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Changes in the karyotype of the cell line, DSIR-HA-1179, and a comparison with that of its parent insect, *Heteronychus arator* (F.) (Coleoptera: Scarabaeidae)

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be similar, but the number of chromosomes per cell was much more variable (range, 12-73; 42 metaphase cells). The modal chromosome number of testicular tissues from *H. arator* was $2n = 20$ — including an Xy pair — which is characteristic of the Scarabaeidae. The karyotype of DSIR-HA-1179 cells is unstable, with the number of chromosomes becoming more variable with continued propagation.

Keywords DSIR-HA-1179 cell line; *Heteronychus arator*; Coleoptera; Scarabaeidae; karyotype; chromosomes

Abstract Eighteen months after establishment of the primary cell culture, 36 metaphase nuclei of cell line DSIR-HA-1179, derived from the black beetle, *Heteronychus arator* (F.), had between 32 and 43 chromosomes; one cell contained 67 chromosomes. Structural abnormalities such as double minute chromosomes, ring and di- and tri-centric chromosomes were observed. Thirty-six months after establishment the chromosomes appeared to

INTRODUCTION

Of the 114 insect cell lines listed by Hink (1976) the karyotypes are known for 57. There is considerable variation among the karyotypes which have been analysed. In the Diptera, *Drosophila melanogaster* Meigen cell lines whose karyotypes have been determined are tetraploid, aneuploid, or a combination of diploid and heteroploid, and cell

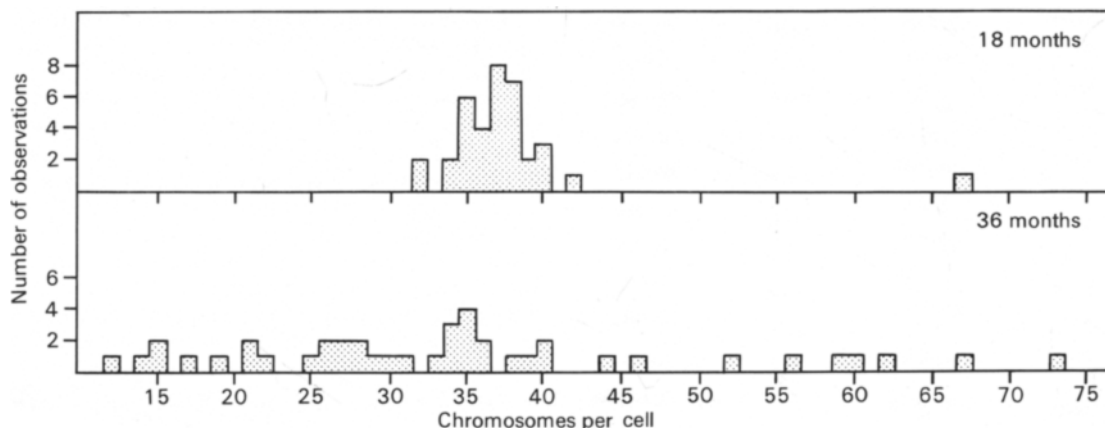
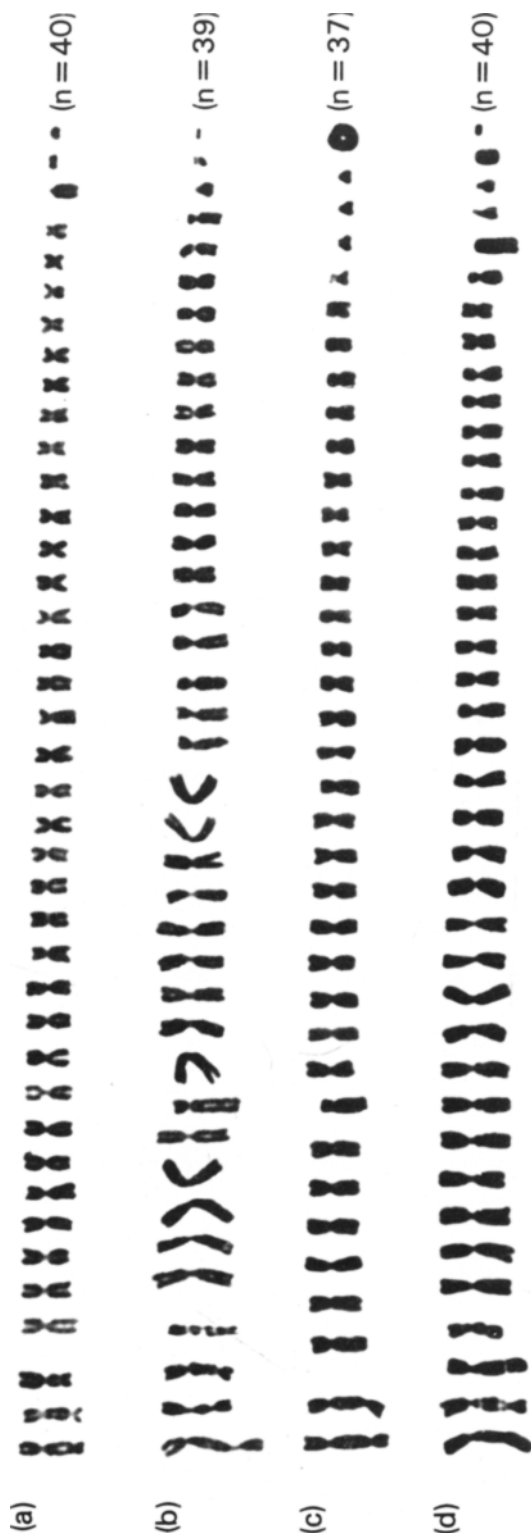


