Presence of highly repetitive DNA sequences in *Tribolium* flour-beetles

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Digestion of genomic DNA from seven species of *Tribolium* (Coleoptera) with *Sau3AI*, *TaqI* and *ClaI* restriction enzymes shows the presence of remarkable amounts of highly repetitive DNA sequences in these species. In *Tribolium freemani* the sequences are tandemly repeated with a satellite monomer of 166 bp, A-T rich (70.5 per cent), representing 31 per cent of the total genome and located in centromeric chromosome areas as demonstrated by *in situ* hybridization. The sequence has the potential to form secondary structures such as stems or cruciforms due to the presence of frequent inverted repeats. *Tribolium castaneum*, *T. anaphe* and *T. madens* show homologous sequences to *T. freemani* satellite DNA but *T. confusum*, *T. audax*, *T. brevicornis* and other tenebrionid beetles, such as *Tenebrio molitor* and *Misolampus goudoti*, do not. A phylogenetic dendrogram, based on the homology and abundance of highly repetitive sequences deduced by dot-blot hybridization, chemotaxonomic and karyological characters, is proposed for the seven studied species of *Tribolium*.

Keywords: Coleoptera, genus *Tribolium*, satellite DNA, *T. freemani*.

Introduction

The genomes of eukaryotic organisms usually contain highly repetitive sequences that may constitute from less than 1 per cent to more than 50 per cent of the total nuclear DNA (Skinner, 1977). These sequences can be interspersed through the genome or, more often, tandemly repeated in large clusters of DNA. These highly repetitive tandem sequences have been called 'satellite DNAs' because, initially, they were recognized through their differential buoyant density compared to that of the 'main band DNA'. The chromosomal location of these sequences is, in most cases, pericentromeric and no clear functions have been demonstrated for them although some hypotheses suggest a possible role related to chromosome pairing (Wu *et al.*, 1988).

The comparison of satellite DNAs among groups of different mammals such as primates (Musich *et al.*, 1980; Fanning *et al.*, 1989), rodents (Dod *et al.*, 1989; Redi *et al.*, 1990) and carnivores (Fanning *et al.*, 1988)

has shown that they can differ considerably in copy number and DNA sequence. However, some groups such as cetaceans and pinnipeds (Arnason & Widegren, 1986, 1989; Arnason *et al.*, 1984) share satellite DNAs with a high degree of sequence conservation among species of different genera, and this is accompanied by very slow karyotypic evolution. In these latter taxa the study of highly repeated DNA sequences has become a valuable taxonomic character even at the suborder level (Arnason & Widegren, 1986). At the intraspecific level, concerted evolution (Dover, 1982), operating through mechanisms of amplification and homogenization of the DNA sequences, has been suggested to explain the low degree of variation of the satellite DNA families observed.

The Coleoptera constitute the largest animal group with more than 350,000 described species (Lawrence, 1982). The family Tenebrionidae is very conservative in terms of karyological evolution; nearly 60 per cent of the 200 species studied have $2n=20$ chromosomes (Juan & Petitpierre, 1990) but some species are known to contain large blocks of heteropycnotic chromatin in meiocytes (Smith, 1952) and variable amounts of

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C-banding positive chromosomal material (Juan & Petitpierre, 1989). *Tenebrio molitor* is the only beetle where the satellite DNA has been characterized (Petitpierre *et al.* 1988; Davis & Wyatt, 1989; Ugarkovic *et al.*, 1989). In this species a large portion of the genome (50–60 per cent) comprises a highly homogenous and chromosomally equilocated satellite DNA (Davis & Wyatt, 1989; Juan *et al.*, 1990, 1991) with a monomeric repeat unit of 142 bp.

The genus *Tribolium*, belonging to the same family, the Tenebrionidae, has been extensively used in genetic analysis (Sokoloff, 1972) and contains several serious pest species of stored products but little is known about it at the DNA level (Brown *et al.*, 1990). Furthermore, the phylogenetic relationships among different *Tribolium* species, based on morphological, cytogenetic and allozymic data, are not in agreement with each other (Wool, 1982), and their genome sizes show no correspondence with the currently accepted taxonomic groups (Alvarez-Fuster *et al.*, 1991).

