wild-type and dahlia females, dahlia males.

A vermilion lethal-x gene: *vm*-809 (+.*vm.lx*) in compound with *oy*-423 produces abundant offspring and therefore the deleterious factors are complementary. Its lethal incidence is delayed so that the majority of haploid males are able to eclose but very few or none emerge from the puparium through the small hole gnawed by their sibs. In gross structure they appear not to be abnormal. The deleterious factor may be thought of as bordering on male sterile but has been called a lethal because none is able to walk about, feed or mate, and they soon die. Eye color appears dark red. There is a full amount of scarlet pigment but considerable black clumped in masses throughout the eye tissue. In this aggregation of the black it differs from *vm*-MK. The color factor is *S*, giving complementary relationships similar to *st*-DR and scarlet color in compounds with peach and other genes *st* in *S*. For this reason the gene was at first called *st*-809.

Early counts of sons from mothers compound for a normal allele (*pe*, *ap*-837, *oy*-DR, *oy*-250) totalled only 389 against 2882. These 389 had fallen or had been pushed by their brothers out of the small hole pricked in the puparium. Daughters of compounds crossed with normal males were 519 compounds against 583 homozygous normal suggesting a dominant effect reducing viability slightly.

A stock was maintained with genes *vm*-809 (+.*vm.lx*) and *oy*-250 (*oy.st*.+). The scarlet compound females were selected for breeding after they had mated with their oyster brothers. Scarlet females were crossed with mahogany-819 males (*mh*.+.+, wild phenotype) and a Merry-Go-Round was run for several

generations, wild-type (*vm-809/mh-819*) and mahogany (*oy-250/mh-819*) females, oyster viable, vermilion inviable males, alternating with scarlet (*vm-809/oy-250*) and mahogany females, wild-type (*mh-819*) viable, vermilion inviable males.

The influence of the gene black-424 segregating independently of *R* is to remove all bright pigment, orange-336 (*R*-locus *or.st*) *bk-424* being white. *vm-809/bk-424* males are dark lavender and the scarlet compound females, *vm-809/or-336*, are changed to pale lavender if homozygous for black. The lavender color is probably due to reduced black pigment, absence of red, and a structural effect. Counts from pale lavender females (*vm-809/bk-424/or-336/bk-424*) crossed with white males (*or-336/bk-424*) totalled 577 pale lavender, 1647 white females and 69 dark lavender, 249 white males. The deficiency of pale lavender females, consistently found in the separate vials, suggests that reduction in viability of the compounds may be increased by *bk-424*.

Scarlet lethal-x genes: Eight of the scarlet lethal genes (*+st.lx*) tested with *oy-423* proved complementary, the compounds producing abundant offspring. Five of these have incidence in the egg stage. *st-474* is the only *R*-locus spontaneous mutation ever found associated with a lethal. Occurring in 1952, it has been bred ever since in compound with several other *R*-locus genes without loss of the lethal. *st-821* and *st-824* were obtained in the X-ray experiments of CASPARI. *st-855* and *st-856* are two of five scarlets obtained at the Summer Institute for High School Teachers held at the Duke University Marine Biological Station, Beaufort, N. C. in 1959. The plan of this experiment was similar to CASPARI's except that 3000 gamma from a cobalt source were used in the treatment of wild-type males. Of the other three of the five Beaufort scarlets, two were normal without a deleterious factor and the third, failing to sting the host pupae and being kept alive for 12 days by honey feeding, was possibly a dominant sterile (compare CASPARI's, 1958, "unknown" mutants).

The remaining three of the eight *+st.lx* scarlets are late pupal lethals. The haploid males of *st-811*, *st-813* and *st-830* differ from each other structurally but all appear less normal than *vm-809*. Counted sons from females compound for a normal allele were from *st-811*, 195 normal, 93 scarlet inviable; from *st-813*, 477 normal, 329 scarlet inviable and from *st-830*, 96 normal, 33 scarlet inviable. In all cases those counted as scarlet inviable had definitely recognizable scarlet eyes. Immature with indeterminate eye color are omitted. Before making the counts it is convenient to immerse the puparial contents in alcohol in a petri dish and to separate the specimens from the remnants of the fly pupa to which they tend to adhere. In this respect they differ from the males of *vm-809*.

