

ACKNOWLEDGMENT

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The author is also indebted to the National Research Council for a financial grant, and to Dr. C.K. Yin for constructive criticism.

Introduction

Centrifugal force as a mutagen has moved into focus as a result of the great upsurge of missile research. Accelerations in the launching of space projectiles become a biological problem when living things are placed into the nose cone.

Differences in densities of particles within the cell result in a reorientation of these particles, as was shown by Kasaguchi (1936). At sufficient forces chromosomal aberrations are to be expected.

Two possible effects are physiological damage including death of the cell or of the whole organism, and chromosomal aberrations affecting successive generations.

Very little experimental work has been done in this field. The centrifuge is a suitable device for the study of such mutagenic forces.

An embryological study was made by Pauli (1927) on Musca domestica, using centrifugal force in connection with other mechanical manipulations. No genetical studies followed. There appears to be no other work done with diptera.

Tanaka and Kasaguchi (1932) have produced a triploid silkworm by centrifuging eggs. More aberrations in the silkworm are reported by Kasaguchi (1936). With plants, centrifugation was used in connection with radiation on Tradescantia and Vicia by Sax (1943) and by Woff and van Herstel (1954). These workers found that preirradiation centrifugation decreases the number of aberrations whereas postirradiation

centrifugation increases aberrations. C.H. Yu (1960) centrifuged barley seed at forces up to 25000 g for as long as 24 hours, using caryocytes that had just begun to germinate. He found this to be near the lethal limit. There was a linear decrease of germination and a linear increase of chromosomal aberrations with increasing magnitude of centrifugal force.

It seems surprising that no experimental work had been done with Drosophila using centrifugal force, considering the vast array of genetical and cytological research on this genus. The advantages of this experimental animal offered in so many fields of biological work are equally great for the present study: ease of handling, fast reproduction, large progeny, and conspicuous phenotypical differences.

Materials and Methods

The stock cultures used for this study besides wild type (+) were scarlet eye (g), white eye (w), yellow body (y) and miniature wing (m). These are all sex-linked recessives. The stock was obtained from Furtak, Chicago. The following culture medium was found to be the best:

For 24 half-pint bottles:

Water	1115	cc
Agar	22.5	g
Corn syrup	202.5	g
Oatmeal cereal	10	g
Cornmeal	110	g
Casain	3-5	g
Holdax	2.25 g in 7.5 cc abs. alc.	

The addition of casain offset the depressing effect of the Holdax. The oatmeal smoothes the surface of the medium, which facilitates the observation of larval activities.

The flies were sucked out of the culture bottles into a thin glass tube where the sex could be determined and other preliminary observations could be made. They were then blown into the vials for centrifugation. When microscopic examination was indicated, they were blown into a Burco polyethylene etherizer and, after examination, centrifuged shortly before awakening started. The vials were closed with a rubber stopper through which a fine hole

was used. At speeds with a thrust of over 5000 g this stopper tended to be pressed into the vial. The hole in the stopper allowed for equalizing the air pressure.

An International Refrigerated Centrifuge, Model 7B-1 was used. Timing was started when the desired rotational speed was reached. The centrifuge was operated at room temperature or slightly higher (due to friction). The range was 23 - 24° C.

After centrifugation the flies were sorted out and transferred to culture bottles. At higher forces the flies had to be shaken loose from each other or separated with needles. Eggs were transferred with silver needles to which they tend to adhere on contact. Steel needles caused discoloration of eggs.

The progenies of treated flies were then examined for possible mutants. When these were obtained crosses were made to determine the genetic behavior of the mutants.

Experimental Results.

I. Lethal Doses.

Experiments were undertaken initially to determine how much centrifugal force the flies could stand.

a. Adults.

20 adult flies, 5 females and 5 males of each of the five stock strains were examined for phenotypical aberrations. Only normal ones were then subjected to 2000, 4000, 6000, 8000 or 10000 g for 20 minutes, 40 minutes or 60 minutes.

Table 1

Survival of Adult Flies

	20 min.					40 min.					60 min.				
	ε	w	y	m	+	ε	w	y	m	+	ε	w	y	m	+
2000 g	10	10	10	10	10	10	10	7	10	10	10	10	10	9	10
4000 g	10	10	9	10	10	10	10	10	9	10	10	10	9	9	10
6000 g	10	10	7	9	8	9	10	10	9	9	10	9	9	7	8
8000 g	8	7	8	7	8	9	8	7	9	8	9	9	10	7	7
10000g	7	7	8	6	9	6	4	6	6	7	3	2	1	5	5

At 6000 g and higher the flies lump together at the distal end of the vial, and at 10000g some are crushed. Their body fluids glue the whole group together so that the individuals have to be pried loose. In an evaluation of the survival figures it has to be kept in mind that there are a number of chance factors involved. E.g. if large females have been crushed there will be enough liquid for flies to drown. It was necessary to avoid any temperature shock when the vials were placed into the centrifuge head so that no condensate could form inside. It is also a matter of chance in what position the flies come to lie when the highest speed is reached. Furthermore, the concept of survival is relative. Some of the survivors die one hour later or the next day or they linger for two weeks. In these experiments a "survivor" is an animal that has not

died within a few hours after the treatment and is in general active the next day.

