HEREDITARY CHANGE IN BOMBYX AFTER TREATMENT WITH DNA¹

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EVIDENCE has accumulated concerning the uptake of DNA by living cells of higher organisms. Most investigations have been carried out on isolated cells cultivated or maintained *in vitro*. The generality of this phenomenon [extensively reviewed by LEDOUX (1965)] has been demonstrated in several laboratories with different systems (BENDICH, WILCZOK and BOBENFREUND 1965; KONG and FICQ 1967; SCHELL 1968; STROUN *et al.* 1967). The possibility that exogenous DNA can be integrated into the recipient genome has also been suggested (AYAD and FOX 1968; LEDOUX and HUART 1969; MEIZEL and KAY 1965; ROBINS and TAYLOR 1968). Evidence suggesting genetic transformation in a mammalian cell under conditions in which a considerable amount of DNA is known to be integrated into the recipient genome has been presented (FOX, FOX and AYAD 1969). Furthermore, transformation by DNA in somatic cells of the intact mouse has been demonstrated (OTTOLENGHI-NIGHTINGALE 1969). Thus, it is plausible that genomes of eukaryotes can integrate DNA and that it can then be expressed.

In *Ephestia*, CASPARI and NAWA (1965) obtained wing scales with wild-type coloration after injection of wild-type DNA into recessive *ml/ml* larvae. NAWA and YAMADA (1968) have shown that treatment of *Ephestia* larvae or eggs having a recessive eye-color genotype with DNA from wild type produced wild-type individuals in the treated generation and in subsequent generations. It has also been verified that the newly acquired eye color is transmitted through several generations and does not disappear. The experiment reported in the present communication demonstrates suggestive evidence for the induction of specific heritable changes by treatment with DNA in the silkworm, *Bombyx*.

MATERIALS AND METHODS

Strains: Two strains, Sw-1 and KH-19, carrying the recessive genotype white-1 (w_1 , locus 0.0 of the tenth linkage group) inbred in this Institute were used: KH-19 originally bred by KIKKAWA as a specific riboflavin-accumulating strain, and Sw-1 established by a cross between KH-19 and wild-type strain Chinese 108. The source of wild-type DNA was strain Bm or strain Chinese 108.

In white-1, as well as in cinnabar of *Drosophila*, 3-hydroxykynurenine synthesis from kynurenine is blocked (the white eye or egg color is due to the lack of the brown pigment formation). White-1 is nonautonomous for the brown pigment (KIKKAWA 1937, 1941).

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Preparation of DNA: DNA was prepared from fully grown larvae of the last instar which had been stored in a freezer (-20°C). Frozen whole larvae were minced with a knife. A batch of the minced larvae was crushed in saline-EDTA solution with mortar and pestle and the mixture was centrifuged. The precipitate was suspended in saline-EDTA solution, made 2.5% in sodium dodecylsulfate, kept at 60°C in a water bath for 10 min with occasional shaking, and then cooled. To the lysate was added 5 N sodium perchlorate to a final concentration of 1 N, and the suspension was shaken for 10 min with an equal volume of chloroform + isoamyl alcohol (24:1). After centrifugation, the aqueous phase was collected, and crude fibrous DNA was precipitated from it by adding 1.5 volumes of alcohol. The DNA wound on a glass rod was dissolved in SSC (0.14 M NaCl, 0.014 M trisodium citrate), and the solution was then made 1 M in NaCl by addition of solid NaCl. Deproteinization was completed by two or three cycles of chloroform + isoamyl alcohol washing and reprecipitation. The DNA was further purified by treatment with heated pancreatic RNase (Worthington) and α -amylase crystallized from *Bacillus subtilis* (Sigma), followed by further chloroform + isoamyl alcohol deproteinization. The purified DNA was finally dissolved in 0.9% NaCl and used within a few days. The DNA samples prepared in this way were contaminated with 13–17% RNA. The RNA was not removed by repeated treatment with RNase. The molecular extinction coefficient at 260 m μ with respect to phosphorus was from 7,500 to 8,300. On heating the DNA solution to 100°C , the increase in optical density was from 28 to 30%. The hyperchromatic shift after treatment with DNase was approximately 34%. In one specimen, the intrinsic viscosity was 45 and the sedimentation velocity in 0.1 M NaCl was $S_{20w} = 21$. A molecular weight of 7.0×10^6 was calculated for this DNA, using the relationship given by CROTHERS and ZIMM (1965).