A dominant semisterile, recessive lethal gene: Females compound for *st-823* are of very low fecundity. The stock was bred for a year through 19 generations. It was found necessary to set for breeding a large number of females after they had been exposed to their brothers. No records were kept of numbers used but usually almost all of the compounds were set in order to produce the next generation. The number of host pupae was reduced because of the few offspring ex-

pected. The gene was run with peach and *oy*-423 giving scarlet compound females and with *oy*-DR and *ap*-837 giving wild type.

During the first half of the year, recorded offspring totalled 910 compound and 1003 pale females, 1337 pale males, 39 diapause larvae. Incidence of the recessive lethal was assumed to be at the egg stage because no inviable larvae or pupae were observed.

It was then noticed that occasionally there was present a small pink- or scarlet-eyed pupa which failed to mature. Similar pupae were found with colorless eyes and young white pupae or prepupae were also noted. It became evident that these were the haploid *st*-823 males, dying in different developmental stages and hitherto unnoticed because of their low frequency and minute size. In bodily proportions they are not abnormal, but coloration appears anteriorly only.

Recorded offspring for the second half year were 949 compound and 954 pale females, 2671 pale males, 54 diapause larvae and 193 of the minute pupae with eyes ranging from scarlet through pink to colorless. Total lethal-bearing compounds, 1859, recorded for the year are but slightly lower in number than their pale sisters, 1957, indicating that dominant factors depress viability little if at all.

Percentage of females is low in the first half year, about 60 percent, and much lower in the second half, about 40 percent. The reason for the difference is not understood as the females were exposed equally to mating with their brothers at all times. It is probable that the gene, besides containing a recessive lethal, *l*, and a dominant semisterile, *Ss*, carries some factor, *Rf*, restricting fertilization of the eggs.

Tests made of pale sisters of the lethal bearers showed normal fecundity in all cases, indicating the dominant factor *Ss* to be inherent in gene 823 rather than something isolated by chance in the stock. Eighty-nine scarlet females compound for *oy*-423 were set producing 213 progeny (44 wild type, 53 apricot females, 90 oyster males, 26 diapause), 2.4 per *st*-823/*oy*-423 mother. Total recorded progeny from lethal bearers, compounds of *st*-823, are 8110. There were 1859 lethal bearers, most but not all of which were set for maintaining the stock. If all were set, offspring per mother would be 4.36. This is almost twice the average for the *oy*-423 compounds. Probably not more than 1500 were set which would give 5.4 offspring per mother. It seems highly probable, therefore, that *st*-823 carries the factor *fsa*.

There may, therefore, be four or more separate deleterious factors in the gene *st*-823 (+*st.fsa.lx.Ss.Rf*). Gene 823 is now being maintained on the diplo-triploid level (WHITING 1960a). The diploid males, *st*-823/*oy*-DR for example, (+*st.fsa.lx.Ss.Rf/oy*.+.+.+.+) are of normal fertility making it easy to maintain the stock and showing that the incidence of factor *Ss* is limited to the female.

Male steriles: The males of *st*-814 are very active, apparently accomplishing mating successfully. However, no offspring result. In earlier tests in seven different cultures totalling 125 peach females set with 293 *st*-814 males there were no daughters. Over 5000 peach sons were examined. In more recent tests involv-

ing 20 peach females set with 44 *st*-814 males in which many connections were observed, there were 1347 peach sons produced, no daughters. All cultures set of (scarlet) compounds with *oy*-423, totalling 129 females, produced an abundance of progeny indicating that the male-sterile of *st*-814 (*msx*) does not mask *fsa*. Counted sons of these compounds were 854 scarlet, 812 oyster and counted sons of (wild-type) compounds with *oy*-DR were 243 scarlet, 280 oyster, showing good viability of *st*-814 males.