Many flies suffer bodily distortions and are unable to go through the mechanics of mating. Some appear morphologically normal but are abnormal in their behaviour, e.g. are closed down in their movements. Some are sterile, probably because of injuries to their gonads. Generally, the results are parallel to the ones of *Tu* for barley seeds, namely: the greater the force and the longer the duration of application, the less survival.

Only after the mildest treatment (2000g/20 min.) was survival 100 per cent, and survival decreased gradually up to 8000g/60 min. Thus, 56 per cent of the flies survived 6000g/20 min. and 84 per cent survived 8000g/60 min. Above this speed, however, damage was much more severe, for only 32 per cent of the flies survived 10000g/60 min. In most cases 10000g for one hour left some fertile survivors. When adult flies were centrifuged singly, the cushioning effect of the neighbour fly bodies was removed and more flies died. In one experiment it was shown that 13000g applied for two hours can be survived by some flies, but no progeny could be obtained.

Differences in survival between the sexes and between the five different strains were not significant.

b. Pupae.

20 pupae of the *y* and *w* strains were centrifuged for 60 minutes at 10000g on the paper to which they were attached. 17 hatched. The

paper may have shortened the centrifugation radius slightly, and this may have been significant, since the radius was only 55 mm. Thus the actual force may have been slightly less than calculated. However the survival rate is high. The pupa may well be the ideal stage in the life cycle for this treatment because of its resistant construction.

c. Larvae and eggs.

First, second, and third instar larvae and eggs were centrifuged together with the lump of food medium in and on which they lived. This was done at least 20 times. Adult flies were obtained in most cases from these larvae and eggs. Twice adult flies were obtained from larvae and eggs that received over 1300g for one hour. It was not established whether the survivors of this treatment had been eggs or larvae or both.

d. Eggs.

Sometimes etherized flies were caught in the process of laying an egg. These eggs, which protruded for part of their length from the ovipositor, were removed after centrifugation and raised successfully in a culture bottle. A method of collecting eggs in greater numbers could be worked out profitably.

II. Phenotypical Aberrations.

In over 200 experiments treated individuals and their F_1 and F_2 were raised. Each individual fly was examined for conspicuous

aberrations. Any mutation on chromosome I, whether dominant or recessive should show in the male progeny. Some of the aberrations on II, III, or IV would be evident in the F_2 from random matings even if recessive. In many cases the abnormal was not bred at random, but was isolated and mated with parents, similar abnormal, or, in the case of males, with virgin females. Previous experience with ants and fruitflies allowed estimation of the age of young imagoes from 0 minutes to about 15 hours on the basis of wing development, degree of pigmentation on the thorax and abdomen, and mode of movements. So it was possible to use virgins when indicated without removing an entire population and waiting for the emergence of virgins.

When non-virgins had been treated and an aberration was detected, it could not be determined whether the chromosomal mutation had occurred in oogenesis, in unfertilised eggs, in zygotes, or young embryos, or even to sperms in the spermatheca.

A great many "aberrations" proved to be the result of some physical accident during the ontogeny of the individual or even in handling after hatching. Leg deficiencies, malformations of body parts, club-foot, wing distortions, especially when present on only one side, were usually found to be non-inheritable. A number of "dwarfs" proved to be just undernourished or otherwise disturbed and did not transfer this characteristic to subsequent generations.

Any dominant lethals can, of course, not be detected directly.

A number of matings yielded very small progenies, possibly because of dominant lethal mutations.

1. Unusual sex ratios.

Experiment No. 1:

w ♀♀ virg. untreated x + ♂♂ 4000/20

White eye virgin females were mated with two wild-type males that had been given 4000g for 20 minutes. The F_1 (with possibly some F_2) comprised 15 ♀♀ 33 ♂♂. Since only the fathers had been centrifuged, one of them may have suffered a lethal dominant mutation on the X chromosome of his sperm killing all his daughters and none of his sons. The other treated male used as father did not suffer this mutation so that he produced the same number of daughters as sons. Another explanation would be, that a portion of the sperm of both fathers had been affected. A support for both these hypotheses seems to lie in the fact that in later counts of this population at random mating the ratio between the sexes became normal. Dominant lethals could not have been present in the surviving F_1 .

Experiment No. 2:

♀ virg. 6000/20 x w ♂♂ 6000/20

A wild-type virgin female and two white eye males were given 6000g for 20 minutes and then mated.

