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Restriction endonuclease chromosome banding in *Tapinoma nigerrimum* (Hymenoptera, Formicidae).

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Fixed metaphase chromosomes and isolated genomic DNA of the ant *Tapinoma nigerrimum* were treated with the restriction endonucleases *Eco*RI, *Hae*III and *Tru*9I. *Eco*RI and *Hae*III induced a banding pattern similar to G-banding, whereas *Tru*9I induced a C-band-like pattern. There is only a partial accord between the action of these enzymes on isolated genomic DNA and on DNA in fixed chromosomes. Our results indicate that the presence of target sequences along the chromosomes is necessary but not sufficient to produce chromosome bands. The chromosomal organisation also seems to be important in producing longitudinal differentiation on chromosomes.

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Over the last years a few techniques have been developed to improve identification of complement chromosomes (reviewed by SUMNER, 1994). Among a number of applications, restriction endonucleases (REs) have proved useful for cytogenetic studies, since they have allowed the study of particular areas of eukaryotic chromosomes in terms of DNA base composition and the structure on fixed chromosomes, thus revealing a large variability in different chromosomal regions (KAMISUGI et al. 1992, FERNÁNDEZ-GARCÍA et al. 1998; PIECZARKA et al. 1998).

The effects of the digestion with restriction endonucleases of type II on fixed chromosomes showed the existence of regions resistant to these enzymes, which led to differential banding patterns. The action of a restriction endonuclease depends on the amount of target sites at a given chromosomal area: when the number of the targets is high, short DNA fragments (less than 1 kb in length) are extracted during the treatment, while low frequencies of recognition sites produce long DNA fragments (more than 1 kb in length) which are retained by chromosomal proteins. The observation of regions with different staining intensity points to different sensitivity to enzyme digestion, even showing areas that have been completely extracted (gaps) (BIANCHI et al. 1990).

In a previous paper, we reported the existence of G-banding pattern in the ant *Tapinoma nigerrimum* (LORITE et al. 1996a). The aim of the present study is to examine the possible relationship between G-banding pattern, restriction endonuclease-banding and base composition of DNA in this species. For this purpose we have used the cytological data obtained by in situ digestion of fixed chromosomes and the molecular data obtained from the study of extracted DNA, digested with the same enzymes. We used REs with different AT and GC rich base target sequences, *Hae*III (GG↓CC), *Tru*9I (T↓TAA) and *Eco*RI (G↓AATTC).

MATERIAL AND METHODS

Chromosome preparations

Various samples of the ant *T. nigerrimum* (Nylander, 1886) (Hymenoptera, Formicidae) were collected in the province of Jaén (Spain). Chromosome preparations were prepared from testes of early pupae, using the technique described by LORITE et al. (1996b).

Isolation and digestion of genomic DNA

Worker pupae individuals were used for genomic DNA extraction according to the technique of HEINZE et al. (1994). Digestion of isolated DNA with REs (*Hae*III-GG↓CC, *Tru*9I-T↓TAA, and *Eco*RI-G↓AATTC) was made according to the supplier's recommendations using 4 U/μg DNA. The digested DNA was analysed by electrophoresis in 1% agarose gel.

Digestion on fixed chromosomes with restriction endonucleases

Incubation was carried out in a moist chamber for 16 h with 10 to 40 U of each enzyme in 100 μL of the buffer furnished by the supplier (Boehringer), per slide. The slides were washed in distilled water and stained with Giemsa or propidium iodide. Control

