Further Studies on the Salivary Gland Chromosomes of *Drosophila virilis*

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Contents

	Page
Introduction	435
Descriptions of the chromosomal aberrations used in the present study	436
The effect of the heterozygous translocations on crossing over	444
Locating genes on the salivary gland chromosomes	446
Genetical nature of certain structures of the salivary gland chromosome	448
Summary	453
Literature	453
Appendix	456

Introduction

The first salivary gland chromosome maps of *Drosophila virilis* were published by Hughes (1936) and myself (Fujii 1936) nearly simultaneously. Later, Hughes (1939) revised his maps in his report on the analysis of the hybrid between *D. virilis virilis* and *D. virilis americana*. More recently, Patterson, Stone and Griffen (1940) have completed more detailed maps of the chromosomes of wild stock of *D. virilis virilis* from Henly, Texas.

These author's studies have elucidated the morphology of the chromosomes in great detail. The recognized bands have been considerably increased; the presence or absence of the β -heterochromatin in each chromosome has been elucidated; the existence of the bands in the β -heterochromatin has been confirmed and the composition of the α -heterochromatin has been worked out, besides some other less important things. The genetical side of the study of these chromosomes, however, has called relatively little attention of these investigators.

I have carried out genetical and cytological studies on various strains of translocations and other chromosomal aberrations in *D. virilis* so far available to me. These studies have enabled me, first, to determine the effects of these translocations on crossing over, second, to locate a number of genes on the salivary gland chromosomes, and third, to elucidate to some extent the genetical significance of the structure of the chromosomes. This paper deals with these findings.

Before going further, I wish to acknowledge my indebtedness to Prof. Taku Komai through whose kind guidance and encouragement the com-

pletion of this work has been possible. My thanks are also due to Dr. Mitsushige Chino and Dr. Hideo Kikkawa for valuable advice and precious materials.

Descriptions of the chromosomal aberrations used in the present study

The following chromosomal aberrations have so far been reported in *D. virilis*. Chino and Kikkawa (1933), in their paper on the cytological demonstration of crossing over in this species, used two strains of chromosomal aberrations, namely, an attached third and fifth chromosome strain and a translocation strain involving the first and the fifth chromosomes. Kikkawa (1933a) worked on the viability and fertility of some hypo- and hyperploid flies produced in the translocation strain involving the third and fourth chromosomes. Chino (1936) discovered a spontaneous inversion in the fifth chromosome, and estimated the degree of reduction of crossing over by this inversion existing in the heterozygous state. Komai and Takaku (1938, 1940a, 1940b) obtained two independent inversions in the X-chromosome and studied their effect upon crossing over and disjunction. Besides, some other strains of chromosomal aberrations had been secured and preserved in our laboratory. All these strains were used for the present study. Patterson, Stone and Griffen's descriptive method has been followed. For example, a translocation in which the third chromosome is broken between III12I and III12I₁ and the fourth chromosome between IV10G and IV10H, is designated as "III12I; IV10H+IV10G; III12I₁", the semicolon indicating the point of reattachment. Similarly, the inversion whose distal breakage is between V1G and V1G₁, and proximal breakage between V22B and V22C, is described as "V1G₁-V22B". The name of the discoverer of the strain is shown in the parenthesis.

In(X)Sd and In(X)Sp (Komai and Takaku)

I10I-I18G and I21K-I30G. Cyto-genetical studies on these inversions have been carried out by the discoverers (1940b). In(X)Sd includes *singed* locus, and its proximal break is approximately at the locus of *miniature* or that of *dusky*. In(X)Sp includes *white*, *small bristles*, *apricot* and probably *ragged* also. Accordingly, the loci of *yellow*, *echinus*, *cherry*, *crossveinless* and *vermillion* are involved in the region distal to both inversions, while *forked* is present in the intercalary uninverted segment. In(X)Sp, whose proximal break occurs between I30G and β -heterochromatin, does not include the *bobbed* locus.

529 Y-IItr. (Fujii)

Y; II2H+II2G; Y (Fig. 1). This is a mutual translocation involv-

ing the Y and the second chromosomes, the latter carrying the dominant gene *Barb*. In the salivary-gland cell nuclei the distal part of the second chromosome is attached to the *a*-heterochromatin. In *D. melanogaster* it has been demonstrated that the Y chromosome undergoes synapsis with the inert region of the X-chromosome (Prokofyeva 1935, 1937, Tiniakof 1936). The same seems to be true in *D. hydei* also (Frolova 1936). If the Y chromosome of *D. virilis* behaves similarly in synapsis, we should find a region between the *a*-heterochromatin and the distal half of the second chromosome conjugating with the X-chromosome. This, however, is not the case; the second chromosome is directly attached to the *a*-heterochromatin, as shown in Fig. 1.

Ordinarily this translocation is transmitted only to the male line, since it involves the Y chromosome. Occasionally *Barb* females appear in the stocks of this strain. These flies have the translocated chromosomes in addition to the normal diploid chromosome set (Fig. 2). By mating these *Bb* females homozygous for *bobbed* and heterozygous for multiple recessive second chromosome genes with the males carrying *bobbed* and homozygous for these genes, the break point can be determined, since the F_1 flies carrying the Y chromosome fail to develop the *bobbed* character. Thus it has been shown that the second chromosome has a break between *incomplete* and *Barb*.

306 Y-IItr. (Fujii)

Y; II33J+II33I; Y (Fig. 3). The salivary gland nuclei of the fly from this strain shows that the second chromosome is anchored directly to the *a*-heterochromatin at two points, one at its proximal end and the other at some distance from the latter. In the nerve cells of the male larvae heterozygous for this translocation, there are twelve chromosomes, including one short rod (Fig. 3). A male heterozygous for this translocation and also for *bobbed*, *incomplete*, *broken* and *brick* was mated to a female homozygous for these genes, and produced five kinds of exceptional offspring as shown in Table 1.

Table 1. *bb.in.b.bk.* × *bb.in.b.bk.*
306 Y-IItr.

♀			♂			
Expected	Unexpected		Expected	Unexpected		
<i>bb.in.b.bk.</i>	+	<i>bb'.in.b.</i>	+	Abnormal	<i>bb'.in.b.</i>	<i>bb.in.b.bk.</i>
324	10	46	392	21	22	2

The unexpected wild-type females are hyperploids which have the chromosomal constitution X.X.II.Yp;II*d*.IIp;Y*d* (*p* and *d* denote the

proximal and the distal piece of the broken chromosome respectively). The unexpected males *bb.in.b.bk.* are of the XO-type, since they show an exaggerated *bobbed* character. The abnormal males have a shortened abdomen, longer bristles, spread wings and broken post crossveins.

