Breaking down the genome organization and karyotype differentiation through the epifluorescence microscope lens: insects and fish as models

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The use of fluorescent *in situ* hybridization (FISH) has allowed the generation of data concerning the genome structure and chromosomal organization and differentiation of diverse eukaryote organisms. This technique guided to a revolution in the cytogenetic and permitted a more clear visualization of specific DNA sequences onto chromosomes, thus generating detailed physical chromosome maps of species. For studies of genome organization and karyotype ongoing in animals, the most applied sequences are the repeated elements. These elements have been obtained from the genome of distinct species mainly through the use of polymerase chain reaction (PCR) and enzymatic restriction, and have been used for chromosome identification, establishment of chromosomal rearrangements, studies of B and sex chromosomes origin and evolution, and genome organization. Here we summarize the recent advances in the application of the FISH technology in studies of fish and insect chromosomes, under the focus of understanding the organization and evolution of their karyotypes and genomes.

Keywords chromosome; evolution; FISH; molecular cytogenetics

1. Introduction

Prior to the advances on molecular techniques to study the eukaryotic genomes, the cytogenetic provided the first information on the genome organization and location of distinct DNA fractions on chromosomes. For several decades these information were obtained using the classical cytogenetic methods that had permitted only the description of general chromosomal characteristics. Besides the use of classical techniques, some studies were conducted through the use of differential staining, such as C-banding, silver nitrate and fluorochrome staining that led to a better characterization of the chromosomes and their content. Despite these advances, the genome structure under the focus of chromosome analysis remained limited.

In recent decades, the application of *in situ* hybridization using DNA probes onto chromosomes caused a cytogenetic revolution, leading to the transition between the "classical cytogenetic era" to the "molecular cytogenetic era". This methodology has permitted the precise location of specific DNA sequences generating more detailed information concerning chromosomal and genomic organization in several animal groups. Using the *in situ* assay it is possible to integrate the molecular information of DNA sequences to their physical location along chromosomes of all eukaryotic representatives [1, 2].

The hybridization principle is based in the denaturation of chromosomal DNA and its renaturation under the presence of complementary DNA labeled probes, which have the capacity to anneal in the regions with base pair complementarity [3, 4]. Briefly, the *in situ* hybridization consists of four steps, (1) denaturation, (2) hybridization, (3) probe detection, and (4) microscopic analysis (Fig. 1). Since the first application of *in situ* hybridization in chromosomes, this technique has been suffered modifications related to all steps, involving probe achievement, sensitivity increasing in the probe detection, resolution, specificity and quality of the results. Formerly, the DNA probes for *in situ* hybridization were labeled using radioactive isotopes as ^{32}P, ^{125}I, ^{3}H and ^{35}S. On the other hand, nowadays most probes for *in situ* hybridization are visualized/detected through the use of fluorescent molecules under an epifluorescence microscope and the technique is named as fluorescent *in situ* hybridization (FISH).

In animal chromosomes, the FISH has been applied in the detection of distinct sequences allowing the integration of cytogenetic to linkage maps, analysis of chromosomal structure, genome organization, chromosomal ongoing and evolution. For fish and insect chromosomes, the most applied class of probes is represented by the repeated sequences including multigene families of ribosomal RNA (rRNA) genes and satellite DNAs (satDNA), and**,** in a lesser extent**,** transposable elements and microsatellites [for example 5-11].

The repetitive sequences have been obtained by distinct approaches, including for example, polymerase chain reaction (PCR), enzymatic restriction, and chromosome microdissection. Some of the information concerning the type of sequences, methods for probe obtainment, and application of FISH in fish and insect chromosomes will be presented along this chapter, showing the potential of this technique to advance in the knowledge of genome organization and chromosomal differentiation during the evolutionary history of species and groups.

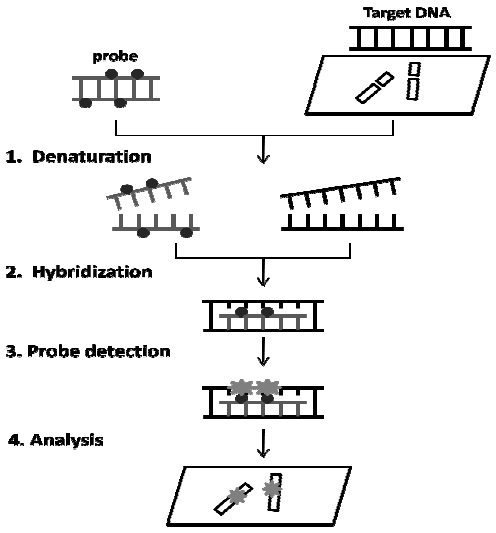

Fig. 1 Basic steps of fluorescent *in situ* hybridization technique. If the probes are labeled directly with fluorescent molecules the step (3) is not necessary.

2. General features of animal genomes and repeated DNAs

The presence of large amount of repetitive DNAs in eukaryotic genomes is a ubiquitous feature and these sequences are characterized by a wide heterogeneity and diversity of repeated families. These sequences can represent a large portion of the genomes and, in some cases, can exceed more than 80% of the cell DNA content [12, 13]. For a long time, no functional action was attributed to some of the repeated sequences, and these elements were known as "junk DNAs". Although in recent years the concept of "junk DNA" has changed mostly due to the discovery of transcribing regions of repeated elements and their involvement in genomic functions, the repeated DNAs have been defined as "encoding" and "non-encoding" sequences. Briefly, the encoding DNAs are represented mainly by the multigenic families, such as rRNAs and histone genes, while the "non-encoding" elements are the satDNAs and transposable elements, besides the micro and minisatelites. These elements can be organized *in tandem*, as for example the satDNAs, or dispersed throughout the genome, as the transposable elements in general. Basically these sequences compose the nuclear genome architecture together with the less repeated sequences represented by the unique and low copy number sequences and low repeated DNAs (Fig. 2).

