SPECIFIC GENETIC EFFECTS OF DNA IN *DROSOPHILA MELANOGASTER*[1]

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THE development of methods for the preparation of native DNA from *Drosophila melanogaster* (MEAD 1964), and of a means of obtaining large numbers of fertilized, permeable eggs by use of the "ovitron" (YOON and FOX 1965a), rendered it profitable to undertake a study of the genetic effects of DNA treatment. Previous attempts to induce DNA-mediated transformations in eukaryotes have not achieved their objective. The most suggestive of these attempts used special screening techniques to detect specific, DNA-induced changes in human cells growing in tissue culture (SZYBALSKA and SZYBALSKI 1962). While changes were detected in one system, none could be demonstrated in a second and the methods used could not exclude mechanisms other than that of transformation. Experiments with Drosophila have yielded no evidence of locus-specific transformations, but have revealed a rather special kind of mutagenesis which will be discussed below (FAHMY and FAHMY 1961, 1965; GERSHENSON 1939, 1965; GERSHENSON and KISSELIOVA 1958; MATHEW 1965). A system of potential value in Ephestia has been reported (CASPARI and NAWA 1965). Reports of specific transformations in the domestic duck have not been confirmed (BENOIT, LEROY, VENDRELY, and VENDRELY 1957, 1960).

The method used in the present work consists of treatment of Drosophila embryos of particular genotypes with "heterologous" DNA prepared from adults of genotypes differing at specified loci, or with "homologous" DNA prepared from adults of the same genotype. After eclosion the treated flies are examined for somatic changes, whole-body or mosaic, for the loci in question. They are also mated to test for transmission of induced changes to subsequent generations.

The results, part of which have been published in abstract form (FOX and YOON 1965; YOON and FOX 1965b), demonstrate that heterologous DNA induces a highly significant increase of somatic mosaicism at the loci differing in treated flies. A similar increase in somatic mosaicism is observed in their progeny and in subsequent generations. No whole-body changes have been observed either among the treated flies or in subsequent generations. Thus, while the induced changes represent cases of "replicating instabilities" (AUERBACH 1946; MATHEW 1964), it is premature to refer to them as involving transformation in the same sense as in bacterial systems.

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MATERIALS AND METHODS

Preparation of DNA: DNA was prepared from adults according to the method of MEAD (1964). The method includes extensive deproteinization, yielding a DNA-RNA complex which is then subjected to RNase digestion (50 μg/ml, 37°C, 30 minutes) followed by further deproteinization. The final product is free of detectable protein and RNA, exhibits a large hyperchromic shift when heated ($T_m = 84°C$ in 0.1M NaCl), has a guanosine + cytosine content of 39.1%, and a molecular weight of 800,000 to 850,000 ($S_{20,w} = 9.0$). Preparations from all of the stocks used in the present work exhibit these properties; a more detailed analysis of the preparations actually used in these experiments is in progress.

Collection and treatment of eggs: The ovitron was used to collect eggs at hourly intervals from inseminated females. Because of their impermeability, eggs collected at the end of the first hour were discarded. Those collected subsequently were immediately dechorionated to increase permeability, and immersed in a modified Ringer solution (YOON and FOX 1965a), or in the appropriate DNA solutions (0.02 mg/ml in modified Ringer) for 7 to 18 hours at 26°C. At the time of collection the eggs range in stage of embryonic development from that immediately preceding pronuclear fusion to that of rapid cleavage divisions (YOON and FOX 1965a). After 7 additional hours they would have completed gastrulation, and after 18 additional hours larval differentiation is nearly complete (POULSON 1950). Within the range studied, the length of exposure to DNA does not seem to affect the results. This agrees with our previous observation (unpublished) that ovitron eggs become impermeable several hours after collection. It is probable that the effective period of treatment ends by the time blastoderm formation is complete (3 to 4 hours).

For each treatment approximately 200 eggs were immersed in 2.0 ml of the appropriate solution. At the end of the treatment the eggs were transferred to standard cornmeal, molasses, agar medium from which mold-inhibitor and living yeast had been omitted; approximately 30 to 40% emerge as adults. The flies were examined as they emerged, and mated as required.