Fig. 1-6. 1. A spermatogonial metaphase plate and the salivary gland chromosomes taken from a young adult male and a larva heterozygous for 529Y-IItr. 2. A oogonial metaphase plate taken from a hyperploid *Barb* female having the translocated chromosome besides the normal chromosome set. 3. Salivary chromosomes and spermatogonial chromosomes from the heterozygotes for 306Y-IItr. 4. Oogonial chromosomes from a hyperploid female of 306Y-IItr. 5. Spermatogonial chromosomes and salivary chromosomes from the heterozygotes for 664Y-IItr. 6. Oogonial chromosomes from a hyperploid female of 664Y-IItr.

Although their genotypes have not been determined because of their complete sterility, it is assumed that they are trisomic X.II.II.IIp; Yd. Yp; IIId. The remaining *bb'* *in.b.* flies are the most interesting ones. Their phenotypes come between *bobbed* and the wild-type, and are to be designated as "*bb'*". The metaphase plate of the fly shows that its chromosome constitution is probably X.X.II. II.IIp;Yd (Fig. 4). Consequently, the peculiar *bb'* character of the fly seems to be due to the presence of Yd. It is known in *D. melanogaster* (Sivertzev-Dobzhansky and Dobzhansky 1933) that the dominance of the wild allele of *bobbed* is reduced when the gene is contained in a translocated chromosome piece. The above finding shows that the same is true in *D. virilis*.

The finding further enables us to locate the *bobbed* gene in the Y-chromosome. Since the fused chromosome, IIp;Yd seems to consist of a large part formed by the inert region of the second chromosome and a very small piece of the Y chromosome, the wild-type allele of *bobbed* must be found near the distal end of the Y chromosome. Moreover, this fly shows the break in the second chromosome to be found between the loci *broken* and *brick*, since IIp;Yd includes the *brick* locus, but not the *broken*.

664 Y-IItr. (Fujii)

Y;III1I+III1H;Y (Fig. 5). This is also a mutual translocation involving the Y and the second chromosomes. The metaphase plate of the nerve cell of the male larva heterozygous for this translocation (Fig. 5), consists of twelve chromosomes indistinguishable from the normal chromosome complex. Occasionally hyperploid females appear as in the case of 529Y-IItr. (Fig. 6). Genetical studies on these hyperploids show that the second chromosome has a break between *incomplete* and *broken*.

40 III-IVtr. (Kikkawa)

III12I;IV10H+IV10G;III12I₁ (Fig. 7). The third chromosome has a break between *telescoped* and *red*, and the fourth between *Scutellar* and *Baroid*. The oögonial metaphase of the young adult fly heterozygous for this translocation (Fig. 7) is similar to that of the wild fly.

134 III-IVtr. (Kikkawa)

III12G;proximal end of IV ? +IV7H;III12H (Fig. 8). This is a rather complicated translocation involving the third and the fourth chromosomes, although in its genetical behaviour it is much similar to 40III-IVtr. In the salivary gland cell, the distal half of the third chromosome is attached to the proximal end of the fourth chromosome instead of its break point. This feature is clearly observable when the fourth chromosome is separated from the α -heterochromatin (Fig. 9). The oögonial

Figs. 7, 8, 10, 11. 7. Salivary chromosomes and oögonial chromosomes from the heterozygotes for 40III-IVtr. 8. Salivary chromosomes and oögonial chromosomes from the heterozygotes for 134III-IVtr. 10. Salivary chromosomes and oögonial chromosomes from the heterozygotes for 83III-IVtr. 11. Oögonial chromosomes from a hyperploid female of 83III-IVtr.

Fig. 9. Salivary chromosomes taken from a larva, heterozygous for 134III-IVtr. The distal part of the fourth chromosome is attached to the break point of the third chromosome, while the distal part of the latter is attached to the proximal end of the former.

metaphase plate of the female carrying the translocation in the heterozygous state, however, is indistinguishable from that of the wild-type female, although a J-shaped chromosome is expected from the feature of the salivary gland chromosome. This discrepancy between the oögonial and the salivary chromosomes has not yet been elucidated.

83 III-IVtr. (Kikkawa)

Inert region of III; IV5C+IV5B; inert region of III (Fig. 10). In

the salivary gland nucleus of the larva heterozygous for this translocation, the proximal end of the third chromosome is attached to the fourth chromosome at IV5C, and the whole composite chromosome has an appearance of a branched chromosome. In the oogonial metaphase plate (Fig. 10), however, there are twelve chromosomes, including a rod shorter than the normal. This indicates that this translocation is also a mutual one (Fujii 1938).

Hyperploid offspring are often obtained in the stock heterozygous for this translocation. In the oogonial metaphase of such a female (Fig. 11) there are thirteen chromosomes, including a short rod like that found in the female heterozygous for this translocation. The genetical analysis of these hyperploid flies has revealed that the loci *plexus* and *Scutellar* are involved in the short rod-like chromosome. This shows that the fourth chromosome has a break on the right side of *Sc*. Kikkawa's unpublished datum further shows that the locus of *Minute IVa* is also involved in this short chromosome.

183 III-Vtr. (Kikkawa)

III12H;V9H+V9G;III12H₁ (Fig. 12). This is also a mutual translocation, in which the third chromosome has a break near *telescoped* and *red*, and the fourth between *eosinoid* and *straw*. Fig. 12 shows a oogonial metaphase plate taken from a female heterozygous for this translocation; it does not present any peculiarity different from that of the wild-type female.

202 III-Vtr. (Kikkawa)

III28A;V8B+V8A;III28B (Fig. 13). The oogonial metaphase plate of the female heterozygous for this translocation (Fig. 13) includes an unequal pair consisting of an abnormally short and an abnormally long chromosome. The former apparently retains the spindle-fiber of the third chromosome and the latter that of the fifth, the breakage in the third chromosome falling close to the proximal end in the salivary gland figure and that of the fifth near its distal end. From the mating $\frac{tb. \quad es.sw.pe.}{tb. \quad es.sw.pe.} \times$

202 III-Vtr.

$tb.sw.pe.$ female flies appear at the rate of 0.25 per cent.
 $tb. \quad es.sw.pe.$