Injection of larvae: Freshly prepared DNA dissolved in 0.9% NaCl to a concentration of 800 $\mu\text{g}/\text{ml}$ was added to 0.1 volumes of spermine solution (500 $\mu\text{g}/\text{ml}$, pH 7). The mixture was then mixed with 0.9 volumes of 0.25% gelatin solution. A substantial amount of precipitate sometimes appeared on mixing but could be dissolved by continuous shaking. The DNA solution (final concentration of DNA, 400 $\mu\text{g}/\text{ml}$) was injected into the body cavity of larvae at indicated stages after the last larval molt, using a 1 ml syringe with an 18-gauge needle. Approximately 0.05 ml of DNA solution per larva (20 μg of DNA) was injected. Thousands of larvae were bred in each experiment and some of them were randomly used for injection. Some of the remaining larvae were randomly raised to adults in order to be used for crosses to the treated animals.

Examination of eye and egg color: Adults raised from injected larvae were scored for pigmentation of the eyes. Each of the unchanged moths, either female or male, was mated to an untreated w_1/w_1 animal in a tin conical box (moth funnel). Each female, after separation from the male, was placed on a paper (egg card), covered by the moth funnel, and laid her eggs in a circle. Eggs of some of the batches were hatched immediately after egg laying, by changing them artificially from hibernating to nonhibernating eggs by immersion in hot dilute hydrochloric acid. Some were allowed to undergo natural diapause and hatched out the next spring. In both cases, egg color was examined several days after egg laying. If an egg had normal color, it was removed from the egg card and reared separately. Some of the white eggs were raised to adults in order to examine possible changes in eye color. Although the egg color of wild type is actually purplish brown and that of w_1/w_1 yellowish white, the words "black" and "white" will be used for the wild type and the white-1 type of egg color, respectively (Figure 4).

RESULTS

Production of black-eyed moths in the treated generation and transmission of the acquired character to subsequent generations: Using several samples of wild-type DNA and w_1 -DNA prepared separately, a series of independent treatments of w_1/w_1 larvae was carried out during four years. Usually injection in the spring gave good survival. Occasionally few or no survivors were obtained in injection experiments done in the summer. The results are summarized in Table 1. In

TABLE 1

Production of moths having pigmented eyes in w_1/w_1 (Sw-1 strain) treated by injection of DNA into larvae

Experiment	Source and preparation no. of DNA	Stage of last instar larvae injected	Number of larvae injected	Number of moths obtained	Number of pigmented moths	Remarks
1	wild-1	mature (female only)	126	67	$\begin{cases} 1 \text{ (M-1)} \\ 2 \end{cases}$	indistinguishable from wild mosaics
2	wild-2	mature	281	252	0	
3	wild-3	5th day	200	120	1 (male)	mosaic
4	wild-4	4th day	100	0	..	
5	wild-5	5th day	130	42	1 (male)	mosaic
6	wild-6	2nd day	300	72	0	
7	wild-6	2nd day	365	7	0	
8	wild-7	7th day	114	65	0	
9	wild-8	5th day	400	143	0	
10	wild-9	5th day	500	135	0	
11	wild-10	5th day	750	282	0	
12	wild-11	5th day	350	10	0	
Total			3,616	1,195	1 + 4	
13	w_1 -1	5th day	137	99	0	
14	w_1 -2	7th day	550	379	0	
15	w_1 -3	7th day	700	250	0	
Total			1,387	728	0	

experiment 1, in which only females were injected with wild-type DNA, one black-eyed and two mosaic-eyed moths were obtained in the treated generation. Two mosaics were also obtained in experiments 3 and 5. No pigmented eyes were obtained by treatment with w_1 -DNA.