Genetic systems: Two experiments have been performed. In the first, *sc cv f; In(3)MRS, M(S)34 ry² Sb/rucuca* females were mated with males of the same genotype and placed in the ovitron. The mutants *sc, cv,* and *f* are sex-linked recessives. One third-chromosome carries an inversion to inhibit crossing over, a dominant Minute mutant, the recessive rosy-2, and the dominant mutant Stubble. The rucuca third-chromosome carries the recessive mutants *ru, h, th, st, cu, sr, e^s,* and *ca* (BRIDGES and BREHME 1944). In the absence of crossing over, the following kinds of zygotes are present among the eggs collected from such females: (1) *sc cv f; In(3)MRS, M(S)34 ry² Sb/In(3)MRS, M(S)34 ry² Sb.* These die as young embryos. (2) *sc cv f*; rucuca/rucuca. These are of low viability, are readily detectable, and are discarded upon emergence. (3) *sc cv f; In(3)MRS, M(S)34 ry² Sb*/rucuca. This is the pertinent class. The heterologous DNA used for treatment was prepared from homozygous rucuca adults. This DNA would carry the following genetic markers: *sc+, cv+, f+, ru, h, th, st, cu, sr, e^s, ca.* Thus, the object was to induce the following changes in treated embryos. (1) On the X chromosome: *sc* to *sc+*, *cv* to *cv+*, *f* to *f+*. Since these changes are to dominant alleles, they would be detectable both in males and females. (2) On the *In(3)MRS, M(S)34 ry² Sb* chromosome: *ru+* to *ru*, *h+* to *h*, *th+* to *th*, *st+* to *st*, *cu+* to *cu*, *sr+* to *sr*, *e+* to *e^s*, *ca+* to *ca*. Although these are changes to recessives, they would be opposite the corresponding recessives on the rucuca chromosome and would be detectable.

In the second experiment, *y w sn^3* eggs were treated with heterologous Oregon-R-EL2 DNA. This DNA should carry the dominant wild-type alleles for the three sex-linked loci: *y+*, *w+*, and *sn+*. The changes sought were *y* to *y+*, *w* to *w+*, and *sn^3* to *sn+*. They would be detectable both in male and female flies.

Scoring: Crossing over between the heterozygous third chromosomes was observed in the first experiment in spite of the presence of *In(3)MRS*, and this rendered useless the scoring of whole-body changes for any of the rucuca recessives. Classification difficulties in that experiment also rendered useless scoring for changes at *f, sr,* and *e^s*. Otherwise, all flies were scored both for whole-body and mosaic changes. The phenotypic criteria used for distinguishing mosaics are given in Table 1.

TABLE 1

Phenotypic criteria used for scoring mosaicism

Change	Phenotypic criteria for mosaicism
sc→*sc*+	Four scutellar bristles but with bristles missing in any of following areas: orbital, notopleural, postvertical.
cv→*cv*+	Partial or complete crossvein, unilateral.
y→*y*+	Patch of black bristles (one or more), or patch of pigmented hypoderm, or both.
w→*w*+	Patch of red ommatidia (one or more), or red ocellus, or both.
sn^s→*sn*+	Patch of straight bristles (one or more).
ru+→*ru*	Patch of disarranged facets or complete disarranged eye, unilateral.
h+→*h*	Patch of extra hairs (one or more) on one of following: scutellum, veins, pleurae, head.
th+→*th*	Unilateral or partial curled wing, or erect and crossed post-
st+→*st*	Patch of scarlet ommatidia (one or more), or colorless ocellus (one or both), or both.
cu+→*cu*	Unilateral or partial curled wing, or erect and crossed postscutellar bristles, or both.
ca+→*ca*	Patch of claret ommatidia (one or more), or reduced ocellar pigmentation (one or more), or both.

A coding system was used to prevent the observer from knowing the treatment received by the flies under examination. In the first experiment all of the scoring was performed by one observer (S.B.Y.), but a large number of mosaics were examined by the second observer (A.S.F.) with no disagreement about classification. In the second experiment, the two observers scored approximately equal numbers of flies, and their results exhibited no significant differences when they were decoded at the end of the experiment. The data of both experiments exhibit no evidence of temporal shifts in classification.

RESULTS

Frequency of somatic mosaicism among treated flies: No whole-body changes were observed among treated flies for the sex-linked loci studied in experiments 1 and 2. Whole-body changes observed for the third-chromosome loci in experiment 1 were no more frequent among DNA-treated flies than among Ringer-treated controls, and could be attributed to crossing over in the female parents. The absence of whole-body changes is not unexpected: such changes would arise only as a result of genetic alterations occurring prior to the initiation of cleavage.

The observed frequency of mosaics among treated flies is given in Table 2. In experiment 1, the frequency of mosacism among flies treated with rucuca DNA was more than twice that among Ringer-treated controls. No treatment with homologous DNA was performed. In experiment 2, treatment with heterologous Oregon-R DNA produced about four times as many mosaics as did treatment with homologous *y w* sn^s DNA, and about 20 times as many as did Ringer treatment. Chi-square analysis in 2 × 2 contingency tables discloses that heterologous DNA induces a highly significant increase in the frequency of mosaics both in comparison with homologous DNA and with Ringer ($P < 0.001$). In experiment

TABLE 2

Frequency of mosaics among treated flies

Experiment	Treatment	Mosaics		Total number of flies examined
		Number	Frequency	
1	rucuca DNA	150	0.026	5,666
	Ringer	16	0.011	1,405
2	Oregon-R DNA	47	0.040	1,173
	y w sn^3 DNA	16	0.009	1,839
	Ringer	2	0.002	980

2, on the other hand, while homologous *y w sn*^{3} DNA induced a higher frequency of mosaicism than did Ringer the difference is of doubtful significance ($x^2 = 3.5$, $P = 0.05 - 0.10$).