Fig. 1 Histogram showing the variation in chromosome number found in DSIR-HA-1179 cells at 18 and 36 months after the establishment of the primary cell culture.



lines from *Aedes* and *Culex* mosquitoes are mostly diploid or polyploid. In the Orthoptera the cell lines derived from *Blattella germanica* (L.) (German cockroach) are diploid, polyploid, or a combination of diploid and tetraploid (Hink 1976). Lepidopteran cell lines contain many small chromosomes — in a *Bombyx mori* (L.) cell line many cells contain over 100 (Grace 1967) — and the number often varies widely within the same cell line.

The stability of karyotypes in tissue from whole insects contrasts with the karyotype variability in cell lines. Yadav & Pillai (1979) found that 145 of 194 scarab beetles studied possessed the karyotype $2n = 20$, Xy male, indicating that the normal scarabaeid haploid number is $n = 10$. Crawford (1982) recently established a cell line from neonate larval tissues of the scarab *Heteronychus arator*. The karyotype of this species and its cell line DSIR-HA-1179 are reported and compared in this paper.

MATERIALS AND METHODS

Cells

DSIR-HA-1179 cells were grown in Schneider's *Drosophila* medium (Gibco) in 25 cm² flasks (Falcon) at 27°C and subcultured as described by Crawford (1982). Haploid and diploid cells were obtained in vivo from the testes of newly emerged *H. arator* male adults.

Chromosome preparation and staining

DSIR-HA-1179 cells were incubated with colchicine (0.1 µg ml⁻¹ of culture medium) for 4 hours, and harvested using trypsin. They were treated with hypotonic 0.075M KCl fixative for 25 minutes, then fixed with 3 changes of cold Carnoy's solution (methanol: glacial acetic acid, 3:1 v/v), air dried on slides, and stained with 7% Giemsa (Gurrs Improved R66) for 8 minutes.

The testicular tissue was incubated in Schneider's *Drosophila* medium with colchicine (0.1 mg ml⁻¹) for 4 hours, and then teased into small fragments, using fine needles. The fragments were further disrupted by being forced up and down through a hypodermic needle, and were then treated with a hypotonic solution of KCl, fixed, and stained, as above.

