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Torus shaped bands at the 2R telomere region and at the region 68 of the salivary gland chromosomes of *Drosophila auraria*[1]

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Summary. The telomere of the 2R arm of the salivary gland chromosomes of *D. auraria* exhibits a definite toroidal structure in routine squashed preparations, stained either by propionic orcein-carmine or by fluorescent dyes. There is evidence that a band (or bands) of region 68 (possibly homologous to that of *D. melanogaster)* of the 3L chromosome arm also exhibits a toroidal structure. These toroids are associated with heterochromatin, but it is not certain that they are themselves heterochromatic.
Key words. Toroidal bands; *Drosophila*; polytene chromosomes.

Polytene chromosomes are an ideal system to study the interphase chromosomal structure. Since these chromosomes are the results of 10 rounds (2^{10}–1024 C) of DNA replication[2], they have a large size, visible under the light microscope, exhibiting a characteristic banding pattern, which represents a cytological map of the genome. Many genes can be placed on the map and a large number of these exhibit, during their activity, the phenomenon of puff formation. Looking at the literature concerning the structure of the bands, we can see that the main idea which has been proposed is that the band has a disk-like structure[3]. Recently, studies have been published proposing a toroidal structure for bands of these chromosomes in *Drosophila melanogaster*. These studies were performed by using thin sections examined under the light or the electron microscope after specific treatments[4,5].

In this report we show that certain bands of the polytene chromosomes of *D. auraria,* when observed under the light microscope in routinely fixed squashed preparations, exhibit torus-shaped configurations.

Materials and methods. A stock of *Drosophila auraria,* originally collected in Kirishima, Japan, and maintained in the Department of Zoology, University of Texas, Austin, as stock no. 3040.11b, and three sublines (nos. 17, 8 and 2) derived from the original stock by sibling matings[6] were used in this study.

The routine squashed preparation method was used for the observation of salivary gland polytene nuclei of animals at various developmental stages. Salivary glands were dissected in a Ringer-type solution[7], fixed in 3:1 ethanol:propionic acid, stained in propionic orcein-carmine and observed under a phase contrast microscope. Furthermore, the chromosomes were stained with fluorescent dyes (Quinacrine dihydrochloride[8] and Hoechst 33258[9]) and observed using a BG 12 excitation filter and a 53 barrier filter under a Zeiss fluorescence microscope.

For the detection of late replicating patterns of the genetic materials, a routine ^{3}H-thymidine (250 μCi/ml, s.a. 2 Ci/mM, The Radiochemical Centre Amersham) autoradiographic method[10] was used during several developmental stages.

Results and discussion. During the examination of the salivary gland polytene chromosomes of all strains of *D. auraria* used in this study, we observed that the bands which form the telomere of the 2R chromosome arm (two heavy plus a few fainter bands) exhibit a definite resemblance to a toroidal structure; this is the only region of the polytene complement which shows this structure more often than not, under the conditions described above. Figure 1 illustrates the torus shaped telomere of the 2R chromosome arm.

Beyond the 2R telomere, a band (or bands) of region 68 of the polytene chromosomes of the stocks examined also seems to show a toroidal structure (figs 2b, 2c). This region of the *D. aura-*

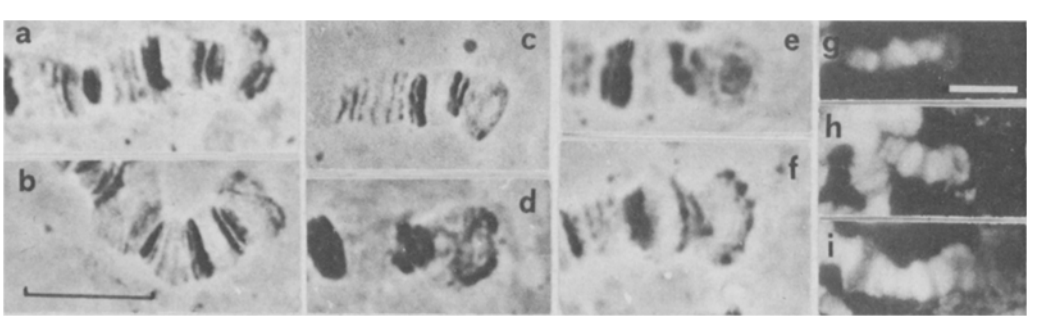

Figure 1. Toroidal structure of the 2R chromosome telomere of *D. auraria. a–f* Stained with propionic orcein-carmine; *g* stained with Quinocrine dihydrochloride; *h–i* stained with Hoechst 33258. *a* Asynapsed chromatids indicate toroidal structure; *b* figure eight type appearance of torus shaped bands; *c, d, g, h* toroidal structure of the telomere; *e* tight configuration of the telomere; *f, i* distorted configuration. Bars in all figures represent 10 μm.