In this paper we deal with the characterization, cloning and sequencing of the monomeric satellite DNA of *Tribolium freemani*, and a search for homologous sequences in other tenebrionid species related to *Tribolium*, like *Tenebrio molitor* and *Misolampus goudoti*, which can be taken as outgroup species. In this paper we examine the evolution of satellite DNAs and attempt to clarify the phylogeny of the group.

Materials and methods

Source of specimens

The sources of the studied specimens were as follows. *Tribolium audax* Halstead, *T. brevicornis* Lec., *T. confusum* Duval, *T. castaneum* Herbst, *T. freemani* Hinton and *T. madens* (Charp.) were obtained from the *Tribolium* Stock Center at California State University, San Bernardino (CA, U.S.A.). *Tribolium anaphe* Hinton was obtained from ADAS Central Science Laboratory (Slough, U.K.). *Tenebrio molitor* L. was obtained from a commercial dealer and *Misolampus goudoti* Guér. was collected from the wild in Mallorca (Spain).

Isolation of genomic DNA

Total genomic DNA was isolated from 250 mg of pooled larvae or adults homogenized in 0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA, 0.5 per cent SDS, incubated at 65°C for 30 min, then placed on ice for 1 h after adding potassium acetate to a final concentration of 1 M. The homogenate was then centrifuged at 5000 g for 10 min. The resultant supernatant was extracted

with 1 vol. of phenol saturated with 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (TE), 1 vol. of phenol-chloroform-isoamyl alcohol (50:48:2) and 1 vol. of chloroform-isoamyl alcohol (24:1) and precipitated with 2 vol. of cold ethanol. The precipitated nucleic acids were finally washed with 70 per cent ethanol, dried and dissolved in TE at a concentration of 0.6 mg ml⁻¹.

Restriction analysis

Restriction enzymes were provided by Boehringer-Mannheim and Gibco BRL and the digestion conditions were as recommended by the manufacturers. Between 3 and 10 µg of DNA were digested with the restriction enzyme and RNAase, and run on 1.5–2 per cent agarose gels under standard conditions (Maniatis *et al.*, 1982).

Cloning and sequencing of satellite monomers

Whole DNA of *Tribolium freemani* was completely digested with *Sau3AI* and the band corresponding to the monomeric repeat unit was eluted from the agarose gel slice and purified according to the method described by Koenen (1988). This monomer was cloned into the *Bam*HI site of the bacteriophage M13 and subsequently sequenced by the chain-terminating dideoxy method (Sanger *et al.*, 1977).

Labelling of probes

DNA genomic bands from different *Tribolium* species corresponding to repetitive DNA were eluted from the agarose as above and radiolabelled with ³²P-dCTP by random priming (Feinberg & Vogelstein, 1983). The same method was used to label RF-DNA of recombinant M13 containing *T. freemani* monomeric satellite DNA.

Dot-blot and Southern hybridizations

Serial dilutions of the same amount of total DNA of each *Tribolium* species, *Tenebrio molitor*, *Misolampus goudoti*, plus lambda DNA as a negative control, were first blotted onto Hybond-N membranes. The blotted DNAs were then denatured with 0.5 N NaOH and cross-linked to the membrane by exposure to ultraviolet light. Secondly, DNA digests from 1.5 per cent agarose gels were transferred to nylon membranes according to the capillary method of Southern (1975) and the DNA fixed to the membrane as above. Southern transfers and dot-blot membranes were pre-hybridized for 2 h with 2 × SSC, 5 × Denhardt's solution, 0.5 per cent SDS and 100 µg ml⁻¹ denatured

herring sperm DNA at hybridization temperature. The radiolabelled probe was added and hybridization performed at 55°C for 16 h. Following hybridization the filters were extensively washed with 2 × SSC, 0.1 per cent SDS and 0.1 × SSC, 0.1 per cent SDS at 37°C. The filters were exposed to X-ray film with intensifying screens at -80°C for 1-24 h. In a hybridization reaction the melting temperature (T_m) of the hybrid can be calculated by an empirical formula as described by Beltz *et al.* (1983). From the G + C content of the *T. freemani* satellite of 29.5 per cent and in the hybridization conditions used we can estimate that a sequence similarity between probe and target, equal to or greater than 76 per cent, would allow the maintenance of hybrids.