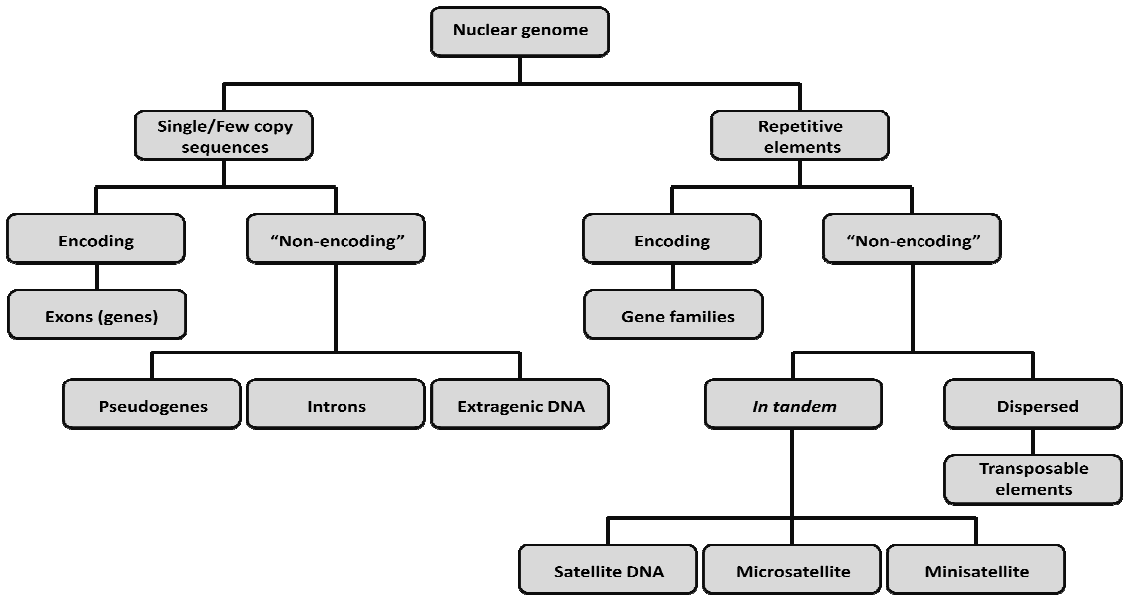

Fig. 2 Nuclear genomic organization in eukaryotes. The "non-encoding" remits to the recent discovery of transcription of these elements (more details can be found in text).

The term "multigene family" is used to indicate groups of DNA sequences (genes) that have descended from a common ancestral gene and show notably structural similarity and function [14]. For chromosomal studies, the most used multigene families are the rRNA genes (major 45S and minor 5S transcribing units) and, in a lesser extent, the histone genes. The ribosomal DNAs (rDNA) are organized *in tandem* arrays with variable number of repetitions in distinct genomes. The 45S rDNA repeats contain units separated from each other by intergenic spacers (IGS) and transcribes for the 18S, 5.8S and 28S rRNAs. The rRNA transcribing regions are separated from each other by internal transcribed spacers (ITS) (Fig. 3a). On the other hand, the minor 5S rRNA sequences are transcribed by the highly conserved 5S rRNA genes with 120 base pairs (bp) interspersed from each other by a non-transcribed spacer (NTS) with variable nucleotide sequence (Fig. 3b) [15]. The multigene family that codes for the histone proteins in general can be organized in one cluster formed by all intronless tandemly organized histone sequences (H1, H2a, H2b, H3 and H4), spaced by noncoding DNA sequences, as observed in the genome of *Drosophila melanogaster* (Figure 3c) [16]. These same genes can be distributed as single genes or group of histone genes, as in *Gallus domesticus*, man and mouse. Moreover, the two kinds of organization can be found in the same genome, which is the case of *Xenopus laevis* [17, 18].

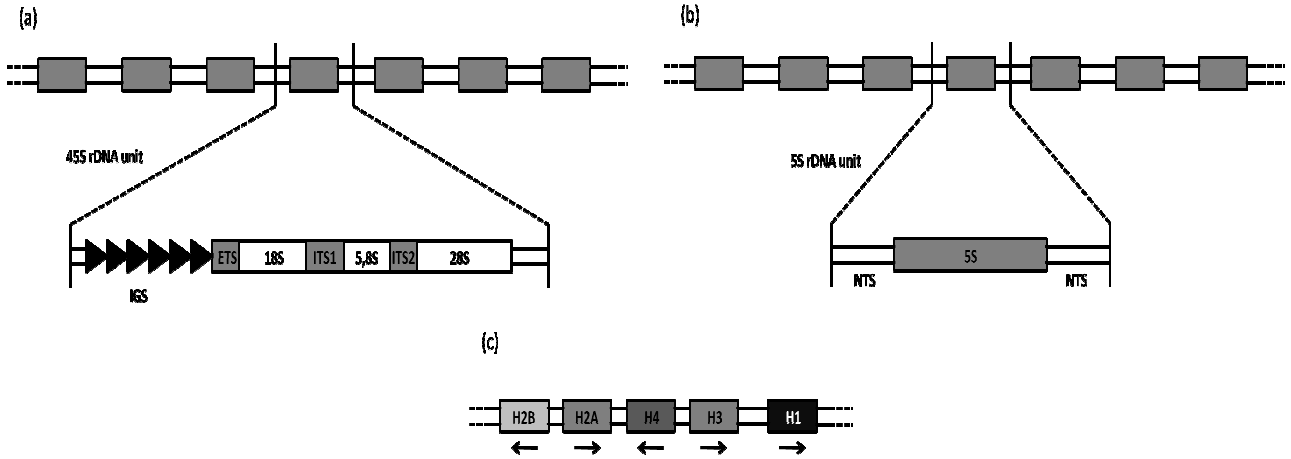

Fig. 3 Genomic organization of the eukaryotic multigenic families of (a) 45S rRNA, (b) 5S rRNA and (c) histones in fruit fly.

