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Gene transfer in *Drosophila melanogaster*: genetic transformations induced by the DNA of transformed stocks

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SUMMARY

DNA prepared from transformed stocks of *Drosophila melanogaster* induces second-step transformations resembling the original. The gross yield of transformants induced by transformed DNA is several times higher than that induced by the original allo-DNA, but much of this high frequency is attributable to a few exceptionally large clusters of transformants among flies treated with transformed DNA. When these large clusters are omitted from the data, the frequency of transformants induced by DNA from transformed stocks is the same as that induced by allo-DNA. The data therefore support the conclusion that the original DNA-induced alterations resulted from the transfer of genetic material capable of indefinite replication.

1. INTRODUCTION

A previous series of papers has suggested that the specific genetic effects induced by DNA in *Drosophila melanogaster* may properly be regarded as transformations resulting from gene transfer (Fox & Yoon, 1966, 1970; Fox *et al.* 1970; Fox, Yoon & Gelbart, 1971*a*; Fox *et al.* 1971*b*). Genetic analysis of transformed stocks has shown that the position of the introduced genetic information may be mapped to specific locations by standard genetic techniques.

More recently we have reported the presence of cytological alterations in salivary chromosomes of transformed stocks (Fox & Valencia, 1973, 1975). In every stock examined, except untreated controls, extra chromatin has been observed in a small proportion of salivary gland chromosomes, associated with the chromosome band corresponding to the map position of the genetic alteration.

This report presents evidence indicating that DNA prepared from transformed stocks induces transformations resembling those induced by the original wild-type ('allo') DNA.

2. MATERIALS AND METHODS

(i) *Stocks*

The transformed stocks used in this work resulted from the treatment of $v^1;bw$ embryos with DNA prepared from wild-type adults (Fox & Yoon, 1970). In contrast with the tinged, white-eyed phenotype of $v^1;bw$, they exhibit coloured eyes resulting from the presence of variable, but less than wild-type, amounts of ommochrome. Their larval fat bodies exhibit kynurenine-fluorescence (T. M. Rizki, personal communication), and extracts contain tryptophan pyrrolase activity (Tobler, 1975).

The stock *6203♂4* exhibits a genetic alteration which maps at $1\text{–}32{\cdot}4 \pm 0{\cdot}7$ (Fox & Yoon, 1970; Fox *et al.* 1971*a*), a position not statistically different from that of the *vermilion* (*v*) locus (1–33·0; see Lindsley & Grell (1968) for gene symbols and map positions). Like the $v^1;bw$ stock from which it originated, it is homozygous for the autosomal mutant *brown* (*bw*, 2–104·5) which blocks the formation of red eye-pigments. It is regarded as a dominant transformant because females heterozygous for the transformed *X* and an *X* carrying v^1, but homozygous for *bw*, exhibit coloured eyes. Eye colour of this stock is described below. The exosome model suggests that the transformed *X* of this stock carries the mutant v^1 at 1–33·0 on the chromosome (salivary band 10A1: Lefevre, 1969), and a v^+ exosome associated with that site (for a diagrammatic representation see fig. 1, Fox & Valencia, 1975).

The transformed stock *e5·6* exhibits a genetic alteration which maps at 1–0·7 (Fox & Yoon, 1970; Fox *et al.* 1971*a*), a position not statistically different from that of the *suppressor-of-sable* [*su*(*s*)] locus (1–0·0). It too is homozygous for *bw*, but is regarded as a recessive transformant because heterozygotes for an *X* carrying v^1 (homozygous for *bw*) exhibit off-white eyes like those of $v^1;bw$. Eye colour of the stock is described below. The exosome model suggests that the transformed *X* of this stock carries the mutant v^1 at 1–33·0 and $su(s)^+$ at 1–0·0 on the *X* chromosome (salivary band 1B11; E. Grell, personal communication), and a v^+ exosome associated with the latter site (see fig. 2, Fox & Valencia, 1975).

Each of these stocks provided a source of *transformed DNA*. *Iso-DNA* (i.e. DNA prepared from flies of the same genotype as those treated) was prepared from flies of the $v^1;bw$ stock. *Allo-DNA* (i.e. DNA from flies differing at a specified target locus from those treated) was prepared from flies of a wild-type Urbana stock.

(ii) *DNA preparation*

DNA is prepared by a modification of the method of Mead (1964). Since we have not provided a full description in previous publications, details are given here.

