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A transposing forked-duplication with position effect variegation in *Drosophila*

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SUMMARY

In the course of an X-ray experiment, the normal allele of forked was transposed to the second chromosome, where it acts as a suppressor of forked. In this position, which is near the centromere, the duplication (*Dp-f*$^+$) is subject to a variegated position effect. This was studied in its dependence on the hetero-euchromatin balance; the results agree with and extend those found for other position effects. In addition, we found regional preferences for variegation in the individual flies. The most interesting aspect of *Dp-f*$^+$ is its tendency to transpose either to the homologous second chromosome or to Chromosome IV. In the latter position, *Dp-f*$^+$ acts as a dominant near-lethal, so that the apparent selectivity of insertion sites is at least in part due to deleterious effects at insertion sites other than its original one. In a new, and presumably, centromere-far position of *Dp-f*$^+$ on Chromosome II the variegated position effect disappeared and transposition was reduced in frequency or wholly abolished. The frequency of losses of *Dp-f*$^+$ approximately equalled that of transpositions. Since there is good evidence that transpositions occurred pre-meiotically, the apparent losses of *Dp-f*$^+$ may have been due to meiotic segregation separating the loss from the new insertion.

1. INTRODUCTION

Evidence for the existence of transposable elements in *Drosophila* has been reviewed by Green (1977). One case has been studied in detail by Ising & Ramel (1976). In the course of analysing a forked duplication for details of its variegated position effect, we realized that it has a tendency to transpose between chromosomes. In the following, we describe and discuss the origin, genetics and position effect variegation of this duplication (*Dp-f*$^+$), and present evidence for its transposable nature.

2. RESULTS

I. *Origin of the duplication*

The duplication (*Dp-f*$^+$) was discovered as a suppressor of forked in an experiment (Shukla, Sankaranarayanan & Sobels, 1979) in which X-rayed ♂♂ were mated to ♀♀ whose *X*-chromosome carried a number of recessive markers in addition to a recessive lethal. The markers of interest in the present context are

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yellow (y) and forked (f); the chromosome will therefore be symbolized as yfl, where l stands for the recessive lethal. The treated chromosome, too, carried marker genes; but as these are of no significance in the present context, the chromosome will be considered wild-type ($+\ +\ +$). The original cross can therefore be expressed as

$$♂♂+\ +\ +(3000\ \mathrm{R}) \times ♀♀ \frac{yfl}{+\ +\ +}.$$

The exceptional ♀ had the genotype

$$\frac{yfl}{\text{treated } X}.$$

Phenotypically, she was forked in about half her body and non-forked in the remainder. She was mated to a ♂ of genotype yf and the result is shown in Table 1. In this table, 'variegated' indicates flies with a few scattered forked bristles on an otherwise non-forked background. The number of these flies may have been underestimated, since only the dorsal sides of the flies were carefully inspected.

Table 1. *Progeny test on the forked-mosaic female*

F_1 ♀ $\frac{yfl}{\text{treated X}} \times ♂yf$

F_2 No ♂♂ of either type

	Genotypes		Phenotypes: Forked	Variegated	Non-forked
(A)	$\frac{yf}{\text{treated X}}$	29	15	8	6
♀♀ (B)	$\frac{yf}{yfl}$	23	11	8	4

In further tests, all forked ♀♀ bred true for f, while all variegated and non-forked ♀♀ yielded again the three types of progeny: forked, variegated and non-forked, with the ratio between the first class and the sum of the two others approximately 1:1. Moreover, when the originally induced forked-lethal had been replaced by forked from a different strain, ♂♂ with Dp-f^+ were also obtained, and these were of the same three types as the ♀♀.

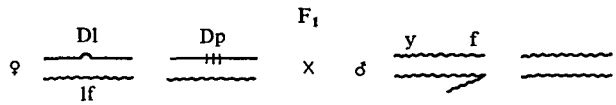
A number of conclusions could be drawn from these findings.

(1) The complete absence of ♂♂ in the F_2 showed that not only the untreated but also the treated X carried a lethal. Thus, irradiation had produced a sex-linked lethal.

(2) The large forked area in the original ♀ showed that the treated X carried also a mutation to forked. The simplest assumption was that (1) and (2) were due to the same cause, namely a deletion in the forked region which was lethal in hemizygotes but viable in heterozygotes.

(3) Phenotypically, the F_1 ♀ was a mosaic for forked. Her gonads, however,

were derived wholly from the non-forked part of her body and carried throughout a dominant suppressor of forked on one of the autosomes. This can be most clearly seen from her B daughters. These did not carry the treated X and should all have been forked. Instead, half of them were either non-forked or variegated for forked. The latter observation suggested that the suppressor was subject to a variegated



Males		F_2			Females		
Phenotype	Genotype	Sperm	Ova	Sperm	Genotype		Phenotype
Inviable	$\frac{Dl}{Y}\ \frac{Dp}{+}$	Y	Dl; Dp	yf	$\frac{Dl}{yf}\ \frac{Dp}{+}$	(A)	Wild-type or variegated
Inviable	$\frac{lf}{Y}\ \frac{Dp}{+}$	Y	lf; Dp	yf	$\frac{lf}{yf}\ \frac{Dp}{+}$	(B)	Wild-type or variegated
Inviable	$\frac{Dl}{Y}\ \frac{+}{+}$	Y	Dl; +	yf	$\frac{Dl}{yf}\ \frac{+}{+}$	(A)	Forked
Inviable	$\frac{lf}{Y}\ \frac{+}{+}$	Y	lf; +	yf	$\frac{lf}{yf}\ \frac{+}{+}$	(B)	Forked