The satDNA, minisatellite and microsatellite are tandemly arrayed and highly repeated sequences found in the eukaryotic genomes. These sequences are variable in their structure, repeated unit and cluster repetition sizes. The satDNAs are organized in large clusters that are usually located in the telomeric and centromeric heterochromatic regions of the chromosomes. The size of a basic motif or repeated unit of the satDNAs can vary from 100 to 1,000 nucleotides and it can be represented in the genome from 1,000 to over 100,000 copies [12, 19]. The minisatellite DNAs include all moderately repeated DNAs with size variation from 10bp to 100bp distributed throughout the eukaryotic genomes with a variable number of repeats called VNTR (Variable Number of Tandem Repeats). These sequences can be used as markers in forensic analysis and show discriminatory capacity leading to specific individual patterns, due to their intense evolutionary dynamic generating complex arrays in the genomes [20]. The last group, the microsatellites, is formed by short sequences composed by 1-6 nucleotides per unit. They are highly polymorphic markers, constituted by ubiquitous simple sequence tandem repeats (SSR) in the eukaryotic genomes. Although the microsatellites are neutral, some of these repetitive sequences can be linked to *loci* under strong selection in a wide range of taxa, and have been applied in cytogenetic mapping and as molecular markers [11, 21, 22].

Most part of repeated elements in animal genomes is derived from the transposable elements (TEs), and these sequences can represent a large portion of the genomes, i.e. around 40% of human genome. These sequences are differentiated from the other genome sequences by their capacity of mobilization and spread. This mobilization is capable to generate polymorphisms as consequence of the variability in the copy number of inserted sequences. Previously, these elements were considered genomic parasites or "junk" DNA, but this view have changed in the last years by the observation of distinct roles of mobile DNA in genomic structure, function and evolution [23, 24]. The transposable elements are divided in two main classes, which differ in their mode of transposition. There are elements in which the transposition is mediated by a RNA molecule, called retrotransposable elements or class I, while the transposons or elements of class II are transposed by a DNA molecule [23]. Moreover, these sequences are subdivided into subclasses, orders, superfamilies, families, and subfamilies according to their enzymology, structural similarities, and sequence relationships. Mechanisms of sequence duplications and transposition are some of the causes of the variation in the quantity for the distinct elements in some genomes and the variation in genome size for distinct groups [23, 25].

3. Strategies for isolation of repeated DNAs to be used as probes in fluorescence *in situ* hybridization

Keeping in mind that the repeated elements are abundant in the genome of distinct animal groups, it would be easy to think that these sequences represent interesting chromosome markers for cytogenetic mapping purposes. In fact, most part of the studies related to chromosomal organization and karyotype evolution under the molecular cytogenetics focus has been conducted by means of repeated elements mapping. They are useful for this propose due their *in tandem* array or enrichment in specific chromosome regions, that allow the easily visualization of distinct blocks along the chromosomes.

Nowadays, with the advent of molecular biology and improvement in the cell biology manipulation, the isolation and characterization of distinct repeated elements turn to be available, generating a range of probes to be used for cytogenetic mapping. Among the cellular and molecular techniques for repeated DNA accessing/obtaining, the most used are the Polymerase Chain Reaction (PCR), enzymatic restriction, and chromosome microdissection, besides the use of BAC (Bacterial Artificial Chromosomes) libraries containing repeated DNAs. These techniques have already been applied in distinct studies for analysis of genome organization and karyotype evolution in fish and insects, although the application of some of them, such as chromosome microdissection and BAC libraries, remains still restrict to few works. These technologies have permitted the acquirement of probes for whole chromosomes or sub chromosomal regions (centromere, telomeres, specific arms), specific chromosomal locus or dispersed elements.

Although there are distinct approaches to isolate repeated DNAs, the most used is the PCR, due to the low cost and the simplicity to be performed. The most common elements isolated by PCR are the multigene families such as ribosomal DNAs (rDNAs) and histone genes, although some transposable elements and telomeric sequences have also been accessed in this manner. The limitation of this technique is related to the possibility of isolation of only known sequences, when specific primers are used. There are some conserved and universal primers described in the literature for amplification of repeated DNAs by PCR that can be used in distantly related taxa. Moreover, for less conserved sequences it is possible to design primers exploring the sequences deposited in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov).

The use of restriction enzymes has permitted the isolation of highly abundant sequences, such as satDNA and, in a lesser extent, disperse transposable elements. This method is based in the use of a specific enzyme that cuts repetitive DNA family members in specific regions, generating similar size and nucleotide composition fragments. It is a more laborious methodology in comparison to PCR, in both time consuming and costs, due the necessity of testing distinct enzymes, and DNA cloning and sequencing. The main difference in relation to PCR is that in this approach unknown repeated DNAs are isolated that are more useful in studies of related taxa, due the intense variation in the sequences.

The chromosome microdissection for analysis of fish and insect chromosomes until now is restricted to few works. It consists in the isolation of entire chromosomes or specific regions of interest directly from the metaphasic plates using an inverted microscopy and a micromanipulation apparatus. The main difficulty of this technique is related to the recognition of the target chromosome or a chromosomal region due to the similarity in the chromosome morphology and size in the chromosome complement of most species. The quantity of isolated chromosomes to be used in the amplification process is also critics. These difficulties have been minimized by improvement in the methods of microdissected-DNA amplification and the use of C-banded and meiotic chromosomes for microdissection [26-28].