Like males of *st*-814, *oy*-816 males are very active but have not been observed actually to accomplish mating. No daughters have been sired when *oy*-816 males have been set with many freshly eclosed females. Viability of males is normal compared with their brothers. Sons of compounds with scarlet, *st*-DR and *st*-689, total scarlet 999, oyster 1011. Among these, the sons of compounds with *st*-689 totalling scarlet 716, oyster 717 and many (uncounted oyster) offspring bred from compounds with *oy*-423 show that the male-sterile of *oy*-816 (*msx*) does not mask *fsa*.

Near steriles: Males of *st*-841 have viability practically equal to their normal brothers. Females compound for a pale eye color (*oy*-423, *pe*, *oy*-DR) produced 2285 scarlet and 2409 pale sons.

It is difficult to obtain homozygous females. The males are very active and aggressive, vigorously competing in attempts to mate, which they rarely accomplish. In the earlier work "many" cultures were set with scarlet males, none of which sired offspring. Later, a few successes were attained. In mass cultures reared from peach females that had been exposed to peach and scarlet brothers from compound mothers there were only 19 scarlet females among over 3200 peach females and males. These may have resulted from a single mating. A culture reared from 14 peach females exposed to 24 scarlet males produced 53 scarlet females, 732 peach males. A culture from 50 wild-type, *st*-841/*oy*-DR, virgins set with many scarlet males produced about 3000 sons (scarlet and oyster), but only five daughters and these were found in a single vial. Mass cultures from similar crosses were then made up and progeny examined but not counted. There were collected 84 scarlet females (homozygous). These, set with host pupae, died in less than four days being unable to feed, except that in one vial there were produced 19 offspring, seven wild-type females, 12 scarlet males.

It is suggested that only rarely is a scarlet male able to accomplish a mating, but if this occurs he sires daughters normally. The near-sterility of *st*-841 applies to both sexes. This near-sterility is *nsx*, nonhomologous with *fsa*. Many scarlet sons were produced from scarlet compound females, *st*-841/*st*-689, and the gene is conveniently kept in stock with *oy*-423 *pu*.

st-841 prevents crossing-over with purple body color. No undoubted crossovers have yet been found in the *st*-841/*oy*-423 *pu* cultures. Among sons bred from *st*-841/*pe pu* mothers there were 6520 parentals (*st* or *pe pu*) and five anomalous. Three of the latter were mosaics and two were probably crossovers with peach eyes and wild-type body although mosaicism is possible here also.

oy-840 was at first thought to be male-sterile. After several unsuccessful attempts to get matings of wild-type females compound for *st*-DR with *oy*-840

males, daughters were produced in six cultures. From three cultures, 62 oyster females were obtained. These were set with about 40 host pupae, but 35 flies eclosed so that most of the pupae were not stung. Only five progeny were produced, oyster males in one culture.

The gene causes near sterility in both sexes, but the males that accomplish mating sire a normal number of daughters. The compounds with *oy*-423 and *st*-689, *fsa*-bearing genes, are highly fertile. The stock is conveniently kept with *st*-689, the fertile females being wild type (*oy*.+.+.*nsx*/+.*st.fsa*.+).

Semilethals: Three scarlet semilethals found by MRS. CASPARI are being maintained. All are highly fertile and viable in compound with *oy*-423 and are, therefore, semilethal-*x*, *slx*.

st-808 can be bred in pure stock but deformed specimens are not infrequent in both sexes. Compounds for pale (*pe*, *oy*-DR) produced 849 scarlet, 1545 pale sons, 35.5 percent scarlet.

Homozygotes of *st*-810 produce few progeny and often fail to sting the pupae supplied. Compounds for pale (*pe*, *oy*-423, *ap*-837) produced 376 scarlet, 1547 pale sons, 19.55 percent pale. Many dead larvae and pupae were found and many scarlet adults had difficulty in eclosing.

Homozygotes of *st*-834 are of low viability. Two cultures from wild-type females, *st*-834/*oy*-DR, by *st*-834 males produced only 24 scarlet including five defectives, as against 177 wild-type daughters. Only three of these homozygotes were fertile producing 36 progeny. Compounds for pale (*pe*, *ap*-837, *oy*-DR) produced 177 scarlet, 3229 pale sons, 5.2 percent scarlet. There were many defectives among the scarlet.

