MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B, VOL. XIX, NO. 1, Article 3, 1947.

Overlapping Inversions in the X-chromosome of *D. virilis* Hybrids

By

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I. Introduction

Cytogenetic studies on the hybrids between *Drosophila virilis* subspecies, *virilis*, *americana* and *texana*, were carried out by Spencer (1938), Hughes (1939), and Patterson, Stone and Griffen (1940). The sexual isolation between them was studied by Stalker (1941). Chino also published his genetic studies on the three subspecies (1940, 1941). Since last year, I have worked on the genetics and cytology of the same material under the direction of Dr. M. Chino. The results obtained so far are included in the present paper.

II. Cytological results

All the *Drosophila virilis* subspecies show a pair of rod-shaped sexchromosomes on the metaphase plate. In *americana* the X-chromosome is fused with an autosome and the Y-chromosome is free. In his study on salivary gland chromosomes, Hughes has comfirmed that *americana* differs from *virilis* in the arrangement of genes in various ways, especially its X-chromosome contains rather complex inversions. According to Patterson, Stone and Griffen, the X-chromosome of *americana* has three inversions when it is compared with that of *virilis*, while *texana* has two of them, lacking the third one. The X-chromosome of the salivary gland cells of the hybrid either *virilis*×*texana* or *virilis* × *americana* shows an incomplete synapsis, and there are overlapping inversions occupying the proximal half of the chromosome.

The extents of these inversions approximately estimated are as follows:

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Hybrid	From	То	Мар	(Author)	
v×t	90 16 ID5e	170 30 IG3c	Genetical Salivary "	(Chino (Fujii (Patterson	'36) '36) '40)
v×a.	75 12 IC7d 8DI	170 30 IG30 19A2	Genetical Salivary "	(Chino (Fujii (Patterson (Hughes	'36) '36) '40) '39)

a=americana, v=virilis, t=texana

The $v \times t$ overlapping inversions have the distal break approximately at *forked* (f, 89.0), and include *eosin* (w^e , 105.0) *scalloped* (*sd*, 114.0) and *small bristles* (*sb*, 131.5). The distal break of $v \times a$ overlapping inversions seems to be approximately at *minuature* (*mt*, 78.0). Therefore, *yellow* (y, 2.9), *cross-veinless* (c, 25.0) and *miniature* (*mt*, 78.0) are located in the univerted segment. The proximal break of these overlapping inversions falls very near the inert region of the chromosome.

III. Effect of the overlapping inversions on crossing over in X-chromosome

The effect of the inversions in the heterozygous state on crossing over in the X-chromosome is shown in Table I.

Regions		Control values for uninverted			
	v×t	Total flies	v×a	Total flies	X-chromosome obtained by Chino
j'с	38 .7	333	21.6	93	22.2
y-mt	48.8	168	50.0	128	44.8
c-mt	44.7	341	46.6	203	39.4
c—f	41.4	333	22.2	48	42.7
mt—f	0.005	195	-		10.8
mt-we	0.1	762	100.0	651	25.7
f	0.0	101	0.0	104	15.8
mt-sd	0.2	487	0.0	179	
mt—sb	0 .0	239	-	. —	40.2
\u00er_sd	0.0	762	0.0	149	7.4
rvc—sb	0.0	341	·		29.1

Table I. Recombination values for heterozygous overlapping inversions.

All of the data presented in Table I show that (1) the crossing over in the region included in the overlapping inversions is suppressed to almost zero; (2) the crossing over in the distal region of the chromosome beyond *mt*, hardly presents any such supressing effect. The crossing over in the region at the distal end of the chromosome (*y-mt*) shows even a tendency to increase, much as in the case of the two independent inversions XSpd worked out by Komai & Takaku (1940).

IV. Primary non-disjunction

Like in various strains of inversions recorded in the X-chromosomes of *D. virilis* as well as of *D. melanogaster*, the present hybrids produce a considerable number of exceptional males and females, when the X-chromosomes are heterozygous for the inversions. This is especially the case in the $v \times a$ hybrid (Table 2).

Table 2. Frequencies of primary exceptions in the hybrids having the overlapping inversions in heterozygous state together with those obtained by previous authors for *D. virilis virilis* X-chromosome. The primary exceptions were obtained from the mating $mt\text{-}vv^c/+\times y\text{-}mt\text{-}vv^c$ or $mt\text{-}vv^e/+\times mt\text{-}vv^e$.