A BAC (Bacterial Artificial Chromosomes) library is a collection of cloned DNA sequences usually in *Escherichia coli* with sizes larger than 100 kilobases (kb). As well as for microdissection, the BAC libraries with repeated elements have not been extensively used in studies of fish and insect chromosomes. This technology has permitted the integration of genetic maps to physical location of some sequences into chromosomes, being useful for cytogenetic and genomic comparisons. On the other hand, this methodology has encountered difficulty in application in chromosome mapping due the necessity of available libraries of BAC clones, which in general depends of genome sequencing projects that frequently are restricted to few species of interest.

Besides of the strategies explained above, the repeated elements can be isolated using simple modifications in distinct methodologies that in general permit the isolation of a pool of repeated elements. For example, the C_0t-1 DNA fraction, DOP-PCR and total genomic DNA can also be used as probes. The C_0t-1 DNA is a fraction of genomic DNA elements enriched for highly and moderately repeated DNAs. This methodology is based in the reassociation kinetic of DNA strands, and consists basically in the denaturation of fragmented genomic DNA, reannealing and treatment using the enzyme S1 nuclease that is active against single-stranded DNAs [29, 30]. In general, the regions labeled by this genomic fraction correspond to the heterochromatic areas, such as centromeres and telomeres. In the same way, the isolation of repeated elements using the DOP-PCR (Degenerate Oligonucleotide Primed-PCR) that is based in the use of degenerated oligonucleotides, can also be useful. Another method using unknown sequences for comparative analysis is the use of the whole genome of one species as a probe in order to be hybridized to the karyotype of another related organism. This modification in the FISH technique is named genomic *in situ* hybridization (GISH). Although most part of the studies using GISH has been conducted in plants, there are few but interesting examples in animals, including fishes and insects [31, 32]. The disadvantage of these methodologies is related to the isolation of a large number of unknown sequences. On the other hand, they represent low cost assays without necessity of DNA cloning or

sequencing. These methodologies have shown to be promising for isolation of non-specific repeated DNAs to be used as probes in comparative analysis and genomic organization of animal chromosomes.

The strategies revisited above are currently in use to isolate repeated DNAs in insect and fish genomes for use in studies of genome organization and karyotype comparisons among distinct taxa. These strategies have encountered applicability in the analysis of the whole genome structure as well as in studies that focus in the origin and evolution of parasitic elements (B chromosomes) and sex chromosomes, heterochromatin differentiation and a fine scale identification of chromosome rearrangements. In the next sections, general aspects and examples of cytogenetic mapping of repeated DNAs in insects and fishes will be presented under the focus of fluorescence *in situ* hybridization.

4. Chromosomal mapping of repeated DNAs in insect chromosomes

Although insects correspond to the most diverse animal group and have high diversity of karyotypes including presence of diverse diploid numbers in related species, absence or presence of different sex chromosome systems, presence of parasitic elements and occurrence of monocentric and holocentric chromosomes, the cytogenetic studies in this group are still scarce. Moreover, the studies in this group for long time have been concentrated in classical analysis, with description of basic features of the karyotypes. On the other hand, in the recent decades the use of FISH in insects has been useful in studies of genome organization and chromosomal evolution. Among the repeated sequences that have been mapped in animal chromosomes, the most applied sequences in insects are the multigene families followed by satDNAs and, in a lesser extent, transposable elements and microsatellites. These studies are concentrated in representatives belonging to the orders Coleoptera, Diptera, Hemiptera, Lepdoptera and Orthoptera.

4.1 Chromosomal mapping of multigene families

In fact, as in other animal groups, the most applied chromosomal marker obtained through FISH in insects is the major rDNA multigenic family (Fig. 4a,c) followed by the 5S rDNA (Fig. 4a) and histone (Fig. 4b) multigene families that were mapped in few species of insects belonging to Coleoptera, Diptera, Hemiptera and Orthoptera. A wide variation of quantity and chromosomal location for the major rDNA has been observed in all groups. For example, in grasshoppers belonging to the families Acrididae, Romaleidae and Proscopiidae, it was observed one to eight pairs bearing major rDNA sites, including the presence of this sequence in B chromosomes [33, 34]. This wide variation has been more common for Acrididae, that is the most studied group, while for Proscopiidae and Romaleidae a lesser variation was described [34, 35]. Moreover, this sequence has been found in B chromosomes of distinct species, such as *Eyprepocnemis plorans* and *Omocestus bolivari*. In *E. plorans* the B chromosome of distinct population has a remarkable variation related to the quantity of rDNA that in general is inactive [33, 36]. According to Cabrero and Camacho 2008 [33], the intra and interespecific variation for 45S rDNA in some grasshoppers can be explained by structural chromosome rearrangements, such as translocations or inversions, ectopic recombination and transposition.

For Coleoptera there are results of mapping major rDNA for few representatives belonging, for example, to the families Tenebrionidae, Scarabaeidae, Chrysomelidae, Carabidae, Cicindelidae and Buprestidae. The studies in the family Cicindelidae have shown an interesting pattern with correlation of the number and location of rDNA with distinct sex mechanisms and diploid number reduction. More basal species with single sex chromosome systems and higher chromosomal numbers present high number of rDNA sites located in the autosomes, while in species with lower chromosome numbers and presence of multiple sex chromosome systems have rDNA associated to heterosomal elements, or to the both autosomal chromosomes and heterosomes [37]. For the 16 species of Scarabaeidae so far studied the results have shown a wide variability for this marker related to number and location in autosomes or sex chromosomes. The highest variability is observed in representatives of the subfamily Scarabaeinae, with species harboring rDNAs in both autosomes and sex chromosomes; *Bubas bison* with 8 sites, which is one of the highest numbers for the family; *Gymnopleurus sturmi* with polimorphism related to the number of sites (4 or 5); *Eurysternus caribaeus* with rDNA in the X and Y chromosomes, apparently resulted from a fusion along the karyotype differentiation in this species that has involved extensive chromosome fusions [9, 38, 39]. In lepdopterans, the rDNA/NORs were determined in 32 species belonging to six superfamilies. Some distinct patterns were observed inside and between the superfamilies, related to the number of sites and precise position in the chromosomes. Only in Noctuoidea, the pattern of rDNA organization was conserved, in contrast with the high variability observed for Pyraloidea and Papilonoidea [10]. According to Nguyen et al. 2010 [10], the variability for this order can be explained by fusions, multiplication of rDNA sites, chromosome fissions and mainly by ectopic recombination.

