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SHORT COMMUNICATION

G-banding and chromosome condensation in the ant, *Tapinoma nigerrimum*

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Well-defined G-bands were obtained on metaphase chromosomes from *Tapinoma nigerrimum* using trypsin and warm 2 × SSC in sequence. The G-banded pattern allowed the identification of all chromosomes. Evidence for asynchronous condensation of the chromosomes of this species is provided. Different banding patterns were obtained when metaphase chromosomes were stained with DA/DAPI alone and with DA/DAPI after a standard G-banding procedure. The G-banding phenomenon is discussed using the result obtained.

Key words: chromosome condensation, DA/DAPI, fluorochrome, Formicidae, G-banding

Introduction

Previous cytogenetic studies in ants have been carried out by Crozier (1975), Palomeque *et al.* (1988, 1990, 1993), Hirai *et al.* (1994) and Imai *et al.* (1994), among others. Until now only C-banding and nucleolar organizer region (NOR) banding have been applied in ants.

Since G-banding was discovered, reports have provided clear evidence only for metaphase chromosomes from warm-blooded vertebrates. In insects, G-banding has been applied in various orders; in the majority of these a few bands occur in the chromosomes, but in *A. domesticus* (Warchalowska-Sliwa *et al.* 1978) and in *E. berlesei* (Odierna *et al.* 1993) a good pattern of G-banding has been obtained.

The aim of this paper is to determine the pattern of G-banding in the ant, *Tapinoma nigerrimum*. Our results confirm previous reports of G-bands, showing that it is possible to obtain G-bands in metaphase chromosomes from invertebrates. This staining, allowing accurate identification of all pairs of chromosomes and their linkage gene groups, is useful in basic as well as more advanced genetic studies. This study also shows that, in *T. nigerrimum*, chromosomal condensation is an asynchronous process that apparently follows a precise pattern. Chromosome study using fluorescent staining with dA + dT- specific DAPI fluorochrome dye has also been carried out. The G-banding phenomenon is discussed using the results obtained. In previous cytogenetic studies we have applied the C-banding and silver impregnation techniques to this species (Palomeque *et al.* 1988, 1990).

Material and methods

Chromosome preparations were made from testes using the technique described by Meredith (1969). G-banding was performed essentially following the method of Burgos *et al.* (1986). These slides were decolorized and stained with DA/DAPI using the technique described by Schweizer (1980). Slides that did not receive G-banding treatment were also stained with DA/DAPI. The chromosome length and the length of G-bands were measured using an image analyzer (Videoplan Kontron) in 20 metaphases with different chromosomal condensation. In chromosome 8, various stages (from a to d) were considered in relation to the degree of chromosome condensation and existing numbers of bands.

Results and discussion

After G-banding, the chromosomes of *Tapinoma nigerrimum* showed a distinctive and reproducible pattern of bands. An idiogram of the banding pattern is shown in Figure 1. All centromeric regions show a negative G-banding response. The telomeric regions show a positive G-banding response except the telomeric regions of chromosome 8. Figure 1 also shows a composite G-banded karyotype with different degrees of chromosome condensation and different banding patterns. The number of bands per chromosome decreases as the degree of condensation increases. This study shows that, in *T. nigerrimum*, chromosomal condensation is an asynchronous process that apparently follows a precise pattern that changes the banding patterns.

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The condensation of chromosome 8 has been analyzed in detail. In Figure 2a several chromosomes 8 with different chromosomal condensation and different banding pattern are shown. The corresponding band idiograms for each of the chromosomes are also represented. This figure depicts the relation between band number and chromosome condensation. Late metaphase chromosomes show almost uniform staining. The lengths of each chromosomal region (short arm, centromeric region and long arm) in the various stages considered are also represented in Figure 2b, expressed as fractions of the length measured at the least condensed stage. The short arm is more rapidly condensed in the first stage of the condensation cycle with the corresponding band fusion; little condensation in other stages was observed. In contrast, the centromeric region is especially condensed at the final stage of the condensation cycle. A more uniform condensation was observed in the long chromosome arm. Chromosome 8 of *T. nigerrimum* carries C-banded heterochromatin in part or almost all of its short arm (Palomeque *et al.* 1988, 1993). We suggest that the faster condensation observed in this region may be due to the presence of a C-banded heterochromatic block. Differential arm condensation in chromosomes with heterochromatic block has also been reported by other authors (Ponce de Leon *et al.* 1992; Kakeda & Fukui 1994).

After staining with dA + dT-specific DAPI fluorochrome dye, the chromosomes of *T. nigerrimum* appear stained uniformly fluorescent apart from a region of the short arm of chromosome 6 (Figure 3a). However, a similar G-banding chromosomal pattern was observed following staining with DAPI after trypsin + 2 × SSC treatment (Figure 3b).