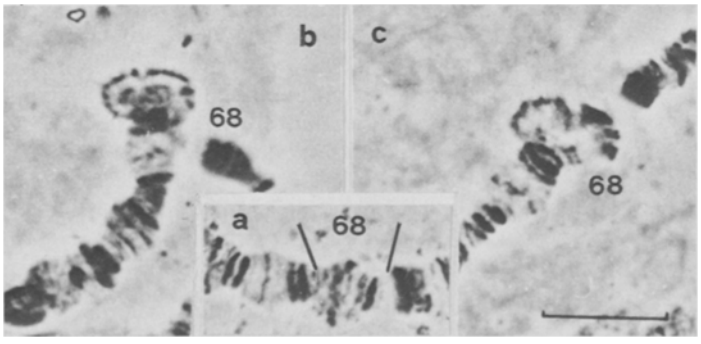

Figure 2. Region 68 of the 3L chromosome arm of *D. auraria*. *a* Usual configuration; *b–c* toroidal appearance of bands in the region.

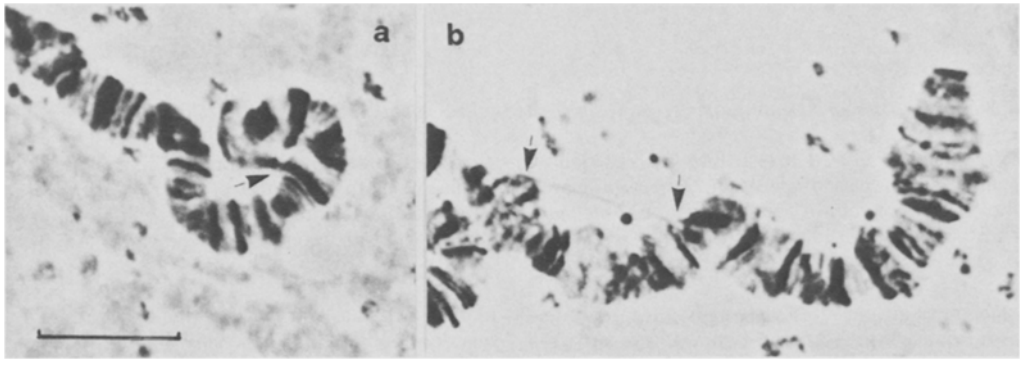

Figure 3. Ectopic pairing (arrows) involving the 2R telomere (a) and bands of region 68 (b).

ria chromosomes is very similar to the respective region 68 of *D. melanogaster*[6].

Recent research on toroidal bands has been presented by Mortin and Sedat[4], and Sorsa[5] who, by using a number of light and electron microscopic techniques, have attempted to show that these bands are either a generalized[4] or a special[5] phenomenon concerning polytene chromosome structure.

The data presented in this report show that toroidal type structures can be observed under the light microscope by routine techniques where acid fixation is the rule. This comes into disagreement with Mortin and Sedat[4] who claim that the reason why toroidal structures are not usually observed is the severe treatment that the chromosomes receive during the preparation of the slides. As mentioned earlier, at least as far as the tip of the *D. auraria* 2R chromosome arm is concerned, the appearance of a toroidal structure is the rule rather than the exception, and this holds true both with propionic porcein-carmine staining and with fluorescent staining.

Sorsa[5] proposes that the toroidal structure is associated with less polytenization of the regions where these structures are observed. Although our autoradiographic data do not indicate late replicating patterns of the toroidal regions of the *D. auraria* chromosome complement, our data on ectopic pairing of these regions (fig. 3) seem to indicate that Sorsa's idea may be correct in our case; both regions, in which bands exhibiting toroidal structure are found, are seen to pair ectopically which means that they contain elements of intercalary heterochromatin. The existence of this heterochromatin in the case of region 68 of *D. auraria* is substantiated further by the fact that the homologous region of *D. melanogaster* also pairs ectopically[11,12], but it does not exhibit a late replicating pattern as well[12]. Although Sorsa[5] proposes that the toroid structure is observed in heterochromatic bands, our data (as evidenced by ectopic pairing) do not allow us to decide whether the bands themselves exhibiting the torus shaped structure or other neighboring bands are heterochromatic. In the case of the 2R telomere, however, there are indications that the ectopic pairing observed involves a terminal entity. In some cases this entity may be broken up (if there is puffing in the preceding region) creating a funnel-like structure (fig. 1f, 1i); tight pairing of this entity does not allow the observation of an open torus type configuration (fig. 1e).

From the data presented above, it becomes evident that toroidal band structure is real enough, and seems to be somehow associated with heterochromatic components. If this is the general case, it is difficult to see how all (or most) bands of the polytene chromosomes can be toroids. Perhaps a study of the bands known to form ectopic associations would provide more information.

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