The oogonial metaphase plate of these flies (Fig. 14) shows thirteen chromosomes including one short chromosome mentioned above. This extra chromosome has the wild allele of *es.*, but not that of *tb.* This fact indicates that the fifth chromosome has a break between *es.* and *sw.* From

the mating $\frac{202 \text{ III-Vtr.}}{re.ba. \quad CVa.es.} \times \frac{re.ba. \quad es.}{re.ba. \quad es.}$, F₁ flies having the phenotype

re.es. or *ba.* appear as a result of double crossing over. This means that the third chromosome has a break on the right side of *ba.* The fact that the extra short chromosome does not include the *tb.* locus, indicates that the third chromosome is broken between *tb.* and the proximal end. In addition to the hyperploid flies mentioned above, there appear three different kinds of exceptional flies with the phenotype *tb.*, *pe.* and *tb.es.sw.* respectively. Furthermore, from the mating $\frac{cn.}{cn.} \frac{es.}{es.} \times \frac{202 \text{ III-Vtr.}}{cn. \quad es.}$, some exceptional F_1 flies having the phenotype *cn.* or *es.* appear. The

Figs. 12-15. 12. Oögonial chromosomes and salivary chromosomes from the heterozygotes for 183III-Vtr. 13. Salivary chromosomes and oögonial chromosomes from the heterozygotes for 202III-Vtr. 14. Oögonial chromosomes from a hyperploid female of 202III-Vtr. 15. Salivary chromosomes and oögonial chromosomes from the heterozygotes for 126III-Vtr.

cn. fly from the latter mating is a hyperploid which corresponds to the *tb.sw.pe.* from the former mating. The cytological studies of the *es.* flies from the latter mating, as well as of the three kinds of the exceptionals

from the former mating have shown that they are not hyperploid, but have apparently normal chromosomal sets as shown in Fig. 13. No analysis of these exceptionals as to their chromosomal constitutions, however, has been accomplished.

126 III-Vtr. (Kikkawa)

Inert region of III; V16A+V15G; inert region of III (Fig. 15). In the salivary gland nuclei the proximal end of the third chromosome is

Figs. 16-17. 16. Salivary chromosomes and oögonial chromosomes from the heterozygotes for 3500IV-Vtr. 17. Salivary chromosomes from the heterozygote for CVa.

attached to V16A. In the oögonial metaphase plate there are twelve chromosomes, of which two are distinguishable from the rest by their abnormal lengths, one being shorter and the other longer (Fig. 15). Thus the strain has a chromosome configuration similar to that of 83III-IVtr. Minute studies on this strain have revealed that the third chromosome has a break in the inert region and the fifth chromosome has one on the right side of *es*.

3500 IV-Vtr. (Chino)

IV20H; V21B + V21A; IV20I (Fig. 16).

The fourth chromosome has a break between *veinlet* and *dachsous*, while the fifth chromosome has one between *eosinoid* and *peach*. The oögonial metaphase plate of the female heterozygous for this translocation (Fig. 16) can not be distinguished from that of the wild fly.

C Va (Chino)

V1G₁-V22B (Fig. 17). This inversion has been analyzed genetically

by the discoverer (1936). He has found that the suppressing effect of the inversion on crossing over extends on the whole length of the fifth chromosome, but it is stronger in the region distal to *straw* than on the more proximal region. Cytological studies carried out by myself have shown that this inversion involves *sw.* locus.

Bar (Br.) (Chino)

II2J-II4E. This is a dominant mutant belonging to the second linkage group. It reduces the crossing over of the genes located in its neighbourhood. Chino has assumed that this peculiarity is due to the presence of an inversion. A careful study of the salivary chromosome of this strain has revealed a small inversion near the distal end of the chromosome.

Baroid (Ba.) (Chino)

IV10J-IV13F. This is a dominant mutant in the fourth linkage group and reduces the crossing over in its neighbourhood. The salivary gland chromosome of this strain carries an inversion.

Glued (Gl.) (Chino)

V21C-V22B. This is a dominant mutant in the fifth linkage group and reduces the crossing over in its neighbourhood, exactly like *Br.* and *Ba.* This is also due to a small inversion found in this chromosome.

The effect of the heterozygous translocations on crossing over

To find the effect of the various kinds of translocations mentioned above on crossing over, the females heterozygous for the translocations and also for the several genes in the chromosomes concerned, were mated to the males homozygous for the same genes. The recombination values obtained are summarized in Table 2, with the corresponding standard values according to Chino (1936-1937).

Since some crossing over remains in both the chromosomes concerned in all of these translocations, it is difficult to make out by genetical analysis the exact loci of the breakages. So, for an accurate analysis, it is necessary to suppress crossing over in one of the chromosomes by means of an inversion. I have one such strain CVA, that suppresses almost entirely the crossing over in the fifth chromosome. By using this inversion, the three III-Vtr. and one IV-Vtr. strains have been analyzed very nearly to their break points. The hyperploid individuals, which have the translocated chromosome in addition to the normal chromosome set, are also suitable for this purpose. Y-IItr., 83III-Vtr. and 202III-Vtr. have been analyzed by using such hyperploid flies.

Table 2. Recombination values in the heterozygous translocations.

* Indicates that the break point falls within the limit of the region under consideration.

** Indicates that the break point is found in the neighbourhood of the region under consideration but outside of its limit.

The second chromosome

Regions	<i>in-Bb</i>	<i>in-b</i>	<i>Bb-b</i>	<i>b-bk</i>	<i>bb-in</i>	<i>bb-b</i>	<i>bb-bk</i>
Standard values	31.0	48.1	50.0	41.8	—	—	—
529 Y-IItr.	16.4*	43.9	45.3	—	16.2	45.4	—
664 Y-IItr.	—	45.2*	—	46.0	22.6	42.6	53.0
306 Y-IItr.	—	48.3	—	32.6*	43.2	38.2	32.6

The third chromosome

Regions	<i>cn-sv</i>	<i>cn-t</i>	<i>sv-t</i>	<i>sv-re</i>	<i>t-ba</i>	<i>re-ba</i>	<i>re-tb</i>
Standard values	11.8	41.6	30.7	35.3	23.7	23.2	36.2
40 III-IVtr.	15.4	32.7	—	21.2**	—	7.6**	18.4
134 III-IVtr.	16.1	—	22.7**	25.3**	—	—	23.8
83 III-IVtr.	19.1	—	—	37.6	—	—	36.3
183 III-Vtr.	13.1	—	—	21.7**	—	14.1**	23.2
183 III-Vtr. with CVa.	—	—	—	—	6.0**	9.1**	—
202 III-Vtr.	17.7	—	—	43.8	—	29.4	31.8**
202 III-Vtr. with CVa.	—	—	—	—	23.5	26.1	—
126 III-Vtr.	14.8	—	—	36.5	—	—	40.1
126 III-Vtr. with CVa.	—	—	—	—	24.7	21.5	—