In spite of many years' research, the functional significance and the molecular mechanism of G-banding and other classes of chromosome bands are still unknown (reviewed by Sumner 1994). It has been suggested that in non-vertebrate metaphase chromosomes G-banding is poor because the genome does not allow compartmentalization into discrete domains or bands (Holmquist 1989). Recent scanning electron microscopy

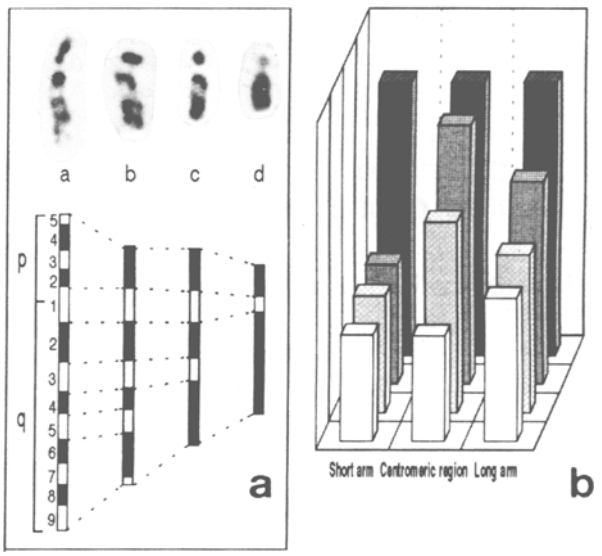

Figure 2. a Chromosome 8 showing different chromosomal condensation and different G-banding patterns, the corresponding band idiograms and a possible sequence in the process of band fusion. **b** Representative graph of the lengths of each chromosomal region in the various stages considered expressed as fractions of the length measured at the least condensed stage. ■, stage A; ■, stage B; □, stage C; □, stage D.

of trypsin-pretreated insect chromosomes has shown that these chromosomes are segmented into blocks, like mammalian chromosomes (Wolf *et al.* 1994). Furthermore, compositional compartmentalization has also been found in non-vertebrates (Isacchi *et al.* 1993). It has been proposed that G-banding is a result of local rearrangements on the chromatin and/or altered proteins (Babu & Verma 1987). G-banding has also been explained as a consequence of the division of the chromosomes into segments that are A + T rich or G + C rich, corresponding to G-positive and G-negative bands respectively.

To explain our results we suggest two possible hypotheses. In the first hypothesis, we suggest that only one segment of the short arm of chromosome 6 is

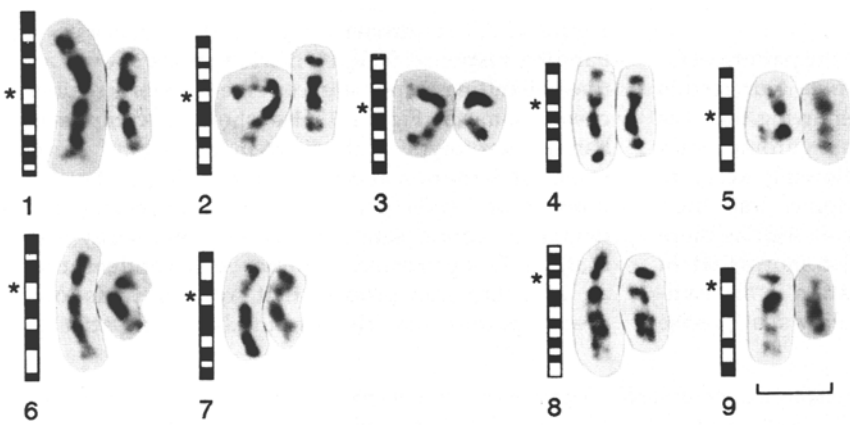

Figure 1. Idiogram and composite G-banded karyotype of *Tapinoma nigerrimum* showing different degrees of chromosome condensation and different banding patterns. Bar = 5 μm.

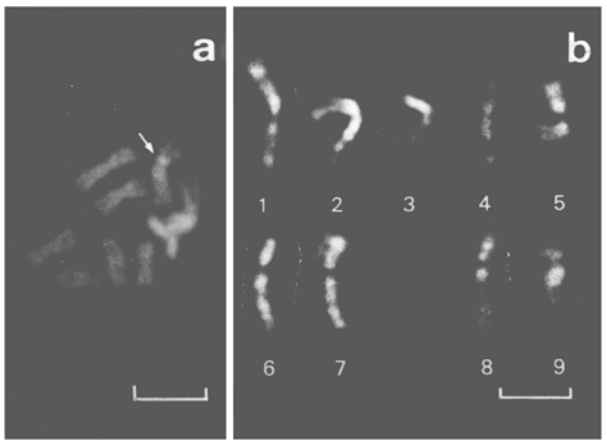

Figure 3. Staining with DA/DAPI. **a** Stain in untreated chromosomes showing a band on chromosome 6 (arrow). **b** Haploid karyotype of chromosomes stained with DA/DAPI after treatment by G-banding showing a similar G-banding pattern. Bar = 5 μm.

