Satellite DNA from the beetle *Tenebrio molitor*

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Summary. A 142 base-pair satellite DNA from the mealworm beetle, *Tenebrio molitor,* has been cloned and sequenced. The satellite DNA is revealed by making a restriction digest of genomic DNA with either EcoRI or HinfI, and constitutes approximately 49% of the genomic DNA. The presence of huge amounts of satellite DNA correlates well with the prominent blocks of heterochromatin found in tenebrionid beetles. A similar restriction digest of *Xanthogaleruca luteola* genomic DNA does not release a prominent satellite component.
Key words. Beetle; satellite; DNA.

Highly repetitive DNA varies greatly among closely related species. Within the genus *Drosophila, D. nasutoides* has about 60%, *D. virilis* 41%, and *D. melanogaster* 25%, while no satellite DNA has been detected in *D. ezoana*[1, 2]. Several functions have been tentatively attributed to the satellite DNA in cell metabolism or chromosome organization and pairing, or in speciation, though none of them have been clearly validated after a close and detailed scrutiny[1]. Although the exact function is unknown, satellite DNA is valuable in interspecies phylogenetic comparisons. Since no beetle DNA sequences have been reported, our study is the first step towards an evolutionary survey of satellite DNA in beetles.

Material and methods. Larvae of the mealworm beetle *Tenebrio molitor* were obtained from a dealer. We also collected a sample of the elm leaf-beetle, *Xanthogaleruca luteola,* in the wild (Sacramento, CA). DNA was extracted from the beetle samples and purified using methods given by Blin and Stafford[3] and Maniatis et al.[4] with minor modifications.

The genomic DNA was digested with various restriction enzymes and size-separated on agarose gels. Negatives of the DNA fluorescence photographs were scanned at 500 nm using a Cary spectrophotometer in order to obtain an approximate estimate of the satellite percentage in the beetle genome. The DNA was either extracted from the gel for cloning purposes or transferred to nitrocellulose[5]. The conspicuous satellite bands resulting from the EcoRI digest (fig. 1, lane 3) were inserted into pUC 18 plasmid. Selected clones were then labeled with ^{32}P by nick translation[4] and used as hybridization probes of the DNA bound to nitrocellulose. The nitrocellulose filters were prehybridized overnight at 60 °C in 3X saline sodium citrate (SSC), 5X Denhardt's solution, and 0.5 sodium dodecyl sulphate (SDS) containing 200 µg/ml denatured salmon sperm DNA[4]. Hybridization was conducted for 4 h under the same conditions. The filter was washed in 3X SSC, 1X SSC, and finally in 0.5X SSC. The extent of hybridization was recorded by autoradiography.

The satellite sequence was released from a pUC clone by restriction with EcoRI and transferred to the phage vector M13mp19. Both orientations of the insert were sequenced using the Sanger dideoxy method[6].

Results and discussion. As seen in figure 1, the satellite sequence is revealed by restriction of *Tenebrio molitor* DNA with EcoRI (lane 3) and HinfI (lane 5). The monomeric unit is 142 base-pairs (bp) long with dimers and trimers at approximately 284 bp and 426 bp. Depending on the position of the baseline the satellite constitutes 44 to 56% of the DNA with the monomer constitution approximately 38% of the genome and the dimer and trimer contributing 9% and 2%,

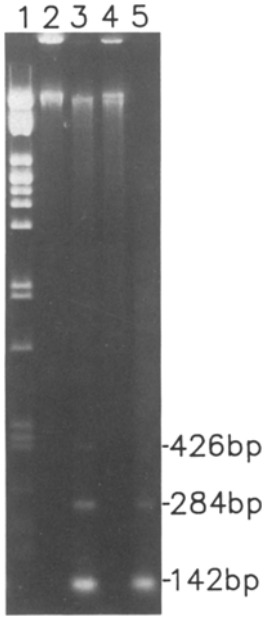

Figure 1. Restriction digest of *Tenebrio molitor* DNA. The genomic DNA was restricted with BamHI (lane 2), EcoRI (lane 3), HindIII (lane 4), and HinfI (lane 5). The DNA was size separated on a 1.4% agarose gel. Lane 1 contains lambda phage DNA restricted with PstI as a marker.