The mapping of 5S rDNA and histone genes is restrict to few species belonging to grasshoppers, beetles, dipterans and hemipterans, although the results until now are incipient. In grasshoppers, the 5S was mapped in seven species and some variations were reported for Acrididae, including sites in the B chromosome [40-43]. On the other hand, the four species belonging to an ancient family (Proscopiidae) showed only one site of 5S located in the chromosome four [34]. For Coleoptera and Hemiptera, one species for each order was studied and in both cases this multigene family was located in one chromosome, which represents the same bivalent bearing the 18S rDNA in *Acyrthosiphon pisum* (Hemiptera), and a distinct 18S rDNA bearing chromosome in *Dichotomius geminatus* (Coleoptera) [42, 44].

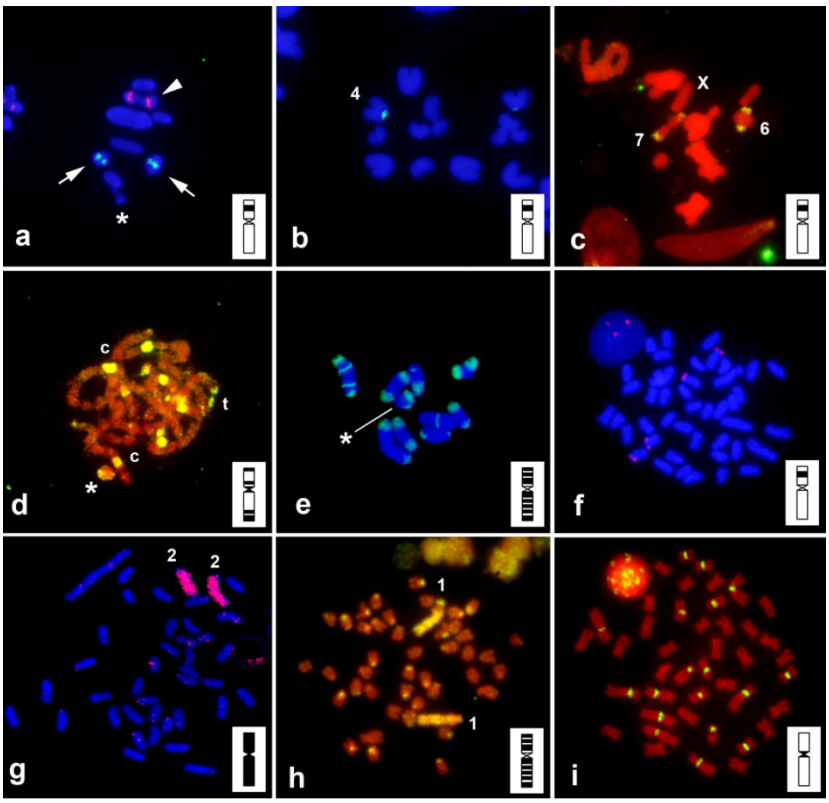

Fig. 4 Examples of FISH in chromosomes of insects (a-e) and fish (f-i). (a-c,e,f) Probes obtained by PCR: (a) 18S rDNA (green, arrow) and 5S rDNA (red, arrowhead) in metaphase I of the beetle *Dichotomius geminatus*; (b) H3 histone gene in metaphase II of the grasshoper *Stiphra robusta*; (c) 18S rDNA in metaphase I of grasshopper *Tetanorhynchus silvai*; (e) Mariner transposable element in metaphase I of the beetle *Diabroctis mimas*; (f) 18S rDNA in mitotic chromosome of the fish *Haplochromis obliquidens*. (d) C_0t-1 DNA fraction in pachytene of the beetle *D. geminatus*; (g) chromosome paint probe obtained by chromosome microdissection of chromosome pair 2 of the fish *Oreochromis niloticus*; (h) FISH in *O. niloticus* using a BAC enriched of repetitive DNAs; (i) satellite DNA 5S*Hind*III obtained by enzymatic restriction in the fish *Hoplias malabaricus*. Asterisks in (a), (d) and (e) indicate the sex bivalent; in (d), c = centromere and t = telomere; the scheme in each figure indicates the hybridization pattern that can be obtained using distinct kinds of probes. Image (i) is courtesy of Cioffi MB and Bertollo LAC (Universidade Federal de São Carlos, Brazil), and image (e) Sárah G Oliveira. Scales are not shown to the micrographs.

The histone genes (mostly the H3) were mapped in 19 chironomid midges, 11 fruit flies, one beetle, and 39 grasshoppers. It is a common pattern the presence of only one site for this sequence that moreover presents a remarkable conservation in relation to the position. More than one site was described for some dipterans [7, 34, 45-47]. Moreover, an association for the histone genes and 5S rDNA was observed in the beetle *D. geminatus* and for the four ancient grasshopper species studied [34]. These findings could indicate a linked organization for these genes, as observed for other invertebrates such as one mussel and two crustaceans [48, 49], but other experiments, such as fiber-FISH and southern blotting need to be performed to confirm this statement.

Besides the use of multigenic families in studies of genomic organization and karyotypic comparisons they are also informative for studies of some B chromosome origin and evolution in insects. Good examples have been reported for grasshoppers in the species *E. plorans*, *Locusta migratoria* and *Rammathocerus brasiliensis* [40, 41, 43]. In beetles, an example of the use of multigene family as a tentative of elucidation of B chromosome origin was done by Cabral-de-Mello et al 2010b [42] in the beetle *Dichotomius geminatus*.