—— Treated chromosome

~~~~ untreated chromosome

(A) and (B) refer to Table 1

Dl = deletion of forked locus

Dp = duplication of forked locus

Dp does not cover whole of Dl

Dl Dp ♂♂ are inviable

Fig. 1. Genetical interpretation of the data shown in Table 1.

position effect. In the A daughters, the suppressor acted on the heterozygotes between forked and the newly induced forked deletion.

(4) The deletion of forked and the dominant autosomal suppressor of forked had arisen in the same irradiated spermatozoon. The simplest assumption is that both were due to the same event, transposition of a piece of X with the normal allele of forked into one of the autosomes. Since, however, the viability of ♂♂ with the treated X was not restored by the presence of the duplication, it must be assumed that the transposed piece had lost some of its original material before or during integration into an autosome. As about half of the original fly did not carry *Dp-f*$^+$ in its soma, it seems that the transposed piece spent a short time in the free state before being integrated into an embryonic cell that subsequently gave rise to half the soma and the whole of the gonad.

Fig. 1 shows our interpretation of the data.

II. *Further genetical tests*

(1) *The forked-deletion.* Attempts to separate the lethal from forked were unsuccessful. Among nearly 5000 sons of ♀♀ heterozygous for the treated X, no forked

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♂♂ were found. This agrees with the assumption that both the lethal and the forked mutation were due to a deletion in the forked region.

(2) *The forked⁺-duplication.* The *Dp-f⁺* covered forked (56.7) in all tested strains. It did not cover either of the two nearest available flanking markers, rudimentary (54.5) and fused (59.5). Nor did it cover the known forked deficiency *Df* $(1)^{t\ 257\text{-}5}$ (Lindsey & Grell, 1968), which lacks three bands to the left and four to the right of forked. This agrees with the assumption that *Dp* had lost some of its material before incorporation into an autosome.

(3) *Location of Dp-f⁺.* With forked serving as marker gene for the presence or absence of *Dp-f⁺*, a standard test for locating a gene on a chromosome was performed. It showed that *Dp-f⁺* is carried on Choromosome II. A more accurate location test was carried out on 100 individual ♂♂, keeping their progeny separate. The idea behind this scheme was the possibility that *Dp-f⁺* might be an unstable element, with a tendency to change its location. The marker genes used were *cn* and *bw*, both on Chromosome II, at 57.5 and 104.5 respectively. Both affect eye colour: homozygotes for *cn* (cinnabar) have bright red eyes, homozygotes for *bw* have brown eyes, and double homozygotes have white eyes. Recombinants were scored in the progeny of *f/f*; *Dp-f⁺/cn bw* ♀♀ and *f*; *cn bw/cn bw* ♂♂. If, in one or more of the 100 P_1 ♂♂ *Dp-f⁺* had transposed to a different chromosome, it would have segregated independently of *cn bw* in these progenies; but this was not the case. *Dp-f⁺* was found to lie between *cn* and *bw*. Altogether 129 recombinants with *cn* were obtained in a total of about 8000 offspring, which puts *Dp-f⁺* at 59 on Chromosome II. A similar test carried out some months later located it at the same place. Further, successful, experiments on the transposable nature of *Dp-f⁺* will be described in a later section.

III. *The variegated effect*

In variegated flies, forked bristles occur singly or in small clusters in one or more areas of the body. Table 2 shows the distribution and frequency of forked bristles on the dorsal side of 400 variegated flies. 200 of these were ♂♂, and 200 ♀♀. In each group, half the flies were heterozygous for *Dp-f⁺* and half were homozygous. The bristles on the two sides of the body were scored separately as left (L) and right (R). The four first rows represent bristles on the head (H), the remaining ones, bristles on the thorax (T); there are no macrochaetae on the abdominal tergites.

A number of features should be noted in this table.

(1) In both sexes, variegation was stronger in heterozygotes for *Dp-f⁺* than in homozygotes: the difference was more pronounced in ♀♀ (419/121) than in ♂♂ (168/140). The highest degree of variegation was found in heterozygous ♀♀ (4·2 bristles per fly).

(2) Heterozygous ♀♀ showed more than twice as much variegation as did heterozygous ♂♂ (419/168); in homozygotes, this difference between the sexes had disappeared.

(3) In all classes, variegation was much more pronounced on the thorax than on

the head. The ratio between the numbers of bristles per fly that can be scored in these regions (26/16 = 1·4) was significantly exceeded in all classes, the discrepancy being highest in homozygous ♂♂ (140/0) and lowest in heterozygous ♀♀ (about 4).