The mosaicism was not inherited, since crosses of the four obtained mosaics to untreated w_1/w_1 produced only white-eyed moths. The black-eyed female (M-1) from experiment 1, which was indistinguishable from wild type in eye color, produced slightly pigmented eggs (less pigmented than intermediately colored eggs) when crossed to an untreated w_1/w_1 male. The moths (F_1 of M-1) developing from the slightly pigmented eggs were all black-eyed. Although the F_1 of M-1 produced segregating black- and white-eyed progenies, unusual patterns of egg color were obtained: When 121 sister-brother matings between the F_1 of M-1 were carried out, 49 crosses produced egg batches consisting of only black eggs, while 72 crosses produced batches consisting of intermediately colored eggs which were not expected since normally they are the product of a maternal effect. In the crosses between females (F_1 of M-1) and w_1/w_1 males or between w_1/w_1 females and males of the F_1 of M-1, production of eggs with unexpected colors was observed. However, the eggs, independently of whether they showed normal or unusual color, developed into black- and white-eyed adults in the proportions expected from simple Mendelian segregation. The situation is illustrated, in comparison with control crosses, in Figure 1.

Production of black eggs and black eyes in the progeny of treated moths and

TABLE 2

Production of black eggs and black-eyed moths in BF_1 derived from white-eyed moths injected with DNA as larvae

Experiment*	Egg		Adult		
	Number of batches examined	Number of black eggs	Number of moths examined	Black-eyed moths	mosaics
1	51	0	..	..	..
2	120	3† (M-2,3,4)	..	..	..
3	72	0	..	..	..
5	39	0	..	..	..
6	67	0	..	..	..
7	7	0	..	..	..
8	57	0	..	..	..
9	56	0	..	..	..
10	50	0	..	..	..
11	100	0	8,119	2 (M-5,6)	4
12	7	0	165	1 (M-7)	0
Total	626	3	8,284	3	4
13	87	0	..	..	..
14	341	0	..	..	..
15	100	0	6,715	0	0
Total	528	0	6,715	0	0

* The same numbers as in Table 1.

† Obtained from the same parent.

were obtained. These appeared in a single batch (269 eggs, Figure 4) from a treated female crossed to an untreated male. M-2 developed into a male, and the cross to an untreated female produced a mixture of white and intermediately colored eggs, as shown in Figures 2 and 4. (The control cross of a wild-type male to a w_1/w_1 female gave only intermediately colored eggs.) However, all of the white eggs as well as the intermediately colored eggs developed into black-eyed moths (F_1 of M-2). Among 104 batches from sister-brother matings between the F_1 of M-2, 66 egg batches consisted of black eggs only, 13 batches were mixtures of black and white eggs, 10 were mixtures of black and intermediate, and 15 were mixtures of all three kinds of color. Control crosses (103 crosses of $+/w_1 \times +/w_1$, Figure 2) gave only batches of black eggs. M-3 was also a male, and its progeny when crossed to a w_1/w_1 female segregated in a similar manner to M-2 (not shown here). The M-4 egg did not hatch.

Some of the white eggs in experiments 11 and 12 were raised to adults. As shown in Table 2, three black-eyed mutants (M-5, M-6, and M-7) and four mosaics were obtained among 8,284 adults. The size of the mosaic patches ranged from a great part of one eye in the largest mosaic to a small spot of an eye. All of the mosaics gave only white-eyed moths when crossed to w_1/w_1 . The M-5 male behaved as a w_1^+/w_1^+ homozygote as shown in Figure 3, since it gave only black-eyed progeny (F_1 of M-5) when crossed to a w_1/w_1 female. A cross of females (F_1 of M-5) to w_1/w_1 males (3 pairs) gave 523 black and 389 white-eyed

FIGURE 2.—Segregation of M-2 in Table 2. Control crosses are shown in broken lines.

progeny (referred to as A hereafter), indicating a significantly higher number of black-eyed offspring ($\chi^2 = 20$, $P < 0.001$). A cross (24 pairs) between white-eyed progeny from A again produced some black eggs: three among 445 in one

FIGURE 3.—Segregation of M-5 in Table 2.

batch (referred to as B), and one among 322 in another batch (C) as shown in Figure 3 and Table 3. Other kinds of crosses between A, white female $\times$ black male (30 pairs), black females $\times$ white male (22), and black female $\times$ black male (20), were carried out. Four pairs out of the 30 crosses of white female $\times$ black male produced both white and intermediately colored eggs, and one pair out of the 22 crosses of black female $\times$ white male produced intermediately colored eggs together with black ones (as illustrated by underlining in Table 3). Such egg colors were not obtained in corresponding control crosses, such as $w_1/w_1 \text{♀} \times +/w_1 \text{♂}$ (40 pairs), $+/w_1 \text{♀} \times w_1/w_1 \text{♂}$ (51), and $+/w_1 \text{♀} \times +/w_1 \text{♂}$ (70).