Fig. 2 Typical karyotypes of the *Heteronychus arator* (F.) cell line, DSIR-HA-1179; note the di- and tri-centric chromosomes in all karyotypes, the double minute chromosomes in (a), (b), and (d), and the ring chromosome in (c).

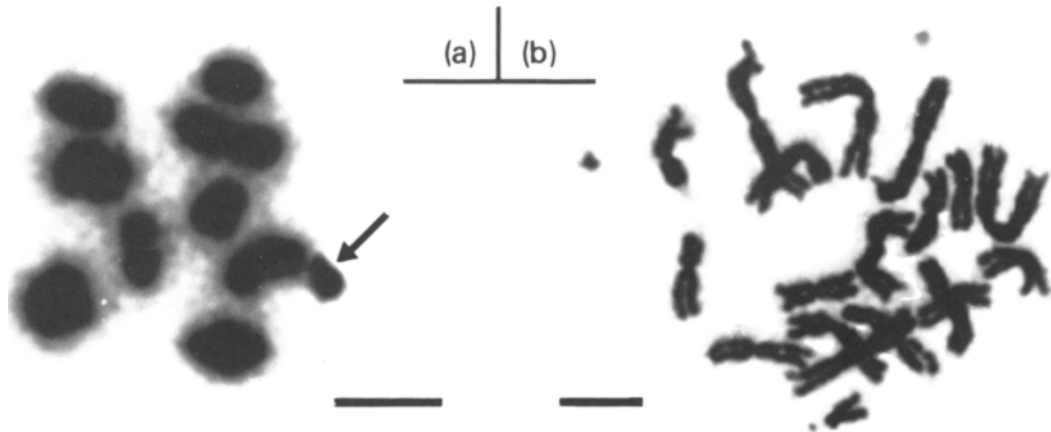


Fig. 3 Karyotype of *Heteronychus arator* (F.): (a) haploid chromosomes from testicular tissue; note the unpaired bivalent containing the small male sex chromosome (arrowed); (b) diploid chromosomes from testicular tissue.

RESULTS AND DISCUSSION

Of 36 DSIR-HA-1179 cells examined 18 months after the primary culture was established, 35 had from 36 to 42 chromosomes (modal number = 37), and one cell contain 67 chromosomes (Fig. 1). The karyotype consisted predominantly of metacentric chromosomes; the number of di- and tri-centric chromosomes, ring chromosomes, and double minutes varied markedly. The karyotypes of 4 representative cells are shown in Fig. 2.

After a further 18 months in culture the cell line chromosome number was highly variable (Fig. 1), but the appearance of the chromosomes was little changed, with the di- and tri-centric chromosomes, characteristics of the earlier analysis, still present in virtually all mitotic figures. Double minute and ring chromosomes were occasionally present.

It is clear that the variability in the chromosome number present after 18 months in culture had increased markedly after 36 months and that chromosome number is of no use as an identification character for these cells. The continued presence of characteristic di- and tri-centric chromosomes, which are normally found only in continuous cell lines is the only distinguishing karyotypic feature of this line. The essentially unstable nature of polycentric chromosomes means that methods other than karyotypic analysis (e.g., serology) should be used to confirm cellular identity.

All meiotic figures of *H. arator* testicular cells observed ($n > 15$) had 10 bivalents (Fig. 3a), the

characteristic number for scarabs (Yadav & Pillai 1979). The sex chromosome bivalent was distinguishable in most cells (Fig. 3a). One mitotic figure contained 9 pairs of chromosomes (metacentric and submetacentric), plus a small acrocentric x, and a tiny y chromosome (Fig. 3b).

We conclude that the cell line DSIR-HA-1179 is, after 36 months of culture, an unstable polyploid with a highly variable chromosome number. The chromosomes of DSIR-HA-1179 cells will be studied in a further 18 months to determine the effect of continued propagation.

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