Table 3 gives the frequences of mosaics induced by the individual preparations of heterologous and homologous DNA used in experiment 2. Although there is considerable variation from preparation to preparation, much of this is probably attributable to the small number of flies treated with some preparations, and the frequency of mosaicism induced by homologous DNA never exceeds that induced by heterologous DNA. Even if the results obtained with *y w sn*^{3} DNA preparation number 12 are omitted, the difference between the effects of heterologous and homologous DNA is highly significant ($P < 0.001$).

If the "target" hit by heterologous DNA is chromosomal, and all other factors are equal, twice as many sex-linked mosaics should be induced in females with two X chromosomes as in males with one. On the other hand, the frequency of third-chromosome mosaics should be equal in the two sexes, since both have a single target chromosome. In point of fact, for the sex-linked loci involved in both experiments, 20 sex-linked mosaics were observed among 3,371 males treated with heterologous DNA (frequency = 0.0059), while 37 out of 3,468 females exhibited sex-linked mosaics (0.0107). No such difference between the sexes was observed among flies treated with homologous DNA or Ringer. For the third-

TABLE 3

Effects of individual DNA preparations used in experiment 2

Source of DNA	Preparation No.	Total No. of flies examined	Mosaics	
			Number	Frequency
Oregon-R	2	867	38	0.044
	3	158	5	0.032
	4	148	4	0.027
y w sn^3	1	131	2	0.016
	2	65	0	0
	4	12	0	0
	5	264	7	0.026
	7	151	1	0.007
	12	1216	6	0.005

TABLE 4

*Distribution of mosaics among loci scored in experiment 1**

Treatment	Mosaics								
	sc+	*cv*+	*ru*	*h*	*th*	*st*	*cu*	*ca*	Total
rucuca DNA	8	2	80	45	0	3	6	6	150
	(0.0014)	(0.0003)	(0.0141)	(0.0079)	(0)	(0.0005)	(0.0011)	(0.0011)	(0.0265)
Ringer	0	0	12	4	0	0	0	0	16
	(0)	(0)	(0.0085	(0.0028)	(0)	(0)	(0)	(0)	(0.0114)

* Frequencies given in parentheses.

chromosome loci involved in experiment 1, 61 out of 2,753 males were mosaics (0.022) while 79 out of 2,913 females exhibited mosaicism (0.027).

The distribution of mosaics among the eight loci scored in experiment 1 is given in Table 4. Among flies treated with rucuca DNA, mosaics were observed for all loci except *th*. Only *ru* and *h* mosaics were observed among Ringer-treated controls. The frequency of rucuca DNA-induced mosaicism for all eight loci, taking into consideration the difference between males and females in number of sex-linked genes, is 2.88×10^{-3} per treated gene. The 95% confidence interval around this value, calculated by a modification of the method of KIMBALL (1956), ranges from 1.68×10^{-3} to 4.88×10^{-3}. The method assumes, however, that the distribution of mosaics among loci is a negative binomial distribution, and a chi-square test of this assumption reveals that the frequencies exhibited by *ru* and *h* are too high. They may, therefore, be especially susceptible to treatment with heterologous DNA. If they are omitted, the frequency of mosaicism induced by rucuca DNA among the remaining six loci is 0.57×10^{-3}. The 95% confidence interval for this value has an upper limit of 1.16×10^{-3}, and a lower limit of 0.26×10^{-3}. It is, therefore, significantly higher than zero, which is the frequency of mosaics among Ringer controls if *ru* and *h* are omitted.

The distribution of mosaics among the three loci scored in experiment 2 is given in Table 5. Although no w^+ mosaics were observed among the flies treated with heterologous DNA, data presented below show that these flies transmitted

TABLE 5

*Distribution of mosaics among loci scored in experiment 2**

Treatment	Mosaics			
	y+	*w*+	*sn*+	Total
Oregon-R DNA	30	0	17	47
	(0.026)	(0)	(0.014)	(0.040)
y w sn^s DNA	6	2	8	16
	(0.003)	(0.001)	(0.004)	(0.009)
Ringer	1	0	1	2
	(0.001)	(0)	(0.001)	(0.002)

* Frequencies given in parentheses.

w^+ mosaicism to their progeny. The frequency of induced mosaicism for all three loci, adjusted for the difference between sexes in number of sex-linked genes, is 8.2×10^{-3} per treated gene, with a 95% confidence interval ranging from 3.4×10^{-3} to 18.6×10^{-3}. If *w* is omitted, the corresponding frequency for the two remaining loci is 12.41×10^{-3}, with a 95% confidence interval between the limits of 3.91×10^{-3} and 36.14×10^{-3}.