Table 2. *Number of forked bristles on the dorsal side of variegated flies (in brackets: overall numbers)*

	♂♂*Dp*/+		♂♂*Dp*/*Dp*		♀♀*Dp*/+		♀♀*Dp*/*Dp*	
	L	R	L	R	L	R	L	R
Orbitals (6)	1	0	0	0	19	19	0	0
Ocellars (2)	2	0	0	0	18	10	0	0
Verticals (4)	3	1	0	0	13	16	0	0
Post-verticals (2)	5	4	0	0	2	3	3	0
Σ Head (14)	11	5	0	0	52	48	3	0
	16		0		100		3	
Humerals (4)	15	9	5	1	13	6	0	6
Pre-suturals (2)	6	8	7	4	11	9	3	2
Notopleurals (4)	25	15	23	20	39	22	24	19
Supra-alars (4)	13	12	16	4	27	14	5	2
Dorsocentrals (4)	5	3	3	4	31	13	3	2
Post-alars (4)	4	2	4	4	31	21	6	2
Scutellars(4)	15	20	19	26	50	32	31	13
Σ Thorax (26)	83	69	77	64	202	117	72	46
Total	94	74	77	63	254	165	75	46
	168		140		419		121	
Forked bristles per variegated fly	1·7		1·4		4·2		1·2	

(4) In all classes, variegation was more pronounced on the left than on the right, with the ratio between left and right about 1·6 in ♀♀ and about 1·3 in ♂♂.

A special analysis of forked bristles on the scutellum showed that these arise independently on the left and right side of the body. The few cases in which one left and one right scutellar bristle were forked were not in excess of what would be expected from a coincidence of two single forked scutellars. On the contrary, there was a more than expected frequency of cases in which two left or two right scutellar bristles were forked, indicating that the event resulting in variegation had occurred prior to the separation of the two bristle anlagen from each other.

The effects of one or two doses of *Dp-f*$^+$, of an extra *Y*-chromosome, and of the origin of *Y* and *Dp-f*$^+$ from father or mother were further studied in a number of crosses listed in Table 3. The degree of variegation in the progeny of these crosses is shown in Table 4. In all six crosses, variegation was measured by the proportion of variegated flies among the non-forked progeny. In crosses (a) and (e), the mean numbers of forked areas per fly (as defined in Table 2, one area occasionally

overlapping two bristle groups), the mean numbers of forked bristles per area, and the mean number of forked bristes per fly were determined for 100 ♂♂ and 100 ♀♀. In contrast to the last line of Table 2, the mean number of forked bristles per fly refers to all non-forked flies, including the non-variegated ones; this has resulted in some differences.

Table 3. *Crosses for determining the effect of the residual genotype on variegation**

(a) ♂*f*; *Dp*/+ × ♀*f*/*f*; +/+
(b) ♂*f*; +/+ × ♀*f*/*f*; *Dp*/+
(c) ♂*f*; *Dp*/+ × ♀*ff*/Y; +/+
(d) ♂*f*; +/+ × ♀*ff*/Y; *Dp*/+
(e) ♂*f*; *Dp*/*Dp* × ♀*f*/*f*; *Dp*/*Dp*
(f) ♂*f*; *Dp*/*Dp* × ♀*ff*/Y; *Dp*/*Dp*

* *ff*/Y = ♀ with attached X's both carrying *f*.

Table 4. *Analysis of the non-forked progeny in crosses (a) to (f) in relation to the numbers of Y and Dp-f⁺ and their maternal (m) or paternal (p) origin*

Cross	Sex	Genotype	Variegated (%)	Forked areas per fly	Forked bristles per area	Forked bristles per fly
(a)	♂	Y_p; Dp_p	59 (504)*	0·9	1·6	1·4
	♀	No Y; Dp_p	94 (528)	4·0	1·2	4·8
(b)	♂	Y_p; Dp_m	93 (786)	—†	—	—
	♀	No Y; Dp_m	96 (856)	—	—	—
(c)	♂	Y_m; Dp_p	66 (843)	—	—	—
	♀	Extra Y_p; Dp_p	47 (717)	—	—	—
(d)	♂	Y_m; Dp_m	34 (673)	—	—	—
	♀	Extra Y_p; Dp_m	38 (557)	—	—	—
(e)	♂	Y_p; *Dp*/*Dp*	33 (285)	0·5	1·0	0·5
	♀	No Y; D_p/D_p	66 (300)	0·8	1·0	0·8
(f)	♂	Y_m; D_p/D_p	42 (740)	—	—	—
	♀	Extra Y_p; D_p/D_p	20 (535)	—	—	—

* Number of flies examined.
† No scored.

The following conclusions can be drawn from Table 4.

(1) In *females*, variegation was most pronounced when *Dp-f*⁺ was present in heterozygous condition, independent of its origin from father or mother (a, b). Homozygosity for *Dp-f*⁺ reduced the percentage of variegated flies as well as the number of forked bristles per fly (a; e). An extra Y reduced variegation even further (c,d). The lowest degree of variegation was found in ♀♀ that were homozygous for *Dp-f*⁺ and carried an extra *Y* (f).

(2) In *males*, too, homozygosity for *Dp-f*⁺ reduced variegation (a and b *versus* e and f); but here the situation was complicated by an apparent influence of the maternal or paternal origin of *Dp-f*⁺ and *Y*. The lowest percentage of variegated

♂♂ occurred in heterozygotes for *Dp-f*$^+$ in which both *Y* and *Dp-f*$^+$ were of maternal origin (d), and the highest in heterozygotes for *Dp-f*$^+$ in which *Y* was paternal and *Dp-f*$^+$ maternal (b). Other crosses failed to show an influence of the origin of *Y* on variegation (e.g. e and f); but such an influence has been reported by several other workers (Spoffard, 1976).

Table 5. *The effect of the* Y*-chromosome on the action of* Dp *in heterozygotes*

	P_1 (genotypes)		F_1 (phenotypes)			
Cross	♂	♀	Sex	No *Y*	1*Y*	2*Y*
(a)	$X\hat{Y}/O$	× *XX*; *Dp/Dp*	♂	149 var	—	—
				46 f	—	—
			♀	—	245+′	—
(b)	*X/Y*; *Dp/Dp*	× $\hat{XY}/\hat{XY}$	♂	—	—	523+′
