Morphology of the Chromosomes of *Drosophila ananassae*

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Introduction

*Drosophila ananassae* Doleschall (*D. caribbea* Sturtevant) is widely distributed in tropical regions of both the Eastern and Western Hemispheres (Kikkawa, 1936). Sturtevant (1921) in his study of the North American Drosophilinae recorded the occurrence of *D. caribbea* in Brazil, Central America, and the islands of the Caribbean Sea. In the autumn of 1933, and in subsequent years, this species was collected at Tuscaloosa, Alabama, which is considerably north of the southern limit of that area in which freezing temperatures may be expected. The repeated occurrence of the species over the period indicated suggests that it may survive the winter in Alabama, rather than be reintroduced annually. Temporary importation of *D. ananassae* from the tropics to a more temperate region has been reported by Moriwaki (1935), who collected the flies in Tokyo in 1931, but was unsuccessful in subsequent attempts.

The flies breed well in the laboratory under the same culture conditions commonly employed for *D. melanogaster*. Stocks of the Tuscaloosa material which have been maintained over the past three years were used predominantly in the present study, another stock, secured from Japan, serving for comparison.

The chromosomes of *D. caribbea* were first studied by Metz (1916) from material collected in Panama and Cuba. He described the female complement (oogonial) as consisting of four pairs of V-shaped chromosomes, one of which is shorter than the other three. His figures and diagrams of spermatogonial chromosomes show a V-shaped X and a rod-shaped Y. Kaufmann (1936a, 1936b) reported that ganglion cells of the larvae of both the Alabama and the Japanese stocks possess an unequal-armed, J-shaped Y-chromosome. Kikkawa (1936) likewise found a J-shaped Y in spermatogonial cells of his material. In the present article there will be presented a more detailed account of the chromosomes of the ganglia and the salivary glands.
For the study of these cells temporary aceto-carmine preparations were used. Salivary glands remained in the stain about one-half hour, the ganglia for 1–2 hours prior to transferring to a slide. The cells were flattened by rolling a glass vial heavily weighted with mercury across the cover. It was found that if the customary paraffin, gum-mastic seal was supplemented by the occasional application of a thick solution of gum shellac or lacquer, the preparations could be kept in a condition suitable for study over a period of several weeks. Such slides may be made permanent by removing the seal and exposing them to the vapor of dioxan in a closed chamber for a few days.1) A thin solution of gum dammar is then permitted to seep beneath the cover. By this method removal of the cover is unnecessary, and the relocation of a given cell in the smear is facilitated; it is especially useful in the study of the neurocytes. Observations and drawings were made using a 1.4 condenser, a 1.4 apochromatic objective, and compensating oculars.

Chromosomes of the Ganglia

The Constrictions—The chromosome complement consists of three pairs of V-shaped autosomes and the sex chromosomes. These are a pair of V-shaped X-chromosomes in the female, an X and a J-shaped Y in the male. The three pairs of autosomes are designated in this paper as A (the longest at metaphase), B (of intermediate size), and 4 (the shortest), since it is uncertain which of pairs A and B correspond with the second and third chromosome linkage groups determined by Moriwaki (1935) and Kikkawa (1936).

The X-chromosomes and all of the autosomes have a median to submedian primary constriction or spindle attachment region (text-figs. 1–22). The secondary constrictions vary considerably in the degree of their visible expression in different nuclei, but a survey of several cells leads to the following interpretation.

Autosomes A in favorable late prophase and early metaphase figures show two distinct secondary constrictions in each arm. Of these the proximal exist at about one fourth of the distance along the longer arm, and one third of the distance along the shorter arm, where they delimit the heteropyknotic areas bordering the primary constriction region. The distal portion of each arm is bisected by another constriction (text-figs. 3, 9). Suggestions of the existence of subterminal constrictions occasionally have been seen (text-figs. 16, 18).

1) The use of dioxan was recommended by Elizabeth G. Lawrence in Drosophila Information Service No. 6, 1936.
In the B chromosomes the most pronounced constriction is submedian in the longer arm, and during the prophase frequently divides the chromosome into two widely separated portions (see especially text-figs. 5–7). This constriction appears to flank the heteropyknotic area adjacent to the spindle attachment region, as does also a less...
pronounced constriction in a similar position in the shorter arm (text-figs. 1, 3).