Transmission of mosaicism to F_1 and subsequent generations in experiment 2: If the mosaicism induced by heterologous DNA is of the conventional sort, it should sometimes extend into the gonads and treated flies should give rise to clusters of offspring exhibiting whole-body changes. The most intensive test for the transmission of the effects of heterologous DNA was performed in experiment 2. In that experiment, treated (mosaic and nonmosaic) *y w sn*^{3} males were mated either to untreated doubly attached-X females (*y f* : =/*Y*) or to untreated *y w sn*^{3} females, while treated (mosaic and nonmosaic) *y w sn*^{3} females were mated with untreated *y w sn*^{3} males. The results of these matings are given in Table 6.

The most striking feature of these results was the absence of whole-body changes among the F_1 progeny. Instead, the progeny of flies treated with heterologous DNA exhibited a marked increase of mosaicism. The pooled data are given in Table 7, where it may be seen that the frequency of mosaicism among the progeny of flies treated with Oregon-R DNA is 3 to 4 times as high as the frequency among the progeny of flies treated with *y w sn*^{3} DNA or Ringer. This difference is highly significant in both cases ($P<0.001$), while the difference between the F_1 of *y w sn*^{3} DNA-treated and Ringer-treated flies is not significant ($P = 0.50$–0.70). It may be concluded that the mosaicism induced by heterologous DNA is transmitted *per se* to the progeny of treated flies. This transmission occurs even if the treated fly does not itself exhibit mosaicism.

TABLE 6

Frequency of mosaicism in F_1 of experiment 2

Treatment	No. treated flies tested*	Mosaics in F_1						Total no. F_1 examined
		y$^+$		*w*$^+$		*sn*$^+$		
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
(a) Treated *y w sn*^{3} ♂ × *y f* : =/Y ♀ ♀.								
Oregon-R DNA	8	0	0	4	0	0	0	612
Ringer	3	0	0	0	0	0	0	168
(b) Treated *y w sn*^{3} ♂ × *y w sn*^{3} ♀ ♀.								
Oregon-R DNA	313	0	22	1	3	1	1	21,413
y w sn^3 DNA	258	2	2	0	1	0	2	15,506
Ringer	162	2	2	1	0	1	1	12,958
(c) Treated *y w sn*^{3} ♀ × y w sn^3 ♂ ♂.								
Oregon-R DNA	245	3	12	7	5	5	8	13,258
y w sn^3 DNA	192	0	1	0	2	5	1	8,480
Ringer	132	0	0	2	0	1	0	7,329

* Treated males were tested in (a) and (b); treated females were tested in (c).

TABLE 7

Summary of mosaicism observed in F_1 of experiment 2

Treatment of parents	Mosaics in F_1		Total number of F_1 flies examined
	Number	Frequency	
Oregon-R DNA	72	0.0020	35,283
y w sn^3 DNA	16	0.0007	23,986
Ringer	10	0.0005	20,455

It should be noted that frequency of mosaicism in the F_1 of flies treated with heterologous Oregon-R DNA (0.002) is well below the frequency in the treated parents themselves (0.040). This reduced frequency is of the same order of magnitude as was observed among the homologous-DNA and Ringer controls in the treated generation (0.009 and 0.002, respectively). Furthermore, the frequency of mosaicism in the F_1 of homologous DNA-treated and Ringer-treated flies (0.0007 and 0.0005) is well below that exhibited by their parents. It seems possible, therefore, that the collection of eggs in the ovitron and their immersion during the treatment period results in an increased incidence of mosaicism. This does not obviate the effects of heterologous DNA.

The locus distribution of mosaics observed in the F_1 is given in Table 8. Among the progeny of Oregon-R DNA-treated flies, the frequency of y^+ mosaicism was highest while that of sn^+ mosaicism was lowest. w^+ mosaics were observed in the F_1, even though none were seen in the treated flies themselves. This also indicates that the effect produced by heterologous DNA is transmitted to the progeny even if it is not expressed in their treated parents.