Adult flies are collected from unyeasted mass cultures and are stored in a deep-freeze. The following procedure utilizes 25 g of frozen flies; 45 g may be utilized by increasing the volumes of all extracting solutions proportionately. The flies are thoroughly homogenized in parts at 4 °C in 200 ml of saline-citrate solution

(0·15 M-NaCl and 0·015 M-$Na_3C_6H_5O_7.2H_2O$), using a large, all-glass (Kontes-Duall) tissue grinder. The homogenate is centrifuged at 22000 *g* for 15 min at 4 °C. The supernatant is discarded and the pellet is re-homogenized in parts in 200 ml of the saline-citrate solution and re-centrifuged. This process of homogenization and centrifugation is repeated five more times (a total of seven homogenizations and centrifugations), and the final combined pellet is suspended in 130 ml of a 1:1 (vol:vol) mixture of 0·1 M-NaCl and 5% of the detergent Aerosol OT (Fisher Scientific). The suspension is then stirred in a flask for 10–16 h on a magnetic stirrer at 4 °C. At the end of this period the solution is made 1 M for NaCl by the addition of solid salt, and stirred for 10 additional minutes. It is then centrifuged for 15 min at 22000 *g*, 4 °C, and the supernatant is decanted. The pellet is discarded. Two volumes (approximately 260 ml) of chilled (–20 °C) absolute ethanol are added to the supernatant, and the mixture is stored in the deep-freeze for at least 30 min. It is then centrifuged at 15000 *g*, 4 °C, for 15 min to sediment the precipitated nucleoprotein.

The pellet is dissolved in 18 ml of 0·15 M-NaCl to which is added 2 ml of chloroform–octanol (5:1, vol:vol), shaken vigorously for 10 min at room temperature and centrifuged at 10000 *g* (4 °C) for 15 min. The supernatant is collected, 1 ml of chloroform–octanol is added, the mixture is shaken for 10 min, and centrifuged at 10000 *g* (4 °C) for 15 min. The supernatant is collected again, 0·5 ml of chloroform–octanol is added, the mixture is shaken for 10 min, and centrifuged at 10000 *g* (4 °C). The final supernatant which is now deproteinized, is dialysed against 3 l of 0·15 M-NaCl for 10–16 h at 4 °C.

Residual RNA is now removed by digestion with ribonuclease. Bovine pancreatic ribonuclease (Worthington), 500 μg/ml in 0·15 M-NaCl, is heated at 80 °C for 10 min and is added to the dialysed preparation in the ratio of 0·1 ml of ribonuclease to 1 ml of the preparation. The mixture is incubated at 37 °C for 30 min. It is then deproteinized three times with chloroform–octanol as outlined above. After the final centrifugation, the supernatant is applied to a Sephadex G-50 column which has been equilibrated either with a modified insect Ringer solution (Yoon & Fox, 1965) or with 0·226 M sucrose; elution is performed with the corresponding solution. The void volume of the column is collected in one fraction and 5 ml fractions are taken thereafter. The optical density of these at 260 nm is determined, and the fractions containing the bulk of the DNA are combined. DNA concentration is determined by the diphenylamine test, and adjusted to 0·02 mg/ml by dilution with the eluting solution. The DNA solution is divided into aliquots of a size sufficient for one experiment, and stored at – 20 °C until use.

The product contains no protein or RNA detectable by the Folin or orcinol tests, respectively. It is double-stranded, with a melting point of 84 °C in 0·195 M-NaCl and a G + G content of 39·1%. It is dispersed in molecular size, with a modal molecular weight of about 10^6.

(iii) *Collection and treatment of eggs*

All of the experiments reported in this paper were performed with eggs from v^1;*bw* females inseminated by v^1;*bw* males, collected in the ovitron and dechorionated as previously described (Yoon & Fox, 1965; Fox & Yoon, 1966). Treatments were performed with 0·226 M sucrose or with appropriate DNA solutions (0·02 mg/ml in 0·226 M sucrose).