The F_2 counted:

Aug. 24	59 ♀♀	: 30 ♂♂
Aug. 26	33 ♀♀	: 14 ♂♂
Aug. 30	47 ♀♀	: 37 ♂♂
<hr style="width: 50%; margin-left: 0;"/>		
Total	139 ♀♀	: 81 ♂♂

An hypothesis was made that this is a ratio of 2 females to 1 male because of a sex-linked recessive lethal mutation. A chi-square test gave a P value greater than 0.20, so that the data are not inconsistent with the hypothesis. Thus it seems clear that sex-linked recessive mutations were induced on the X-chromosomes of the mother.

	observed	calculated (2:1)	d	d ²	d ² /c
females	139	147	-8	64	0.435
males	81	73	8	64	0.877
	<hr style="width: 50%; margin-left: 0;"/>				<hr style="width: 50%; margin-left: 0;"/>
	220				1.312 = χ^2
$\chi^2=1$ <u>$P > 0.20$</u>					

2. Short wings.

Another mutation on the X-chromosomes was induced in the following experiment.

Experiment No. 3:

Eggs and larvae from the g stock were centrifuged at 10000g for 60 minutes. One female and one male hatched. They were mated. 14 days

later there was one male in the F_2 with short wings. This character bred exactly like the sex-linked "r". With normal females this male produced phenotypically normal F_2 $\frac{1}{2}$ $\frac{1}{2}$. In a backcross with the father some of his daughters yielded short wings and normal in the ratio 1:1 in both sexes. In later interbreedings with a stock no difference between this mutation and "r" could be detected.

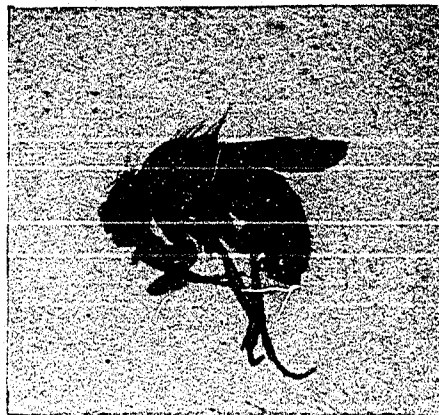


Figure 1: Short wing character in male of F_2 from centrifuged F_1 larvae and eggs.

3. Bent wings.

Experiment No. 4:

100 virg. untreated x g of 6000/20

Wild-type virgin females were mated to garnet eye males which had been centrifuged at 6000g for 20 min. After the F_1 was reared, the

later there was one male in the F_1 with short wings. This character bred exactly like the sex-linked "m". With normal females this male produced phenotypically normal F_1 's. In a backcross with the father some of his daughters yielded short wings and normal in the ratio 1:1 in both sexes. In later interbreedings with a stock no difference between this mutation and "m" could be detected.



Figure 1: Short wing character in male of F_1 from centrifuged F_1 larvae and eggs.

3. Bent wings.

Experiment No. 4:

+♀ virg. untreated x ♂ 63 6000/20

Wild-type virgin females were mated to garnet eye males which had been centrifuged at 6000g for 20 min. After the P_1 was removed, the

P_1 , which appeared all wild-type, were allowed to mate at random. 35 days later the population contained one male and two females whose wings were bent upwards, the strongest curvature occurring slightly more distally. These flies were not as active as those with normal wings. They were isolated and mated with normals:

Experiment No. 5:

2 ♀♀ virg. x bw ♂

All flies in the progeny were phenotypically normal. They were allowed to interbreed. Three gravid females from this culture were then isolated. They yielded the ratios 14:4, 25:9, and 26:8 normal: bw, total 65 normal: 21bw. The sexes were about equal in numbers. A series of backcrosses of these bw flies to males and virgin females of the parental generation yielded 1:1 ratios, again regardless of sex. This bw mutation is therefore an autosomal recessive. Subsequently this character was bred into the m strain.



Figure 2: bw mutation bred into a male of the m strain.



Figure 3: bw bred into a ♀ of the m strain.

F_1 , which appeared all wild-type, were allowed to mate at random. 35 days later the population contained one male and two females whose wings were bent upwards, the strongest curvature occurring slightly more distally. These flies were not as active fliers as those with normal wings. They were isolated and mated with normals:

Experiment No. 5:

2 ♀♀ virg. × bw ♂

All flies in the progeny were phenotypically normal. They were allowed to interbreed. Three gravid females from this culture were then isolated. They yielded the ratios 14:4, 25:9, and 26:8 normal: bw, total 65 normal: 21bw. The sexes were about equal in numbers. A series of backcrosses of these bw flies to males and virgin females of the parental generation yielded 1:1 ratios, again regardless of sex. This bw mutation is therefore an autosomal recessive. Subsequently this character was bred into the m strain.



Figure 2: bw mutation bred into a male of the m strain.



Figure 3: bw bred into a ♀ of the m strain.