There was, however, a correlation between mosaicism in the treated generation and that observed in the F_1. Among 2,318 progeny from 36 Oregon-R DNA-treated mosaics, 19 were mosaic (frequency = 0.0082). Among 32,965 progeny from 530 Oregon-R-DNA-treated nonmosaics, only 53 were mosaic (0.0016). This difference is highly significant. Furthermore, 24 y^+ mosaics yielded 13 y^+ and 5 w^+ mosaic progeny (out of 1,614), while 12 sn^+ mosaics yielded 1 sn^+ mosaic

TABLE 8

*Distribution of mosaics among loci in F_1 of experiment 2**

Treatment of parents	Mosaics in F_1			
	y^+	w^+	sn^+	Total
Oregon-R DNA	37	20	15	72
	(0.0010)	(0.0006)	(0.0004)	(0.0020)
y w sn^3 DNA	5	3	8	16
	(0.0002)	(0.0001)	(0.0003)	(0.0007)
Ringer	4	3	3	10
	(0.0002)	(0.0001)	(0.0001)	(0.0005)

* Frequencies given in parentheses.

and no y^+ or w^+ mosaics (out of 704). Thus, there seems to be a tendency for heterologous DNA-treated mosaics to transmit their own type of mosaicism.

Further information regarding the transmission of mosaicism by treated flies to their F_1 progeny may be derived from Table 6. Restricting attention to the progeny of Oregon-R DNA-treated flies, all four of the F_1 mosaics obtained from the matings of treated *y w sn*^{3} males to attached-X females were males. Of the 28 F_1 mosaics obtained from matings of treated males with *y w sn*^{3} females, 26 were females. Among the 40 F_1 mosaics obtained from treated females, 15 were males and 25 were females. Thus, the transmission of mosaicism by heterologous DNA-treated flies parallels the transmission of the treated X chromosomes. The effect produced by heterologous DNA appears to be transmitted with the chromosome carrying the locus which is ultimately affected. The data discussed in the previous paragraph suggest that this association is with the affected locus itself.

The transmission of y^+ and w^+ mosaicism was followed for three additional generations (i.e., to the F_4) by mating mosaic flies with untreated *y w sn*^{3}. The data are summarized in Table 9. No whole-body changes were observed. Both y^+ and w^+ mosaicism were transmitted to the end of the experiment. It is interesting to note that w^+ mosaicism was observed in the F_2 and F_3 of lines in which it had not previously been evident.

Transmission of mosaicism in experiment 1: In experiment 1, rucuca DNA-treated *sc cv f; In(3)MRS, M(S)34 ry*^{2} *Sb*/rucuca flies were mated with rucuca homozygotes. All of the progeny were examined for DNA-induced effects transmitted in association with the treated *In(3)MRS, M(S)34 ry*^{2} *Sb* chromosomes, while the sons of treated females were examined for effects transmitted in association with treated *sc cv f* chromosomes. Whole-body changes were observed among the progeny, but these were always associated with crossing over between the *In(3)MRS, M(S)34 ry*^{2} *Sb* chromosome and its rucuca homologue. Mosaic progeny were also observed, and some of these were subjected to appropriate matings to follow transmission into subsequent generations.

168 rucuca DNA-treated flies, of which 48 were mosaics, were tested for transmission. Sixteen yielded one or more mosaic offspring; these are listed separately

TABLE 9

Transmission of Oregon-R DNA-induced mosaicism to subsequent generations in experiment 2

Mosaics tested*		Mosaics among progeny			Total number of progeny examined
Generation	Number	Generation	y^+	w^+	
(a) Transmission by y^+ mosaics.					
F_1	26	F_2	24	1	1,214
F_2	17	F_3	10	1	544
F_3	2	F_4	1	0	150
(b) Transmission by w^+ mosaics.					
F_1	13	F_2	0	4	1,926
F_2	2	F_3	0	1	59
F_3	1	F_4	0	1	114

* y^+ mosaics were tested in (a), w^+ mosaics in (b).

TABLE 10

Transmission of rucuca *DNA-induced mosaicism to subsequent generations in experiment 1*

Mosaicism in treated fly	Mosaicism transmitted	Number generations transmitted	Number generations followed
ru	*ru*	1	2
sc+	*sc*+	1	1
sc+	*sc*+	1	1
sc+	*sc*+	2	2
sc+	*sc*+	2	2
sc+	*sc*+	2	2
ru	*cu*	7	7
h	*sc*+	1	1
ca	*ru* and *sc*+	1	1
none	*sc*+	1	1
none	*ru*	1	2
none	*ru*	1	2
none	*ru*	1	2
none	*ru*	1	1
none	*h*	1	1
none	*cu*	1	1