			Percentage	Total flies
v×t		ô	2.27	747
$v \times a$	{	₽ 8	6.06 10.45	33 727
In(X)Std	• {	ç. S	0.20 1.63	17608 5890
No inversion, spontaneous	{	₽ ô	0.0124 0.12	
No inversion, X-rayed	{	₽ ô	0.I0 0.12	

The *virilis* \times *americana* hybrid shows about 5 times as many nondisjunctional males as the *virilis* \times *texana* hybrid. This difference is probable due to that the X-chromosome in *americana* is fused with one of the autosomes, IV, while it is free in *texana* and *virilis*, and also that a long included inversion is present in the IV chromosome of *americana*.

V. Lethal effect

The inversions in the X-chromosome so far found in this species have a lethal effect on the male, irrespective of their length and locus. The present overlapping inversions have no such effect, in spite of their complexity. In the F_6 of the hybrid of $v \times t$, however, a recessive lethal gene was discovered. This is located in the region included in

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the overlapping invsrsions, as shown by the experiments presented in Table 3.

Cross	$\mathbb{P}\left(\frac{mt\text{-}\tau\omega^2}{l}\right)\times \mathfrak{F}(mt\text{-}\tau\omega^e)$	$\operatorname{P}\left(\frac{mt-sd}{l}\right) \times \operatorname{O}\left(mt-w^{e}\right)$	$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \\ \end{array} $	
F1	$\begin{array}{c} \varphi + mt - \tau v^{e} & \Im & mt - \tau v^{e} \\ 252 & 320 & 282 \end{array}$	$\begin{array}{rrrr} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \end{array}\\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \end{array} \begin{array}{r} \begin{array}{c} \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \begin{array}{c} \\ \end{array} \end{array} \begin{array}{c} \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \begin{array}{c} \\ \end{array} \end{array} \begin{array}{c} \begin{array}{c} \\ \end{array} \\ \begin{array}{c} \\ \end{array} \end{array} \end{array} \end{array} \end{array} \begin{array}{c} \\ \end{array} \end{array} \end{array} \end{array} \end{array} \begin{array}{c} \\ \end{array} $	♀+ mt 8 mt-f f 314 369 195 1	

Table 3. Experiments for locating the lethal gene on the X-chromosome $(v \times t)$.

The examination of salivary gland chromosome has revealed, however, nothing suggesting a deficiency in the corresponding region. In the F_2 — F_6 hybrids of the $v \times a$, the lethal gene has not been discovered.

VI. Feasibility of the cross and vitality and fertility of hybrids

(a) texana $(\varphi) \times virilis (\diamond)$

This cross is very difficult, but the hybrid female is always fertile. For this cross *virilis* males having several recessive mutant characters were used, and the hybrid females $(X^{\nu}X')$ were backcrossed with the *virilis* male $(X^{\nu}Y')$.

Sex-chromos	somes	$X^{i}X^{v}$	XvXv	$X^{t}X^{v}:X^{v}X^{v}$ (%)	Xt Yv	Xv Yv	$\begin{array}{c} X^{t}Y^{v} \colon X^{v}Y^{v} \\ (\%) \end{array}$
	F_2	295	278	106.1	160	306	52.2
	\mathbf{F}_3	735	735	100.0	528	678	77.8
(a) $t \times v$	\mathbf{F}_4	404	411	98.2	241	434	55-5
	\mathbf{F}_5 '	384	412	93.9	197	388	50.7
	\mathbf{F}_{6}	189	184	102.7	II2	190	58.9
Sex-chromos	somes	X ^a X ^v	XvXv	$\begin{array}{c} X^a X^v : X^v X^v \\ (\%) \end{array}$	XaYv	$X^{v}Y^{v}$	XaYv:XvYv (%) ,
	Fg	48 [°]	37	129.7	47	26	180.7
	F_3	193	179	107.8	130	190	68.4
(b) $a \times v$	\mathbf{F}_4	334	2 40	139.1	204	261	78.1
	\mathbf{F}_5	446	401	111.2	245	369	67.4
	\mathbf{F}_6	661	593	111.4	354	539	65.6
Sex-chromo:	somes	X"Xt	$X^{v}X^{v}$		X^rYt	XtYt	$\frac{X^{v}Y^{t}:X^{t}Y^{t}}{(\%)}$
	F_2	139	121	114.8	91	117	77.7
(c) v×t	\mathbf{F}_3	101	84	120.2 *	- 78	82	95.1
	\mathbf{F}_4 .	87	71	122.5	76	78	97-4
	\mathbf{F}_5	44	43	102.3	43	39	110.2
5	\mathbf{F}_{6}	81	95	85.2	77	91	84.6

Table 4. Number of hybrid progeny

(**b**) americana $(\bigcirc) \times virilis (\diamondsuit)$

This cross is even more difficult than (a). The hybrid female, however, is invariably fertile. The strains used for this cross were the same as in (a).