In situ hybridization

Satellite DNA monomers and dimers of *Tribolium freemani* were non-radioactively labelled with biotin-16-UTP (Manuelidis *et al.*, 1982) by nick translation and hybridized with chromosome spreads from the same species obtained by a technique described elsewhere (Juan *et al.*, 1990). Signal detection was performed by treatment with a streptavidin-peroxidase complex and staining with diaminobenzidine (DAB) (Ambros *et al.*, 1986). We used preparations treated as mentioned but without a probe for *in situ* hybridization as a negative control.

Results

Presence of highly repetitive sequences in *Tribolium*

The total DNA of the seven *Tribolium* species used in this study was completely digested with *EcoRI*, *HaeIII*,

HinfI, *Sau3AI*, *PstI* and *ClaI* restriction enzymes. Three of these endonucleases induced conspicuous bands after gel electrophoresis, ethidium bromide staining and ultraviolet transillumination. *Sau3AI* gave a band of about 170 bp and multiples thereof in *Tribolium freemani*, *T. anaphe* and *T. brevicornis* DNAs, suggesting a tandem arrangement of highly repetitive DNA in these species (Fig. 1a). *ClaI* also induced bands of similar sizes in *T. freemani*, *T. anaphe* and in *T. madens*, but in this last case with a less clear ladder pattern (Fig. 1b). *TaqI* produced clear-cut bands in all the studied species; the bands of *T. anaphe* were identical in pattern but different in relative intensity to those obtained with both *Sau3AI* and *ClaI*. In *T. freemani*, however, there were some smaller bands and several extra bands (Fig. 1c). This points to the existence of more than one target site for *TaqI* in the *T. freemani* monomeric band.

Cloning and sequencing of *T. freemani* monomeric satellite DNA

We chose the 170 bp monomeric band of *T. freemani* produced by *Sau3AI* for cloning and subsequent sequence analysis. Five recombinants were randomly used to sequence in both directions. The sequence is 166 bp long, with target sites for *Sau3AI*, *ClaI* and *TaqI* as expected from the restriction analysis. A consensus sequence was derived from five sequenced monomers and each individual sequence compared with the consensus to determine the number and type of substitutions that had occurred (Fig. 2). Twenty-five independent substitutions were observed and their characteristics are given in Tables 1 and 2. The substitution rate in the five variants is 3 per cent, which

Fig. 1 Agarose gel electrophoresis of genomic DNA from different *Tribolium* species. Digestion with *Sau3AI* (a), *ClaI* (b) and *TaqI* (c). *Tribolium anaphe* (1); *T. audax* (2); *T. brevicornis* (3); *T. castaneum* (4); *T. confusum* (5); *T. freemani* (6) and *T. madens* (7). Monomers, dimers and trimers of 166, 322 and 498 bp respectively are indicated by arrowheads.

Fig. 2 Nucleotide consensus sequence of *Tribolium freemani* satellite DNA monomeric unit. The beginning and the end of the sequence are delimited by the restriction site *Sau3AI*. Four regions of dyad symmetry are indicated by arrows and clusters of three or more runs of A or T nucleotides by dots.

corresponds to an average of five changes per monomer, insertion/deletion events account for 3.6 shifts per monomer (2 per cent). The sequence is AT-rich (70.5 per cent) and a computer search found a remarkable richness in inverted repeats. A total of four were identified with loops of 20 bp or less, with an homology of 100 per cent between stems (see Fig. 2) and many with lower homologies and a capacity to form cruciforms. Moreover, the satellite repeat shows 12 runs of $d(A-T)n \geq 3$ residues (Fig. 2). No significant similarity was found after a maximum alignment with the previously described satellite DNA of *Tenebrio molitor* (Petitpierre *et al.*, 1988) or with other sequences from insects in the data bank.

