Original Article

Cytogenetic and Genome Research

Cytogenet Genome Res 317476
DOI: 10.1159/000317476

Accepted: March 1, 2010
by M. Schmid
Published online: ■■■

© S. Karger AG, Basel
PROOF Copy for personal use only
ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.

Cytogenetic Mapping of 5S and 18S rRNAs and H3 Histone Genes in 4 Ancient Proscopiidae Grasshopper Species: Contribution to Understanding the Evolutionary Dynamics of Multigene Families

D.C. Cabral-de-Mello[a] C. Martins[a] M.J. Souza[b] R.C. Moura[c]

[a]UNESP – Universidade Estadual Paulista, Instituto de Biociências/IB, Departamento de Morfologia, Botucatu,
[b]UFPE – Universidade Federal de Pernambuco, Centro de Ciências Biológicas/CCB, Departamento de Genética,
[c]UPE – Universidade de Pernambuco, Instituto de Ciências Biológicas/ICB, Departamento de Biologia, Recife, Brazil

Key Words
Chromosomal evolution · FISH · Genome organization · Multigene families · Repetitive DNA

Abstract
This paper reports on the chromosomal location of 18S rRNA, 5S rRNA and H3 histone multigene families in 4 species of a relatively ancient and diversified group of grasshoppers belonging to the family Proscopiidae. The 5S rRNA and H3 histone genes were highly conserved in the number of sites and chromosomal position in the 4th chromosome pair in all species analyzed, whereas the 18S rRNA genes showed slightly more variation because they were present on one or 2 chromosome pairs, depending on the species. The 5S and 18S rRNA gene families occurred in different chromosomes; in contrast, H3 histone and 5S rRNA genes co-localized in the same chromosomal position, with an apparently interspersed organization. Considering that the Proscopiidae family is a relatively ancient group compared with the Acrididae family, the association of the H3 histone and 5S rRNA multigene families can represent a basal condition for grasshoppers, although more research is needed on other representatives of this insect group to confirm this statement. The presence of such an association of 5S rDNA and H3 histone in mussels and arthropods (beetles, grasshoppers and crustaceans) suggests that this linked configuration could represent an ancestral pattern for invertebrates. These results provide new insights into the understanding of the genome organization and the evolution of multigene families in grasshoppers and in insects as a whole.

Copyright © 2010 S. Karger AG, Basel

Repetitive DNA elements constitute a large portion of eukaryotic genomes, including tandem arrays and dispersed repeats. Tandem array repeats comprise mainly satellite DNAs and multigene families [Charlesworth et al., 1994]. The ribosomal RNA (rRNA) and the histone protein gene families include a variable number of copies and locations across the genomes. The use of fluorescence in situ hybridization (FISH) for rRNA and histone genes has provided useful chromosomal markers for comparative analysis, elucidation of genome organization and identification of chromosomal rearrangements in many organisms. Among invertebrates, for instance, mapping

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2010 S. Karger AG, Basel
1424–8581/10/0000–0000$26.00/0

Accessible online at:
www.karger.com/cgr

D.C. Cabral-de-Mello
UNESP – Universidade Estadual Paulista
Instituto de Biociências/IB Departamento de Morfologia
Botucatu, SP 18618-000 (Brazil)
Tel./Fax +55 14 3811 6264, E-Mail dccdm84@yahoo.com.br

of rRNA genes has been performed in many groups, such as worms, insects, mollusks, echinoderms and others [Vitturi et al., 2002; Wang and Guo, 2004; Caradonna et al., 2007; Cabrero and Camacho, 2008]. On the other hand, mapping of histone genes has been restricted to 19 chironomid midges [Hankeln et al., 1993], 11 fruit flies [Schienman et al., 1998; Ranz et al., 2003], 5 mollusks [Eirín-López et al., 2004; Zhang et al., 2007], 35 acridid grasshoppers [Cabrero et al., 2009] and one beetle species [Cabral-de-Mello et al., 2010].

The family Proscopiidae comprises about 266 species that have been arranged into 3 subfamilies: Hybusinae, Xeniinae and Proscopiinae, all of which are distributed exclusively throughout Central and South America [Liana, 1980; Otte et al., 2003]. The taxonomic status and the phylogenetic position of this family remain controversial, although it represents a relatively ancient and diversified group if compared with the acridids, forming a quite old Acridomorpha group [Descamps, 1973; Matt et al., 2008]. Proscopiidae are characterized by diploid chromosome numbers (2n♂ = 15, 17, 19) lower than those observed in the Acrididae grasshoppers (most species with 2n♂ = 23), although both families coincide in sharing the XO/XX sex chromosome system and acrocentric chromosomes in most species [Mesa and Ferreira, 1981; Moura et al., 1996; Souza and Moura, 2000]. According to Mesa and Ferreira [1981], fusions, fissions and inversions are responsible for the chromosomal diversity observed in the Proscopiidae family. Other aspects of the chromosomal organization, e.g., the location of repetitive DNA, have scarcely been studied, with only 4 species analyzed for heterochromatin and nucleolus organizer region location, through classical cytogenetic methods [Moura et al., 1996; Souza and Moura, 2000].

With the aim of contributing to the knowledge of grasshopper genomes, we investigated the chromosome location of 3 multigene families (5S rRNA, 18S rRNA and H3 histone genes) in 4 Proscopiidae species, using single and double FISH. Our results revealed the colocalization of 5S rRNA and the H3 histone genes as well as the independent location of the 18S rDNA. These data contribute to a better understanding of the genome organization of multigene families in grasshoppers and in insects as a whole.