In the fourth chromosomes both limbs often reveal subterminal constrictions (text-figs. 2, 16, 18). That of the longer limb represents the position at which the development of the nucleolus normally occurs. This may be determined from a consideration of the pro-phases, when the nucleolus separates a chromomere-like satellite from the remainder of the longer arm. Such detail, which is visible only in deeply stained preparations because of the small size of the satellite, is shown in text-figure 8. The fourth chromosomes alone are associated with the nucleolus in the prophase cells of the female, but in the male three chromosomes maintain such association, since the long arm of the Y also possesses a nucleolus-forming region. As in the autosomes, the satellite is separated from the bulk of the chromosome,

Text-Figs. 10–18. Anaphases (figs. 10–12), late pro-phases and metaphases (figs. 13–18) of neurocytes of larvae of D. ananassae. 10, 16–18 from female larvae; 11–15 from male larvae. Legend as for figs. 1–9. × ca. 3500.
a delicate connecting chromatic thread traversing the nucleolus (text-
fig. 8). Another constriction occurs in the long arm of the Y, about
one-fourth of the distance from spindle attachment to distal end
(text-figs. 4, 7, 8, 13).

In the X-chromosomes, submedian secondary constrictions border
the heteropyknotic regions on each side of the spindle attachment
region. Other constrictions are subterminal in these chromosomes.

**Heteropyknosis**—In the resting nuclei and in the early prophase,
before the chromosome complement becomes defined clearly, certain
deeply staining bodies may be recognized. The largest, which lie in
contact with the nucleoli, often reveal the tightly coiled chromonemata
characteristic of contracted chromosomes. They have been inter-
preted accordingly as the chromosomes concerned with nucleolus
formation, namely, the fourth chromosomes in the female, the fourths
plus the Y in the male. Other smaller, deeply staining bodies probably
represent those heteropyknotic regions which lie adjacent to the
spindle attachment regions in the X and the longer autosomes. Thus,
in some male nuclei five pairs of dot-like or rod-like bodies were seen,
which may be interpreted as the proximal portions of the chromosomes
A, B, and X. At this stage the more distal portions of these chromo-
somes stain weakly, and are indistinguishable.

**Heteroploid Nuclei**—In several of the ganglia, both male and
female, tetraploid cells were observed (text-figs. 21, 22). Usually
but one or two such cells per ganglion were seen; occasion-
ally, however, large patches of tetraploid tissue oc-
curred.

Two of the ganglia studied sug-
gested that the indi-
viduals from which
they were dissected
had arisen follow-
ing non-disjunction.
Thus, in one case,
all of the cells were
trisomic for the fourth
chromosome (text-fig.
19); in the other case, only one sex chromosome was present, an XO
individual (text-fig. 20).
The Salivary Chromosomes

Kikkawa (1935) first indicated that the number of strands radiating from the chromocenter in salivary gland nuclei of *D. ananassae* was six, rather than the eight to be expected from the conjugation of four pairs of V-shaped chromosomes. More recently, Kikkawa (1936) and Kaufmann (1936a) have determined independently, employing different criteria, that the six strands represent the arms of the four longer autosomes and the X-chromosomes, the fourth chromosomes being reduced to a small heterochromatin mass forming part of the chromocenter.

*The Structure of the Chromocenter*—Salivary glands were selected mostly from female larvae preparing to pupate. In the nuclei of these glands the proximal regions of the paired homologues form a compact chromocenter, from which the more distal portions of the chromosome arms radiate into the nuclear cavity (Plate 40, figs. 1, 2). The chromocenter frequently has been disrupted into its component parts (Plate 40, figs. 3, 4) by use of the method of smearing described in the first section of this paper. When the connections between the chromosome arms are thus revealed, it is found that the six strands possessing euchromatic sections represent the right and left limbs of three of the conjugated pairs of V-shaped chromosomes. The regions of these chromosomes adjacent to the primary constrictions contribute, therefore, to the formation of the chromocenter. But in addition thereto, the chromocenter discloses, following its fragmentation, a small, bipartite mass, frequently associated with the nucleolus (text-fig. 24; Plate 40, figs. 3, 4). That this heterochromatic mass represents the fourth chromosomes is suggested by the similar nucleolus-chromosome relationship of the ganglion cells. The validity