4.2 The satellite DNAs on insect chromosomes

Studies concerning the satDNAs sequences in insects are scarce, related to all aspects, such as molecular organization, sequences and precise chromosomal location. Moreover, the studies are fragmented and restricted to representatives belonging to eight orders out of 32. In fact, the studies are concentrated in Diptera (~40 species), Coleoptera (~55 species) and Hymenoptera (~30 species), while for the other orders (Hemiptera, Isoptera, Lepdoptera, Orthoptera and Phasmida) few sequences from lesser than ten species for each group were isolated and characterized [reviewed by 6].

The satDNAs isolated in insects are concentrated in two groups related to the length size of the sequences, one large with variation ranging from 300bp to 400bp, and one small with 140-190bp, although variations have been reported with larger and small sequences. As observed in other invertebrate and vertebrate groups, the heterochromatin and

satDNAs in insects are mainly concentrated in the centromeric and/or telomeric regions of the chromosomes, although some satDNAs have been reported in other regions. Among the isolated sequences, some are specie-specific and other are shared among some species including non related taxa. Moreover, the same type of these elements can be shared by all chromosomes of one species, can be shared only by a subset of them or can be chromosome specific, including autosome or sex chromosome specificity [reviewed by 6]. Examples of satDNAs shared by all chromosomes or by the majority of them were described in species of the genus *Drosophila* for the sequence ATOC180 in *D. ambigua*, *D. obscura* and *D. tristis* [50]; in three species belonging to the family Chrysomelidae (Coeloptera) - *Chrysolina americana*, *C. carnifex* and *Xanthogaleruca luteola*, with the sequences CAMA, CCAH and XALU, respectively, located in the pericentromeric regions [6, 51]; in two species of *Cicindela* for the sequence CM383 which is located in centromeric region of all chromosomes, including the sex multivalent (four X), and in the short arm of the Y chromosome [52].

In general, sequences specific for one or few chromosomes are located in the sex elements, associated to the X or Y chromosomes, or to both, although specific elements for one or few autosomes were described, for example, in *Anopheles gambiae* (Ag53C in centromeric regions of chromosomes 2 and 3), *Chironomus pallidivitatus* (a 375bp in the centromere 3) and in *Drosophila melanogaster* (dodeca satellite on centromere of chromosome 3). In *D. melanogaster*, each centromeric region presents distinct repeated DNAs. Sequences located out from centromere/pericentromere or telomere/subtelomere are lesser frequent and were described only for few species of Orthoptera (*Caledia captiva*) and Diptera (*Drosophila ezoana*, *D. littoralis*, *D. virilis*, *Sarcophaga bullata*) [reviewed by 6].

The heterochromatic nature of B chromosomes is a common pattern indicating that this elements harbor satDNAs, although in insects the use of satDNA sequences for the study of these elements is restrict to few species as exemplified for *Eyprepocnemis plorans* (Orthoptera) and *Drosophila subsilvestris* (Diptera). In both species the isolated satDNA, pSP for *D. subsilvestris* and a satDNA with 180bp for *E. plorans*, are present in the B chromosome [36, 53]. The satDNA from *E. plorans* is also shared by some autosomes and it was observed in a related subspecies, *E. plorans meridionalis*, but it was absent in other studied species of Eyprepocnemidinae. In the B chromosome, the quantity of this sequence is variable in distinct populations, and the use of this element with 45S and 5S rDNA probes was useful to determine the B multiregional origin in *E. plorans* [36, 40]. On the other hand, the satDNAs apparently are not the most informative sequences to establish supernumerary chromosome origin, due their presence in some chromosomes of the A complement.

4.3 Transposable elements and microsatellite DNAs mapping

In a lesser extent, in comparison with the multigenic families and satDNAs, the transposable elements and microsatellites have been mapped in insect chromosomes. Among the transposable elements, the *R2*, *P* elements, *I* elements, *Bari1*, *TART* and *rolled* are examples of mapped sequences (Fig. 4e) [54-58], and for microsatellite the few studies were performed using distinct repetitions [11, 59]. The results concerning these elements are fragmented and insufficient; moreover, they are concentrated in few Dipteran species. A general pattern is the location of transposable elements in few chromosomes or chromosomal regions, including sex chromosomes, evidencing specific signals indicating a compartmentalized organization. These elements are in general associated to the heterochromatin or rDNA regions, including interspersion with satellite DNAs being identified as the main component of certain satDNA families. Moreover, it has been suggested the participation of TEs in some cases of satDNA formation and spreading.

As the satDNAs and transposable elements, the microsatellites can also be associated to the heterochromatic regions, however some di- and trinucleotide repeats are distributed through euchromatic regions. A good example of utility of microsatellites for chromosomal and genomic comparisons in insects was recently reported by Santos et al. (2010) [11]. These authors described the location of 72 dinucleotide microsatellite by FISH on polytene chromosomes of *Drosophila subobscura*, comparing their density between the sex chromosomes and autosomes. Analyses were also conducted *in silico* using the complete genomes of *D. melanogaster* and *D. pseudoobscura* inferring the loci conservation and synteny. All 72 dinucleotides mapped in specific bands in distinct chromosomes and were more conserved between *D. suboscura* and *D. pseudoobscura*, being located in the same chromosomal region. On the contrary to compartimentalized organization, in the orthopteran *E. plorans* a dispersed pattern of distribution for the microsatellite $(AG)_{10}$ was observed. This element was scattered in the euchromatic regions and was absent in centromeric heterochromatin and in the B chromosome [59].