TABLE 3

Production of egg batches of unusual color† in crosses between (A) in Figure 3

Experiment	Cross	Egg color		
	♀ $\times$ ♂	White	Intermediate	Black
1	W $\times$ W*	442	0	3
2	W $\times$ W	321	0	<u>1</u>
3	W $\times$ B	167	68	<u>45</u>
4	W $\times$ B	226	123	<u>69</u>
5	W $\times$ B	215	80	<u>23</u>
6	W $\times$ B	143	117	<u>36</u>
7	B $\times$ W	0	<u>110</u>	117

* W, white eyed; B, black eyed.

† Eggs of unusual color are shown by underlines.

All three black eggs in batch B developed into black-eyed moths, two males and one female. All of these behaved again like w_1^+/w_1^+ homozygotes, since the cross to w_1/w_1 gave only black-eyed progenies. The black egg in batch C was a male, and was considered to be a w_1^+/w_1 heterozygote, since its offspring segregated both black-eyed and white-eyed moths in a 1 : 1 ratio when crossed to a w_1/w_1 female. The M-6 female behaved like a w_1^+/w_1 heterozygote in the cross to a w_1/w_1 male, but gave a significantly higher number of black-eyed offspring than expected (158 black and 87 white, $\chi^2 = 20.5$, $P < 0.001$). The M-7 male also showed an abnormal segregation ratio when crossed to a w_1/w_1 female (50 black and 18 white, $\chi^2 = 15$, $P < 0.001$).

No pigmented eggs or mosaic eyes were obtained in experiments 13, 14, and 15 (Table 1) where w_1 -DNA was injected.

Animals from another strain of white-1, KH-19, were also treated with wild-type DNA. As shown in Table 4, no black eyes or eggs were obtained in the treated and next generation.

DISCUSSION

The results described in this paper are fundamentally the same as the results obtained with *Ephestia* by NAWA and YAMADA (1968). There are several advantages in the use of silkworms for this kind of experiment. These are as follows: It is easy to get a large number of larvae in the same stage at the same time. The injection of larvae is simple. A male can mate with two or three females. The egg color of white-1 not only is determined by its own genotype but also reflects that of its mother. Of most importance is that several hundred eggs are deposited within a short time on a paper (egg card) by a female placed in a separate box (moth funnel), making contamination of eggs almost impossible. The main disadvantage is that it is only possible to obtain three or four generations in one year. Because of diapause, eggs must be treated by an artificial hatching method if eggs are to complete development within the same year. The eggs laid by females injected during their larval stage were, in general, sensitive to this treatment. Therefore, it was necessary to wait until the following spring if good hatchability was required. In our experiments, when black eggs were obtained they were in most cases allowed to hatch without treatment in the following spring.

TABLE 4

Treatment of larvae on 5th day of the last instar of KH-19 strain with DNA

Experiment	Source and preparation no. of DNA	Treated generation			BF ₁			
		Number of larvae injected	Number of moths obtained	Black-eyed moths	Number of egg batches examined	Black eggs	Number of moths examined	Black-eyed moths
16	wild-8	500	213	0	..	.		.
17	wild-9	750	135	0	..	.		.
18	wild-10	1,500	155	0	70	0	7,234	0
Total		2,750	503	0	70	0	7,234	0

The present data are suggestive of genetic alterations induced by the injection of DNA, but they do not exclude other possibilities. It will be necessary to get more instances of positive effects together with large numbers of controls before this conclusion can be established. The experiments will therefore be repeated with larger numbers of controls. However, most of the experimental results represented exceptional cases which have never been observed in control crosses involving untreated white-eyed animals.