DNA hybridization analysis

The total digested DNA of *Tribolium* species did not show hybridization with a satellite DNA probe from *Tenebrio molitor*, as expected from the lack of sequence homology mentioned above. *Sau3AI* digests of *Tribolium freemani*, transferred to a membrane and hybridized with the cloned 170 bp *Sau3AI* labelled band (pTfr probe), gave almost no signal outside the ladder of repetitive bands (Fig. 3a). A total digestion of *T. freemani* DNA with *TaqI* and similarly probed gave an extensive ladder with multiples of 170 bp (Fig. 3b).

On a filter containing DNA digested with *TaqI* of all *Tribolium* species, only a band of 370 bp of *T. castaneum* showed cross-hybridization with the pTfr probe (Fig. 3b). Another probe from *T. anaphe*, composed of monomeric and dimeric *ClaI* bands (pTan), was used in Southern hybridizations (Fig. 3c), this probe hybridizes with *T. anaphe* satellite DNA up to the tetramer and all the repeat ladder of *T. freemani* bands.

Dot-blot hybridization experiments were performed to deduce the gross homology and abundance of the highly repetitive sequences among the nine surveyed species of tenebrionids. Three more probes were used for this purpose: two *TaqI*-bands of 430 and 450 bp from *T. brevicornis* (pTbr), a *ClaI*-band of 170 bp from *T. madens* (pTma) and a *TaqI*-band of 370 bp from *T. castaneum* (pTca). All five *Tribolium* DNA probes were used in dot-blot hybridizations with total

Table 1 Base substitutions in *Tribolium freemani* satellite sequence

New base	Original base			
	G	A	T	C
G	—	3	2	1
A	3	—	2	2
T	2	3	—	2
C	4	—	1	—

Table 2 Base changes in *Tribolium freemani* satellite sequence

Change	Number	Ratio (a)/(b)
(a) G, C — X	14	1.27
(b) A, T — X	11	
(a) Transversions	16	1.77
(b) Transitions	9	

G, C = G or C; A, T = A or T; X = any base.

denatured DNA from the seven *Tribolium* species plus *Tenebrio molitor* and *Misolampus goudoti*. The results are shown in Fig. 4. Only pTfr probe hybridized with all *Tribolium* DNAs, while none of the probes shared sufficient homology under the conditions used with the DNA from the two species of different genera. As expected from Southern analysis, the hybridization signals were slight in most cases except for the pTan probe with *T. freemani* DNA.

In situ hybridization

To detect the chromosomal localization of *T. freemani* satellite DNA we hybridized *in situ* the pTfr probe with chromosome spreads of the same species. Although the chromosomes of *T. freemani* are very small (1–3 μ m), the signal is seen to be approximately coincident with the C-bands (Fig. 4 a–c). The C-bands are pericentromeric in this species, and so too is the satellite DNA.

Fig. 3 (a) *Tribolium freemani* genomic DNA digested with *Sau3AI* and hybridized with pTfr probe. (b) *Tribolium* genomic DNAs digested with *TaqI* and hybridized with pTfr probe and (c) with pTan probe. (See text for details). AN: *T. anaphe*; CA: *T. castaneum*; F: *T. freemani*.

Fig. 4 Dot-blot hybridization results. Genomic DNAs (3 µg) from *Tribolium freemani* (1); *T. audax* (2); *T. brevicornis* (3); *T. madens* (4); *T. confusum* (5); *T. castaneum* (6); *T. anaphe* (7); *Tenebrio molitor* (8) and *Misolampus goudoti* (9) were denatured and hybridized on a nylon filter with pTan (a); pTbr (b); pTca (c); pTfr (d) and pTma (e) labelled probes.