![Text-Figs. 23-24. Nucleolus-satellite relationship in salivary gland nuclei of *D. ananassae*. 23 satellite with ten visible bands. 24 satellite attached to heterochromatic body representing one arm of the paired fourth chromosomes. × 2250.](image-url)
of such an interpretation receives further support from the existence in the salivary nuclei of a small, cone-shaped, banded body associated with the nucleolus, and also connected with one of the two arms of the bipartite section of the chromocenter (text-figs. 23, 24, Plate 40, figs. 3, 5, 6). The conical, banded body may be regarded, therefore, as the chromomere-like terminal segment of the longer arm of the fourth chromosomes. Its chromatic nature, together with that of the strand with which it is connected to the chromocenter, has been determined by the Feulgen technique. That the satellite represents conjugated sections of two homologous chromosomes is revealed when failure of pairing occurs in certain regions (see text-fig. 23). As indicated in text-figures 23 and 24, the satellite has about ten distinct visible bands. In some nuclei, however, the banding is less clearly defined because the chromonemata and the chromomeres are widely spaced.

The shortest of the chromosome arms with euchromatic sections represent the X-chromosomes. Such identification follows comparison of male and female cells, in the former of which the X exists in the slender haploid condition (Plate 40, fig. 3) readily distinguishable from the paired condition in the female. It is uncertain at present which of the four longer arms represent the second and third chromosomes. Experiments to determine this point are now in progress.

Discussion

The Chromocenter—In D. ananassae, as in other species of Drosophila, there exists both in the resting stages of mitotic cells and in the salivary gland nuclei, that type of deeply staining chromatic region, which Heitz has designated as the chromocenter. That such regions of resting mitotic nuclei represent heteropyknotic chromosomes, or portions thereof, is evident from the contained chromonemata which they sometimes reveal (Kaufmann, 1934) as well as from their behavior through the mitotic cycle. When in close contact the heteropyknotic regions form a “Sammelchromozentrum”, which is to be regarded, therefore, as a close approximation of these bodies rather than as an amorphous, vacuolated aggregate.

That the chromocenter of the salivary gland nucleus represents the heteropyknotic regions of the mitotic chromosomes was suggested by Heitz (1933a). There is at present, however, no uniformity of opinion concerning the structure of the chromocenter of the salivary gland nucleus. The concept of an amorphous, or undifferentiated chromocenter has been presented (Heitz, 1933a, 1933b; Painter, 1935; Painter and Stone, 1935; Koller, 1935). Contrasting with such inter-
pretations are those involving specific organization within the chromocenter. Several of the recent studies have indicated that the chromocenter is composed primarily of the proximal regions of the contributing chromosomes. Such structure is especially well demonstrated by dissociation of the chromocenter following pressure in smearing (Frolowa, 1936; Bauer, 1936c; Plate 40, fig. 4 of the present paper). The pressure not only separates the chromosomes from each other but reveals the heterochromatic connections between the arms of the V-shaped members of the complex. At the same time the chromomeric; banded nature of the heterochromatin is disclosed most strikingly (cf. Prokofjeva-Belgovskaja, 1935a, 1935b; Muller and Prokofjeva, 1936; Frolowa, 1936; Bauer, 1936b, 1936c). Bauer's searching analysis of this problem reveals that the heterochromatic regions of the chromosomes of Drosophila and the Chironomidae contain the same number of chromonemata as the euchromatin, but that heterochromomereres differ in structure from the euchromomereres.