The fourth chromosome

Regions	<i>px-Sc</i>	<i>Sc-ve</i>	<i>Ba-ve</i>	<i>ve-ds</i>	<i>ds-ir</i>
Standard values	22.3	41.8	12.9	42.6	25.0
40 III-IVtr.	21.9	27.1*	1.4	—	—
134 III-IVtr.	18.3	37.7*	5.2	—	16.7
83 III-IVtr.	14.5*	41.7	—	—	23.8
3500 IV-Vtr.	—	—	—	10.8*	8.4
3500 IV-Vtr. with C5a.	—	—	—	15.0*	4.2

The fifth chromosome

Regions	<i>sh-st</i>	<i>st-a</i>	<i>a-es</i>	<i>es-sw</i>	<i>sw-pe</i>
Standard values	5.0	7.8	11.3	39.4	44.7
183 III-Vtr.	5.0	8.0	7.2	31.6*	—
202 III-Vtr.	0.7	2.0	1.0	38.9*	—
126 III-Vtr.	6.5	8.4	14.5	32.5*	41.3
3500 IV-Vtr.	—	—	—	29.4	16.7*

Among the translocations involving the second chromosome, 664Y-IItr. suppresses the crossing over in its neighbouring region more than does 529 or 306 Y-IItr. This is apparently due to the fact that the section *in-b* is longer than the section either *in-Bb* or *b-bk*.

Among the translocations involving the third chromosome, 40 III-IVtr., 134III-IVtr. and 183III-Vtr. which all have the break points near *t*. and *re*. reduce considerably the crossing over in the region *t-re-ba*. 202III-Vtr, on the other hand, reduces slightly the crossing over between *re*. and *tb*. only, showing that the break point is far apart from the locus of *tb*. Both 83III-IVtr. and 126III-Vtr., which have a break in the inert

region of the third chromosome, show the normal recombination values throughout the whole length of the chromosome.

All the translocations involving the fourth chromosome, except 83III-IVtr., considerably reduce the crossing over in the regions involving the breakage. 40III-IVtr., and 3500 IV-Vtr. show such an effect not only on the region including the breakage, but also on the more proximal regions, while 83III-IVtr. in which the breakage is found at a more distal locus, greatly affects the crossing over in the region distal to it.

The translocations involving the fifth chromosome, except 3500IV-Vtr., give scarcely any effect on the crossing over in the neighbourhood of the break. In 202III-Vtr. the distal region *sh-st-a-es* shows a greater effect than the region *es-sw* where the breakage occurs. This may be due to the fact that *es-sw* is too long to reveal such an effect.

Reviewing all the data on the effect of the heterozygous translocations on crossing over presented above, the conclusion seems justified that the effect is most pronounced in the vicinity of the breakage wherever it occurs. Apart from this fact, there is scarcely anything to be recognized as the effect of the translocations on crossing over. Even the presence of an inversion in one of the translocated chromosomes causes hardly any significant change of the crossing over value. All these are probably owing to the much greater genetical length of the *virilis* chromosomes as compared with the *melanogaster* chromosomes.

Locating genes on the salivary gland chromosomes

The genetical data on the chromosomal aberrations presented above, can be used for locating the genes on the salivary gland chromosomes. Thus the maps on Plate 1 have been prepared.

The first chromosome genes have been located on the basis of Komai and Takaku's data on In(X)Spd (1938, 1940a, 1940b). It is worth notice that the active gene *bobbed* is located in the β -heterochromatin.

The second chromosome genes have been located by means of the three different Y-II translocations and the *Bar* inversion. In the genetical map the *Bar* locus is assigned to the region between *incomplete* and *Barb*, but the exact location is hardly possible, because of the nearly complete suppression of crossing over in the neighbouring region. *Incomplete* and *Bar* are located at the extreme distal end of the salivary chromosome, while their loci on the genetical chromosome map are assigned to 45.2 and near 64.5 (Puffed locus) respectively, namely, near the distal one-third of the second chromosome. This discrepancy in the locations of these genes between the genetical and salivary chromosome maps remains to be solved.

Of the third chromosome genes, *telescoped* and *red* can not be located accurately, because the break points of the three translocations used for

the analysis (134,40III-IVtr. and 183III-Vtr.) reduce the crossing over in the neighbouring regions so much as to allow no double to occur. The break points of both 83III-IVtr. and 126III-Vtr. which are in the β -hetero-chromatin, are put, for convenience' sake, at the distal end of the third chromosome.

The location of genes on the fourth chromosome has been performed by using the four translocations and one inversion *Baroid* stated above. I have nothing to add to what is illustrated on the plate.

On the fifth chromosome *straw* can not be located by the genetical data relating to 3500IV-Vtr. It is very likely, however, that it is situated on the left side of V21A, since the *Glued* inversion has its distal breakage in the region distal to that of 3500IV-Vtr. The genes used in the present experiment, *shaggy*, *scarlet*, *approximated* and *eosinoid*, are located on the genetical chromosome map in the region from 62.5 to 91.4. On the salivary chromosome map, however, they are to be located near its distal end. This discrepancy between the genetical and salivary chromosome maps is left for a future study.

We have no chromosomal aberration involving the sixth chromosome. But the sixth salivary-gland chromosome of the New Orleans strain originally sent to us by Dr. M. Demerec some years ago, has an abnormal staining capacity (Fujii 1940a). From the mating $\frac{\textit{Gap glossy}}{\text{New Orleans}} \times \textit{glossy}$,

a *glossy* female was obtained. This female was mated to a male having the normal sixth chromosome, and some of the F_1 larvae were examined for their salivary-gland chromosomes. About half of them showed the sixth chromosome of a peculiar constitution, namely, one longitudinal half has the normal staining capacity, while the other half is made up of a lightly staining distal quarter (VI1A-VI1G), and a deeply staining proximal quarter (VI2A-proximal end). There is no doubt that this constitution is due to crossing over between the normal and the New Orleans chromosomes. The locus of *gl.* must, therefore, be located to the right of VI2A and that of *Gp.* to the left of VI1G. Similarly, the genes *acute*, *abdomen-rotatum* and *hump* have been located to the left of VI1G, and *stubby* to the right of VI2A.

The observations stated about, also give us the clue to the loci of the spindle-fibers on the chromosomes of this species. Komai and Takaku's data mentioned above, show that the point of spindle-fiber attachment of the X chromosome is found on the side of *bobbed*, and further that this gene is included in the inert region. The data presented above verify the previous decision of Chino on the spindle-fiber attachments of the second, fifth and sixth chromosomes, based on the effect of high temperature on crossing over, as well as on other genetical grounds. For the

third and fourth chromosomes, the present data have disclosed the loci of the spindle-fiber for first time.

Genetical nature of certain structures of the salivary gland chromosome

The cyto-genetical data on the chromosomal aberrations dealt with above help us to disclose the genetical nature of certain structures of the salivary-gland chromosomes found in *D. virilis*.