The phenotypic expression of *bw* was clear in the shorter wings of the *m* strain also. Again the strongest curvature occurred slightly nearer the distal end of the wing.

But there was a pronounced difference in the intensity of expression of this mutation between the two sexes, in miniature flies. In the females the curvature of the wings was much stronger than in the males, but it was always strong enough in the males to be differentiated from normal.

There were no intermediate stages between normal and *bw* even in the males. The fact that the intensity of the phenotypical expression varies with sex only in the *m* strain, not in flies of normal wing length, may be a matter of variable expression, influenced in this case by the *m* genotype in combination with some factor derived from the difference between the sexes.

Since the *m* population as a whole exhibits a more static overall picture in a culture bottle due to the shorter wings and the thereby impaired flying skill, the *bw* homozygotes in such an *m* population cannot be singled out at first glance without anaesthetization and close examination, whereas there is a marked difference in flying agility between the *bw* and straight wings in the flies with normal wing length.

The interaction of the two independent characters *m* and *bw* throws some light on the mechanism of the bending of the wings: Waddington (1940), in a study of curly wings, explains the curling by assuming different rates of contraction on the upper and lower surfaces of the

wings in drying after hatching from the pupa. This difference must be greater where the curvature is most pronounced. Since the morphological features of the pupa in the *m* strain do not differ from those in the pupa of normal wing length flies, except in the size of the future wing, the location of the strongest curvature must be genetically determined, not mechanically by environmental factors during emergence, e.g. the shape of the pupal case.

In the *m* strain this spot of greatest curvature is not found at the same absolute distance from one end of the wing but divides it in the same ratio as in the long wing. In the long wing as in the short wing the bending appeared strongest slightly distal to the middle of the wing.

4. Pink eyes.

The history of this mutation was as follows:

Experiment No. 6:

+ ♀♀ virg. untreated x + ♂♂ 10000/20

Two wild-type virgin females were mated to three wild-type males after the latter had been centrifuged at 10000 g for 20 min. The F_1 was normal.

Experiment No. 7:

1 ♀ virg. F_1 10000x/60 ♂♂ F_1 10000/60

Of the F_1 from Experiment No. 6 one virgin female and several males were given 10000g for 60 min., and the one female survivor was mated

against two surviving males. In the progeny from this cross there were an unusually high number of phenotypical aberrations. Among others there was found one male whose eyes were a watered-down pink instead of the bright red of the wild type. Some of the peripheral ommatidia were missing. That area was brown like the rest of the chitin on the head, so that the eyes were reduced in size.

Spontaneous "pink eye" mutations have been reported by Brehms and Demerec (1942), Bridges (1923), Richards (1916), and Bridges and Morgan (1923). But the phenotypical details of our artificially induced aberration do not match the ones in the literature, except the one described by Bridges. In no case, however, was a reduction in size connected with it. This reduction in the size of the eye did not persist as the following experiments showed.

Experiment No. 8:

2 ♀♀ virg. x 1 pink eye ♂

The pink eye male was mated to normal virgin females. The progeny was normal with regard to eye colour. Two virgin females of this progeny were backcrossed to their father and yielded 48 normal and 40 pink eye flies of both sexes. The pink eye aberration is therefore recessive. It was not determined whether it was sex-linked or autosomal.

It was found that the reduction in size of the eyes was not as consistent as the distinct difference in coloration. Some pink eye females and males had normal size eyes. The variation was independent of sex. But it occurred only in flies of the pink eye phenotype.

The eye pigmentation did not change markedly during the lifetime of the fly as it does in a number of other eye colour mutants, e.g. in the garnet eye strain.

If this pink eye character is autosomal, the first discovered pink-eyed male must have been homozygous, and the mutation must have occurred in his grandfather, one of the males that originally received a dose of 10000g for 20 minutes in Experiment No. 6. Then both of his parents must have been heterozygous for this new recessive character. The additional and more severe centrifugation of both his parents was not the cause of this aberration, nor did it interfere with it. Since there were a number of other aberrations emerging in the same experiment, this initial treatment of the grandfathers with 10000g for 20 minutes must have been unusually antagonic on at least one of these male flies.

It was observed that the progenies involving pink eye were rather small as compared with other crosses under the same conditions. This suggested a detrimental effect of this mutation on viability or fertility.