5. Fish genomes under the focus of fluorescence *in situ* hybridization

The chromosome location of DNA sequences and genes represents an important tool in the elucidation of several aspects of karyotype characterization, chromosome rearrangements, comparative studies and integrative genomics among fish species. In this topic, information related to physical mapping of repetitive genes and DNA sequences in the fish chromosomes under the focus of the epifluorescence microscopy is organized and discussed. Since the fish are in a basal position in the vertebrate phylogeny, the cytogenetic mapping contributes to the clarification of several aspects

related to the genome organization and evolution of vertebrates. The molecular cytogenetics had bring considerable contributions to the knowledge of fish genomes throughout the application of FISH procedures to the resolution of several aspects involving chromosome structure, sex chromosomes, supernumerary chromosomes and evolution of karyotypes.

Fishes are characterized by a high level of genome plasticity not evidenced in any other vertebrate group. The DNA content of haploid cells can vary from 0,39 picograms (pg) to 248 pg among species and the chromosome number can vary from 2n = 16 to 2n = 446. Polymorphisms at the chromosomal level involving the presence of supernumerary chromosomes, polyploidy and structural variations are also frequent in the group. The genomic plasticity of fishes can be clearly illustrated by the range of sex determination mechanisms that occur in the group: (i) several species are hermaphrodite and change the sex in a specific stage of its life cycle; (ii) the sex determination mechanisms can vary from the classical female or male heterogametic sex to hermaphroditism, sex reversal and environmental or hormonal sex determination; (iii) although most species do not have identified sex chromosomes, diverse sex chromosome systems XX/XY, ZZ/ZW, $X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2, ZZ/ZW_1W_2 have been described for representative species of diverse groups.

In the last two decades, a high amount of data has accumulated concerning the cytogenetic mapping DNA sequences in the fish chromosomes. Most part of available data is related to the mapping of repetitive DNAs such rRNA genes, satDNAs and transposable elements. Just few single copy genes have been mapped in the chromosomes of these vertebrates. In the same way, chromosome painting is also a powerful technique that had also find application in the fish cytogenetics. The cytogenetic mapping had added new findings related to the diversity of chromosomal and genomic organization among fish species.

5.1 Cytogenetic mapping of rRNA genes

The first mapping sequences on the chromosomes of most animal species were represented by rRNA genes. The two rDNA classes (45S rDNA and 5S rDNA) (Fig. 4f) have both been extensively applied as chromosome probes in cytogenetic studies of fish species. Both rDNA classes could present extensive variation in relation to the number of sites among species, even among related species. The variation observed in the chromosomal distribution of rDNA sites is not informative in relation to the relationship of members of most groups. Repeated DNAs in general, as the ribosomal RNA multigene families, are subject to the action of several molecular mechanisms and are thought to be the most rapidly evolving components of eukaryotic genomes [60, 61]. The high dynamic evolutionary rate of repeated elements generated patterns of chromosomal organization that can not follow the phylogeny of the groups. Among fishes, the chromosomal distribution of rDNA clusters can be informative in relation to comparative analysis of close related species or even to the characterization of population variations. In this way, a special attention should be exercised in phylogenetic interpretation based on rDNA cytogenetic map data, at least for comparative analysis involving higher taxa.

The integration of cytogenetic (FISH) and genomic tolls (nucleotide sequencing, Southern blot and bioinformatics) has allowed an interesting interpretation on the organization and evolution of 5S rRNA genes on fish genomes. Variant classes of 5S rDNA repeats are common in the genome of fishes and seem to reflect mechanisms of birth-death and concerted evolution [14] that act in this genomic region. Some variant copies of 5S rDNA seem to be favored during selection and spread in specific chromosomal regions allowing the formation of major classes of 5S rDNA.

5.2 Cytogenetic mapping of satellite DNAs and transposons

Tandem repeat DNAs have been mapped in the chromosomes of several fish species (around 60 species) and for most of them they are frequently accumulated in the centromeric area. The satellite DNA has been investigated in a range of species representative of diverse fish taxa including Acipenseridae, Adrianichthyidae, Anostomidae, Channichthydae, Characidae, Cichlidae, Cyprinidae, Erythrinidae, Gobiidae, Heptapteridae, Loricariidae, Parodontidae, Pimelodidae, Poecilidae, Prochilodontidae, Salmonidae, Sparidae, Tetraodontidae and Cyclostomata [for review 62]. Studies on satellite DNAs have proved to be useful in clarifying a myriad of questions, including centromeric structure, and origin and evolution of sex and supernumerary chromosomes. Satellite DNAs could also find applications in the physical mapping of the genome, contributing to the development of genetic markers of significant importance to fundamental and applied biology of fish species. Satellite DNAs are very dynamic regions in the genome that differentiated rapidly during the evolutionary time. Different species generally present high divergence among satellite DNA families as a result of concerted evolution mechanisms [63], leading to species-specific satellite DNA sequences, although there are few exceptions, in which a group of species, or even a whole family or order, shares a same satellite DNA family.

One example to illustrate the intense evolutionary dynamic of satellite DNAs in the fish genome can be visualized in *Hoplias malabaricus*, a "species complex" of Erythrinidae family (Fig. 4i). A satellite DNA family named 5S*Hin*dIII, which shares similarities to 5S rDNA 'true' repeats [64], occurs in the genome of this species. This sequence is located in the centromeric region of several chromosomes and it is not shared by other Erythrinidae genera, and it is exclusive to this species [65]. Seven karyomorphs (A-G) were previously characterized and were included in two major groups, Group I (karyomorphs A, B, C, D) and Group II (karyomorphs E, F, G), based on their gross karyotype structure [66].

The physical mapping of 5S*Hind*III satellite in the karyomorphs A-B and C-D showed that several chromosomal markers had conserved location in the four karyomorphs. In addition, some other markers were only conserved in corresponding chromosomes of karyomorphs A-B and C-D. These data therefore reinforced and confirmed the proposed grouping of karyomorphs A-D in Group I and highlight a closer relationship between karyomorphs A-B and C-D. Moreover, 5S*Hind*III also mapped in the XY and X_1X_2Y sex chromosome systems, providing new evidences concerning the origin of the different sex chromosome system in the species [67]. Besides the variation on the chromosomal distribution of 5S*Hind*III satellite among the karyomorphs, there were also evident variations within distinct populations of karyomorph A [68].