Discussion

Satellite DNAs of *Tribolium*

Species of the genus *Tribolium* have remarkable amounts of satellite DNAs detected by digestion with *Sau3AI*, *ClaI* or *TaqI* restriction enzymes. In *T. freemani* the contribution of this satellite DNA to the total genome is estimated at 31 per cent from densitometric scanning of photographs of agarose gels (C. Juan & E. Petitpierre unpublished data). The great abundance of these sequences is in agreement with the cytological observations of male meiocytes described by Smith (1952). The prophase I bivalents in *T. castaneum* and *T. confusum* show large blocks of heteropycnotic pericentromeric heterochromatin. Similar blocks are present in *T. freemani* chromosomes and are the sites where the satellite DNA is actually located, as can be inferred from the *in situ* hybridization with a *T. freemani* satellite probe. *Tenebrio molitor*, a member of the same subfamily within Tenebrionidae, has half of the genome composed of one 142 bp unique sequence tandemly repeated and localized in the pericentromeric regions of all chromosomes (Petitpierre *et al.*, 1988; Davis & Wyatt, 1989; Juan *et al.*, 1990). A comparison between *Tenebrio molitor* and *Tribolium freemani* satellites failed to reveal any homology between them, suggesting a high degree of divergence in the repeated sequences of the two species. *Misolampus goudoti*, another somewhat related species with large C-bands constituting about 58 per cent of the total chromosome length (Juan & Petitpierre, 1989), does not show hybridization with a *T. freemani* satellite DNA probe either.

The sequence analysis of the *T. freemani* satellite monomer shows an apparently higher substitution rate and a number of insertions/deletions than the recorded in 22 different cloned monomers of *Tenebrio molitor* satellite sequence, which displayed only 2.5 changes per monomer (1.8 per cent), without insertions and deletions (Ugarkovic *et al.*, 1989). In *T. freemani*, we have found an average of five changes per monomer corresponding to a substitution rate of 3 per cent. Nevertheless, we cannot exclude the possibility that some monomeric variants were cloned with a higher probability than others and/or random effects caused by the relatively low number of variants sequenced.

The changes involving A or T nucleotides are less than expected if we consider the high A + T richness of the sequence (about 70 per cent); on the other hand the transversion/transition ratio is 1.8, similar to that found in the satellite of the primate *Callimico goeldii* (Cgo satellite) with equivalent A + T content and rate of sequence divergence (Fanning *et al.*, 1989).

The richness in AT pairs as in the *T. molitor* satellite sequence is in agreement with the DAPI positive nature of the pericentromeric regions in both species (data not shown). The frequent short direct repeats, such as AAAAT or AAAAAATTAA, could presumably have originated by saltatory replication with accumulation of mutations of these or other related AT-rich motifs, as proposed by Southern (1975) for the satellite DNA of mouse.

It has been hypothesized that secondary structures in the satellite DNA sequences, such as cruciforms, can be related to a tendency of the length of the repeat unit to fit the DNA organized in the core particle of the nucleosome (Israelewski, 1983; Fowler & Skinner, 1985). *Tenebrio molitor* satellite DNA has few possibilities to form cruciforms, although its length is very close (142 bp) to the DNA in the core particle (Ugarkovic *et al.*, 1989). Recently Plohl *et al.* (1990) proposed a sequence-induced DNA curvature in *T. molitor* satellite resulting in a left-handed superhelix. This would be related to DNA condensation and constitutive heterochromatin folding in this species. We do not have direct evidence of curvature in the *Tribolium freemani* satellite DNA but the sequence is rich in (A-T) $n \geq 3$ residues. Martínez-Balbás *et al.* (1989) have shown that in mouse, rat and alpha-monkey satellites, the presence of such (A-T) $n \geq 3$ residues is probably responsible for the observed DNA curvature. Moreover, there is the possibility of forming four perfect loops in the *T. freemani* satellite 166 bp sequence, which is rather longer than the nucleosomal core particle sequence; the mentioned secondary structures could be localized reducing the length to about 140 bp.

Southern blot analysis using a *T. freemani* satellite probe shows that all these repetitive sequences are arranged in tandem clusters in the *T. freemani* genome because no hybridization is present outside the ladder of repetitive bands. Moreover, frequent mutations in the *TaqI* target sites give rise to multiple bands up to the 15-mer (~2500 bp), demonstrating a high degree of tandem repetition in the satellite DNA of this species.