α -heterochromatin. There is a great deal of controversy among the workers on the salivary gland chromosomes of *D. melanogaster*, as to the nature of the so-called chromocenter. Painter (1935) is of the opinion that it is an aggregate of accessory materials of all chromosomes. Muller and Prokofjeva (1935) hold that it has the same regular banding as the euchromatic parts of the chromosomes. Koller (1935) takes the view that it is only an undifferentiated magma. Bauer (1936), lastly, maintains that the chromocenter is completely chromosomal and composed of heterochromomeres. As far as my observations go, although no compact structure suggesting a chromocenter occurs in the salivary gland chromosomes of *D. ananassae*, *D. montium* or of *D. melanogaster*, in the chromosomes of *D. virilis*, a compact mass of small granules to be identified as the α -heterochromatin, is distinctly observable at the point to which all the roots of the salivary gland chromosomes assemble, as illustrated in Fig. 1. The mass is variable in shape, and may be spherical, fusiform or linear. All the chromosomes anchor on this body. Frolova (1936) who has studied the salivary gland chromosomes of *D. funebris*, *D. hydei*, *D. repleta*, *D. lugubrina* and *D. virilis*, recognizes a distinct α -heterochromatin mass in all these species. The granular structure, according to her, is due to the gathering of all "Leitkörperchen" in the telophase of the last mitosis of the salivary gland nucleus. Tiniakov (1936) maintains that the chromocenter is no more than "chromatic ringlets" occurring at the inert proximal end of the chromosomes, which are liable to be torn off by the pressure of the cover slip in smear preparations. Patterson, Stone and Griffen (1940) take the view that each chromatid of the salivary gland chromosome has two centromeres at its proximal end, and these centromeres construct together the α -heterochromatin.

Heitz (1933) finds a similar body formed by fusion of inert materials in the resting nuclei of *D. virilis* and some other species, and calls it "Chromozentrum". The α -heterochromatin of the salivary gland chromosome, according to him, corresponds to the inert region of the mitotic chromosome. Makino, more recently (1940), worked on the behaviour of the chromocenter during the mitosis of the oesophageal ganglion nucleus of the *D. virilis* larva, and found that the heterochromatic regions which

occupy the proximal halves of the mitotic chromosomes fuse into a chromocenter in the course of the change from telophase to prophase.

In the three translocations involving the second and Y chromosomes (529, 664 and 306) mentioned above, the second chromosome is attached to the α -heterochromatin with its break point (Figs. 1, 3, 5). The Y chromosome which carries no active gene, except the allele of *bobbed* and some genes relating to fertility, does not conjugate with the proximal inert region of the X chromosome, as mentioned in the previous section. Thus, it seems natural to conclude that the Y chromosome is involved in the α -heterochromatin, as is also the inert regions of all the other chromosomes. This view is fully supported by Heitz's and Makino's observations stated above.

The salivary gland nucleus of *Drosophila* contains a large nucleolus. The α -heterochromatin of *D. virilis* is united with the nucleolus by a

Fig. 18. Spiral threads connecting the α -heterochromatin with the nucleolus.

bundle of irregular spiral threads, as shown in Fig. 18. Heitz (1934) also gives a figure (his fig. 4) showing a thread stretching between the β -heterochromatin and the nucleolus, although he gives no statement of the chromosome to which this β -heterochromatin belongs. Frolova (1936) also, based on her observations on the salivary gland chromosomes in *D. virilis* as well as in some other species, holds that the α -heterochromatin is united with the nucleolus by a thread.

Tiniakov (1936) gives a similar figure for *D. virilis*. For *D. melanogaster*, *D. funebris* and *D. obscura*, however, he maintains that the X chromosome is directly connected with the nucleolus. A similar connection of the X-chromosome with the nucleolus is reported in *D. pseudoobscura* by Bauer (1936) and in *D. melanogaster*, *D. funebris*, *D. immigrans* and *D. subobscura* by Emmens (1937). Kaufman (1938) finds a nucleolus-forming region in the X-chromosome in the salivary gland cell of *D. melanogaster*. Thus, there is a distinct tendency that in the species having distinct compact α -heterochromatin there is a thread or a bundle of threads uniting the nucleolus with the α -heterochromatin, whereas in the species having no α -heterochromatin the nucleolus is directly connected with the X chromosome. Griffen and Stone's recent work (1940) shows an obvious centromere at the proximal end of the shorter arm of the fourth salivary gland chromosome of *D. melanogaster*. They are of the opinion that the centro-

meres fuse to form the α -heterochromatin in *D. virilis*, so in *D. melanogaster* they must make up the α -heterochromatin. If this is true, the X chromosome is united with the nucleolus invariably by the mediation of the α -heterochromatin, even if this is not verified by actual observation. At any rate, the question as to whether the α -heterochromatin actually exists or not in the salivary gland chromosomes of *D. melanogaster* and some other species remains to be solved.

β -heterochromatin. I have reported in my 1936 paper that the salivary gland chromosomes of *D. virilis*, except the third and the sixth, carry at their bases the so-called β -heterochromatin wherein some bands are recognisable as illustrated precisely in Hughes' revised maps (1939) and also in Patterson, Stone and Griffen's Henly maps (1940). Based on the fact that the β -heterochromatin of the X chromosome conjugates with the Y chromosome in *D. melanogaster* (Prokofjeva 1935, 1937, Tiniakov 1936), the β -heterochromatin is generally considered to represent the inert region of the mitotic chromosome. If this were the case, the distal half of the second chromosome of my Y-IItr. should be found to be attached to the β -heterochromatin of the X chromosome. In all the cases observed, however, the second chromosome is directly attached to the α -heterochromatin at its break point. Careful examination of the salivary gland chromosome of the male larva has never revealed any diploid feature of the β -heterochromatin in the X chromosome (Fig. 3). Furthermore, both in 83III-IVtr. and 126III-Vtr. the third chromosome is broken at its inert region. Since this chromosome has no β -heterochromatin, as previously noted, it is certain that the β -heterochromatin does not represent the inert region of the mitotic chromosome. In *D. melanogaster* the *bobbed* gene is located in the β -heterochromatin of the X chromosome, and the same seems to be true in *D. virilis* according to Komai and Takaku's data (1940). Thus, it is possible that the bands found in the β -heterochromatin represent the active genes occurring in the inert region, as previously suggested by Frolova (1936).