Loss of deleterious factors from the markers and "crossing-over" within the genes: *st*-842 was shown by MRS. CASPARI to be female-sterile. Homozygous scarlet females, from wild-type, *st*-842/*oy*-DR, compound females crossed with *st*-842 males, were set at five different times from January to March 1956, but in none of the tests were offspring produced. Three different settings of scarlet females, *st*-842/*oy*-423 (+.*st*.+."*fsc*"/*oy.st.fsa*.+) produced abundant scarlet and oyster sons, showing that the female-sterility factor, "*fsc*" of CASPARI, was not *fsa*. Nonhomology was also shown with near-sterile-*x*, *nsx*, in *st*-841, "*fsb*" of CASPARI. Scarlet compound females, *st*-842/*st*-841 (+.*st*.+."*fsc*"/+.*st*.*fsb*.+) were obtained from wild-type, *st*-842/*oy*-DR, females crossed with *st*-841 males and from wild-type, *st*-841/*oy*-DR, females crossed with *st*-842 males. From both crosses the scarlet compound daughters were highly fertile, producing many scarlet sons. It may then be concluded that *fsa* and CASPARI's two factors "*fsb*" and "*fsc*" are nonhomologous.

In two tests made several months later, homozygous *st*-842 females proved sterile, failing to sting the pupae, but in others, scarlet sons were produced. It was then found that all scarlet females tested in the stock were fertile so that the sterility factor, "*fsc*" of CASPARI, was lost.

Two explanations for the loss of "*fsc*" may be considered. (1) It may have been at a distance from the eye-color factors, too far to be integrated into the gene so that it crossed over. (2) *st*-842 had been run with peach from the original mutant

female which was *pe/st*-842. It is possible that a spontaneous mutation in the *O* factor, unstable *O.st*, of peach occurred, changing *pe.st* to *+.st*. By chance this may have replaced *st*-842 in the stock.

CASPARI's tests with *st*-815 indicated male sterility, but later, fertile scarlet males appeared and the male sterility was lost. Many cultures were bred from females compound for peach or for apricot-837 but males were counted from only 17. In each of 12 of these there was a marked deficiency of *st* below the equality expected. These totalled 514 *st*, 995 pale (*pe* and *ap*). The other five cultures each showed near equality, totalling 247 *st*, 252 *pe*. Loss of deleterious factors by crossing-over may here be suggested. Male sterility may have been lost by crossing-over during the first few generations and a lethal or semilethal more closely linked to the marker, scarlet, retained for a longer period and subsequently lost.

A single wild-type brother of the expected oyster sons of *st*-474/*oy*-DR (*+.st.lx/oy.+.+*) females, proved to be diploid (WHITING 1960a, p. 954), not a crossover between *O* and *S*.

Two "wild-type" males occurred among the dahlia sons of an *oy*-805/*da*-GF (*oy.st.lx/da.+.+*) female. Each of these bred to *oy*-250 (*oy.st*) females sired many *da* daughters. Total counted offspring of the females mating with their *oy.st* brothers were 912 red, 770 oyster females; 623 "wild type," 597 red, 1194 oyster males. These are the results to be expected if the two "wild-type" males were *da*-GF ebony, which closely resembles wild type. This new spontaneous mutation has been called *eb*-577.

Three normal scarlet males appeared in one of the cultures from the *st*-823/*pe* stock. They were tested by mating with *ap*-837 females and bred like normal *st*-DR (*+.st*). It is possible that they may have resulted from a crossover between the color factors and the deleterious factors of 823 or the *O* factor of peach may have mutated to *+*, unstable-*O* (WHITING 1960a) changing *pe.st* to *+.st*.

Recently there were found in *oy*-803/*st*-DR stock, 21 white males in addition to 152 of the expected scarlet color suggesting that the oyster-white gene had lost the lethal factor by crossing-over. Analysis showed that the viable white had nothing to do with *oy*-803 but was a mutation to black, *bk*-425, which gives a white phenotype, *st*-DR *bk*-425, in combination with scarlet.