The hybridization between sequences of repetitive DNA of different *Tribolium* species was very dependent on the stringency conditions used and on the homology and relative abundance of such sequences in the genome. This is probably the reason why no reciprocal hybridization was observed between probes and specific DNA bands in some cases, such as in *T. freemani* and *T. anaphe* satellites. In fact, the relatively low G+C content of these satellites makes it necessary to use very relaxed hybridization conditions allowing the maintenance of the hybrid between the probe and the target DNA.

The different patterns of bands obtained with the various restriction enzymes demonstrates differences in the repeat sequences between species. Full sequence analysis of clones of these various repeats will allow an accurate comparison of these changes.

Phylogenetic relationships of Tribolium based on highly repetitive sequences

Hinton (1948) classified the extant species of *Tribolium* into five different groups based on morphological characters. Considering our seven species of *Tribolium*, they can be grouped under Hinton's criterion as follows: *confusum* species-group with *T. confusum* and *T. anaphe*, *castaneum* species group with *T. castaneum*, *T. madens*, *T. freemani* and *T. audax*, and the *brevicornis* group with *T. brevicornis*. The latter is considered the most primitive and well differentiated from the others. Based on cytogenetic data, Smith (1952) claimed that *T. confusum* with $2n=18$ chromosomes was derived from an ancestor similar to *T. castaneum* ($2n=20$) by a translocation of one autosome to the X-chromosome; later Samollow *et al.* (1983) provided genetic support for this hypothesis. In another study, Howard (1987) analysed the chemical components of the defensive secretions as characters for a cladistic analysis of *Tribolium*. Under these criteria he suggests that *brevicornis* and *castaneum* are sister species-groups without synapomorphies in common with the

Fig. 5 C-banded spermatogonial metaphase (a) and metaphase I (b) of *Tribolium freemani*. *In situ* hybridization of *Tribolium freemani* metaphase I chromosomes with a satellite DNA probe from the same species (c). $\times 2500$.

Fig. 6 Phylogeny of seven species of the genus *Tribolium* based on DNA hybridization data (■) gain, (□) loss. Chemotaxonomic characters given by Howard (1987) (●) gain, (○) loss and diploid chromosome numbers (Shimeld, 1989). *Tenebrio molitor* is an outgroup species.

confusum species-group and that *T. brevicornis* and *T. audax* are members of the same species-group.

The data herein, derived from the dot-blot hybridization patterns of DNA, are a simplified measure of the cross-hybridization among the different satellites. A phylogenetic analysis using PAUP (Phylogenetic Analysis Using Parsimony), based only on the hybridization data, showed a low level of evolutionary information, but if we consider the chemical compounds of defensive secretions (Howard, 1987), karyological data (Shimeld, 1989), plus the DNA hybridization data as three independent character sets, a cladogram can be produced (Fig. 6). The *castaneum* species-group is well supported by the acquisition of two synapomorphies in *T. castaneum*, *T. freemani* and *T. madens*. On the other hand, the *confusum* species-group is defined by the gain of two chemical characters and the loss of two DNA characters. The *brevicornis* species-group, in contrast to Howard's proposed phylogeny, would be a sister of the *confusum* group by the shared loss of two DNA characters and the acquisition of a karyotype of $2n = 18$ derived from the ancestral $2n = 20$ (assuming reversion from $2n = 18$ to $2n = 20$ as highly unlikely). *T. audax* would be paraphyletic with respect to the other species-groups.