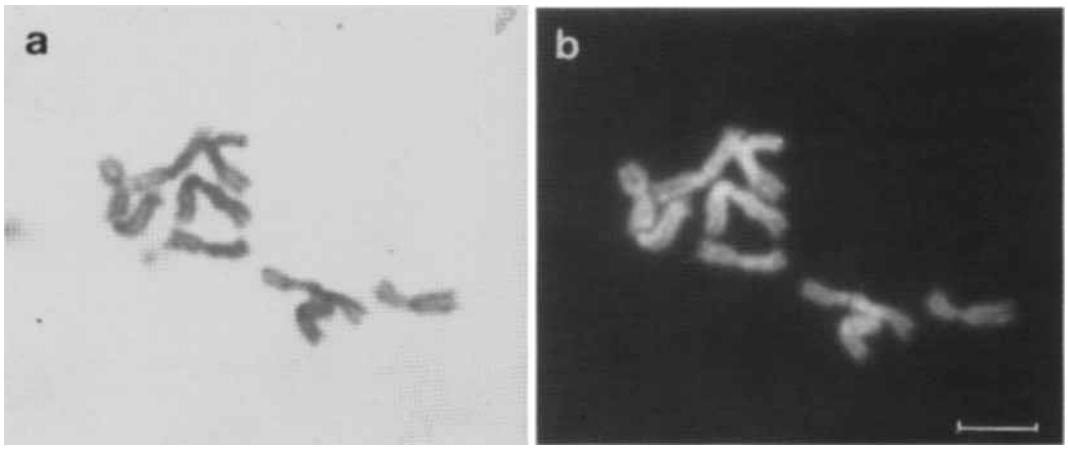

Fig. 1. a and b. *Tapinoma nigerrimum*. Haploid metaphase plates after incubation in the RE-buffer (without enzyme) and after staining with Giemsa **(a)** or propidium iodide **(b)**. Bar = 5 μm.

preparations were incubated in the corresponding buffer (M buffer and H buffer) without the enzyme. Five chromosome preparations were used to determine the restriction endonuclease banding pattern for each enzyme; 20 metaphase plates were analysed in each chromosome preparation.