5. Curly wings.

Experiment No. 9:

+ ♀♀ virg. 10000x/60 + ♂♂ 10000/60

Virgin females and males of the wild-type strain were centrifuged for 60 minutes at 10000g. The survivors were mated. In the F₁ there were three males and one female whose wings were strongly

curled upwards and slightly outwards. They were wrinkled in some individuals of later generations. Their flight was erratic and consisted of short hops. They were isolated and bred as follows:

1. 1♀ Curly x ♂♂ normal

F₁: 13 Curly (6♀♀, 7♂♂)

ratio 1:1

13 normal (6♀♀, 5♂♂)

2. + ♀♀ virg. normal x 1♂ Curly

F₁: 51 Curly (38♀♀, 13♂♂)

ratio 1:1

85 normal (45♀♀, 40♂♂)

3. + ♀♀ Curly x 1♂ Curly (backcross to father)

F₂: 69 Curly (38♀♀, 31♂♂)

ratio 3:1

25 normal (12♀♀, 13♂♂)

	observed	calculated	d	d ²	d ² /c
Curly	69	70.5	1.5	2.25	0.0319
Normal	25	23.5	1.5	2.25	0.0962
					<hr/> 0.1281 = χ^2
	N = 1	<u>P > 0.70</u>			

The deviation of the observed ratio from the expected is not significant.

These ratios are the inheritance pattern of a dominant autosomal character. In a number of subsequent breedings some of the Curly males and females of the F₂ yielded 100% Curly progeny with normal

mates, showing homozygosity.

Several Curly animals of the F_2 had their wings strongly distorted, almost shrivelled, and it was first believed that they were the 25% homozygous flies expected in the F_2 . But their number was much smaller than that, and two of them were shown to be heterozygous by yielding a 1:1 ratio when mated with normals. This was perplexing because the population was decidedly bimodal, with no gradations between Curly and Curly-shrivelled. The shrivelled flies did not produce more shrivelled progeny than the Curly.

The curvature in the Curly wings occurred throughout the length of the wings and differed in that respect from the recessive autosomal bent wing character induced in Experiment No. 4, where the strongest bending occurs slightly distal to the middle.

The complete independence of the bw character from Curly was shown in the following dihybrid cross:

C = Curly wing (dom.)	}	alleles
c = normal wing (rec.)		
B = normal wing (dom.)	}	alleles
b = bent wing (rec.)		
P ₁ : CCBB x cobb		

Three Curly females were isolated and mated each to a bent wing male. It was hoped that by chance one of the females was homozygous for Curly. This proved to be the case: two of the F_1 's contained 50% normals showing that the mothers had been

heterozygous (CcBb) for Curly. The F_1 of the third female was all Curly. They were allowed to mate at random. The F_2 counted:

65 Curly

18 normal

5 bent wing

This is a ratio of 13 : 3.6 : 1

	observed	calculated (12:3:1)	d	d ²	d ² /o
Curly	65	66	1	1	0.015
normal	18	16.5	1.5	2.25	0.136
bent	5	5.5	0.5	0.25	0.045
	88				0.196 = χ^2
N = 2		P > 0.20			

The data are not inconsistent with the hypothesis of independent assortment, with Curly epistatic over bent wing.

P_1 CcBb x ccbb

gametes CB cb

F_1 CcBb (all Curly)

F_2

	CB	Cb	cB	cb
CB	CCBB	CCBb	CcBB	CcBb
Cb	CCBb	CCbb	CcBb	Ccbb
cB	CcBB	CcBb	ccBB	ccBb
cb	CcBb	Ccbb	ccBb	ccbb

9 Curly

3 Curly, bent wing

3 normal

1 bent wing

The genotypes *CCbb* and *Ccbb*, which should show Curly due to its dominance and bent wing due to its homozygosity could not be distinguished phenotypically, obviously because Curly is epistatic over bent wing without being allelic. It would have been interesting to look for secondary phenotypical manifestations like bristle characteristics of these two independent genes and to sort the F_2 of this dihybrid cross with regard to them.

A "Curly wing" autosomal dominant character was obtained spontaneously by Hard (1923), who analyzed it cytologically and showed that it was due to an inversion on the left arm of the second chromosome. Startsev in 1931 found a "Curly wing" in wild-type flies caught at Ann Arbor. Bridges and Li, in 1936 also made a salivary gland analysis. But this mutation, in the homozygous form, showed more extreme wing characteristics or was even lethal. A cytological study might prove or disprove the identity of the previously reported "Curly wing" mutation with ours. Crosses between the two would also be relevant.

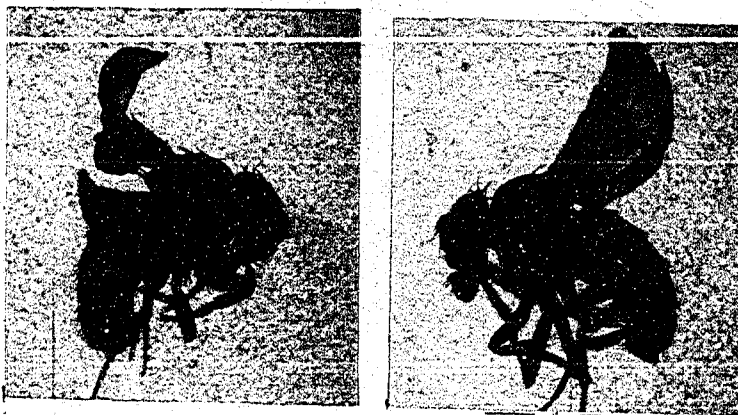


Figure 4, 5: Two Curly males.