			♀	—	534+′	—
(c)	$\hat{XY}/O$	× $\hat{XY}/X$; *Dp/+*	♂	59 var	100 var	—
				(35 f*)		
			♀	—	63+′	65+′
(d)	*X/Y*	× $\hat{XY}/X$; *Dp/+*	♂	—	45 var	50+′
			♀	37+′	54+′	—
				19 var		

Abbreviations: var = variegated for forked; $\hat{XY} = Y^S.X.Y^L$, carrying *f*, other marker genes, and an inversion.

* See test.

The effect of the *Y* chromosome on variegation was further studied in crosses which allowed a comparison between males and females with 0, 1 and 2 *Y*-chromosomes. The strain used in these crosses had a compound *XY* chromosome, with the long and short arms of the *Y* attached to the two ends of the *X* ($Y^S.X.Y^L$; in Table 5 abbreviated as $\hat{XY}$). All *X*-chromosomes in the crosses carried *f*; the compound *X* carried, in addition, marker genes and an inversion. Males and females with either $\hat{XY}$ or a free *X* were crossed with each other; *Dp-f*$^+$ was present in one of the sexes in homozygous or heterozygous conditions. Only progeny heterozygous for *Dp-f*$^+$ are listed in Table 5; in crosses with heterozygotes the +/+ offspring was recognized by being forked all over (but see discussion of Cross c).

The data in Table 5 complement those of Table 4 and have to be compared with them. This leads to the following conclusions.

(1) *Females without* Y (cross d). Variegation was found in 19 out of 56, i.e. in 33 %. Thus the position effect in these ♀♀ was less pronounced than in the *XX* ♀♀ of Table 4 (a, b), in which over 90 % were variegated. A possible reason for this difference may be the fact that the ♀♀ in cross 5 d had developed from eggs carrying both *Dp-f*$^+$ and *Y*, in which there may have been some effects of *Y* on *Dp-f*$^+$ already before fertilization.

(2) *Females with one* Y (crosses a, b, c, d). In all of these, nearly 900, suppression of *f* by *Dp-f*$^+$ was complete, i.e., there was no position effect at all. This has to be

compared with crosses c and d in Table 4, in which about 40% of $X\hat{X}/Y$; $Dp/+$ ♀♀ were variegated. The reason for the difference may lie in the fact that the attached X's in the latter crosses lacked part of the centromeric heterochromatin, so that the overall amount of heterochromatin in these ♀♀ was less than in the $X/X\hat{Y}$ ones.

(3) *Females with two* Y (cross c). All of them were $+^{t}$; thus the position effect was completely inhibited.

Table 6. *Phenotypes and genotypes among males without Y in cross c*

Phenotypes		Genotypes: Original classification	Genotypes: Re-classification
Variegated	59	*Dp*/+	59 *Dp*/+
Forked	125	+/+	(appr.) 35 *Dp*/+
			(appr.) 90 +/+

(4) *Males with one* Y (crosses c and d). All 145 ♂♂ were variegated, indicating a strong position effect. Since in c both their Y and their *Dp-f*$^{+}$ were maternally derived, the corresponding cross in Table 4 is d, in which only 34% of the ♂♂ were variegated. There is no obvious explanation for this difference, except that the free Y in 4 d may have acted differently from the attached one in 5 c. The result of d, in which Y was paternal and *Dp-f*$^{+}$ maternal, does not differ materially from the corresponding cross 4 b.

(5) *Males without* Y (crosses a and c). In cross a, the mother had been homozygous for *Dp-f*$^{+}$ so that all her sons were $Dp/+$. Out of 195, 149, i.e. 77%, were variegated, while the remaining 23% were forked. In the latter ♂♂, *Dp-f*$^{+}$ was completely inactive or, expressed differently, the position effect was complete. In cross c, a difficulty arose from the fact that the mother had been heterozygous for *Dp-f*$^{+}$. Thus, among her progeny, half would be expected to carry *Dp-f*$^{+}$ and be phenotypically forked, and half not to carry it and be phenotypically wild-type or variegated. This was indeed found to be true for the female progeny and for sons with a Y-chromosome. Among sons without Y, however, there were about twice as many forked as variegated ones. If all of the former are classified as *Dp*-free, the segregation ratio for *Dp-f*$^{+}$ would be grossly distorted in just this one group of progeny. We prefer to assume that, as in cross a, inactivation of *Dp-f*$^{+}$ was complete in a proportion of the ♂♂, which therefore appeared forked even in the presence of *Dp-f*$^{+}$. Table 6 shows the actual numbers and the two ways of attributing genotypes to them. The re-classification has been used in Table 5 and Fig. 2. Since ♂♂ without Y are sterile, proof for its correctness could not be obtained.

(6) *Males with two Y-chromosomes* (crosses b and d). All 573 of these were wild-type, showing that *Dp-f*$^{+}$ was fully active in them, i.e. that the position effect was completely inhibited.

The effects of the *Y*-chromosome on *Dp-f*$^+$ in heterozygous condition can be summarized as follows.