The fourth chromosomes of ganglion cells of D. ananassae are unique among the autosomes of species of Drosophila so far described in that they are totally heteropyknotic, and stand, therefore, as an exception to the generalization that sex chromosomes are heteropyknotic to a far greater extent than the autosomes of the same group (cf. Heitz, 1935). Moreover, the fourth chromosomes are essentially "inert", since only three linkage groups have been described in D. ananassae, those of the X-chromosomes and of the two pairs of longer autosomes (Moriwaki, 1935; Kikkawa, 1936). In salivary gland nuclei, however, the fourth chromosomes are represented by a sizeable mass of heterochromatin, plus the satellite, contrasting thereby with the more restricted expression of such "inert" chromosomes as the Y of this and other species. If the amount of banding is an indication of the genic content of heterochromatin regions, as Frolowa (1936) has suggested, it is probable that further genetic studies will reveal genes in the fourth chromosomes. The satellite alone possesses at least ten bands, and many others exist in the arms which form part of the chromocenter. It would not to be expected that if the fourth chromosomes were totally inert, they would have been retained in all populations of a species so widely distributed as D. ananassae. It is now known that the term "inert" is purely relative, and is essentially a misnomer as applied to heterochromatin (cf. Schultz, 1936).

The exact portions of the fourth chromosomes which form the banded heterochromatin of the salivary gland nucleus could not be determined. The general organization of the chromocenter suggests that the spindle fiber attachment regions of the chromosomes may be
represented. In addition the satellite and the adjoining region of the long arms seem to be present. If this interpretation is correct, it is interesting to note that the satellite, which comprises about one fifteenth to one twentieth of the length of the mitotic prophase chromosome, is in the salivary gland nucleus more than half as long as the remainder of the long arm.

Kikkawa (1936) refers to the fourth chromosomes of his material as J-shaped, suggesting thereby the existence of a type differing from the V-shaped chromosomes of the present study. The occurrence of different types of Y-chromosomes in D. ananassae has been indicated in an earlier publication (Kaufmann, 1936a). That considerable variation may occur in the shape of the "inert" Y-chromosome of a species, has been shown by Dobzhansky's studies of D. pseudoobscura.

The Position of the Nucleolus—The existence of nucleoli in the autosomes of D. ananassae contrasts with the situation in other species of Drosophila in which their development occurs in the sex chromosomes. Association of the nucleoli with the X- and Y-chromosomes in mitotic cells of Drosophila was first reported for D. melanogaster (Kaufmann, 1933, 1934), and independently by Heitz for this and other species (1933a, 1933b). Although in D. ananassae the Y-chromosome possesses a nucleolus-forming region, the X-chromosome normally is not associated with the nucleolus. It is tempting, therefore, to postulate that this unique situation may have resulted from translocation of the nucleolus-forming region from the X to an autosome during the processes of speciation. But beyond the fact that the nucleoli of all species of Drosophila so far studied develop in heteropyknotic regions of chromosomes, there is little evidence to support such an assumption. Heitz (1933b) has emphasized that the chromosome-nucleolus relationship is independent of heteropyknosis and of the nature of the sex chromosomes, and Bauer's studies lead him to the conclusion that fundamentally many chromosome sections have the capacity for nucleolus formation (1933b).

In mitotic cells delicate chromatic threads connect those parts of the chromosome dissociated by the development of the nucleolus. That similar strands exist in the salivary gland nuclei of Drosophila has been reported prior to the present publication (Heitz, 1934; Kaufmann, 1934; Frolowa, 1936). When tested by the Feulgen method, I have found, as has Frolowa, that such threads give the characteristic reaction of chromatin, although Heitz (1935) defines them as "anukleal." On the basis of Bauer's recent studies, the threads may be regarded as aggregates of chromonemata. The connection between the chromosome and its satellite in the salivary gland nucleus of D. ananassae, for example, appears frequently as a loose
association of individual threads. In the satellite also, the nature of
distribution of the chromonemata of the homologues, with their con-
stituent chromomeres, determines whether the aspect of banding, or
some less precise pattern, results.

