マウスおよび他種動物における動原体“ヘテロクロマチン"ふさの簡便染色法

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SHORT COMMUNICATION

A SIMPLE TECHNIQUE TO DEMONSTRATE THE CENTROMERIC 'HETEROCHROMATIN' IN THE MOUSE AND OTHER ANIMALS[1)]

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Pardue and Gall (1970) observed that the centromeric areas of mouse chromosomes were heterochromatic in their cytological preparations following the *in situ* hybridization procedure with the satellite DNA and referred to these regions as centromeric heterochromatin. In confirmation of the above observation, Arrighi *et al.* (1970) demonstrated that the constitutive heterochromatin of *Microtus agrestis* was revealed by the essentially similar procedure. *In situ* hybridization techniques, however, require relatively long time including the treatment of the preparations with NaOH or thermal denaturation in a buffer which often destroy the integrity of the chromosome morphology. The present communication describes a simple and consistent technique to reveal the centromeric 'heterochromatin' of the mouse chromosomes. The technique proved applicable to grasshoppers as well as various mammals including man.

The procedure which incorporates steps of the lactic acid and heat treatment to the conventional air-drying and Giemsa staining is as follows:

(1) Fix cells with 3 : 1 methanol-acetic acid in a usual manner.

(2) Place a small drop (ca. 0.005-0.01 ml) of the concentrated cell suspension on a slide and immediately add 2 drops of 3 : 1 glacial acetic acid-50% lactic acid.

(3) Apply, 1-2 min later, a large drop (ca. 0.02 ml) of methanol-acetic acid followed by 3 additional drops of the fixative when the previously applied fixative spreads over the slide. Then, dry up the slide in the air. Mild flaming ensures good spreading of chromosomes.

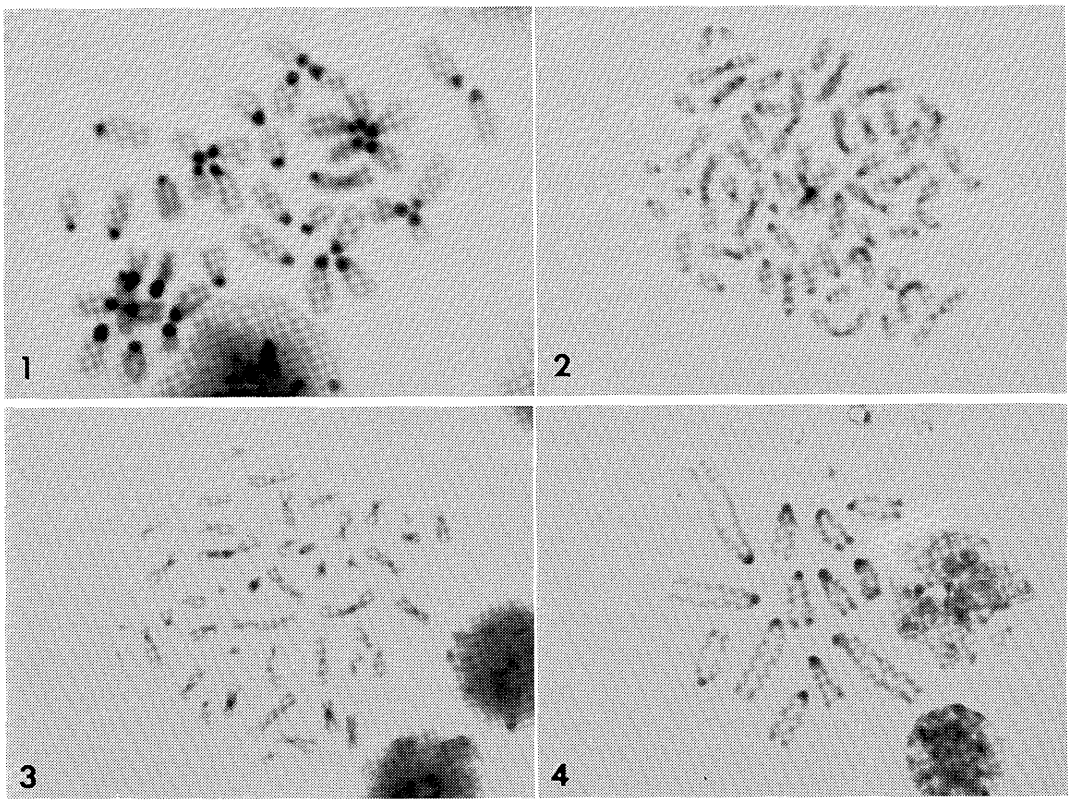
(4) Immerse the dried slides in hot distilled water kept at 92±2°C for 8 min and then in 2×SSC at room temperature for 2-3 min.

(5) Stain 10-30 min with Giemsa diluted (ca. 1%) in 0.01 M phosphate buffer, pH 6.4.

(6) Rinse with tap water and air dry.

Fig. 1 represents a metaphase from a female mouse embryo prepared by the present procedure. The darkly stained regions localized near the centromere appear reddish violet while chromosome arms bluish green with the aid of a green filter. As noticed by Pardue and Gall (1970) the Y did not show a differential pattern along the length.

1) Contributions from the Chromosome Research Unit, Hokkaido University.



Figs. 1–4. Metaphase plates prepared with the technique described.

Fig. 1, a female mouse embryonic cell. Fig. 2, a cultured mouse A9 (L) cell. Fig. 3, a human male lymphocyte. Fig. 4, a spermatogonium of *Acridium japonicum*.

The double structure of the heteropycnotic region is apparent in the biarmed chromosomes of the mouse A9 cell (Fig. 2). It is also evident that the loss or gain of the heteropycnotic region occurs in the course of karyotypic evolution. Figs. 3–4 show that the secondary constrictions of man and telomeric areas in some chromosomes of the grasshopper *Acridium japonicum* are differentially stained, in addition to the centromeric regions.

Although a similar pattern is occasionally demonstrated in conventional air-dried preparations after the thermal treatment described above, a clear and consistent result is obtained after the acetic-lactic acid treatment. Since the size and staining intensity of the differentially stained regions are different among species, the technique would have potential usefulness in the investigation of somatic hybrids. Further studies are in progress to characterize the region demonstrated by the present technique.

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LITERATURE CITED

Arrighi, F. E., T. C. Hsu, P. Saunders, and G. F. Saunders, 1970 Localization of repetitive DNA in the chromosomes of *Microtus agrestis* by means of *in situ* hybridization. Chromosoma **32**: 224-236.

Pardue, M. L., and J. G. Gall, 1970 Chromosomal localization of mouse satellite DNA. Science **168**: 1356-1358.