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References

- ALVAREZ-FUSTER, A., JUAN, C. AND PETITPIERRE, E. 1991. Genome size in *Tribolium* flour-beetles: inter- and intraspecific variation. *Genet. Res.*, **58**, 1-5.
- AMBROS, P. F., MATZKE, M. A. AND MATZKE, A. J. M. 1986. Detection of a 17 kb unique sequence (T-DNA) in plant chromosomes. *Chromosoma*, **94**, 11-18.
- ARNASON, U., HÖGLUND, M. AND WIDEGREN, B. 1984. Conservation of a highly repetitive DNA in cetaceans. *Chromosoma*, **89**, 238-242.
- ARNASON, U. AND WIDEGREN, B. 1986. Pinniped phylogeny enlightened by molecular hybridizations using highly repetitive DNA. *Mol. Biol. Evol.*, **3**, 356-365.
- ARNASON, U. AND WIDEGREN, B. 1989. Composition and chromosomal localization of cetacean highly repetitive DNA with special reference to the blue whale, *Balenoptera musculus*. *Chromosoma*, **98**, 323-329.
- BELTZ, G. A., JACOBS, K. A., EICKBUSH, T. H., CHERBAS, P. T. AND KAFATOS, F. C. 1983. Isolation of multigene families and determination of homologies by filter hybridization methods. *Meth. Enzymol.*, **100**, 266-285.