The 'normal' sexes, i.e. ♂♂ with 1 *Y* and ♀♀ without *Y*, contain a varying proportion of flies in which *Dp-f*$^+$ is either fully active (wild-type) or partially suppressed (variegated). In ♂♂, removal of the *Y* results in such a strong position effect on *Dp-f*$^+$ that a large proportion of the flies are wholly forked. On the contrary, addition of a second *Y* to the male complement counteracts the position effect so effectively that *Dp-f*$^+$ is fully active and the flies are wild-type. In ♀♀ addition of one *Y* inhibits the position effect on *Dp-f*$^+$, so that many or all flies are wild-type; in ♀♀ with two *Y* chromosomes there is no position effect; *Dp-f*$^+$ is fully active and all flies are wild-type.

IV. *Transpositions of* Dp-f$^+$

As has been mentioned before, the location tests gave no indication for transposition of *Dp-f*$^+$ from its original position on Chromosome II. In fact, transpositions would have to be very frequent for one of them to be found in a sample of only 100 ♂♂. Further experiments were carried out with the sole aim of testing for loss or transposition of *Dp-f*$^+$ in large numbers of flies. Two mating schemes were used, A and B. In both of them, all flies were genotypically forked, so that the presence of *Dp-f*$^+$ in a fly could be detected by its being phenotypically non-forked or variegated.

Scheme A utilized the same markers that had been used in the location tests *cn* (cinnabar) and *bw* (brown).

P_1 ♂♂ *f*; *cn Dp/cn bw* × ♀♀ *f/f*; *cn bw/cn bw*

Keeping in mind that no crossing-over is expected in ♂♂ and that the interaction of *cn* and *bw* yields white eyes, the expected F_1 is as follows:

50 % *f*; *cn Dp/cn bw* cinnabar eyes, normal or variegated bristles

50 % *f*; *cn bw/cn bw* white eyes, forked bristles.

Loss of *Dp-f*$^+$ will be detected in the cinnabar flies. If such a fly has not received *Dp-f*$^+$ either because it has been lost altogether or because it has been transposed to a chromosome that did not segregate into the same zygote as the *cn* chromosome, its bristles will no longer be forked-variegated but forked throughout. Transposition of *Dp-f*$^+$ will be detected in the white-eyed flies. If such a fly carries a transposed *Dp-f*$^+$ on any one of its chromosomes, it will no longer be forked but variegated or wild-type for bristles. Table 7 (*a*) shows the results of three experiments.

There were 5 exceptional flies among a little over 12000 offspring. Since, however, losses could be detected in only one half of the flies and transpositions only in the other half, the number of flies used for ascertainment was only half that counted, i.e. about 6000. Four of the exceptional flies had lost *Dp-f*$^+$ and this was confirmed in further tests. The fifth might have been due to transposition of *Dp-f*$^+$ to the homologous chromosome or to another chromosome. Location showed that the first was the case. As far as could be judged by crossover data, the transposed *Dp-f*$^+$ occupied the same locus on the *cn bw* chromosome as it did originally on the *cn* chromosome. This suggests that it might have arisen from a

rare spermatogonial crossover. In fact, it is possible to make the same assumption for the four flies that had lost *Dp-f*$^+$ from the *cn* chromosome; alternatively, they might have resulted from transpositions of *Dp-f*$^+$ to another chromosome, which was not included in the exceptional flies.

Scheme B. In order to decide between these possibilities, a mating scheme was used that prevented crossing-over and made it possible to follow transposition to any one of the chromosomes. Four dominant marker genes were used, all of them lethal in homozygous condition: *Cy* (curly; wings) and *Pm* (plum; eye colour) on

Table 7(*a*). *Tests for meiotic loss or transposition of* Dp-f$^+$

Exp.	No. P_1 ♂♂	Total offspring	Average no. offspring per male	Exceptions (phenotype) cn; f (loss)	Exceptions (phenotype) w; f ^{var} (transposition)
I	25	3175	127	1	0
II	20	2958	147	1	0
III	45	6068	134	2	1
		12201		4	1

* Variegated for forked.

Chromosome II, and *Sb* (stubble; bristles) and *D* (dichaete; wings and bristles) on Chromosome III. *Cy* is connected with a system of inversions that prevents crossing-over in the whole chromosome.

P_0 ♂♂*f*; *Dp/Dp*; +/+ × ♀♀*f/f*: *Cy/Pm*; *Sb/D*

P_1 ♂♂ or ♀♀*f* (or *f/f*); *Cy/Dp*; *D*/+ mated singly to *f/f* ♀♀ or *f* ♂♂.

F_1. If *Dp-f*$^+$ has retained its position, all *Cy* progeny will be forked, and all non-*Cy* progeny will be non-forked or variegated. Exceptional flies will be of two types. One of these, due to loss of *Dp-f*$^+$, can be detected among the non-*Cy* flies by being forked instead of variegated. The other, due to transposition, can be detected among the *Cy* flies by being non-forked or variegated for forked. Table 7*b* shows the results of an experiment in which exceptions were scored separately in the progeny of 40 *Dp*-carrying ♂♂ and 30 *Dp*-carrying ♀♀.