It is surprising that a black-eyed moth (M-1) appeared among adults developed from w_1/w_1 larvae injected with wild-type DNA. Since M-1 behaved as if it were a w_1^+/w_1^+ homozygote as far as eye color is concerned, one may ask whether it was a contaminant. However, the feature of pigmentation of the eggs produced by M-1 seemed to contradict this possibility. The biochemical basis of the maternal effect of white-1 has been clarified by KIKKAWA (1941) and well reviewed (KIKKAWA 1953; TAZIMA 1964). The egg produced by the cross of a $+/+$ or $+/w_1$ female to a w_1/w_1 male contains a sufficient amount of 3-hydroxykynurenine from the mother. Therefore the egg with genotype $+/w_1$ or w_1/w_1 is black since it has the enzyme converting 3-hydroxykynurenine into black pigments, ommochromes. In other words, a black-eyed female always produces black eggs only. Contrary to this general rule, female M-1 (black eyed) produced no black eggs but slightly colored ones when crossed to an untreated w_1/w_1 male. It may be that the M-1 female could not supply a sufficient amount of 3-hydroxykynurenine to her eggs, although the eyes of M-1 were pigmented so that they were indistinguishable from wild type. All of these slightly colored eggs were raised to become black-eyed moths (F_1 of M-1) which were assumed to be w_1^+/w_1 genotypically. However, about one-half of the F_1 of M-1, in sister-brother matings or in backcrosses to w_1/w_1 males, produced black eggs only while the remaining half produced intermediately colored eggs which are not expected from the general rule of the maternal transmission. In fact, no such instances were observed in the control crosses (246 crosses) of black-eyed females ($+/+$ or $+/w_1$) to $+/w_1$ or w_1/w_1 males, suggesting that appearance of a wild-type female (black eyed) which produces slightly or intermediately colored eggs is an exceptional event. Thus in its breeding behavior M-1 did not behave like w_1^+ and can therefore not be contamination by a w_1^+/w_1^+ animal. Since the breeding behavior of M-1 has never been observed previously, its origin by contamination is highly unlikely. The fact that progeny of M-1 in the cross to w_1/w_1 were all black eyed would represent a massive conversion of the germ cells of M-1. It is reasonably possible to expect the appearance of black eyes in an animal in which the eye disk presumably still contains the original w_1 allele and in which the gonad possesses the mutated gene, since the gonad may produce a sufficient amount of 3-hydroxykynurenine to diffuse into the eye disk and be converted into the dark eye pigments.

A black egg obtained among white eggs of a brood is not considered a contaminant for the following reasons: The injected animals were separated into females and males at the pupal stage. Immediately after hatching, each treated female was mated to an untreated w_1/w_1 male or each treated male was mated to an

FIGURE 4.—Photographs of *Bombyx* eggs. a) A batch of $+/+$ eggs (black). b) A batch of w_1/w_1 eggs (white). c) A batch of $+/w_1$ eggs from the cross of a w_1/w_1 female to a $+/+$ male (intermediate). d) The batch from the cross of a $+DNA$ -treated female to a w_1/w_1 male. Three black eggs (M-2, M-3, and M-4) can be seen among white eggs. e) The batch from the cross of the M-2 male to a w_1/w_1 female. f) Egg batches from sister-brother matings of the black-eyed moths which developed from the eggs of (e): black eggs (f-1); black and white eggs (f-2); black and intermediate eggs (f-3); black, intermediate, and white eggs (f-4).

untreated w_1/w_1 female under separate funnels. The female, after being separated from the male by force, was placed under the funnel on an egg card. Eggs of a brood are deposited one-by-one in a regular order and are firmly glued to the egg card with substances secreted by the female. There would be little possibility that another female could squeeze into such a place in order to deposit her eggs. An egg from the outside, e.g., carried through the air or derived from unclean instruments, could not adhere to the egg card, much less enter secretly in the middle of the regularly arranged eggs. Thus, there is no doubt that the three black eggs M-2, M-3, and M-4 together with 266 white eggs (Figure 4) were derived from the same parents (cross of a treated white-eyed female to an untreated w_1/w_1 male). They behaved fundamentally like w_1^+/w_1^+ homozygotes. The appearance of such homozygotes in BF_1 , from a cross of a treated but phenotypically unchanged w_1/w_1 moth to w_1/w_1 , is an astonishing phenomenon. Even if in a germ cell of the treated animal the w_1 gene were transformed into