A standardized procedure for egg collection and treatment has been used both in present and previous work. Eggs are collected from the ovitron at 1 h intervals. Those collected at the end of the first 2 h are discarded: those collected at the end of hours 3–7 are immediately dechorionated and treated. Treatment is performed at 26 °C in small tissue culture dishes (12 mm deep × 30 mm diam.) containing 2·0 ml of the appropriate solution. The dishes are assigned code numbers drawn from a table of random numbers, and the identity of the solution in each dish is recorded in a log which is not consulted until the whole study is complete and the results are ready for tabulation. Approximately 200 eggs are transferred to each dish; the number of dishes receiving eggs from a single hourly collection depends on the number of eggs collected. Occasionally, if egg number is exceptionally low, a single dish may receive eggs from two successive collections. The dishes are incubated overnight, so that the length of treatment varies from 7 to 18 h; ovitron eggs, however, become impermeable after 3–4 h (Fox & Yoon, 1966). The next morning the eggs from each dish are transferred into an individual culture bottle containing standard cornmeal–molasses–agar–dead yeast medium from which mould inhibitor and living yeast have been omitted. The culture bottle is labelled with the code number of the dish. About 30–50 % of the transferred eggs emerge as adults, and the results are recorded under the same code number. The work of each day is regarded as a single experiment, and experiments are numbered consecutively. Each DNA used in an experiment is derived from a single frozen aliquot.

Careful analysis of data accumulated over a period of almost 10 years reveals no correlation between the occurrence of transformants induced by allo-DNA and the 1 h interval in which the treated eggs were collected from the ovitron. Nevertheless, when sufficient eggs for more than one dish are produced during any one interval, they usually receive different treatments. Every day's experiment almost always includes either sucrose or iso-DNA controls. When several DNA's are being tested for transforming activity, as in the present work, they may not be available at the same time, rendering it impossible to test them simultaneously. However, since more than one preparation of each DNA is usually tested, and since these tests are separated in time, systematic temporal errors would readily be detectable.

(iv) *Transforming phenotypes and scoring criteria*

In previous publications (Fox & Yoon, 1970; Fox *et al.* 1971*b*) the eye colour of v^1;*bw* has been described as a slightly tinged white, while that of the coloured flies resulting from treatment with v^+ allo-DNA varies from fly to fly, involves

the whole eye (i.e. is non-variegated), is bilaterally symmetrical and includes colours ranging from yellows, through reds, to almost full browns. Attempts have also been made to give verbal descriptions of the eye colours of established transformed stocks.

In practice, the eye colour of v^1;*bw* varies from very slightly tinged to definitely tinged while transformed stocks vary over a wide range, sometimes producing flies as light-eyed as the most deeply tinged flies observed in v^1;*bw*. Such off-white flies occurring in transformed stocks, however, almost always behave genetically like their coloured sibs, i.e. they transmit v^+ information to almost all of their progeny (Fox & Yoon, 1970; Fox *et al.* 1971*b*).

Because of this occasional phenotypic overlap a set of criteria has been developed for experiments like those reported here which classifies flies as coloured only if they are definitely darker in eye colour than those which fall in the range of overlap; all others are classified as white. Since eye colour darkens with age, the flies are aged for 24 h before classification. The criteria are such that different observers rarely differ in their diagnosis of individual flies.

Nevertheless it has seemed desirable to describe these criteria in more objective terms, and for this purpose we have resorted to use of the Munsell colour system (Newhall, Nickerson & Judd, 1943). This system identifies colour in terms of three attributes: *hue* (i.e. 100 hues ranging through red, yellow, green, blue, purple and intermediates), *value* (i.e. the degree of lightness or darkness in relation to a neutral gray scale extending from absolute black to absolute white) and *chroma* (i.e. intensity or strength). In terms of this system, *Drosophila* eye colours fall in the hue range extending from 10RP (red-purple), through grades of red (R) and yellow-red (YR), to 2·5Y (yellow).*

To identify the eye colour of an individual fly, several glossy Munsell colour chips of appropriate colours are placed on the stage of a binocular, dissecting microscope illuminated by reflected light as is usual in *Drosophila* work. The anaesthetized fly, which has been aged for 24 h, is placed on one of these chips at the point of maximum illumination, lying on its side with its head oriented toward the light source. The colour of the upper eye is compared with that of the chip and the fly is moved from chip to chip until the closest match is found. The colour notation of the chip providing the closest match is regarded as the colour of the eye.

The modal colour of our v^1;*bw* stock is 5YR 7/4, with the lightest flies exhibiting the colour 5YR 8/4 and the darkest rarely exceeding 5YR 7/6. When the lightest flies classified as coloured in our experiments are matched to colour chips, they rank as 5YR 7/10. This colour is clearly distinguishable from 5YR 7/6. The region of phenotypic overlap is that between 5YR 7/6 and 5YR 7/10, and flies with eyes of these colours are conservatively classified as white.