Table 7(*b*). *Tests for loss or transposition of* Dp-f$^+$. *Scheme B*

No. P_1 flies	Sex of P_1	No. F_1	Exceptions (phenotype) non-Cy; f (loss)	Exceptions (phenotype) Cy; f (transposition)
40	♂	1200	1	2
30	♀	1500	1	5 (4)*
		2700	2	7 (6)

* Two from the same ♀, possibly a cluster.

Again, the number of progeny that could be used for ascertainment was about half that scored, i.e. about 1400. Among these, there were 9 or 8 exceptions, depending on how the cluster of two is counted. These exceptions could not be attributed to crossing-over. The type of cross used made it possible to determine the origin of most of them.

The 2 non-*Cy* forked flies did not carry *Dp-f*$^+$ and could therefore not be analysed. They may have arisen either from loss of *Dp-f*$^+$ from the genome or from its transposition to a chromosome that did not segregate into the zygote. Analysis of the 7 *Cy f*^{var} flies was more revealing.

These were of two genotypes, depending on whether or not they carried *D* on the third chromosome. Those that did not were crossed to flies of the multiply

Table 8. *Analysis of the* Cy f^{var} *exceptional flies*

Fly no.	Sex	Recombination of *Dp-f*$^+$	*Dp-f*+ on chromosomes
		(I) Derived from P_1♂♂	
1	♂	1:1 with sex, *Cy*, *D*	IV
2	♂	linked to *Cy*	II (*Cy*)
		(II) Derived from P_1♀♀	
3	♀	Linked to *Cy*	II (*Cy*)
4	♂ same	Linked to *Cy*	II (*Cy*)
5	♀ mother	Linked to *Cy*	II (*Cy*)
6	♂	1:1 with sex, *Cy*, *D*	IV
7	♀	all progeny forked	Not present in gonads

marked stock used in P_0; those carrying *D* were mated to forked flies, not otherwise marked. In most cases, one generation was sufficient for deriving the chromosome to which *Dp-f*$^+$ had moved; in only one case (6 in Table 8) was a second generation required. The crosses and the conclusions drawn from them are shown in Table 8.

One exceptional fly (7), which carried *Dp-f*$^+$ in its soma, failed to transmit it. Since *Dp-f*$^+$ cannot be scored in the abdomen, this fly may well have been a fore-aft mosaic. It is of interest in this context that the fly in which *Dp-f*$^+$ had been first detected likewise was a mosaic, although in that case the gonads had carried *Dp-f*$^+$.

In the remaining 6 cases, *Dp-f*$^+$ had transposed to another chromosome, with striking selectivity. There was no transposition to either the third chromosome or to one of the sex chromosomes. In four flies, two of which may have formed a cluster (4 and 5), *Dp-f*$^+$ had transposed to the homologous Chromosome II, and in two, to the very small Chromosome IV. In all exceptional flies, the suppression of forked by *Dp-f*$^+$ was complete; there was no variegation for forked bristles. This indicates that *Dp-f*$^+$ had moved away from the centromere-near region. Analogy with the previously found transposition of *Dp-f*$^+$ to a non-inverted second chromosome (Table 7*a*) suggests that also in the present cases, *Dp-f*$^+$ had inserted itself at its original position of 59. Since the *Cy*-inversion of the right arm of Chromosome II has its proximal breakpoint near the centromere in Region 55, insertion of *Dp-f*$^+$ at 59 would indeed have removed it far from the centromere. Unfortunately, this could not be established because no location tests are possible with this multiply inverted chromosome. Location tests for Chromosome IV are difficult and were not carried out.

A possible explanation for the selectivity of transposition was suggested by breeding tests on flies that carried *Dp-f*$^+$ on Chromosome IV. Both ♂♂ and ♀♀ of this type were rather infertile, and very few of their progeny had inherited *Dp-f*$^+$. A number of ♀♀ were individually tested for fecundity, fertility and type of progeny. Fecundity, as measured by the number of eggs laid, was reduced in comparison with *Dp*-free forked ♀♀. More important was the finding that only about 50 % of the eggs hatched, and that almost all flies hatched were forked, i.e.

Table 9. *Frequency of loss of transposition of* Dp-f$^+$ *from a normal Chromosome II and a Cy-chromosome*

		Nbr exceptions	
P_1	Nbr progeny	Loss	Transposition
Dp+/+*Cy*	7400	6	8(7)*
Dp Cy/++	4700	0	0

* If a cluster of 2 is counted as 1.

were free of *Dp-f*$^+$. Thus, in its position on Chromosome IV, *Dp-f*$^+$ acts as dominant near-lethal, resembling in this respect the transposable *his* C4 gene of yeast which, on transposition, created a recessive lethal in the recipient chromosome (Greer & Fink, 1979). If this were the case for many or most sites of transposition, with one special site on Chromosome II forming an exception, the selectivity of transposition would be at least partially explained.