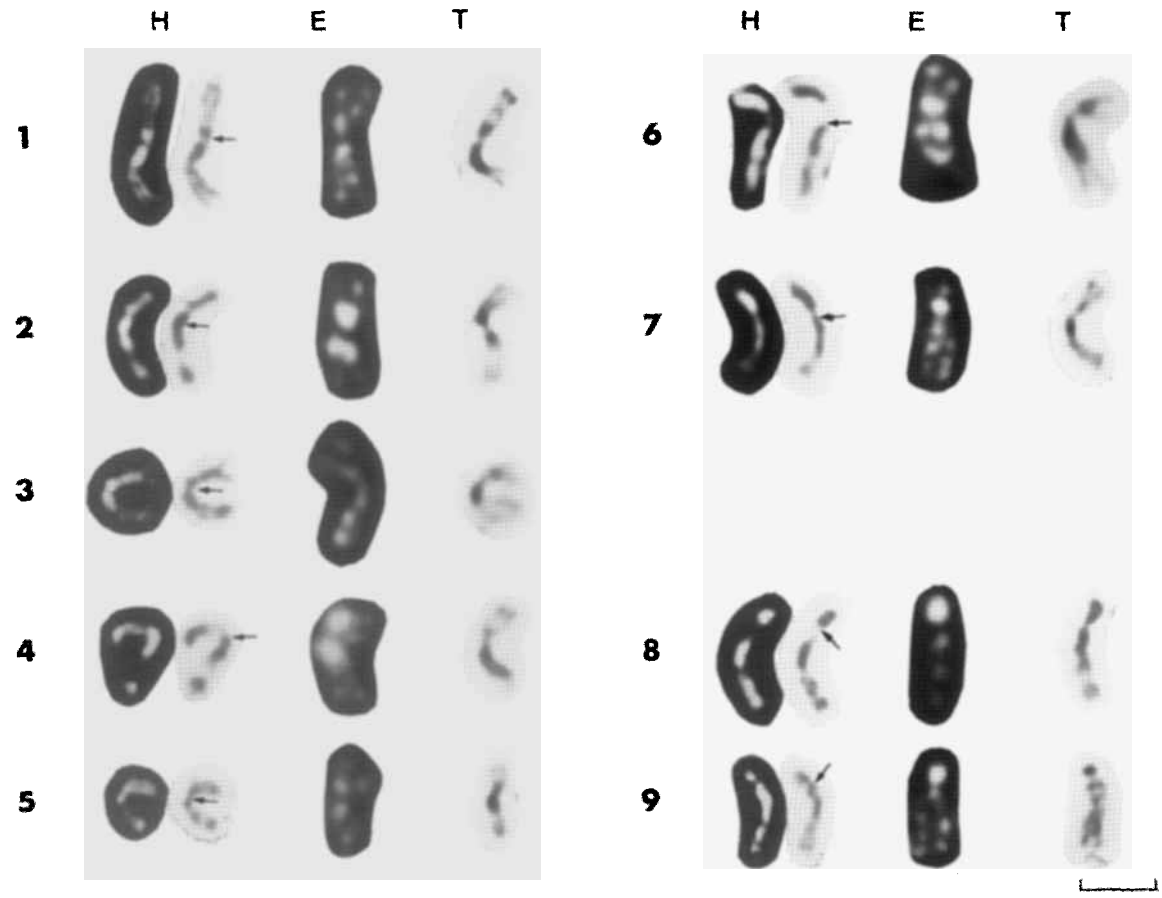

Fig. 2. Banding patterns induced after in situ digestion with restriction endonucleases *Hae*III (H) stained with propidium iodide or Giemsa, *Eco*RI (E) stained with propidium iodide and *Tru*9I (T) stained with Giemsa. Arrows indicate the position of the centromeres. Bar = 5 μm.

RESULTS AND DISCUSSION

The chromosome number of *T. nigerrimum* is n = 9 in males and 2n = 18 in females and workers, and the standard haploid karyotype formula is 5m + 2sm + 2st (PALOMEQUE et al., 1988). As shown in previous studies, the chromosomes of *T. nigerrimum* had a distinctive and reproducible pattern of bands after G-banding treatment (LORITE et al. 1996a).

In control preparations, the staining reactions with propidium iodide and Giemsa were intense over all the chromosomes. The result obtained with the M buffer is shown in Fig. 1. A similar result was obtained with the H buffer. Incubation in the digestion buffer alone did not produce chromosome banding. However, all three restriction enzymes produced a definite and reproducible banding pattern as 42–92% of treated chromosomes showed typical banding patterns.

When *Hae*III is used on fixed chromosomes a G-band-like pattern was obtained (Fig. 2). This banding pattern is observed with both Giemsa and propidium iodide staining and it can be observed in chromosomes with different degrees of condensation (Fig. 3). In the short arm of chromosome 6 there is a nucleolar organiser region (NOR), detected by in situ hybridisation (ISH), in an Ag-positive and CMA-positive chromosome region (Fig. 3 and LORITE et al., 1997). Fig. 3 also shows that *Hae*III fully digests this chromosomal region, suggesting that a large number

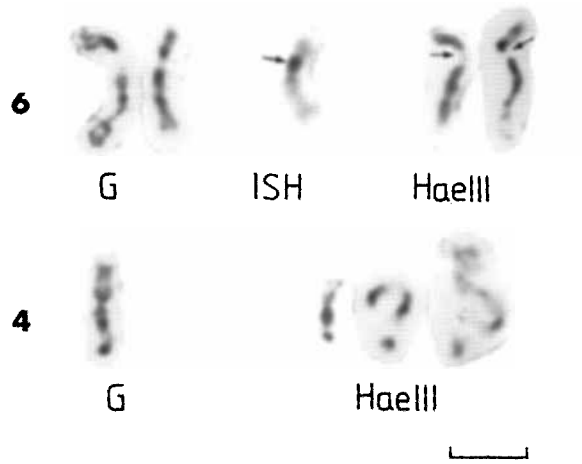

Fig. 3. Chromosome 6 with different degrees of chromosome condensation, after G-banding treatment (right) and after incubation with *Hae*III (left), showing similar banding pattern. Chromosome 6 showing the localisation of the nucleolar organising region detected by in situ hybridisation (ISH) is shown in the center. Arrows indicate the localisation of the nucleolar organising region and the effect of the enzyme on this chromosomal region. Chromosome 4 with different degrees of chromosome condensation after G-banding (left) and after incubation with *Hae*III (right), also showing similar banding patterns. Bar = 5 μm.