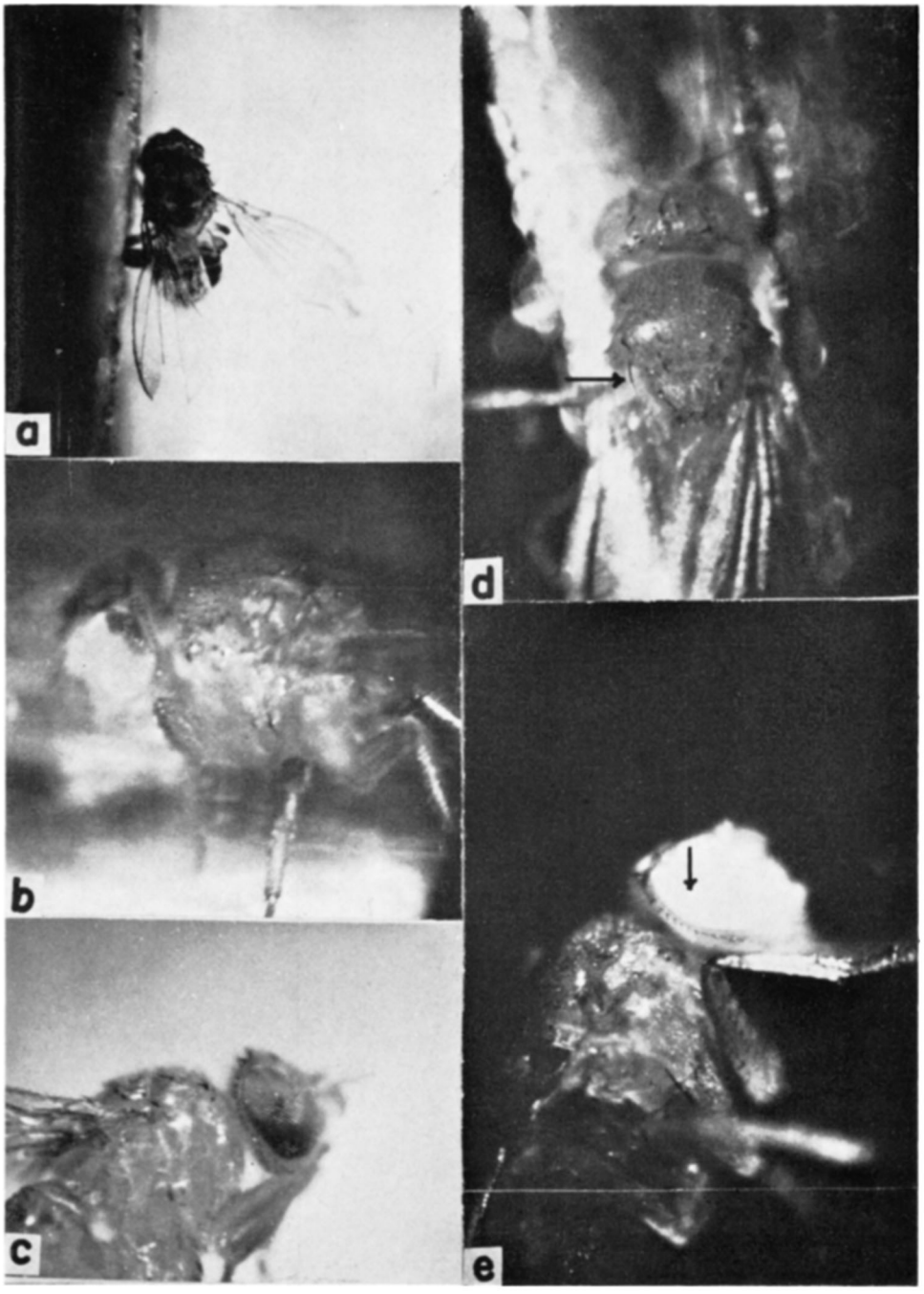
in Table 10. There were six instances of treated mosaics which produced offspring exhibiting the same type of mosaicism. There were three cases of treated mosaics which produced offspring exhibiting a different type of mosaicism. One of these was a *ca* mosaic which yielded one *ru* mosaic and one *sc*+ mosaic among her 75 progeny. The remaining seven cases involved nonmosaic, treated flies which produced mosaic offspring.

When transmission of mosaicism was followed for more than one generation, it frequently persisted to the end of the experiment. In most cases this involved only two generations but in one instance, involving the transmission of *cu* mosaicism which originated in a treated fly which was a *ru* mosaic, persistence was observed for seven generations.

In summary, the transmission of heterologous DNA-induced effects in experiment 1 exhibited the same features as in experiment 2.

Nature of the mosaic patches: The loci used in these two experiments are not of equal value in the scoring of mosaicism or in estimation of the size of mosaic patches. It is difficult to know, for example, how many cells need to be affected in order to detect changes of *cv* to *cv*+, *ru*+ to *ru*, *th*+ to *th*, or *cu*+ to *cu*. In addition, the question of autonomy cannot be answered for all of the loci used. The mutant *y*, for example, is nonautonomous on a *y*+ background (HANNAH 1953), but since we were looking for change in the other direction (*y* to *y*+) this might make the apparent size of mosaic patches larger than their real size. Finally, some of the changes, such as *ru*+ to *ru*, are certainly more susceptible than others to mimicry resulting from nonspecific developmental effects.