In its position on the *Cy*-chromosome, *Dp-f*$^+$ was as viable as previously in its – possibly homologous – position on the normal second chromosome, but was no longer subject to a position effect. Simultaneously it appeared to have become stabilized in its chromosomal site. Losses and transpositions were scored in the usual way in the progeny of forked flies, one of the parents transmitting the *Dp Cy* chromosome to its offspring. No exceptions were found in two experiments in which altogether about 9400 F_1 flies were examined. Correcting again for the fact that losses can be detected in only half the flies, and transpositions in the other half, this gives a frequency of 0 in 4700. Table 9 compares this with the frequencies found for a non-*Cy* second chromosome (Tables 7*a* and 7*b*).

The difference is highly significant (χ^2 between 6 and 7). Thus, in its new position *Dp-f*$^+$ not only was no longer subject to a position effect but also showed a strongly diminished tendency for transposition or had lost this tendency altogether.

3. DISCUSSION

Three aspects of the forked-duplication are of interest: its origin, its position-effect variegation, and its tendency to transpose. We shall discuss them in this order.

Origin of Dp-f$^+$

The first fly to carry *Dp-f*$^+$ was a daughter of a ♂ that had received 3000 R of X-rays. The origin of *Dp-f*$^+$ was not a translocation of the types that are readily produced by X-rays and that lead to the exchange or unilateral transposition of fairly long chromosome pieces. In our case, the transposed piece was extremely small, comprising only the normal allele of forked and, at most, very little else. It appears to be the remnant of a larger deletion in the X which, in addition to the forked locus, contained at least one locus for viability. This conclusion is based on three findings: (1) the deletion in the *X* and *Dp-f*$^+$ in Chromosome II arose in the same spermatozoon; (2) the deleted *X* was lethal and lacked the normal allele of forked; (3) *Dp-f*$^+$ covered forked but not the lethal. It also appears that the deleted piece spent some time in the free state before being incorporated into its new position, for the F_1 ♀ carried *Dp-f*$^+$ in the whole of her ovaries but only in about half her soma. The most likely course of events thus was the following. A small piece of the *X*-chromosome surrounding the locus of forked was removed, possibly under the influence of the X-ray treatment. This piece was trimmed down to a smaller one either in the free state or at the moment of integration into its new site.

(i) *Variegated position effect*

In its new position on Chromosome II, *Dp-f*$^+$ is subject to a variegated position effect, variegation being expressed as single forked bristles or small groups of them appearing among the otherwise non-forked dorsal surface of the fly (the ventral one was not studied). Considering the locus of *Dp-f*$^+$, at a distance of 4 c.o. units from the centromere, such a clear position effect is somewhat unexpected. Possibly, a cytological study might reveal the presence of intercalary heterochromatin near the site of *Dp-f*$^+$. Whatever the cause of the position effect, it clearly exists and exhibits the well-known characteristics of similar effects. These concern mainly expression of the variegation in response to differences in the hetero-euchromatin balance of the residual genotype. In general, a low ratio favours expression of variegation, a high ratio inhibits it (Spofford, 1976).

This was found to be true also for the position effect variegation of *Dp-f*$^+$. Keeping in mind that maximum expression of position effect will result in complete inhibition of *Dp-f*$^+$, i.e. in forked flies, while minimum expression of position effect will result in full expression of *Dp-f*$^+$, i.e. in wholly non-forked flies, our results as set out in Fig. 2 will be seen to conform to expectation. The lowest hetero-euchromatin ratio is found in ♂♂ without a *Y*-chromosome, and this is the only genotype in which forked flies appear, i.e. flies in which *Dp-f*$^+$ is fully inhibited. At the other extreme, the highest hetero-euchromatin ratio is found in ♂♂ and ♀♀ with 2 *Y*-chromosomes, and these are wholly non-forked, i.e. have a fully active *Dp-f*$^+$. Wholly non-forked flies occurred also in the progeny of ♀♀ with 1 *Y*-chromosome, provided this was attached to one of the *X*'s. In contrast, ♀♀ with attached X-chromosomes and a free *Y* contained, in addition to wholly non-

forked flies, a varying proportion of variegated ones. As explained before, this may be due to the fact that the attached X's lack part of the central hetero-chromatin, so that the overall hetero-euchromatin balance is reduced. In Fig. 2, these ♀♀ have been included in the middle row, which contains also the normal sexes: ♂♂ with 1 Y, and ♀♀ without Y. All these genotypes yielded mixtures of