6. Distorted Abdominal Sclerite Pattern.

Experiment No. 10:

+ ♀♀ virg., untreated x ♂♂ 6000/20

Virgin females of the wild-type stock were mated to males of the garnet eye stock that had been treated with 6000g for 20 minutes. Of the F_1 one virgin female and two males were the survivors of another centrifugation at 10000g for 60 minutes. They were mated. In the F_2 , a population of about 100 flies, we detected three females and one male with distorted abdominal tergites (dt), and one female whose left half of the thorax with wing was missing. Its whole body was distorted. This latter fly, when mated, was seen to lay eggs, but no larvae hatched. It is likely that copulation was impossible, for this extreme dt type.



Figure 4, 5: Two curly males.

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Figure 6: The *dt* character in severely crippled fly.

The *dt* phenotype shows the abdominal rings in a variety of irregular patterns, more pronounced in females than in males. Judging from the usually unhindered reproduction and normal viability of this type there are probably no serious distortions or malfunctions of abdominal organs, in spite of the derangement of the chitinous rings exteriorly.



Figures 7 and 8: The *dt* character.



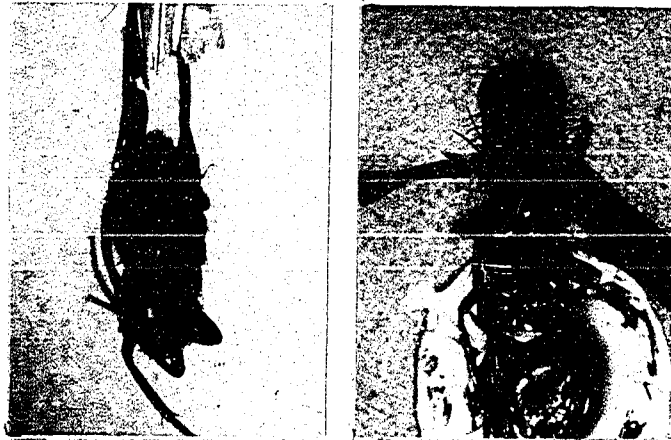
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Figures 7 and 8: The dt character.

The *dt* phenotype was found in many populations descended from centrifuged parents. They were isolated and mated in an extensive series of experiments. But no simple Mendelian ratios were obtained. Since it was suspected that this condition might be an aberration occurring in the Turtex stock or perhaps a frequent spontaneous mutation, a count was made in our untreated stock derived from the Turtex stock.



Figures 9 and 10: Two further *dt* flies.

Table 2

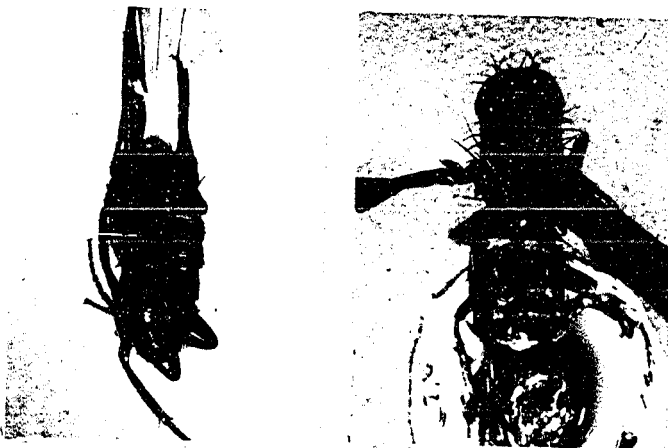
Flies in Untreated Stock.

Strain	normal	♀ <i>dt</i>	♂ <i>dt</i>	% <i>dt</i>
+	855	1	1	.23
g	790	1	-	.13
v	1022	3	2	.50
y	814	-	1	.12
n	566	1	-	.18

average

.23

The dt phenotype was found in many populations descended from centrifuged parents. They were isolated and mated in an extensive series of experiments. But no simple Mendelian ratios were obtained. Since it was suspected that this condition might be an aberration occurring in the Turtax stock or perhaps a frequent spontaneous mutation, a count was made in our untreated stock derived from the Turtax stock.



Figures 9 and 10: Two further dt flies.

Table 2

Flies in Untreated Stock.