* The system is described in a pamphlet entitled *Munsell Color: Notation, Standards, Equipment, Technical Services*, issued by the Munsell Color Company Inc., Baltimore, Md. 21218. The complete Munsell notation for a chromatic colour is written symbolically: Hue Value/Chroma. The complete notation for a sample of 'vermilion' might be 5R 5/14, while that for a sample of 'rose' might be 5R 5/4.

The Munsell notation of eye colours in transformed stocks illustrates the colour range of flies which are classified as coloured. The stock *e5·6* is among those exhibiting the lightest eye colour differing significantly from $v^1;bw$ (Fox & Yoon, 1970). Its eye colour at 24 h of age ranges from 5YR 7/10 or 5YR 6/8 at the lightest, to 5YR 5/10 or 5YR 5/12 at the darkest. Only an occasional fly falls into the region of overlap between 5YR 7/6 and 5YR 7/10. The stock *6203♂4* exhibits the darkest eye colours observed among transformed flies. Originally it exhibited only restricted variability (Fox & Yoon, 1970), but this has increased since its original derivation. Its eye colour at 24 h of age now ranges from 5YR 5/8 at its lightest, to 10R 5/10 at its darkest. With additional ageing, eye colour may deepen to 7·5R 4/12.

Coloured flies produced by treatment with allo-DNA or transformed DNA may exhibit any of the eye colours observed in transformed stocks. It should be noted that the eye colour of *brown* (i.e. $v^+;bw$) classifies as 5R 3/6 to 5R 3/8 at 24 h of age. The eye colours of transformed flies, both in the treated generation and in established stocks, never reach this level.

3. RESULTS

(i) *Effects of Iso-DNA, Allo-DNA and transformed DNA*

In all, 48 experiments were performed in which $v^1;bw$ embryos were subjected to five different treatments. The details of individual experiments are available from the authors on request. Table 1 summarizes the over-all results of these

Table 1. *Number and frequency of coloured flies resulting from egg treatment*

Treatment	No. flies examined	No. coloured	Frequency coloured
1. Sucrose	3095	1	0·0003
2. Iso-DNA (v^1; *bw*)	2378	3	0·0013
3. Allo-DNA (Urbana)	1132	9	0·0079
4. *6203♂4* DNA	3217	156	0·0485
5. *e5·6* DNA	2926	57	0·0195

experiments, giving the number of flies examined and the number and frequency of coloured flies resulting from each of the five treatments. The results of statistical analysis are given in Table 2; comparisons of the effects of the treatments were made in 2×2 contingency tables (2 treatments *v.* 2 phenotypes, white and coloured), using either the Fisher Exact Test (two-tailed) when the marginal total of coloured flies was less than 70, or the χ^2 test when it was 70 or above.

The transforming activity of allo-DNA (treatment 3) and transformed DNA (treatments 4 and 5) is properly evaluated by comparison with the activity of iso-DNA (treatment 2). As in previous experiments, treatment with iso-DNA yields a higher frequency of coloured flies than does sucrose, but as in previous work the difference is not significant (comparison 1 *v.* 2, Table 2).

Allo-DNA (treatment 3) yields coloured flies with a frequency similar to that reported previously (Fox & Yoon, 1970), some 6–7 times higher than that of

iso-DNA. This difference, which is highly significant (comparison 2 *v.* 3, Table 2), measures the transforming activity of allo-DNA.

6203♂4 DNA (treatment 4) produces coloured flies with a frequency nearly 40 times higher than iso-DNA and 6 times higher than allo-DNA. The other transformed DNA, *e5·6* (treatment 5), produces coloured flies with a frequency about 5 times higher than iso-DNA and 2·4 times higher than allo-DNA. In both cases the yield of coloured flies is significantly higher than that of allo-DNA (Table 2: comparisons 3 *v.* 4 and 3 *v.* 5) while the yield obtained with *6203♂4* DNA is significantly higher than that obtained with *e5·6* DNA (comparison 4 *v.* 5).

Table 2. *Statistical analysis of data in Table 1*

Comparison of treatments	P
1 *v.* 2*	$3{\cdot}2 \times 10^{-1}$
2 *v.* 3*	$2{\cdot}8 \times 10^{-3}$
3 *v.* 4†	$8{\cdot}2 \times 10^{-10}$
3 *v.* 5*	$8{\cdot}1 \times 10^{-3}$
4 *v.* 5†	$5{\cdot}4 \times 10^{-10}$

Treatment numbers correspond to those in Table 1. Comparisons were performed by application of * Fisher's Exact Test (two-tailed) or † the χ^2 test to 2×2 contingency tables.