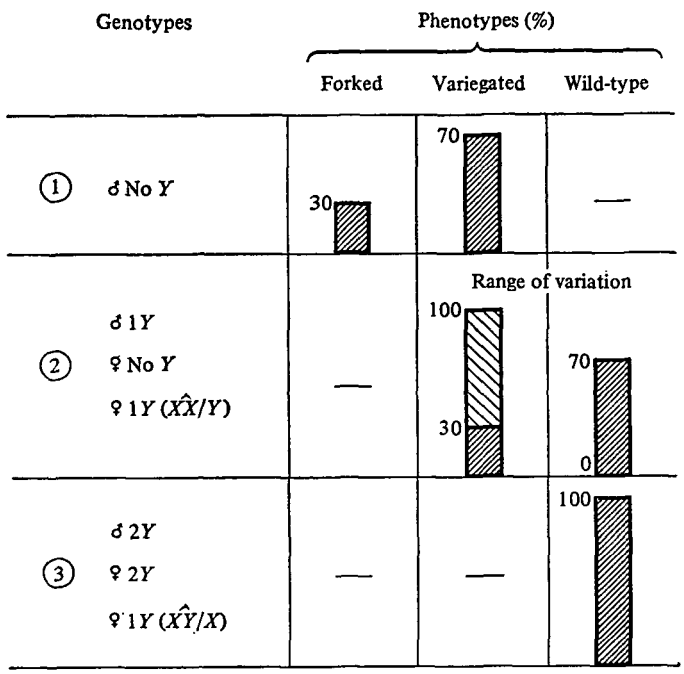

Fig. 2. The effect of the number of Y-chromosomes present on variegation in the two sexes.

wholly non-forked flies without position effect, and variegated flies with position effect. The degrees of position effect in dependence on sex, homo- or heterozygosity for *Dp-f*$^+$, and the maternal or paternal origin of forked and *Dp-f*$^+$ have been discussed; they resemble the variability of other position effects described in the literature. A feature that, to our knowledge, has not formerly been described, was the bilateral asymmetry of the variegation, the left side being the more variegated one in both sexes.

(ii) *Transposition and loss of* Dp-f$^+$

Estimates of the frequencies with which these events happen to *Dp-f*$^+$ in its original position on Chromosome II can be derived from Tables 7(*a*) and (*b*). Adding the data from these tables and taking account of the fact that only half the number of progeny scored could be used for ascertainment (see remarks to Table 7*a*), there were 6 losses and 7 or 8 transpositions in approximately 7400 germ cells, yielding frequencies of 0·09 % for losses and 0·1 % for transpositions. For transpositions, this is an underestimate. The fact that two transpositions

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occurred as a cluster in the progeny of the same ♀, and even more the frequent transpositions of *Dp-f*$^+$ from one second chromosome to its homologue, show that often, possibly always, transposition occurred pre-meiotically; the same conclusion has been drawn by Ising & Ramel (1976) for the transposable element TE 1 of *Drosophila*. In this situation, apparent losses may be transpositions in which the chromosome that had gained *Dp-f*$^+$ did not segregate together with the one that had lost it. It is, therefore, possible that most or even all losses were in reality transpositions. Moreover, the two transpositions to the fourth chromosome were dominant near-lethals; the chance of finding them among the progeny was therefore low. If the same should apply to other sites of integration, many transpositions may have escaped detection. In any case, the frequency of transpositions in our experiments was much higher than that of TE 1, which ranged from 0·5 to 8·5 in 10^5. On the other hand, in contrast to the data of Ising & Ramel, the frequency with which *Dp-f*$^+$ was lost in our experiments was not markedly higher than that of transpositions.

Ising and Ramel interpreted the high frequency of losses compared with transpositions as evidence for an intermediate state, in which TE 1 had left its original site without yet having inserted itself at another one. Although in our experiments there was no excess of losses over translocations, there was some evidence that, for *Dp-f*$^+$ too, insertion in a new site may be preceded by a short period of free existence. Thus, the first fly that showed the presence of *Dp-f*$^+$ carried it only in its gonads and half its soma. A second mosaic, carrying *Dp-f*$^+$ in the soma but not in the gonads, was found among flies with a newly transposed *Dp-f*$^+$.

Transposition of *Dp-f*$^+$ appeared to be site-specific. Out of 10 (or 9, if the cluster is counted as one), 7 were to Chromosome II, and 2 to Chromosome IV. The highly deleterious effect on viability of the two transpositions to IV suggests that this selectivity reflects in part the scarcity of sites at which *Dp-f*$^+$ can be integrated without interfering with the action of a gene or genes required for viability. Probably, this is not the only reason for site specificity. Some degree of sequence recognition might be expected to play a role. This is also suggested by the fact that, in at least one of the 7 cases of transposition to the homologous chromosome, the position of *Dp-f*$^+$ was the same as in the original chromosome. For the 6 transpositions to the *Cy*-chromosome, this could not be ascertained; however, the expectation that, in the inverted *Cy*-chromosome, *Dp-f*$^+$ at its original site would not be subject to a position effect was borne out by observation.

Of special interest is the finding (Table 9) that, in its new and presumably centromere-far position on the *Cy*-chromosome, *Dp-f*$^+$ not only had ceased to show variegation but also seemed to have lost its tendency for transposition. This raises the intriguing possibility that variegated position effect and transposability are in some way related, perhaps by an effect of heterochromatin on both transcription and replication. In the absence of a clear understanding of either position effect or transposition, further speculation on this relationship would at present not be fruitful.

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