Other centromeric satDNAs were mapped in the chromosomes of several fish species. In the Nile tilapia *Oreochromis niloticus*, the SATA satellite family was located in the centromeric region of all chromosomes of the complement [69, 70]. The satDNA isolated from the genome of *Acipenser naccarii* was also preserved in the pericentromeric regions of six species of the same genus and one species of *Huso*. In *Leporinus elongatus* [71], *Chiondraco hamatus* [72], *Poecilia reticulata* [73], and *Oncorhynchus tschawytscha* [74] the isolated satDNAs revealed distinct levels of association with sex chromosomes. For correlation between satDNAs and B chromosomes, a good example was described by Mestriner et al. 2000 [75] in *Astyanax scabripinis*. The satDNA As51 with 51bp isolated from *A. scabripinis* genome showed correlation with supernumerary elements, presenting symmetric distribution in both arms, a feature that associated with meiotic analysis indicated that this an isochromosome.

The other class of repeated sequences explored in the fish genome are the transpososable elements (TEs). The fish genome contains all types of TEs and some of them have been already studied under the focus of cytogenetics. The molecular cytogenetics of TEs has been explored in *Tetraodon nigroviridis* [76], *Oreochromis niloticus* [77], *Alburnus alburnus* [78], nonothenoid Antarctic fishes [79], and, more recently, in several cichlid species [8, 80]. Besides the dispersed chromosomal distribution of several TE they are also find accumulated in heterochromatic areas.

There are several examples of accumulation of repeated DNAs in specific chromosomes and chromosomal regions, such as centromeric areas and sex chromosomes. In the Antarctic fish *Chionodraco hamatus*, the retrotransposon *Rex3* has also shown an intercalary band in the long arm of the male Y chromosome [79]. According to the authors, the *Rex3* could have accumulated in the autosomes before the fusion that originated the Y chromosome, indicating that the transposable elements could be involved in the molecular differentiation of sexual chromosomes in *C. hamatus*. In the Nile tilapia *O. niloticus*, the largest chromosome pair is enriched of several classes of repeated DNAs including transposons, retrotransposons and satellite DNAs (Fig. 4h) [70]. The largest chromosome pair of the Nile tilapia was probably originated by chromosome fusion [81] and may reflect a prior evolutionary history as a differentiated sex chromosome [82]. The accumulation of repetitive DNAs in its putative sex chromosomes indicates that repetitive elements played important roles in the differentiation of the sex chromosomes in fish species.

6. Mapping of repeated DNA pools, GISH and chromosome painting

Other assays using repetitive DNAs, whole genome or chromosome content have been encountered application for studies of genome organization and chromosomal ongoing in insects and fishes, such as GISH, CGH (Comparative Genomic Hybridization) array, the use of C_0t-1 DNA fraction (Fig. 4d) and chromosome paints (Fig.4g). The CGH and GISH have been useful for identification and differentiation of sex chromosomes, as described in Lepidoptera and Heteroptera [31, 83], and in fishes GISH assay was an useful tool to compare the genome of the Nile tilapia (*Oreochromis niloticus*) with other cichlids from Africa and South America [32].

The C_0t-1 DNA fraction was recently used for study of the B chromosome in the beetle *Dichotomius geminatus*, indicating an intraspecific origin for this element [42]. In fishes, Ferreira and Martins 2008 [70] mapped the C_0t-1 DNA fraction in the chromosomes of *O. niloticus*, showing richness for repeated DNAs in the centromeric and telomeric regions and in the entire extension of the largest chromosome, the putativesex bivalent. Although the results using C_0t-1 DNA fraction as probes for chromosome mapping are still restrict, the use of this DNA fraction is a promise tool for analysis of sex chromosomes, heterochromatin differentiation and genomic organization.

Although chromosome paints were performed in few species of fishes and insects, among animals as a whole this methodology has encountered useful application in studies concerning sex chromosomes, B chromosomes and karyotype differentiation. In insects, Willhoeft et al. (1998) [84] performed painting in one species of Diptera, *Ceratitis capitata*, using partial chromosome painting (PCP) of the Y chromosome (five probes, medY1-medY-5) and X chromosome (medX1); Fuková et al. (2007) [85] studied the organization of sex chromosomes in *Cydia pomonella* (Lepdoptera) using a probe for the W chromosome; In grasshoppers, in attempt to elucidate the origin and composition of B chromosomes of *Locusta migratoria* Teruel et al. (2009) [86] used painting probes for X and B chromosomes. For fishes, the chromosome paint was useful for analysis of sex chromosomes in species of *Eignmannia* and *Triportheus* [87, 88]. Recently, Nagamachi et al. (2010) [89] performed the first chromosome paint in fish using all elements of the karyotype (some chromosomes were isolated in groups, due their similarity) obtained from *Gymnotus carapo sensu strictu* to compare distinct karyotypes for this species, with 2n = 40 and 2n = 42. This methodology permitted the description of more chromosome differences between the karyotypes than the observed differences by classical cytogenetic analysis.

7. Concluding remarks

Important information has been acquired using the basic cytogenetic tools, chromosomal banding and FISH in fish, insects and other animals, addressing a better characterization of their genomes. Although several studies have been conducted concerning genome organization and chromosome differentiation in insects and fish, the results until now are scarce and fragmented considering the high diversity of species. Moreover, these two groups are promising experimental models to understand genome and karyotypic evolution related to specific chromosomal rearrangements, supernumerary chromosomes, sex chromosomes, satDNA organization and genome evolution. The integration of molecular cytogenetics and bioinformatic tools using sequenced genomes will be important to clarify the genome organization and chromosomal ongoing among these groups and animals as a whole.

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