Strain	normal	♀ dt	♂ dt	% dt
+	855	1	1	.23
g	790	1	-	.13
w	1022	3	2	.50
y	614	-	1	.12
n	566	1	-	.18

average .23

The percentage of dt flies in the progeny of treated flies was found to be ten to forty times as great as in stock cultures, e.g.:

Experiment No. 11:

a. + ♀♀ virg. 6000/20 x ♂♂ 6000/20

Wild-type virgin females and white eye males were given 6000g for 20 minutes and then mated. The F_1 was transferred to a new bottle. After $\frac{5}{4}$ days a population of 218 contained 6 flies that were dt, 2 ♀♀ and 4 ♂♂. Percentage 2.7.

b. + ♀♀ virg. untreated x ♂♂ 6000/20

Wild-type virgin females were mated to garnet eye males that had been centrifuged at 6000g for 20 minutes. The F_1 , containing 20 to 30 flies, were all given 10000g for 60 minutes, and the survivors mated at random. After $\frac{3}{4}$ days the population of 195 flies contained 9 dt. Percentage 4.6.

c. g ♀♀ 10000/20 x g ♂♂ 10000/20

Garnet eye females and males were given 10000g for 20 minutes and then mated. 2 $\frac{1}{4}$ days later there were 9 dt flies in a population of 94. Percentage 9.6.

When dt phenotypes were isolated and mated with dt flies, the frequency of dt in their F_1 and F_2 could be slightly increased., e.g.

d. g ♀ dt virg. x g ♂ dt

The F_1 contained 1 ♀ dt in 37. When the whole F_1 was mated at

random, the population after 60 days contained 12 dt in 164 flies, i.e. 2.7 times as many dt flies as in the F₁.

If this condition has a genetic basis, as the writer believes, it is clearly not due to a gene with a simple Mendelian inheritance pattern, but perhaps influenced by its genetical environment with a limited penetrance under certain conditions. The very wide variability of the phenotypical expression from one slightly shifted abdominal tergite to a complete distortion and crippling of the whole body (Figure 6 and Figure 9) is another support for the theory of the dependence of this gene on variable conditions in its environment. One of these environmental conditions is sex. The dependence on sex was mentioned above.

Bridges and Fehrer (1944) reported that Bridges in 1911 described a spontaneous sex-linked "bobbed" character as "abnormal abdomen effect in all females and no males." While the phenotype resembles our dt in part, the genetical behaviour does not. Stern (1927) reports a "side abnormal" effect with "tergites of abdomen etched at sides" and "very erratic behaviour of abdominal character." He finds that the male does not show these characteristics because he carries a dominant allele to this sex-linked recessive in his Y chromosome. The dt character however was also found in males, where it is not only less pronounced but also much more difficult to observe because of the strong pigmentation of the posterior coxites.

7. Dwarf.

Experiment No. 12:

+ ♀♀ virg. untreated x ♂♂ 8000/20

Virgin females of the wild-type stock were mated to miniature wing males which had been centrifuged at 8000g for 20 minutes.

The F_1 was normal and was allowed to mate at random. The F_2 contained one male dwarf which was about half the length of a normal fly. It happened to have miniature wings like the grandfather. These wings were proportionately dwarfed like the rest of the body. It was mated to 3 female virgins of the same F_2 . 16 days later the population contained 20 dwarfs in 59. Some of them were still smaller than their father. All dwarfs looked vigorous and behaved normally. They occurred among the π type as well as the wild-type strain in both sexes. The populations were clearly bimodal. These results suggested an autosomal recessive mutation. To prove this - a backcross was impossible because the only parental dwarf was lost - 5 male dwarfs were isolated and mated to stock virgin females. All of their progeny was normal. From each of these progenies two virgin females were backcrossed to their father. Four of these backcrosses yielded:

	17 normal,	15 dwarf
	20 normal,	23 dwarf
	28 normal,	27 dwarf
	28 normal,	25 dwarf
Totals	<u>93 normal,</u>	<u>90 dwarf</u>

This is a good 1:1 ratio. Both sexes were about equally represented. So this dwarf character is autosomal and recessive.

A number of dwarfs have been reported in the literature by several workers. All of these aberrations however are connected with malformations of the body like coarse wings, broad wings, dull surface, general "misshapeness", low viability and female sterility.

None of these associated effects could be found in this dwarf. It did not differ from normal flies except in size and was perfectly proportioned.

DISCUSSION

In this study centrifugal force has been used as a mutagen on adult insects for the first time. Only the most conspicuous phenotypical aberrations have been followed up genetically, and only those whose genetical behaviour could be experimentally established (except dt) were reported.

There were a number of other conspicuous aberrations for which the writer was unable to substantiate his initial hypothesis or to establish a new one. They include gigantism with flies up to twice the normal length; grey body with reduced pigmentation of the body but black bristles; and certain unusual sex-ratios.

It seems certain that a number of induced mutations remained unnoticed in these experiments because they caused only minor phenotypical changes.

This study has established a suitable range of mutagenic forces for use on adult flies, and partially on the other three stages of Drosophila. It has shown for the first time that centrifugation causes mutations in this genus.