- BROWN, S. J., HENRY, J. K., BLACK IV, W. C. AND DENELL, R. E. 1990. Molecular genetic manipulation of the red flour beetle: genome organization and cloning of a ribosomal protein gene. *Insect Biochem.*, **20**, 185–193.
- DAVIS, C. A. AND WYATT, G. R. 1989. Distribution and sequence homogeneity of an abundant satellite in the beetle, *Tenebrio molitor*. *Nucl. Acids Res.*, **17**, 5579–5586.
- DOD, B., MOTTEZ, E., DESMARAIS, E., BONHOMME, F. AND ROIZES, G. 1989. Concerted evolution of light satellite DNA in genus *Mus* implies amplification and homogenization of large blocks of repeats. *Mol. Biol. Evol.*, **6**, 478–491.
- DOVER, G. A. 1982. Molecular drive: a cohesive mode of species evolution. *Nature*, **285**, 111–117.
- FANNING, T. G., MODI, W. S., WAYNE, R. K. AND O'BRIEN, S. J. 1988. Evolution of heterochromatin-associated satellite DNA loci in felids and canids (Carnivora). *Cytogenet. Cell Genet.*, **48**, 214–219.
- FANNING, T. G., SEUANEZ, H. N. AND FORMAN, L. 1989. Satellite DNA sequences in the neotropical marmoset *Callimico goeldii* (Primates, Platyrrhini). *Chromosoma*, **98**, 396–401.
- FEINBERG, A. P. AND VOGELSTEIN, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, **132**, 6–13.
- FOWLER, R. F. AND SKINNER, D. M. 1985. Cryptic satellites rich in inverted repeats comprise 30% of the genome of a hermit crab. *J. Biol. Chem.*, **260**, 1296–1303.
- HINTON, H. E. 1948. A synopsis of the genus *Tribolium* MacLey with some remarks on the evolution of its species groups. *Bull. Entomol. Res.*, **39**, 13–55.
- HOWARD, R. W. 1987. Chemosystematic studies of the Triboliini (Coleoptera: Tenebrionidae): phylogenetic inferences from the defensive chemicals of eight *Tribolium* spp., *Palorus ratzeburgi* (Wissmann), and *Latheticus oryzae* Waterhouse. *Ann. Entomol. Soc. Am.*, **80**, 398–405.
- ISRAELEWSKI, N. 1983. Structure and function of an AT-rich, interspersed repetitive sequence from *Chironomus thummi*: solenoidal DNA, 142 bp palindrome-frame and homologies with the sequence for site specific recombination of bacterial transposons. *Nucl. Acid Res.*, **11**, 6985–6996.
- JUAN, C., GOSALVEZ, J. AND PETITPIERRE, E. 1990. Improving beetle karyotype analysis: restriction endonuclease banding of *Tenebrio molitor* fixed chromosomes. *Heredity*, **65**, 157–162.
- JUAN, C., GOSALVEZ, J., MEZZANOTTE, R. AND PETITPIERRE, E. 1991. Cytological and biochemical characterization of the in situ endonuclease digestion of fixed *Tenebrio molitor* chromosomes. *Chromosoma*, **100**, 432–438.
- JUAN, C. AND PETITPIERRE, E. 1989. C-banding and DNA content in seven species of Tenebrionidae (Coleoptera). *Genome*, **32**, 834–839.
- JUAN, C. AND PETITPIERRE, E. 1990. Karyological differences among Tenebrionidae (Coleoptera). *Genetica*, **80**, 101–108.
- KOENEN, M. 1989. Recovery of DNA fragments from agarose gels using liquid nitrogen. *Trends Genet.*, **5**, 137.
- LAWRENCE, J. F. 1982. Coleoptera. In: Parker J. A. (ed.) *Synopsis and Classification of Living Organisms*. McGraw-Hill, New York.
- MANIATIS, T., FRITSCH, E. F. AND SAMBROOK, J. 1982. *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory, New York.
- MANUELIDIS, L., LANGER-SAFER, P. R. AND WARD, D. C. 1982. High resolution mapping of satellite DNA using biotin-labeled DNA probes. *J. Cell. Biol.*, **95**, 619–625.
- MARTINEZ-BALBAS, A., RODRIGUEZ-CAMPOS, A., GARCIA-RAMIREZ, M., SAINZ, J., CARRERA, J., AYMAMI, J. AND AZORIN, F. 1989. Satellite DNAs contain sequences that induce curvature. *Biochemistry*, **29**, 2342–2348.
- MUSICH, P. R., BROWN, F. L. AND MAJO, J. J. 1980. Highly repetitive component alpha and related alphoid DNAs in man and monkeys. *Chromosoma*, **80**, 331–348.
- PETITPIERRE, E., GATEWOOD, J., AND SCHMID, C. W. 1988. Satellite DNA from the mealworm beetle *Tenebrio molitor*. *Experientia*, **44**, 498–499.
- PLOHL, M., BORSTNIK, B., UGARKOVIC, D. AND GAMULIN, V. 1990. Sequence-induced curvature of *Tenebrio molitor* satellite DNA. *Biochemie*, **72**, 665–670.
- REDI, C. A., GARAGNA, S., DELLA VALLE, G., BOTTIROLI, G., DELL'ORTO, P., VIALE, G., PEVARELI, F. A., RAIMONDI, E. AND FOREJT, J. 1990. Differences in the organization and chromosomal allocation of satellite DNA between the European long tailed house mice *Mus domesticus* and *Mus musculus*. *Chromosoma*, **99**, 11–17.
- SAMALLOW, P. B., DAWSON, P. S. AND RUSSEL, A. R. 1983. X-linked and autosomal inheritance patterns of homologous genes in two species of *Tribolium*. *Biochem. Genet.*, **21**, 167–176.
- SANGER, F., NICKLEN, S. AND COULSON, A. R. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci., U.S.A.*, **74**, 5463–5467.
- SHIMELD, L. 1989. A cytogenetic examination of eight species of *Tribolium*. *Tribolium Inf. Bull.*, **29**, 102–107.
- SKINNER, D. M. 1977. Satellite DNAs. *Bioscience*, **27**, 790–796.
- SMITH, S. G. 1952. The evolution of heterochromatin in the genus *Tribolium* (Tenebrionidae: Coleoptera). *Chromosoma*, **4**, 585–610.
- SOKOLOFF, A. 1972. *The Biology of Tribolium, With Special Emphasis on Genetic Aspects*, Vol I. Oxford University Press, Oxford.
- SOUTHERN, E. M. 1975. Long periodicities in mouse satellite DNA. *J. Mol. Biol.*, **94**, 51–70.
- UGARKOVIC, D., PLOHL, M. AND GAMULIN, V. 1989. Sequence variability of satellite DNA from the mealworm *Tenebrio molitor*. *Gene*, **83**, 181–183.
- WOOL, D. 1982. Critical examination of postulated cladistic relationships among species of flour beetles (*Tribolium*, Tenebrionidae, Coleoptera). *Biochem. Genet.*, **20**, 333–349.
- WU, C. I., LYTTLE, T. W., WU, M. L. AND LIN, G. F. 1988. Association between a satellite DNA sequence and the responder of segregation distorter in *D. melanogaster*. *Cell*, **54**, 179–189.