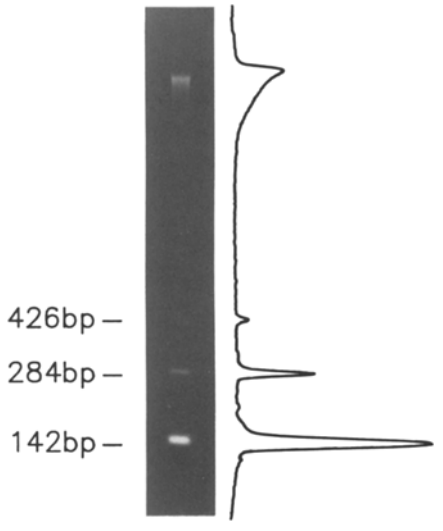

Figure 2. Quantitation of satellite DNA in *Tenebrio molitor*. The satellite DNA was quantitated by scanning a photographic negative of the fluorescence pattern of an EcoRI restriction. The 142 bp monomer constitutes approximately 38% of the genome with the dimer and trimer composing 9% and 2%, respectively.

respectively (fig. 2). The amount of satellite is higher than the average of 5–10% found in most eukaryotes. This finding agrees with a recent analysis on the relative amount of euchromatin and heterochromatin in the mealworm chromosomes [7], and with the chromosomal results obtained on the tenebrionid beetles by using either conventional or C-banding techniques which have evidenced very conspicuous pericentromeric blocks of heterochromatin [8–11].

Restriction of *Tenebrio molitor* DNA with HindIII produced an irregular pattern of faint bands (fig. 1, lane 4). When Southern blots of the restricted DNA are hybridized with the 142 bp satellite (fig. 3), the faint bands in the HindIII restriction (lane 3) show no evidence of hybridization suggesting they result from an unrelated family of repetitive sequences. The autoradiograph also reveals multimers of the satellite in the BamHI restriction that were not apparent in the fluorescence photograph. The 1.7 kbp band corresponds to a 12-repeat unit. A single nucleotide change of T to G at position 81 would generate in the 142 bp monomer sequences a BamHI cleavage site (fig. 4). Sequence polymorphism at this site predicts the ladder of satellite bands appearing in the BamHI restriction digest.

Although the 142 bp sequence contains a number of A and T rich runs, we are unable to resolve it into a tandem array

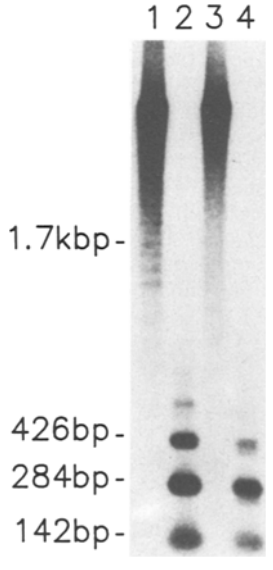

Figure 3. Autoradiograph of *Tenebrio molitor* DNA hybridized with the labeled 142 bp satellite DNA. The genomic DNA was restricted with BamHI (lane 1), EcoRI (lane 2), HindIII (lane3), HinfI (lane 4) and size separated on a 1.3% agarose gel. Following Southern transfer, filter bound DNA was hybridized with ^{32}P labeled satellite DNA. In addition to the monomer, dimer, and trimer bands which appear in the EcoRI and HinfI restrictions, higher multimer bands appear in the BamHI restriction.

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  e      10         20         30         40         50
GAATTCTGTA GTTCTTGCGT CGTTTTACTT CGAAATGTTC AAGTTCCACG
CTTAAGACAT CAAGAACGCA GCAAAATGAA GCTTTACAAG TTCAAGGTGC

        60   h     70         80    s    90         100
ACGAAACTCC GATTCGCACT TAGTTTTTCG TGATCCTACA CAGTTGCGAG
TGCTTTGAGG CTAAGCGTGA ATCAAAAAGC ACTAGGATGT GTCAACGCTC

       110        120        130        140    e
CGAAAAAACG TATTTAGAGG AAAGTTAGCG TCTTGGAACC TGGAATTC
GCTTTTTTGC ATAAATCTCC TTTCAATCGC AGAACCTTGG ACCTTAAG
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Figure 4. Sequence of the 142 bp satellite DNA. The 142 bp fragment was inserted into the phage vector M13mp19, and both orientations were sequenced using Sanger dideoxy method. The restriction sites are: b-BamHI, e-EcoRI, h-HinfI, and s-Sau3A.

of smaller repeat lengths. We therefore conclude that the 142 bp unit is the monomer subunit which is tandemly arrayed to form this satellite DNA. The monomer sequence is not homologous to other published satellite DNAs.

Restriction with EcoRI and HinfI of DNA from the leaf beetle *Xanthogaleruca luteola* failed to reveal a satellite component (data not shown). The two species of beetles, *Tenebrio molitor* and *Xanthogaleruca luteola,* belong to two rather distantly related families, Tenebrionidae and Chrysomelidae, respectively [12, 13]. Further hybridization studies on a wide representation of beetles will be undertaken using the cloned satellite from *Tenebrio molitor* as a hybridization probe. This approach should provide new insights on the evolutionary interrelationships and taxonomy within Coleoptera.

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