Occasionally an oyster male has appeared among the progeny of a wild-type compound female (*oy.+.lx/+.st.+*), suggesting that the lethal may have been lost by crossing-over. Such a one, which produced no progeny, was found among the 1391 scarlet males in a culture from *oy*-801/*st*-DR females. A single oyster male was found among the dahlia brothers from *oy*-805/*da*-GF mothers but this sired no daughters.

In the earlier work with gene *oy*-828 seven oyster sons appeared among "many" scarlet brothers from mothers compound for *st*-689 (*oy.+.+.lx/+.st.fsa.+*). The stock thus became contaminated with oyster lacking the lethal and wild-type females produced many oyster sons. For the most part, scarlets were recorded as "many" but from cultures in which counts were made there were 4404 scarlet, three oyster (0.07 percent oyster), the latter scattered so that

each must have been a separate crossover. Oyster females were obtained by crossing the wild-type compounds with oyster males and a stock was derived similar to *oy-DR*. It appears that for *oy-828* the lethal is at a distance from the color marker so that it may be separated by crossing-over.

DISCUSSION

CASPARI (1958) listed 206 eye color mutants among the 11,062 daughters of wild-type males X-rayed with 1000–5000r which were mated to peach purple females. Successful breeding tests resulted from 153 of these. Thirty-seven did not lay, probably because of being dominant steriles. Seven dying as dark pupae or at eclosion were probably dominant lethals. It cannot be determined how many *R*-region dominant steriles or dominant lethals there were, unmarked by a simultaneous eye-color change or how many dominant lethals occurred potentially marked but dying too young to be detected.

Were a deletion induced including the wild-type alternative to *pe* or to *st*, a lethal would be expected showing the recessive maternal peach eye color but such was not found. One of the five scarlet Beaufort gamma-ray mutants which failed to lay was probably a dominant sterile. The other four (two scarlet normal, two scarlet lethal) may be included with CASPARI'S 153 tested and *st-858* (+.*st*.+.+.*fsa*.+), *da-442* (+.+.+.*da*.+.*fsb*) and *mh-441* (*mh*.+.+.+.+) may be added, making up a total of 160 tested *R*-locus eye-color mutants sired by irradiated wild-type fathers. It will be of interest to consider these in the light of recent tests. CASPARI'S *da-838* has been shown to be a double mutant (*rdh*.+.*da*.+) and her *da-835* should be placed under *S* as *mh-835* (+.*mh*.+.+.*fsa*.*lx*) (see WHITING 1961, pp. 441–442).

These 160 tested genes divide equally into 80 normal and 80 with deleterious factors—recessive lethal 54, recessive semilethal 3, dominant semilethal 2, female-sterile 15, male-sterile 4 and near-sterile 2. At least one of the normal possesses a crossover suppressor and among the deleterious there are some having more than one deleterious factor. It is clear then that when an eye-color mutation occurs it is frequently accompanied by damage in the region close enough to form an integral part of the gene. This frequency differs according to the eye-color factor mutating. Of the 45 *O*-factor changes, 36 were normal (34 oyster, two dark red), nine deleterious (nine oyster). Of the 107 *S*-factor changes, 39 were normal (39 scarlet), 68 deleterious (66 scarlet, two dark red).

Of the five *O S* double mutants (*oy.st*), three were normal, two deleterious. One normal *O M* double mutant (*rdh*.+.*da*.+) and one normal *M* mutant (+.+.*da*.+) occurred and there was a deleterious *P* mutant (+.+.+.*da.fsb*).

These data suggest that a sensitive region subject to deleterious changes may be closer to factor *S* than to factor *O*. Further data respecting factors *M* and *P*, if such become available, will be of much interest.

Five of the genes discussed in the present paper have lethal factors which were designated "*la*." There is evidence that "*la*" differs in different genes, and that this difference consists in *lx* factors which mask *fsa*. It is planned to discuss these

matters in a future paper presenting data drawn from studies at the diplo-triploid level (WHITING 1960a).