'Weak' points in euchromatic region. There are some particular points in the euchromatic region which are more easily broken than the rest, when the material is crushed under the cover slip. Among these weak points, 120A-B, III18C-D, IV6C-D, IV29C-D and V11I-12A are the most prominent ones. Hughes, in his study on the hybrids from *D. virilis virilis* $\times$ *D. virilis americana*, states that "if these weak points have any genetic significance, as for example, the more frequently occurrence of inversion breaks at these points, there is no supporting evidence for this" (1939, p. 815). Catcheside (1933) and Bauer, Demerec and Kaufmann (1938) have worked on the distribution of the breaks on the chromosomes of *D. melanogaster*, and concluded that it is of a random nature within the euchromatic section of the chromosomes, with the exception

of the distal regions where breaks are slightly more frequent, while in the heterochromatic regions the breaks are relatively more frequent. Kaufmann (1939), in a subsequent study on the distribution of induced breaks along the X chromosome of *D. melanogaster*, finds that certain divisions of the euchromatic region show a higher break frequency than that expected on random distribution, and he attributes this to the presence of heterochromatic material intercalated between euchromatic substance.

In all of my 134III-IVtr., 183III-Vtr. and 40III-IVtr., the breaks on the third chromosome fall within a narrow section somewhere between III12G and III12J, although not exactly at the same point. Furthermore, both the CVa and *Glued* inversions have their breaks between V22B-C. I have also demonstrated by utilizing the abnormal staining capacity of the sixth chromosome of the New Orleans strain, that the exchange in this chromosome took place in the small section VI1G-V12A, in 12 out of the 13 cases examined. This observation apparently gives a convincing evidence for the fact that crossing over has a tendency to occur at a definite point instead of being distributed uniformly on the chromosome. Mather (1936) is of the opinion that the scarcity of crossing over in the small fourth chromosome of *D. melanogaster* is due to the influence of the spindle fiber attachment which makes the first chiasma always form within a very limited section near the distal end. According to Charles (1938), if the X chromosomes of *D. melanogaster* cross over just once, the exchange occurs at approximately the middle of the chromosome. Mather's opinion can not explain my data in full, inasmuch as the chiasma should appear in any locus distal of VI1G, if the localized mechanism alone were to determine this. Nor does Charles' interpretation hold here, since exchange is restricted to that single locus, instead of being distributed about it.

It seems that, although the point of crossing over may not be exactly identical in nature with the break point in chromosomal aberrations, yet it has much in common with the latter. It has been assumed that the various chromosome aberrations are due to the so-called illegitimate crossing over between the non-homologous sections of the same or different chromosomes, at some heterochromatic segments intercalated between euchromatic regions. Although the existence of such segments remains to be demonstrated, yet it seems fairly certain that there are some points in the chromosome where crossing over or segmental interchange occurs more frequently than elsewhere.

The distal end of the euchromatic region. The salivary chromosomes have a tendency to gather together at their distal ends as has been observed by Bauer (1935) in *D. virilis*, *D. funebris*, *D. hydei* and in *Chironomus*, and by Frolova (1936) in many species of *Drosophila*. The latter author has noticed in the salivary gland chromosomes in *D.*

hydei and *D. repleta*, that the autosomes are fused at their distal ends into 4 groups, but the X chromosomes usually remain isolated. Such observations lead us to suspect that the distal ends of all the chromosomes are united originally, and separated by the pressure of the coverslip. But

Fig. 19. Section of the normal salivary gland nucleus showing the nucleolus in the centre, the chromosomes cut in transverse section in the periphery and a distal end of a chromosome in the lower left side.

it is also possible that such a union is no more than a secondary association of the chromosomes which are originally independent from one another. A decisive solution of this question may be obtained by the observations of the sections of the salivary glands, which show that the distal ends of the chromosomes are separate, as shown in Fig. 19, thus indicating that the union is only of a secondary occurrence. Emmens (1937) also gives figures showing that the distal ends of the chromosomes observed on sections remain isolated in all the species of *Drosophila* studied by him. Hinton and Sparrow (1941) have calculated the frequencies of the combinations of two associated chromosome ends of *D. melanogaster*, and found

that the observed figure deviated significantly from the expected value based on the assumption of the randomness of the adhesion. They suppose that "it may be due to combination of a mechanical factor such as chromosome length, with a qualitative factor such as a varied amount of terminal adhesion" This statement brings to my mind the fact that the short fourth chromosome of *D. melanogaster* (Bridges 1935), as well as of *D. montium* (Osima 1940), is curved and attached to the chromocenter with its both ends.

This, according to Bridges, is due to the presence of a heterochromatic region at the distal end as well as at the proximal end, of chromosome. Bauer (1936), however, maintains that the association of the chromosomal ends has nothing to do with presence of the heterochromomere-like granules. In his opinion, "this terminal attraction must be effective also in their proximal free ends. The potentially equal attraction of proximal and distal ends to each other can be seen in cases in which chromosomes are only distally united.... . "The difference in amount of proximal and distal union depends probably on the arrangement during the last telophase, in which the proximal ends of all chromosomes come close together, while the position of the distal ends, due to the different length of the chromosomes and the less exact orientation, is more variable." Thus the association of the chromosomal ends can hardly be assigned to any 'mechanical' or 'qualitative' factor. This question also is reserved for a future study.

Summary

1. Genetical and cytological studies on ten translocations and four inversions found in *D. virilis* have been carried out. All the translocations are of the mutual type.

2. The recombination values between the genes located in the vicinity of the loci at which the chromosomes are broken or reattached, are lower than the corresponding standard values.

3. The loci of many genes determined by means of these chromosomal aberrations are illustrated in Plate 1.

4. The loci of the spindle fiber attachment have been determined for all the chromosomes including the second and the fourth for which no genetical data are available.

5. The chromocentral region of *D. virilis* is not a mere aggregate of the proximal ends of the chromosomes, but is composed of very conspicuous granular element—the α -heterochromatin.

6. The β -heterochromatin exists at the proximal end of all the chromosomes, except the third and the sixth.

7. The α -heterochromatin represents the inert regions of the mitotic chromosomes including the Y chromosome, while the β -heterochromatin represents the active genes occurring in the inert regions.

8. The nucleolus is not connected with the β -heterochromatin in the X chromosome, but it is connected with the α -heterochromatin by a bundle of spiral threads.

9. The morphologically 'weak' points found in the euchromatic region seem to have no genetical significance. The existence of some genetically weak points is assumed on the ground that the breakages in chromosome aberrations have a tendency to fall at some definite loci, and crossing over often takes place at a particular space on the sixth chromosome.

10. The fusion of the distal ends of the salivary gland chromosomes frequently observed in the smeared preparations, is no more than a random gathering. Studies on the sections of the salivary glands show that all the distal ends of the chromosomes are separate.