Of the 156 coloured flies resulting from treatment with *6203♂4* DNA, 142 were observed in a single experiment (Expt 51). Omitting Expt 51, the total yield of *6203♂4* DNA was 2535 white-eyed and 14 coloured flies. This result differs significantly from that obtained with *v*^{1}*;bw* DNA ($P_{\text{exact}} = 0{\cdot}01$), but does not differ from that obtained with Urbana DNA ($P_{\text{exact}} = 0{\cdot}37$).

Similarly, of the 57 coloured flies resulting from treatment with *e5·6* DNA, 42 were observed in a single experiment (Expt 56). Omitting Expt 56, the total yield of *e5·6* DNA was 2808 white-eyed and 15 coloured flies. This result differs significantly from that obtained with *v*^{1}*;bw* DNA ($P_{\text{exact}} = 0{\cdot}02$), but not from that obtained with Urbana DNA ($P_{\text{exact}} = 0{\cdot}37$). Omitting the two exceptional experiments, the effects of *6203♂4* and *e5·6* DNA are not significantly different from each other ($P_{\text{exact}} = 1$).

(ii) *Analysis of exceptional experiments involving transformed DNA*

In two experiments (51 and 56) treatment with transformed DNA yielded extraordinarily high frequencies of coloured flies. While omission of the data of these two experiments still leaves results which demonstrate that transformed DNA-induces coloured flies with a frequency not differing from allo-DNA but higher than iso-DNA, these bursts of transformants merit further analysis.

The complete results observed in Expt 51 are given in Table 3. Out of a total of 16 dishes, 7 received treatment with *6203♂4* DNA. These yielded 526 white-eyed and 142 coloured flies. Of the latter, 131 occurred in two dishes (9916 and 9623), which together yielded only 10 white-eyed flies. The remaining five dishes yielded 516 white-eyed and 11 coloured flies; this is significantly higher than the overall

frequency of coloured produced by *6203♂4* DNA omitting Expt 51 ($P_{exact} = 0{\cdot}15 \times 10^{-2}$). Thus, the frequency of coloured flies produced by *6203♂4* DNA is high in general in this experiment, but most of this extraordinary frequency is attributable to the yield of two dishes, the flies from which were almost all coloured. These were two out of three dishes receiving eggs from the fourth hourly collection. The third dish (1883) yielded 104 flies, all of which were white-eyed. Thus, of three dishes receiving random samples of eggs collected during a single hour and treated with the same DNA sample, two yielded virtually all coloured flies and the third yielded none.

Table 3. *Complete results observed in Experiment 51*

Treatment	Dish no.	Hourly interval of egg collection	White	Coloured	Total
Sucrose	7704	5	88	0	88
	7825	6	20	0	20
	4305	7	11	0	11
6203♂4 DNA	9916	4	7	76	83
(preparation no. 1)	1883	4	104	0	104
	9623	4	3	55	58
	4237	6	115	3	118
	3162	6	92	4	96
	4696	6	112	4	116
	7072	7	93	0	93
e5·6 DNA	8737	3	44	0	44
(preparation no. 1)	6744	3	64	0	64
	8930	3,5	111	0	111
	4809	5	84	5	89
	8549	7	78	0	78
	8690	7	89	3	92

Table 4. *Complete results observed in Experiment 56*

Treatment	Dish no.	Hourly interval of egg collection	White	Coloured	Total
v¹;bw DNA	7988	3,4	5	0	5
(preparation no. 3)	3040	4	66	0	66
	5520	7	71	0	71
	8088	7	45	0	45
e5·6 DNA	7077	5,6	0	42	42
(preparation no. 1)	1342	6	61	0	61

The results observed in Expt 56 are given in Table 4. Two dishes received treatment with *e5·6* DNA (7077 and 1342). Together they produced 61 white-eyed flies and 42 coloured. Remarkably, all of the flies produced by one dish were coloured, and all produced by the other were white eyed. The two dishes were treated with the same sample of DNA, and though one dish contained eggs from both the fifth- and sixth-hourly collections, the other also contained eggs from the sixth-hourly collection.