The nucleolus-chromosome relationship of salivary gland nuclei
seems to be defined less clearly in other species of Drosophila,
Painter (1934) notes that in D. melanogaster none of the elements
shows a constant association with the plasmosome. Frolowa (1936)
finds that the nucleoli of D. virilis, D. funebris, and D. melanogaster
are united with the small chromocenter, and through it to all the
chromosomes, although in D. funebris the union seems at times to
be between the nucleolus and the inert part of the X-chromosome.
The latter situation conforms with that of D. ananassae and such
Diptera as Bibio (Heitz and Bauer, 1933), Simulium (Geitler, 1934),
and Chironomus (Bauer, 1935) in which the nucleolus of the salivary
gland cell is associated with a designated section of a certain chromo-

The Constrictions—Pronounced similarities exist between certain
of the chromosomes of D. ananassae and those of D. melanogaster.
Thus, of the three secondary constrictions which have been identified
in each arm of the third chromosomes of D. melanogaster (Kaufmann,
1934), the subterminal ones alone are not pronounced in D. ananassae,
although intimations of their existence have been encountered. More
striking is the resemblance between the pronounced submedian con-
striction in the left arm of the second chromosome of D. melanogaster
(Kaufmann, 1933, 1934; Prokofjeva, 1935), and a similar secondary
constriction in one arm of the B chromosomes of D. ananassae. That
such cytological evidences as similarity of form of chromosomes and
positions of constrictions are inadequate, however, as a test of simi-
lariry of genic content has been emphasized in the recent studies of
Dobzhansky (1935b) and Dobzhansky and Tan (1936). Despite
striking similarity of the somatic metaphase chromosomes of females
of D. pseudoobscura and D. miranda, the salivary glands show that
not a single chromosome of miranda is identical with any chromosome
of pseudoobscura. In light of such information it seems extremely
hazardous, as Dobzhansky and Tan, and Bauer have emphasized, for
cytologists to continue to utilize topographical features of metaphase
chromosomes as the sole criteria in postulating similarities between
the chromosomes of different species.

Summary

1) The chromosome complement of D. ananassae, studied in
the neurocytes, consists of three pairs of V-shaped autosomes and
The sex chromosomes. These are a pair of V-shaped X-chromosomes in the female, an X and a J-shaped Y-chromosome in the male. The chromosomes may be distinguished by relative sizes and characteristic constrictions.

2) The fourth chromosomes and the Y-chromosome appear totally heteropyknotic in resting and early prophase stages of mitotic cells. Short heteropyknotic regions lie adjacent to the spindle attachment regions of the X-chromosomes and the four longer autosomes.

3) In salivary gland nuclei there are but six chromosome arms with euchromatic sections. Four of these represent the paired arms of the longer autosomes, the other two the X-chromosome arms. The fourth chromosomes are represented in salivary gland nuclei by a small bipartite mass of heterochromatin which forms part of the chromocenter.

4) In mitotic prophases of female larvae the fourth chromosomes are associated with the nucleolus, which separates a small satellite-like portion from the remainder of the long arm of these chromosomes. In the male the Y-chromosome forms a third member of the group associated with the nucleolus.

5) The fourth chromosome-nucleolus-satellite relationship is also evident in salivary gland nuclei, the satellite appearing as a banded body associated with the nucleolus, and also connected with the fourth chromosomes by chromatic strands.

6) The chromocenter of mitotic cells and of the salivary gland cells is not amorphous, but discloses, under suitable conditions, the limits of the component chromosomes.

Bibliography


Kaufmann: Morphology of the Chromosomes of *Drosophila ananassae*
Description of Plate 40

Photomicrographs of salivary gland chromosomes of *Drosophila ananassae*. Figs. 1, 2, 4, 5 from female larvae; figs. 3 and 6 from male larvae.

Figs. 1 and 2—Showing the six chromosome arms with euchromatic sections. Four of the six arms are associated at the chromocenter; the other two have been separated. The paired X-chromosomes are represented by the two shortest arms; the four longer arms represent chromosome pairs A and B.

Fig. 3—Chromocenter dissociated by pressure in smearing. The totally heterochromatic fourth chromosomes (4) and the appended satellite are associated with the nucleolus. The unpaired X, left center and crossing the nucleolus in the photograph, exists in the slender haploid condition characteristic of the male.

Fig. 4—Separation of the components of the chromocenter following smearing. The continuity of the arms of the chromosomes is demonstrated. In smearing, the nucleolus has been dragged some distance from the fourth chromosomes (4).

Figs. 5 and 6—The nucleolus and the satellite associated with that portion of the chromocenter representing the fourth chromosomes.