These considerations make comparisons of specific locus rates somewhat uncertain. This is particularly true for the two loci, *ru* and *h*, which exhibited extra-


a
b
c
d
e

ordinarily high mosaic frequency in the heterologous-DNA series of experiment 1. Both on *a priori* grounds and on grounds of the high mosaic frequency observed among Ringer-treated controls, these loci could be especially susceptible to nonspecific effects. For this reason, the conclusion that they are especially susceptible to the specific effects of heterologous DNA must be accepted with caution.

Nevertheless, for those changes where the size of the mosaic patches could best be determined, i.e. for the eye-color changes *st* and w^+ and for the bristle traits y^+ and sn^+, they usually involved just one or a small number of ommatidia or bristles (Figure 1). Indeed, it was possible to score w^+ mosaics where the change affected the pigment cells on only one side of an ommatidium. The largest mosaics observed were a w^+ where one quarter of an eye was red, and a y^+ where the two terminal abdominal segments were affected. In general, the mosaicism induced by heterologous DNA seemed to be very fine-grained. It is not possible to make a firm statement about secular changes in the size of mosaic patches in succeeding generations, but no striking trends were observed.

Only one fly exhibited more than one mosaic patch (y^+ and w^+). In some cases it would have been possible to observe more than one change in the same patch (for example, y^+ and sn^+), but such double changes were never seen.

These observations indicate that the event responsible for the origin of a mosaic patch has a low probability of occurrence. It would be more likely to occur, therefore, late in development when the cell population is large, and more likely in the soma than in the germ line.

Nonspecific changes: Although the genetic systems used in this work were designed to detect changes at specific loci, they were capable of yielding information about some types of nonspecific changes. Thus an increase in the frequency of sex-linked lethals would be reflected in a disturbance of the sex-ratio among the progeny of treated females, but no evidence could be found for such an effect of DNA treatment. Likewise, no dominant Minutes were observed among DNA-treated flies or their progeny, although the scoring of these mutants is subjective and no special search was made. Some nonspecific changes were observed, however, resembling the following mutants: Lobe, lozenge, Bubble, Notch, Blister-like, outstretched, and fringed (BRIDGES and BREHME 1944). These were observed both in homologous and heterologous DNA-treated series. No tests for transmission and allelism were performed.

DISCUSSION

The principal facts disclosed by these experiments are the following: (1) When young embryos are treated with DNA extracted from flies differing at

FIGURE 1.—Photographs of mosaics. (a) *cu* mosaic. rucuca DNA-treated, experiment 1. Right wing partially curled and outstretched, left wing normal. (b) $w+$ mosiac. F_1 male from Oregon-R DNA-treated $y\ w\ sn^3$ nonmosaic female, experiment 2. Upper quarter of left eye is red. (c) *ru* mosaic. rucuca DNA-treated, experiment 1. Note patch of disarranged facets. (d) sn^+ mosaic. F_1 male from Oregon-R DNA-treated $y\ w\ sn^3$ mosaic female, experiment 2. Left anterior postalar bristle is sn^+. (e) w^+ mosaic. F_3 male from a line originating with an Oregon-R DNA-treated nonmosaic female in experiment 2. w^+-mosaicism was observed in F_1 and F_2. Note single w^+ ommatidium in lower left quadrant of eye (as printed).

specified loci (i.e., "heterologous" DNA), they exhibit a high frequency of mosaicism for those loci as adults. (2) The changes responsible for this mosaicism may proceed either from recessive allele to dominant, or from dominant to recessive. (3) The effect of heterologous DNA is locus-specific in the sense that it is not produced as frequently by "homologous" DNA prepared from flies of the same genotype as the treated embryos. (4) In addition to this specific effect, DNA treatment may have a low-order, general mutagenic effect. This is evidenced by the slight and doubtfully significant increase of mosaicism for the test loci among flies treated with homologous DNA, and by the occurrence of nonspecific changes among flies treated with either heterologous or homologous DNA. (5) The frequency of mosaicism induced by heterologous DNA is directly proportional to the number of target chromosomes in the treated flies. Thus, twice as many sex-linked mosaics are induced in females as in males, but the number of autosomal mosaics is the same in the two sexes. (6) There is some evidence of differences in susceptibility to heterologous DNA among the loci studied. (7) The effects of heterologous DNA are transmitted by treated flies to their progeny and subsequent generations, but in the form of mosaicism rather than as whole-body changes. Although a given effect may be lost in some lineages, it may persist as mosaicism in others for as long as seven generations. This is equivalent to 140 to 210 cell generations in the germ line. A given fly, mosaic or nonmosaic, may produce more than one offspring exhibiting mosaicism for the same locus. Thus, the changes induced by heterologous DNA may be regarded as "replicating instabilities". (8) A treated fly need not exhibit the mosaicism which it transmits to its progeny. In one instance, three generations intervened between the time of treatment with heterologous DNA and the observation of mosaicism. (9) Nevertheless, treated mosaics transmit mosaicism to their progeny more frequently than do treated nonmosaics. Furthermore, there seems to be a tendency for treated flies which exhibit mosaicism for a given locus to transmit mosaicism for the same locus more frequently than they transmit mosaicism for other loci. (10) The transmission of mosaicism for a given locus follows the same rules as govern the transmission of the chromosome carrying that locus. Thus, mosaicism for an autosomal locus is transmitted to daughters and sons alike while mosaicism for a sex-linked locus is transmitted according to the rules governing the transmission of X chromosomes. (11) The mosaic patches observed in heterologous DNA-treated flies and their progeny are in general very small. Furthermore, the occurrence of more than one such patch on a single fly is very rare, and no patch has exhibited a change for more than one locus.