It seems most reasonable to assume that the two exceptional dishes encountered in Expt 51 and the one exception in Expt 56 are the result of some condition not present in other dishes of their respective series, and that a general comparison of treatments should leave them out. If this is done for *6203♂4* DNA, the frequency of coloured flies in the remaining data is 0·0081. This is higher than that obtained by omission of all of Expt 51 (see above), but not significantly different from that obtained with Urbana DNA. When dish number 7077 is omitted from the results obtained with *e5·6* DNA the remaining frequency is 0·0052, which is essentially the same as that obtained if the whole of Expt 56 is omitted (see above).

(iii) *Effects of different DNA preparations*

In all, three separate preparations of iso-DNA (v^1;*bw*), two of allo-DNA (Urbana), one of *6203♂4* DNA, and three of *e5·6* DNA were utilized in these experiments. The data are divided according to DNA preparation in Table 5.

The three different preparations of v^1;*bw* DNA produced effects that did not differ significantly. Similarly, the two preparations of Urbana DNA did not yield significantly different effects.

Table 5. *Effects of different DNA preparations*

DNA preparation	No. of experiments	No. of flies examined	No. coloured
y^1;*bw*			
no. 1	7	538	1
no. 2	3	460	1
no. 3	16	1380	1
Total	26	2378	3
Urbana			
no. 1	6	288	4
no. 2	6	844	5
Total	12	1132	9
6203♂4			
no. 1	19	3076	25
e5·6			
no. 1	18	2435	13
no. 2	3	186	1
no. 3	3	263	1
Total	24	2884	15

The two exceptional dishes discussed above have been omitted from the results obtained with the single tested preparation of *6203♂4* DNA. In the case of *e5·6* DNA, the one exceptional dish observed in Expt 56 has been omitted. When this is done, no significant difference exists between the effects of preparation number 1 and the total yield of preparations 2 and 3 ($P_{\text{exact}} = 1$).

4. DISCUSSION

In the present work, as in previous work (Fox & Yoon, 1970), treatment of $v^1;bw$ embryos with allo-DNA produced coloured flies with a frequency which is significantly higher than that observed among flies treated with iso-DNA. The interpretation given to this observation (Fox & Yoon, 1970) is that treatment with allo-DNA introduces v^+ segments of the genome into one or a small number of cells in the young embryo; that these segments are sometimes capable of replication so that clones of cells possessing v^+ information are produced; that this information may be transcribed and translated in some of these cell lineages; and that the kynurenine produced in these mosaic patches may diffuse to the eye, where it serves as substrate for ommochrome desposition (Beadle & Ephrussi, 1937). This interpretation accounts for the intermediate phenotype (between white and full-brown) of coloured flies, for the variability of the coloured phenotype from fly to fly, and for their bilateral symmetry in colour. It is supported by the demonstration of tryptophan pyrrolase activity in extracts of transformed flies (Tobler, 1975), by the presence of kynurenine-fluorescence in their larval fat bodies (T. M. Rizki, personal communication), and by the resemblance of their phenotype to that produced by $T(1;2)v^{65b}$, which involves a V-type position effect of *vermilion*, i.e. phenotypic mosaicism (Fox, unpublished).

If the postulated mosaic patches extend into the gonads of allo-DNA treated flies, transmission to subsequent generations should be observed. Such transmission has been demonstrated, both for the *vermilion* system used in the present work (Fox & Yoon, 1970), and for ten other sex-linked or autosomal loci (Fox & Yoon, 1966; Fox *et al.* 1971*b*). On the other hand, none of the phenotypically altered flies observed among the non-DNA or iso-DNA controls have ever transmitted the alteration to subsequent generations. Transformed stocks have been established from the progeny of allo-DNA treated flies (Fox & Yoon, 1970), and the location of the induced change has been mapped (Fox *et al.* 1971*a*).

The clear result of the present work is that DNA from transformed stocks is as effective as allo-DNA in the production of coloured flies, or more effective if the three cases of extreme clustering are included. Thus, the results suggest that v^+ information, originally introduced by treatment with allo-DNA, is present in flies of transformed stocks many generations after their origin. It follows that coloured flies resulting from treatment with transformed DNA should sometimes transmit the effect to subsequent generations, but this has not yet been tested.

The occurrence of a few large bursts of transformants in isolated dishes treated with transformed DNA is not presently understood. There are many possibilities for variability in the efficiency of transformation inherent in the present experimental system, and future work will be directed toward the establishment of conditions under which transformation is uniformly high. The recent development of methods for the injection of individual, well-staged eggs (Zalokar, 1971; Schubiger & Schneiderman, 1971; Illmensee, 1972; Okada, Kleinman & Schneiderman, 1974) may well afford methods for the solution of these problems.

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