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Appendix

529 Y-IItr.

	$\frac{bb.}{bb.} \frac{in.b.}{in.b.}$	$\times$	$\frac{bb.}{Y} \frac{in.b.}{in.b.}$	
	529 Y-IItr. <i>Bb.</i>			
0	{ <i>bb.in.b.</i>		323	
	{ <i>Bb.</i>		297	
1	{ <i>bb.b.</i>		50	
	{ <i>in.Bb.</i>		47	
2	{ <i>bb.in.Bb.</i>		1	
	{ <i>b.</i>		1	
3	{ <i>bb.in.</i>		247	
	{ <i>Bb.b.</i>		232	
1.2	{ <i>bb.Bb.</i>		1	
	{ <i>in.b.</i>		1	
1.3	{ <i>in.Bb.b.</i>		56	
	{ <i>bb.</i>		59	
2.3	{ <i>bb.in.Bb.b.</i>		2	
	{ +		0	
1.2.3	{ <i>bb.Bb.b.</i>		0	
	{ <i>in.</i>		0	
	Total		1316	

664 Y-IItr.

	$\frac{bb.}{bb.} \frac{in.b.bk.}{in.b.bk.}$	$\times$	$\frac{bb.}{Y} \frac{in.b.bk.}{in.b.bk.}$	
	664 Y-IItr.			
0	{ <i>bb.in.b.bk.</i>		91	
	{ +		99	
1	{ <i>bb.b.bk.</i>		27	
	{ <i>in.</i>		19	
2	{ <i>bb.in.</i>		96	
	{ <i>b.bk.</i>		54	
1.2	{ <i>in.b.bk.</i>		21	
	{ <i>bb.</i>		30	
1.3	{ <i>bb.b.</i>		30	
	{ <i>in.bk.</i>		26	
2.3	{ <i>bb.in.bk.</i>		60	
	{ <i>b.</i>		54	
1.2.3	{ <i>bb.bk.</i>		17	
	{ <i>in.b.</i>		13	
	Total		809	

40 III-IVtr.

	$\frac{cn.t.}{40 \text{ III-IVtr.}} \frac{px.}{px.}$	$\times$	$cn.t.px.$	
0	{ <i>cn.t.px.</i>		56	
	{ +		74	
1	{ <i>t.px.</i>		28	
	{ <i>cn.</i>		38	
2	{ <i>cn.t.</i>		44	
	{ <i>px.</i>		52	
1.2	{ <i>cn.px.</i>		14	
	{ <i>t.</i>		30	
	Total		336	

	$\frac{re.ba.}{40 \text{ III-IVtr.}} \frac{px.Sc.}{px.Sc.}$	$\times$	$re.ba.px.Sc.$	
0	{ <i>re.ba.px.Sc.</i>		114	
	{ +		117	
1	{ <i>re.ba.Sc.</i>		25	
	{ <i>px.</i>		30	
2	{ <i>px.Sc.</i>		12	
	{ <i>re.ba.</i>		9	
3	{ <i>re.px.Sc.</i>		6	
	{ <i>ba.</i>		5	
1.2	{ <i>re.ba.px.</i>		7	
	{ <i>Sc.</i>		2	
1.3	{ <i>ba.px.</i>		4	
	{ <i>re.Sc.</i>		5	
2.3	{ <i>ba.Px.Sc.</i>		3	
	{ <i>re.</i>		1	
1.2.3	{ <i>re.px.</i>		1	
	{ <i>ba.Sc.</i>		1	
	Total		342	

	$\frac{tb.}{40 \text{ III-IVtr.}} \frac{Ba.ve.}{Ba.ve.}$	$\times$	$tb.Ba.ve.$	
0	{ <i>t.Ba.ve.</i>		348	
	{ +		351	
1	{ <i>Ba.ve.</i>		40	
	{ <i>tb.</i>		37	
2	{ <i>tb.Ba.</i>		2	
	{ <i>ve.</i>		6	
1.2	{ <i>tb.ve.</i>		1	
	{ <i>Ba.</i>		2	
	Total		787	

134 III-IVtr.

$\frac{sv.t.}{134 \text{ III-IVtr.}} \times \frac{Sc.}{sv.t.Sc.}$	
0 { <i>sv.t.Sc.</i>	67
{ +	78
1 { <i>t.Sc.</i>	22
{ <i>sv.</i>	19
2 { <i>sv.t.</i>	17
{ <i>Sc.</i>	3
1.2 { <i>sv.Sc.</i>	1
{ <i>t.</i>	5
Total	212

$\frac{re.ba.}{134 \text{ III-IVtr.}} \times \frac{px.Sc.}{re.ba.px.Sc.}$

0 { <i>re.ba.px.Sc.</i>	144
{ +	204
1 { <i>re.ba.Sc.</i>	28
{ <i>px.</i>	32
2 { <i>px.Sc.</i>	2
{ <i>re.ba.</i>	0
3 { <i>re.px.Sc.</i>	18
{ <i>ba.</i>	23
1.2 { <i>re.ba.px.</i>	8
{ <i>Sc.</i>	5
1.3 { <i>ba.px.</i>	7
{ <i>re.Sc.</i>	10
2.3 { <i>ba.px.Sc.</i>	3
{ <i>re.</i>	7
1.2.3 { <i>re.px.</i>	0
{ <i>ba.Sc.</i>	0
Total	491

$\frac{tb.}{134 \text{ III-IVtr.}} \times \frac{Ba.ve.}{tb.Ba.ve.}$

0 { <i>tb.Ba.ve.</i>	140
{ +	88
1 { <i>Ba.ve.</i>	54
{ <i>tb.</i>	31
2 { <i>tb.Ba.</i>	9
{ <i>ve.</i>	6
1.2 { <i>tb.ve.</i>	1
{ <i>Ba.</i>	1
Total	330

183 III-Vtr.

$\frac{re.ba.}{183 \text{ III-Vtr.}} \times \frac{es.}{re.ba.es.}$	
0 { <i>re.ba.es.</i>	247
{ +	321
1 { <i>re.ba.</i>	8
{ <i>es.</i>	5
2 { <i>re.es.</i>	70
{ <i>ba.</i>	24
1.2 { <i>ba.es.</i>	0
{ <i>re.</i>	1
Total	676

$\frac{tb.}{183 \text{ III-Vtr.}} \times \frac{st.a.es.}{tb.st.a.es.}$

0 { <i>tb.st.a.es.</i>	184
{ +	243
1 { <i>tb.a.es.</i>	15
{ <i>st.</i>	24
2 { <i>tb.es.</i>	23
{ <i>st.a.</i>	18
3 { <i>tb.</i>	77
{ <i>st.a.es.</i>	71
1.2 { <i>tb.st.es.</i>	0
{ <i>a.</i>	0
1.3 { <i>tb.st.</i>	8
{ <i>a.es.</i>	7
2.3 { <i>tb.st.a.</i>	4
{ <i>es.</i>	4
1.2.3 { <i>tb.a.</i>	0
{ <i>st.es.</i>	0
Total	678