(c) virilis $(\mathfrak{Q}) \times texana$ (\mathfrak{G})

This cross is reciprocal of (a), and is more difficult. The difference from the cross (a) is that the hybrid male has a *virilis* X-chromosome and a *texana* Y-chromosome.

The details of the crosses a—c are shown in Table 4. In the crosses (a) and (b), the heterozygous male $(X^{t}Y^{v} \text{ or } X^{a}Y^{v} \text{ or } X^{v}Y^{t})$, in spite of their wild phenotype, shows lower vitality than the homo-zygous male $(X^{v}Y^{v} \text{ or } X^{t}Y^{t})$. In females, on the other hand, hetero-zogous wild-type females $(X^{t}X^{v} \text{ or } X^{a}X^{v})$ showed rather high vitality as compared with homozygous mutant females $(X^{v}X^{v})$.

The fertility of the heterozygous and homozygous males was tested by means of the following crosses :

$$\begin{array}{l} \bigcirc X^{v}X^{v} \ (\mathit{mt}\mathchar`v) \times \begin{cases} \diamondsuit & X^{v}Y^{v} \ (\mathit{mt}\mathchar`v) \\ \diamondsuit & X^{t}Y^{v} \ (+) \end{cases} \\ \bigcirc & X^{v}X^{v} \ (\mathit{mt}\mathchar`v) \times \begin{cases} \circlearrowright & X^{v}Y^{v} \ (\mathit{mt}\mathchar`v) \\ \circlearrowright & X^{a}Y^{v} \ (+) \end{cases} \\ \circlearrowright & X^{v}X^{v} \ (\mathit{mt}\mathchar`v) \times \begin{cases} \circlearrowright & X^{v}Y^{v} \ (\mathit{mt}\mathchar`v) \\ \circlearrowright & X^{v}Y^{v} \ (\mathit{mt}\mathchar`v) \\ \circlearrowright & X^{v}Y^{v} \ (+) \end{cases} \end{array}$$

In these crosses the homozygous females were mated with equal numbers of homozygous and heterozygous males. The results showed that the heterozygous males $(X^tY^v \text{ or } X^aY^v)$ had much lower fertility than homozygous males (X^vY^v) , but the difference between the two types of males having the *texana* Y-chromosome (X^vY^t) or X^tY^t was not so remarkable. From this data it is surmised that *americana* and *texana* are both mutant types of *virilis virilis*, and the low vitality and fertility of heterozygous males are due to low fertility and hatchability of the pure strains of these mutant types.

Summary

I. The three subspecies of *D. virilis, virilis, texana* and *americana*, are differentiated from one another by the presence of complex overlapping inversions in the X-chromosome; this can be ascertained by the examination of the hybrid female larva's salivary gland chromosome.

2. The overlapping inversions strongly suppress the crossing over

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in the regions included in the inversions; they have a tendency to increase the crossing over in the distal region of the chromosome not included in the inversions.

3. The heterozygous females having the overlapping inversions, give nearly 18 times (in $v \times t$) and 87 times (in $v \times a$) respectively as many primary exceptional males as the ordinary *virulis* females:

4. A recessive lethal gene was discovered in the region included in the inversions on the *texana* X-chromosome.

5. The heterozygous males $(X^a Y^v, X^t Y^v)$ have lower vitality and fertility than homozygous males $(X^v Y^v)$. This seems to be due to that both *texana* and *americana* arose by mutations from *virilis*. The sexchromosomal isolation between the three subspecies is particularly strong between *texana*, *americana* on the one hand and *virilis* on the other.

Literature

- CHINO, M. 1936-1937. The Genetic of Drosophila virilis. Jap. Journ. Genet. 12: 187-210, 257-277, 13: 100-120.
- -----, 1943. The three subspecies of Drosophila virilis. Jap. Journ. Genet. 18: 88-97.

FUJII, S. 1936. Salivary gland chromosomes of Drosophila virilis. Cytologia 7: 272-275.

- HUGHES, R. D. 1939. An analysis of the chromosomes of the two subspecies Drosophila virilis and Drosophila virilis americana. *Genetics* 24: 811-834.
- PATTERSON, J. T., STONF, W. S. and GRIFFEN, A. B., 1940. Studies in the Genetics of Drosophila XI. Evolution of the virilis group in Drosophila. Univ. Texas publ. 4032: 218-250.
- STALKER, H. D. 1940. Chromosome homologies in two subspecies of Drosophila virilis. Proc. Nat. Acad. Sci. 26: 575-578.