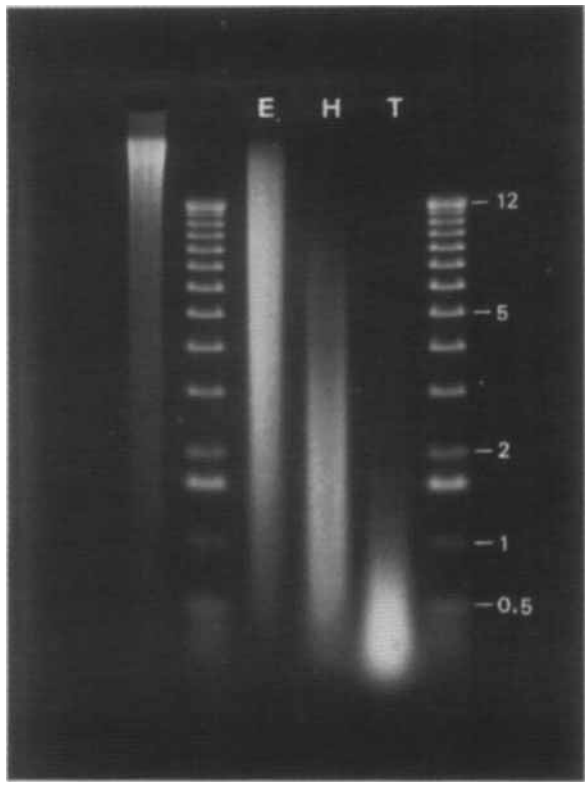

Fig. 4. Electrophoresis of DNA digested which REs. First lane: non-digested DNA as control; second and sixth lanes 1 kb DNA ladder. E), H), and T) Digestions with *Eco*RI, *Hae*III and *Tru*9I, respectively.

of *Hae*III targets are present in this area. This result is not surprising considering that the CMA positive reaction indicates that its DNA is rich in GC-base pairs (SUMNER 1994).

The digestion of isolated genomic DNA with *Hae*III shows the existence of numerous target sequences for the enzyme. A smear consisting of DNA fragments from less than 100 bp to over 10 kb is observed (Fig. 4). The observed effects on fixed chromosomes, especially chromosomes 4 and 6, are in accordance with the results obtained on the isolated DNA.

The restriction endonuclease *Eco*RI also produces a G-band-like pattern. This banding is observed by Giemsa or propidium iodide staining (Fig. 2). *Eco*RI produces a smear with fragments of 500 bp or longer, the majority of them being high molecular weight DNA fragments indicating that the frequency of specific base target sequences of *Eco*RI is lower than the target sequences of *Hae*III (Fig. 4), in spite of these results, the two enzymes produce a G-band-like pattern.

After *Tru*9I digestion, the chromosomes are weakly stained, except for the pericentromeric regions, resulting in a C-band-like pattern in the major-

ity of chromosomes; however, chromosomes 1, 8 and 9 show an intermediate banding pattern between C and G. The NOR on the short arm of chromosome 6, is only partially digested with this enzyme (Fig. 2). When the chromosomes previously digested with *Tru*9I are stained with propidium iodide, no clear differentiation can be observed (data not shown). *Tru*9I produces an intense digestion of the genomic DNA, so the majority of fragments are smaller than 1 kb (Fig. 4), showing the high frequency of the TTAA sequences in the genome of *T. nigerrimum*. Digestion of isolated DNA with *Tru*9I produces very small fragments that, theoretically, can be easily extracted from chromosomes (BIANCHI et al. 1985; MEZZANOTTE et al. 1985, 1992). Therefore, the observed effects on fixed chromosomes are in accordance with the results obtained on isolated DNA. However, there are different chromosomal regions not digested with this enzyme, especially the C- and G- positive pericentromeric regions (PALOMEQUE et al. 1988; LORITE et al. 1996a). No digestion of pericentromeric heterochromatin has been reported by other authors (STUPPIA et al. 1991; PIECZARKA et al. 1998).

In situ digestion with *Tru*9I has been used by other authors with various results. LUDEÑA et al. (1991) reported a R-banding like pattern using *Mse*I (an isoschizomer of *Tru*9I) in human chromosomes. However, VIÑAS et al. (1994) observed a G-band-like pattern using the same RE in eel (*Anguilla anguilla*).

The partial discrepancy between the electrophoretic and cytological data can be explained by the hypothesis that base composition does not represent the only factor affecting the in situ digestion of eukaryotic chromatin by REs. This has also been reported by other authors (BURKHOLDER, 1989; GOSÁLVEZ et al. 1989; JUAN et al. 1991; DE LA TORRE et al. 1991; MANICARDI et al. 1994; FERNÁNDEZ-GARCÍA et al. 1998).

In conclusion, the results obtained on *T. nigerrimum* indicate that the presence and frequency of specific base target sequences are thus necessary but not sufficient to explain the molecular mechanism of DNA cleavage and loss in REs-treated chromosomes. The higher organisation of specific chromosome regions also seems to be important in producing longitudinal differentiation on chromosomes after RE treatments.

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