w_1^+ , the progeny from the cross to w_1/w_1 should be w_1^+/w_1 heterozygotes. The appearance of w_1^+/w_1^+ homozygotes, such as M-2 and M-3, would mean that w_1^+ eggs fertilized by w_1 sperm give rise to w_1^+/w_1^+ homozygotes. In other words, both the w_1 gene in the treated animal and the w_1 allele derived from the untreated parent would be affected. We have not sufficient evidence to present a convincing model to explain these phenomena at this time, but it may be reasonable to imagine replication of the incorporated DNA and its influence on the w_1 locus coming from an untreated parent.

Male M-2 (also M-3) produced a considerable number of white eggs in addition to the expected intermediately colored eggs, but all of the white eggs as well as the black ones developed into black-eyed moths (Figure 2). Some sister-brother matings of the black-eyed progeny produced unexpectedly white or intermediately colored eggs which were not seen in the control crosses (Figures 2 and 4). The appearance of such unusual egg colors (the same was observed in the progenies of M-1 and M-3) may be due to instability of the w_1^+ gene presumably introduced by the injected DNA. If the change from w_1 to w_1^+ were complete in a germ cell, it would give rise to a normal allele at the white-1 locus producing egg color in the normal fashion. If the change is incomplete in some unknown way, the expression of the w_1^+ gene would be incomplete or delayed producing unusually colored eggs. The frequency of the appearance of such egg batches containing eggs with colors not expected from the eye colors of their parents was too high to be considered as due to the factors (such as mutators, modifiers, or incomplete dominance) contributed from the white-1 stocks, particularly since none of the corresponding control crosses (40 batches from the cross of $w_1/w_1 \times +/w_1$, 51 $+/w_1 \times w_1/w_1$, and 185 $+/w_1 \times +/w_1$) produced such egg batches.

When egg color was examined, the appearance of normal-type eggs in experiments involving DNA treatment was rare. In BF_1 of treated animals, 626 egg batches were examined for color changes, but only three black eggs were obtained in a single batch. On the assumption that the average number of eggs in one batch is 300, 626 batches correspond to about 180,000 eggs, giving a very low frequency of 1/60,000. However, when part of the unpigmented eggs were raised, black-eyed adults appeared with a frequency of about 1/3,000. This may represent a case of delayed effect of DNA where the introduced w_1^+ gene is not expressed in the egg stage. The appearance of heterozygotes such as M-6 and M-7 in BF_1 from crosses of treated animals to untreated w_1/w_1 mates can be explained if one assumes that a gonadal cell in the treated larva was affected. However, the fact that they gave a significantly higher number of black-eyed offspring than expected if they were w_1^+/w_1 still represents abnormal breeding. Male M-5 was assumed to be w_1^+/w_1^+ on the basis of segregation of eye color in the cross to a w_1/w_1 female. The cross of M-5 to a w_1/w_1 female produced, however, white eggs only instead of the expected intermediately colored ones. The cross between white-eyed progeny (BF_2 of M-5), which had segregated in successive backcrosses of M-5 to untreated w_1/w_1 , again produced black eggs in a rather high frequency (Figure 3). All four eggs grew into black-eyed adults. The

possibility of contamination in these cases can be excluded by the same reasoning presented above. This may be a case of the persistence of the supposedly transferred dominant marker (w_1^+) in unexpressed form. This is in agreement with the findings of Fox and YOON (1966). They have obtained mosaicism for donor characters by treatment with DNA in *Drosophila*. The effects were shown to be transmitted to subsequent generations to produce repeated mosaicism, suggesting persistence and presumably replication of the effect of DNA through many germ cell generations. The transmission of the effect of DNA as a "replicating instability" to subsequent generations has been found generally in *Drosophila* (GERSHENSON 1940; MATHEW 1965).