Female sterility other than that of the *fsa* type is relatively infrequent. None of the female steriles which have been adequately tested, including the dominant of *st-823* which has been called semisterile, *Ss*, is complete. In all of these cases the incidence of the factor is on the semisterile female herself.

The term semisterile has frequently been applied in plants and in *Drosophila* in which chromosomal interchanges and balanced lethals occur (BURNHAM 1956). Here the incidence is on the offspring, some of which are inviable due to lethal combinations, the parent being affected very little or not at all. Triploids might be called semisterile in the sense of the word.

JOHANSEN (1911, p. 132) failed to distinguish units of heredity from units of segregation. He coined the word "gene" to fit both concepts supposing that "modern Mendelian researches" had demonstrated the existence of "elements" in the gametes. WRIGHT (1958) in discussing the term "gene," called attention to the ambiguity in the frequent use of that term for both the common substrate of alternative units and a particular one of them. He used the term "locus" for the substrate and restricted "gene" to a particular representative of a locus. WRIGHT's "substrate" corresponds to my "region" (WHITING 1961, p. 443) which exists as a part of the germ plasm of the species whether it contains any theoretical "elements" or not and whether or not any units of segregation, "genes," have been formed by mutation. There is no reason to suppose that a "region," a "substrate" for the formation of genes, acts in the wild type as a "locus," a unit of segregation. Mutation strikes a region, gathering miscellaneous genetical materials into a mutant gene which then acts as a unit of meiotic segregation and thus forms a locus, a point in the genetical sense.

There is an ambiguity in the word "factor," somewhat analogous to that indicated by WRIGHT for "gene" in that it may indicate (1) the basis for a unit function whether normal, injured or otherwise changed, or (2) a particular state or condition of that function. Thus a lethal factor or a sterility factor is an injured condition of a factor which previously existed in a state to function normally. The word "factor" standing alone is ambiguous, but the context makes clear what is meant.

I will not dispute the existence of "cistrons" as units of heredity, of "recons" as units of segregation, or of "mutons" as units of mutation (BENZER 1957). These are all of a lower order of magnitude than "genes." "Factors" includes cistrons but also deletions, repeats and inversions which serve to integrate genes and a single mutation may simultaneously affect more than one cistron as well as other factors in the region.

The *R* region in *Mormoniella* is of advantage for genetical study in that it has two factors, cistrons *O* and *S*, which mutate with relatively high frequency to oyster and to scarlet eye color respectively. These colors serve as markers for new mutant genes. It may be supposed that many other regions may also be induced to mutate frequently but unless a marker is simultaneously produced it will be

difficult to detect mutations. Undetected genes, containing deleterious factors only, may soon be lost.

SUMMARY

New data are presented on 37 *R*-locus mutant genes containing deleterious factors or crossover suppressors. Twenty-seven of these genes are among the 206 recognizable eye-color mutants found by MRS. CASPARI (1958) in experiments in which wild-type males were irradiated and mated, siring 11,062 daughters examined, mutation rate 1.86 percent. With the addition of eight mutants from later similar tests there were 38 mutants failing to breed, probably being dominant steriles, and seven immature, probably dominant lethals.

Of the 160 mutants given successful breeding test, 80 were normal and 80 contained deleterious factors of different types—lethals, near-steriles, female-steriles and male-steriles.

Rate of deleterious factor production differs according to the factor mutating to produce the eye-color marker. Among the 107 genes changed in factor *S* alone, 68 were also deleterious (63.6 percent) but for the 45 in *O*, there were only nine (20.0 percent), suggesting a more sensitive region near *S*.

More than one deleterious factor may be produced simultaneously with an eye-color change and one defect may mask others.

The “gene” which forms a temporary unit of segregation in heterozygotes is of a higher order of magnitude than units of heredity (gene elements, cistrons) which may be permanently present in the germ plasm.

Because of the high mutation rate to the marker eye colors scarlet and oyster white, the genetical structure of the *R* region may be easily studied.

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