$\frac{tb.}{183 \text{ III-Vtr.}} \times \frac{es.sw.}{tb.es.sw.}$

0 { <i>tb.es.sw.</i>	50
{ +	69
1 { <i>tb.sw.</i>	16
{ <i>es.</i>	15
2 { <i>tb.es.</i>	17
{ <i>sw.</i>	19
1.2 { <i>es.sw.</i>	12
{ <i>tb.</i>	14
Total	212

$$\frac{re.ba. \text{ CVa.es.}}{183 \text{ III-Vtr.}} \times re.ba.es.$$

0	{	<i>re.ba.es.</i>	517
	}	+	605
1?	{	<i>re.ba.</i>	0
	}	<i>es.</i>	0
2?	{	<i>re.es.</i>	58
	}	<i>ba.</i>	55
1.2?	{	<i>ba.es.</i>	0
	}	<i>re.</i>	0
		Total	1235

$$\frac{t.ba. \text{ CVa.es.}}{183 \text{ III-Vtr.}} \times t.ba.es.$$

0	{	<i>t.ba.es.</i>	393
	}	+	601
1?	{	<i>t.</i>	0
	}	<i>ba.es.</i>	0
2?	{	<i>t.es.</i>	32
	}	<i>ba.</i>	31
1.2?	{	<i>t.ba.</i>	0
	}	<i>es.</i>	0
		Total	1057

202 III-Vtr.

$$\frac{re.ba. \text{ es.}}{202 \text{ III-Vtr.}} \times re.ba.es.$$

0	{	<i>re.ba.es.</i>	142
	}	+	112
1	{	<i>ba.es.</i>	49
	}	<i>re.</i>	40
2	{	<i>re.ba.</i>	56
	}	<i>es.</i>	76
1.2	{	<i>re.es.</i>	42
	}	<i>ba.</i>	30
		Total	547

$$\frac{tb. \text{ st.a.es.}}{202 \text{ III-Vtr.}} \times tb.st.a.es.$$

0	{	<i>tb.st.a.es.</i>	303
	}	+	305
1	{	<i>tb.a.es.</i>	6
	}	<i>st.</i>	7

2	{	<i>tb.es.</i>	5
	}	<i>st.a.</i>	3
3	{	<i>tb.</i>	153
	}	<i>st.a.es.</i>	78
1.2	{	<i>tb.st.es.</i>	0
	}	<i>a.</i>	0
1.3	{	<i>tb.st.</i>	2
	}	<i>a.es.</i>	2
2.3	{	<i>tb.st.a.</i>	0
	}	<i>es.</i>	1
1.2.3	{	<i>tb.a.</i>	0
	}	<i>st.es.</i>	0
		Total	865

$$\frac{re.ba. \text{ CVa.es.}}{202 \text{ III-Vtr.}} \times re.ba.es.$$

0	{	<i>re.ba.es.</i>	162
	}	+	152
1	{	<i>re.</i>	47
	}	<i>ba.es.</i>	52
2	{	<i>re.ba.</i>	64
	}	<i>es.</i>	69
1.2	{	<i>re.es.</i>	23
	}	<i>ba.</i>	36
		Total	605

$$\frac{t.ba. \text{ CVa.es.}}{202 \text{ III-Vtr.}} \times t.ba.es.$$

0	{	<i>t.ba.es.</i>	189
	}	+	203
1	{	<i>t.</i>	76
	}	<i>ba.es.</i>	85
2	{	<i>t.ba.</i>	85
	}	<i>es.</i>	101
1.2	{	<i>t.es.</i>	30
	}	<i>ba.</i>	39
		Total	808

126 III-Vtr.

$$\frac{re.ba. \text{ CVa.es.}}{126 \text{ III-Vtr.}} \times re.ba.es.$$

0	{	<i>re.ba.es.</i>	104
	}	+	101
1	{	<i>re.</i>	27
	}	<i>ba.es.</i>	30

S. Fujii: Further studies on the salivary gland chromosomes of *Drosophila virilis*

2	{	<i>re.ba.</i>	69
	}	<i>es.</i>	99
1.2	{	<i>re.es.</i>	17
	}	<i>ba.</i>	28
Total			475

$\frac{t.ba. \text{ CVa.es.}}{126 \text{ III-Vtr.}} \times t.ba.es.$

0	{	<i>t.ba.es.</i>	48
	}	+	51
1	{	<i>t.</i>	17
	}	<i>ba.es.</i>	18
2	{	<i>t.ba.</i>	28
	}	<i>es.</i>	35
1.2	{	<i>t.es.</i>	10
	}	<i>ba.</i>	8
Total			215

3500 IV-Vtr.

$\frac{ds. \text{ es.sw.pe.}}{3500 \text{ IV-Vtr.}} \times ds.es.sw.pe.$

0	{	<i>ds.es.sw.pe.</i>	114
	}	+	180
1	{	<i>ds.sw.pe.</i>	66
	}	<i>es.</i>	63
2	{	<i>ds.pe.</i>	0
	}	<i>es.sw.</i>	3

3	{	<i>ds.es.sw.</i>	27
	}	<i>pe.</i>	26
1.2	{	<i>ds.es.pe.</i>	1
	}	<i>sw.</i>	0
1.3	{	<i>ds.sw.</i>	6
	}	<i>es.pe.</i>	11
2.3	{	<i>es.sw.pe.</i>	2
	}	<i>ds.</i>	4
1.2.3	{	<i>ds.es.</i>	1
	}	<i>sw.pe.</i>	0
Total			504

$\frac{ve.ds.ir. \text{ CVa.es.}}{3500 \text{ IV-Vtr.}} \times ve.ds.ir.es.$

0	{	<i>ve.ds.ir.es.</i>	282
	}	+	439
1	{	<i>ve.</i>	77
	}	<i>ds.ir.es.</i>	41
2	{	<i>ve.es.</i>	10
	}	<i>ds.ir.</i>	8
3	{	<i>ve.ds.es.</i>	16
	}	<i>ir.</i>	16
1.2	{	<i>ve.ds.ir.</i>	29
	}	<i>es.</i>	34
1.3	{	<i>ve.ir.</i>	4
	}	<i>ds.es.</i>	2
2.3	{	<i>ve.ir.es.</i>	2
	}	<i>ds.</i>	0
1.2.3	{	<i>ve.ds.</i>	0
	}	<i>ir.es.</i>	0
Total			960