Although we have no experimental basis for the identity of the transmissible factor, it must be replicated so that it can produce its effect in further generations with a frequency as high as in the preceding generation. Convincing evidence of the replication of extra DNA introduced into living cells has accumulated recently. LEDOUX and HUART (1969) have found that small fragments of bacterial DNA are joined end-to-end with barley DNA after incorporation into chromosomes of a barley host cell and that the new structure is subsequently replicated. GURDON, BIRNSTIEL and SPEIGHT (1969) have presented evidence for the replication of DNA injected into unfertilized frogs' eggs. They concluded that egg cytoplasm contains an enzyme capable of replicating foreign DNA. Specific gene amplification, in which DNA specifying ribosomal RNA (rDNA) is selectively replicated, has been observed in the oocytes of several amphibians (BROWN and DAWID 1968). They suggested that perhaps the extra replicas of rDNA function as "episomes" in extrachromosomal nuclear organelles and can escape the control mechanisms which function in somatic cells. If DNA carrying the w_1^+ allele is taken up by a germ cell and persists in some way, it would act almost fully as the normal chromosomal gene in some cases or do so to a lesser extent in other cases. If the DNA is able to produce one or more replicas independently of the chromosomes, they would be transmitted to subsequent generations. They would become incorporated as a chromosomal gene in some cells or be maintained as an unstable structure in other cells. Observations in this experiment, such as the appearance of the homozygotes, production of abnormal egg color, or prolonged and delayed effects, might be explained in terms of the existence of the unstable structure of incorporated DNA and its replication followed by transmission to subsequent generations.

Production of normal type by treatment with DNA is a rare event, occurring with a frequency of 10^{-3} – 10^{-4} in this experiment, being of the same order as in the experiment with *Ephestia* (NAWA and YAMADA 1968). This may be characteristic of this kind of experiment in higher organisms, presumably because of the low competence of cells and the heterogeneity of the DNA used where numerous kinds of DNA other than the DNA carrying the marker gene may compete for entrance into the recipient cells and into the nuclei. Even in the case of mammalian culture cells, the yield of transformants is usually small, of the order 10^{-3} – 10^{-4} (SZYBALSKA and SZYBALSKI 1962; FOX, FOX and AYAD 1969).

No black egg or black-eyed moth has been obtained so far in experiments where

DNA from white-1 was injected. This may suggest locus specificity of the effect of DNA, although the number of treatments was not sufficiently large to give definite significance to the difference between the effects of +DNA and w_1 -DNA. Spontaneous back mutation of w_1 to w_1^+ seems to be absent or very rare, since no such observations, at least for egg color, have been reported to date during the maintenance of the strains for many years in our own Institute. The fact that all of the mutants obtained behaved more or less unusually, as described above, suggests that these mutants appeared as a result of the treatment with DNA, apart from the argument whether the effect was specific for +DNA. In an experiment using another strain of white-1, KH-19, no black eggs or black-eyed moths were obtained. It is not known whether this is due to a difference in competence (genetic background) between Sw-1 and KH-19 strains.

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SUMMARY

When larvae of w_1/w_1 (white eyes and white egg color) were treated with DNA prepared from wild-type larvae, black-eyed moths were obtained in the treated generation and in the progeny of backcrosses to untreated w_1/w_1 moths. The change of w_1 to w_1^+ was detected in eggs in some cases by their color, but there were cases when the change was detected only after raising to adults. More than half of the black-eyed moths obtained either in the treated generation or in later generations behaved as if their genotype was w_1^+/w_1^+ . A few behaved as if their genotype was w_1^+/w_1 , but some of them produced significantly higher numbers of black-eyed adults than would be expected, when crossed to untreated w_1/w_1 . Some of these mutants produced eggs of colors not expected from the general rule of maternal transmission, sometimes even in later generations. Crosses between white-eyed moths obtained when one black-eyed mutant which behaved as if its genotype were w_1^+/w_1^+ was backcrossed to w_1/w_1 moths successively until segregation of black and white eyes occurred, again produced black eggs which developed into black-eyed moths. Mechanisms for the unusual features such as the appearance of "homozygotes," production of abnormal egg colors, or prolonged and delayed effects of DNA are discussed in terms of replication of the effect of incorporated DNA in an unstable structure as such, and its transmission to subsequent generations, either in an expressed or unexpressed form, through many cell divisions.

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