sufficiently AT rich to produce a differential fluorescence. In this case the G-bands observed could be considered to be the consequence of local rearrangements of the chromatin and/or altered proteins. According to the second hypothesis, which we favor, the positive G-bands could also be related, at least in part, to a selective accumulation of dA + dT-rich sequences, but they would be inaccessible to the fluorochromes owing to the chromosomal structure and/or the presence of associated proteins. We suggest that in *T. nigerrimum* the standard G-banding procedure would cause structural changes in DNA and/or associated proteins, which could favor the binding of the dye to the DNA. This hypothesis is also in accordance with the results of other authors. Prosperi *et al.* (1994) have observed that the ability to stain DNA with base-specific fluorochromes depends on the DNA topology *in situ*. In addition, human metaphase chromosomes stained with DAPI or CMA3 after other banding procedures show a different banding pattern from those obtained with fluorochrome staining alone (Bella & Gosálvez 1994). Similar results have also been obtained in some grasshoppers (Bella & Gosálvez 1991).

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References

Babu A, Verma RS (1987) Chromosome structure: euchromatin and heterochromatin. *Int Rev Cytol* **108**: 1–60.

Bella JL, Gosálvez J (1991) C-banding with specific fluorescent DNA-ligands: a new approach to constitutive heterochromatin heterogeneity. *Biotech Histochem* **1**: 44–52.

Bella JL, Gosálvez J (1994) Banding human chromosomes using a combined C-banding–fluorochrome staining technique. *Biotech Histochem* **69**: 243–248.

Burgos M, Jiménez R, Díaz de la Guardia R (1986) A rapid, simple and reliable combined method for G-banding mammalian and human chromosomes. *Stain Technol* **61**: 257–260.

Crozier RH (1975) Hymenoptera. In: John B, ed. *Animal Cytogenetics*, Vol. 3. *Insecta 7*. Berlin: Gebrüger Borntraeger.

Hirai H, Yamamoto MT, Ogura K *et al.* (1994) Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the *Myrmecia pilosula* species complex. *Chromosoma* **103**: 171–178.

Holmquist GP (1989) Evolution of chromosome bands: molecular ecology of non-coding DNA. *J Mol Evol* **28**: 469–486.

Imai HT, Taylor RW, Crozier RH (1994) Experimental bases for the minimum interaction theory. I. Chromosome evolution in ants of the *Myrmecia pilosula* species complex (Hymenoptera: Formicidae: Myrmeciinae). *Jpn J Genet* **69**: 137–182.

Isacchi A, Bernardi G, Bernardi G (1993) Compositional compartmentalization of the nuclear genomes of *Trypanosoma brucei* and *Trypanosoma equiperdum*. *FEBS Lett* **335**: 181–183.

Kakeda K, Fukui K (1994) Dynamic changes in the morphology of barley chromosomes during the mitotic metaphase stage. *Jpn J Genet* **69**: 545–554.

Meredith R (1969) A simple method for preparing meiotic chromosomes from mammalian testis. *Chromosoma* **26**: 254–258.

Odierna G, Baldanza F, Aprea G, Olmo E (1993) Occurrence of G-banding in metaphase chromosomes of *Encarsia berlesei* (Hymenoptera: Aphelinidae). *Genome* **36**: 662–667.

Palomeque T, Chica E, Cano MA, Díaz de la Guardia R (1988) Karyotypes, C-banding, and chromosomal location of active nucleolar organizing regions in *Tapinoma* (Hymenoptera, Formicidae). *Genome* **30**: 277–280.

Palomeque T, Chica E, Cano MA, Díaz de la Guardia R (1990) Development of silver stained structures during spermatogenesis in different genera of Formicidae. *Genetica* **81**: 51–58.

Palomeque T, Chica E, Díaz de la Guardia R (1993) Supernumerary chromosome segments in different genera of Formicidae. *Genetica* **90**: 17–29.

Ponce de Leon FA, Li Y, Weng Z (1992). Early and late replicative chromosomal banding patterns of *Gallus domesticus*. *J Hered* **83**: 36–42.

Prosperi E, Giangarè MC, Bottiroli G (1994) DNA stainability with base-specific fluorochromes: dependence on the DNA topology *in situ*. *Histochemistry* **102**: 123–128.

Schweizer D (1980) Simultaneous fluorescent staining of R bands and specific heterochromatin regions (DA-DAPI bands) in human chromosomes. *Cytogenet Cell Genet* **27**: 190–193.

Sumner AT (1994) Functional aspects of the longitudinal differentiation of chromosomes. *Eur J Histochem* **38**: 91–109.

Warchalowska-Sliwa E, Maryanska-Nadachowska A, Jordan M (1978) C- and G-banding in mitotic chromosomes of *Acheta domesticus* (Orthoptera). *Folia Biol* **26**: 295–306.

Wolf KW, Blackman RL, Sumner AT (1994) Scanning electron microscopy of insect chromosomes. *Schistocerca gregaria* (Acrididae, Orthoptera), *Megaselia scalaris* (Phoridae, Diptera), and *Myzus persicae* (Aphididae, Hemiptera). *J Submicrosc Cytol Pathol* **26**: 79–89.