True transformation, as encountered in bacteria, involves the introduction of a DNA segment into a competent cell and actual physical integration of that segment into the host chromosome with replacement of its homologous host segment (LACKS 1962; FOX and ALLEN 1964; BODMER and GANESAN 1964). The present data are suggestive of unstable transformation (IYER 1965), but they do not exclude other possibilities. Therefore, we would not propose a particular model at this time, but it should be noted that any model proposed as an explanation must include the following features: (1) It must provide for an interval of many

cell generations between the time of treatment with heterologous DNA and the expression of its effect. (2) It must provide for replication of the effect during that interval. (3) It should provide for transmission of the effect in close association with the locus which is ultimately affected. (4) The locus-specificity exhibited by the difference between the effects of heterologous and homologous DNA must be explained. (5) The mechanism must be one which would provide for changes from dominant to recessive allele as well as from recessive to dominant. (6) Provision must be made for the extreme rarity, or absence, of whole-body changes.

Although previous work with DNA effects in Drosophila has failed to reveal the locus-specificity which is a major feature of the present work, some important similarities exist. GERSHENSON (1965) and MATHEW (1965) raised larvae on medium containing high concentrations (10 to 13%) of calf thymus DNA and examined progeny of the treated flies for mutations. No appreciable increase in the frequency of sex-linked recessive lethals could be found, but a significant number of complete and mosaic 2nd chromosome lethals were produced. Most of these were probably deletions of varying extent, and complementation tests showed that most were localized in a particular segment of the 2nd chromosome. In addition, it could be demonstrated that the mosaics resulted from replicating instabilities. FAHMY and FAHMY (1961, 1965) injected homologous and heterologous Drosophila DNA into the haemocoel of adult males and tested for the induction of mutations in the germ line. Few sex-linked recessive lethals were induced, but numerous small chromosome deletions resulting in the Minute phenotype were found. Further tests demonstrated that 81% of these were localized to a particular segment of the 4th chromosome (FAHMY and FAHMY 1963). Thus replicating instability and some degree of specificity in localization seem to be characteristic of the effects of DNA in Drosophila. Divergences among the experiments are probably attributable to the source and method of preparation of DNA, to the mode of treatment, and to the genetic systems employed. In particular, the changes encountered in the present work are probably not simple deletions, since they proceed from recessive to dominant as well as from dominant to recessive and are not cell lethal.

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SUMMARY

When young embryos are treated with DNA prepared from adults differing at specified loci (heterologous DNA), they exhibit a high frequency of somatic mosaicism for those loci as adults. Ten out of 11 loci tested have responded to such treatment, and changes from dominant alleles to recessive as well as from recessive to dominant have been observed. This effect of heterologous DNA is locus-specific in the sense that it is not produced as frequently by homologous DNA

prepared from flies of the same genotype. The frequency of mosaicism induced by heterologous DNA is directly proportional to the number of target chromosomes in the treated flies, and the effects are transmitted to subsequent generations in the form of repeated mosaicism rather than as whole-body changes. A treated fly need not exhibit the mosaicism which it transmits to its progeny, but treated mosaics transmit such mosaicism more frequently than do treated nonmosaics. There seems to be a tendency for treated flies which exhibit mosaicism for a given locus to transmit mosaicism for that locus more frequently than for other loci. The transmission of mosaicism for a given locus follows the same rules as govern the transmission of the chromosome carrying that locus. The mosaic patches observed in heterologous DNA-treated flies and their progeny are usually very small, more than one such patch on a single fly is very rare, and no patch has exhibited a change for more than one locus.

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