Some of the aberrations studied may have arisen spontaneously, i.e. by action of cosmic rays or other uncontrolled mutagens. But the mutations reported here (except distorted tergites) have never been observed in the thousands of specimens from untreated stock cultures which were examined under the microscope. The frequency of such spontaneous aberrations must be at least very much smaller

than the ones induced by centrifugation. This was numerically supported by the study of the *ct* character. It is significant that no mutants were observed, other than *ct*, in the control stocks. But truly quantitative studies, perhaps with a *GB* stock, would be highly desirable.

That centrifugation interferes with the rearrangement of chromosomes in certain phases of cell division has been reported by a number of workers when they used other chemical or physical mutagens at the same time or successively.

Only Tanaka and Kawaguchi (1932, 1936), Kawaguchi (1936), Dodson and Yu (1950), and Yu (1960) have dealt with this force as the only or a primary mutagen. Barley seeds survive much greater forces than fruit-flies. But while Yu was able to examine the chromosomal aberrations in somatic cells in root-tips directly in the treated individual, this study is confined to the detection of phenotypical expression in successive generations and the determination of inheritance patterns.

Only one of the aberrations reported here was found to be identical to a well-known mutation, the short-wing character, which behaved like the sex-linked recessive *w*. The others, as discussed above, resemble in some phenotypical or genetical detail spontaneous or induced mutations reported in the literature, but are different in other respects.

For instance there have been a number of pink eye mutations reported by various workers, the first by Richards (1918). But

some of the reported pink eye change colour during the lifetime of the fly. The present mutation remained watery pink throughout life. Furthermore, there was in many flies a reduction in size of the eye, caused by the absence of marginal ommatidia. This occurred only in pink eye flies, but was not a constant effect.

Morgan (1923) made a cytological study of a pink eye mutation which was described as "gull ruby with a purplish tone". It was located on chromosome III at 68.0.

The logical extension of the present study would be in the following fields:

1. An examination of salivary gland chromosomes in heterozygotes with the induced aberration on one homologous. If the aberration is based upon a structural change in the chromosome, it can be seen.
2. Further genetical work with three-point crosses to determine the locus. Only then can it be shown whether the same mutations have been reported before. Crosses with other similar mutants should also be made.
3. A more intense microscopic search for minor phenotypical aberrations, e.g. in the morphology of major and minor bristles, and in the venation of wings. This would yield much more material with the same amount of centrifugation.
4. The speeds of centrifugation of adults could be further increased to the extent that only the occasional fly survives.
5. Centrifugation could be extended to several hours.

6. Intermittent force might have special mutagenic effects.
7. Eggs and larvae, but especially pupae might prove to be better stages for this treatment than the imago.
8. The interaction with centrifugation of other factors like temperature could be studied.
9. Quantitative studies by the G1b method, or by similar methods, should have an especial importance. This would permit more direct comparison with other mutagenic agents.

During a space flight in February 1961 a chimpanzee survived a force of 12g for several minutes as reported by scientists in press interviews. He showed no physiological damage. 12g is a small force as compared with the thousands of g used in this study. Undoubtedly, however, the question of mutagenic effects of smaller accelerations is one of the problems studied in the breedings of animals and plants used in space travel.

Drosophila melanogaster of five strains from Turtov were propagated on a new culture medium containing casain.

The dosage tolerance of flies for centrifugation was determined. It was found that, independent of sex and strain, survival at 10000g applied for one hour ranged from 10 to 50%, with an average of 32% for adult flies. Larvae and eggs survived this treatment also. The highest survival rate was found in pupae.

Survivors were mated with untreated virgins or treated flies. F_2 's were mated at random or with untreated virgins, or they were backcrossed. Phenotypical aberrations were tested for sex-linkage and dominance. The following aberrations occurred:

1. A dominant lethal on the X chromosome was induced in a male.
2. A recessive lethal on the X chromosome was induced in a wild-type virgin female.
3. Short wings, a sex-linked recessive, was induced in a larva and/or an egg.
4. Bent wings, an autosomal recessive, was induced in a garnet eye male. This character was found to be more strongly pronounced in females than in males, when bred into the m strain. In short as well as normal wings the strongest curvature of the occurs, slightly distal to the middle of the wing.

5. Pink eye, a recessive, was induced in a wild-type male. In many pink eye flies there was a reduction in the number of ommatidia. A depression of viability or fertility was observed.
6. Curly wing, a dominant autosomal, was induced in a wild-type male or female. Wings are strongly curved, coiled or shriveled, the curvature not occurring in a limited area of the wing. A dihybrid showed that Curly and bent wing are independent and that Curly is epistatic over bent wing.
7. Distorted abdominal sclerites occurred in normal crosses. A wide range of phenotypical expression was observed in both sexes. It occurred in the untreated stock cultures in 0.2%. In certain progenies of centrifuged parents there were ten to forty times as many. There was no Mendelian inheritance pattern, but probably there was limited penetrance.
8. Dwarfness, an autosomal recessive, was induced in a miniature wing male, with no secondary effects. The length of the homozygotes was about $\frac{1}{2}$ normal.

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