Cloning and characterization of KM190, a specific satellite DNA family of Drosophila kitumensis and D. microlabis

(Recombinant DNA; repetitive DNA; tandem repeats; nucleotide sequence; sequence analysis)

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SUMMARY

The nucleotide sequences of nine clones, pKA191/1–4 from Drosophila kitumensis and pMR190/1–5 from D. microlabis, were determined. They represent a tandemly arranged and highly repetitive satellite DNA family, KM190, which is specific for the two species.

INTRODUCTION

Two East African (Mount Elgon, Kenya) Drosophila species have recently been discovered (D. kitumensis) or rediscovered (D. microlabis) and described as closely related species of the D. obscura group (Tsacas et al., 1985). On the basis of allozyme data, the two species appear to be more closely related to the Palearctic species triad of D. tristis, D. ambigua and D. obscura than to any other of the well studied species of the D. obscura group (Cariou et al., 1988). This view is also supported by comparative analyses of the banding patterns of the polytene chromosomes (Brehm et al., 1990; Brehm and Krimbas, 1990).

The aim of the present study is to clarify the systematic position of D. microlabis and D. kitumensis in the phylogenetic tree of the D. obscura group using a fast evolving, tandemly repeated satDNA as an evolutionary marker. In other recent studies on satDNA from species of the D. obscura group, it has been shown that satDNA can be either species specific, e.g., the pGH290 satDNA family for D. guanche (Bachmann et al., 1989), or characteristic for a subgroup of related species, e.g., the ATOC180 sequence family for D. ambigua, D. tristis and D. obscura (Bachmann and Sperlich, submitted). In addition, species-specific amplification could be observed, too, e.g., the pTET181 satDNA family is species-specifically amplified in the genome of D. tristis and was found with a much lower copy number in D. ambigua, D. obscura, D. microlabis and D. kitumensis (Bachmann et al., 1990).

EXPERIMENTAL AND DISCUSSION

(a) Cloning of satellite DNA

Total genomic DNA of D. microlabis and D. kitumensis, isolated according to Preis et al. (1988), was digested with Alul, AsnI, BamHI, CfoI, ClaI, DraI, EcoRI, HaeIII, HindIII, HpaII, PstI, PvuII, Rsal, SboI, SspI, TaqI, and XbaI, respectively, and subsequently separated on a 5% polyacrylamide gel. After electrophoresis, distinct bands at an approximate fragment length of 190, 380 and 570 bp were observed for the Alul and AsnI digests of both species and for the Rsal digest of D. microlabis, indicating the
presence of a common, tandemly repeated, restriction satDNA in the genome of both species. The 190-bp AluI monomeres from \textit{D. kitunensis} and the 190-bp \textit{RsaI} monomeres from \textit{D. microlabis}, respectively, were isolated from a preparative gel, ligated into the pUC19 plasmid vector and cloned in \textit{Escherichia coli} JM103. The recombinant bacteria were screened for positive colonies with a \textit{\textsuperscript{32}P}-labelled aliquot of the isolated monomere DNA. Nine clones (pKA191/1–4 from \textit{D. kitunensis} and pMR190/1–5 from \textit{D. microlabis}) were selected for further analyses.

(b) Analyses of nt sequences

Fig. 1 shows the alignment of the nt sequences of these clones to pKA191/1. The observed high degree of sequence homology between the repeat units from the nine clones was taken as an argument to group them into a new satDNA family designated \textit{KM190}. The observed average of sequence variability is rather low (ca 5% within the pKA191 clones, ca 6.7% within the pMR190 clones and ca 7% between pKA191 and pMR190 clones) and mainly due to single nt exchanges. Most of them appear to be clone specific (e.g., the C→T at position 9 of pKA191/4), but there are others shared by at least two sequences (e.g., the G at position 75 of pMR190/2 and 3). However, four characteristic differences were found between consensus sequences from \textit{D. kitunensis} and \textit{D. microlabis}: two single nt exchanges at positions 151 (C→T) and 190 (G→A), a single nt insertion/deletion at position 116 and a C→T transition at position 120. (It should be mentioned here that the C in \textit{D. microlabis} must be invariable as it is part of the recognition sequence of \textit{RsaI} which was used to clone the sequences of this species. Nevertheless, Southern-blot analyses of the genomic DNA (data not shown) showed that the \textit{RsaI} site is species specific for the \textit{KM190} repeats of \textit{D. microlabis} but is absent in those of \textit{D. kitunensis}.) \textit{KM190} sequences are moderately A+T-rich (73%) but exhibit otherwise no special characteristics such as internal repeats, duplicated or inverted sequence motives.

(c) Distribution of \textit{KM190} in the \textit{Drosophila obscura} group species

Purified DNA of pKA191/1 was radioactively labelled (Feinberg and Vogelstein, 1983) and probed to \textit{AluI}- digested and blotted genomic DNA of several \textit{Drosophila} species: \textit{D. melanogaster}, \textit{D. subobscura}, \textit{D. guanche}, \textit{D. ambiguа}, \textit{D. tristis}, \textit{D. obscura}, \textit{D. microlabis} and \textit{D. kitunensis} (data not shown). Since no positive signal was obtained in this experiment, the \textit{KM190} satDNA seems to be specific for \textit{D. microlabis} and \textit{D. kitunensis}. It was not possible to determine the phylogenetic origin of \textit{KM190}, since the likely closest relatives of the latter two species, \textit{D. cariouae} and \textit{D. kribiаsі}, could not be analyzed. \textit{D. kribiаsі} was described as a species only on the basis of a single male, and \textit{D. cariouae} could not be established as a laboratory strain (Cariou et al., 1988).

(d) Conclusions

The \textit{KM190} satDNA family was found to be specific for \textit{D. microlabis} and \textit{D. kitunensis}. It consists of tandemly repeated monomeres of 191 bp (\textit{D. kitunensis}) or 190 bp (\textit{D. microlabis}). The repeats are moderately A+T-rich (73%), and an average sequence variability of about 6% was found on the basis of nine sequenced monomeres. In comparison with other satDNA families described in the \textit{D. obscura} group so far, 6% sequence variability appears to be quite low. A variability of 11.3% was found in the pGH290 family of \textit{D. guanche} (Bachmann et al., 1989) and up to 20% in the ATOC180 family of \textit{D. obscura}, \textit{D. ambiguа} and \textit{D. tristis} (Bachmann and Sperlich, submitted). This indicates that the cladogenesis of \textit{D. kitunensis} and \textit{D. microlabis} must have taken place more recently than the speciation events within the species triad of \textit{D. obscura}, \textit{D. ambiguа} and \textit{D. tristis}. It is assumed that these species
separated approximately 3–5 million years ago. This assumption is supported by the small number of only four species-specific characters in the KM190 satDNA family, i.e., three single nt exchanges and a 1-bp insertion/deletion.

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REFERENCES