Materials and Methods

Adult males of 4 species of Proscopiidae grasshoppers, i.e., *Scleratoscopia protopeirae* (7), *S. spinosa* (8), *Stiphra robusta* (12) and *Tetanorhynchus silvai* (6), were collected from countryside regions in the Pernambuco State, Northeast Brazil. The testes were fixed in Carnoy (3:1 ethanol:acetic acid), and the chromosome preparations were made by squashing in a drop of 45% acetic acid and subsequently removing the coverslip with a razorblade after immersion in liquid nitrogen.

DNA probes of the 5S rRNA, 18S rRNA and H3 histone genes were obtained from the genome of the beetle *Dichotomius geminatus* [Cabral-de-Mello et al., 2010]. The 18S rRNA and H3 histone gene probes were labeled by nick translation using biotin-11-dATP (Invitrogen, San Diego, CA, USA), whereas the 5S rRNA gene was labeled with digoxigenin-11-dUTP (Roche, Mannheim, Germany). The FISH procedures were performed according to the method described by Cabral-de-Mello et al. [2010]. Preparations were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted in Vectashield (Vector, Burlingame, CA, USA). Images were captured with the Olympus DP71 digital camera coupled to a BX61 Olympus microscope and were optimized for brightness and contrast using Adobe Photoshop CS2.

Results

The 4 species studied showed 2n♂ = 19 chromosomes, with an XO sex-chromosome system. Acrocentric chromosomes were observed in *Stiphra robusta* and *Tetanorhynchus silvai*, whereas the 2 *Scleratoscopia* species showed 2 submetacentric autosomal pairs (the first and 3rd, in order of decreasing size) and a submetacentric X chromosome. The karyotypes of *Scleratoscopia* species consist of 3 pairs of large chromosomes (L1–L3), 3 pairs of medium chromosomes (M4–M6) and 4 pairs of small chromosomes (S6–S10), the X is a medium element (M5). In *S. robusta* and *T. silvai* the chromosomes showed gradual decreasing size, and were not classified in distinct groups. These results for conventional analysis are in agreement with previous studies [Moura et al., 1996; Souza and Moura, 2000].

FISH with the 18S rDNA probe revealed the presence of sites in the 6th and 7th chromosome pairs of *Stiphra robusta* and *Tetanorhynchus silvai*, whereas it was restricted to the 7th pair in the 2 *Scleratoscopia* species (fig. 1). Furthermore, the chromosome location of the 18S rDNA showed some differences among species because it was interstitial in *S. robusta* (see insets in fig. 1c), but it was pericentromeric in the other 3 species (fig. 1). In addition, the 7th chromosome pair in *S. robusta* showed a heteromorphism for the size of the 18S rDNA cluster (see fig. 1c).

All 4 species showed a similar organization pattern for the 5S rRNA and H3 histone genes. Both types of DNA sequences colocalized in the 4th chromosome pair in the 4 species, but they were located in the pericentromeric

CGR317476.indd 2 13.07.2010 16:20:56

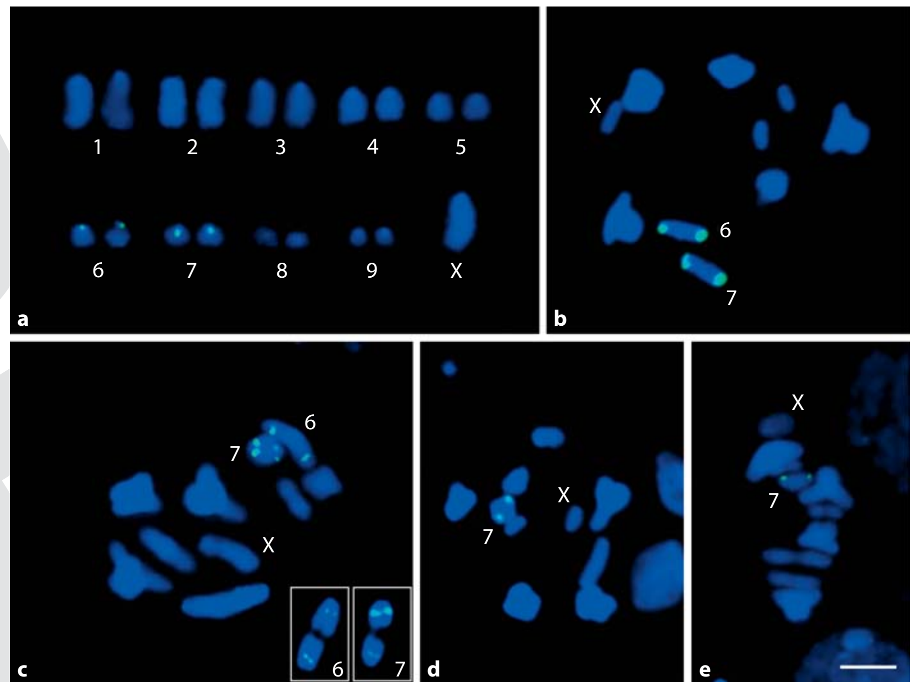

Fig. 1. Fluorescent in situ hybridization of 18S rDNA in the chromosomes of 4 Proscopiidae species. Karyotype (**a**) and metaphase I (**b**) of *Tetanorhynchus silvai*; metaphases I of *Stiphra robusta* (**c**), *Scleratoscopia spinosa* (**d**) and *S. protopeirae* (**e**). The inserts (**c**) show the precise position of the 18S rDNA cluster in chromosomes 6 and 7 in initial anaphase I and the heteromorphism of 18S rDNA clusters between homologues of pair 7. Bar = 5 µm.

region in *Scleratoscopia protopeirae*, *S. spinosa* and *Stiphra robusta* and they were slightly further from the centromere in *T. silvai* (fig. 2).

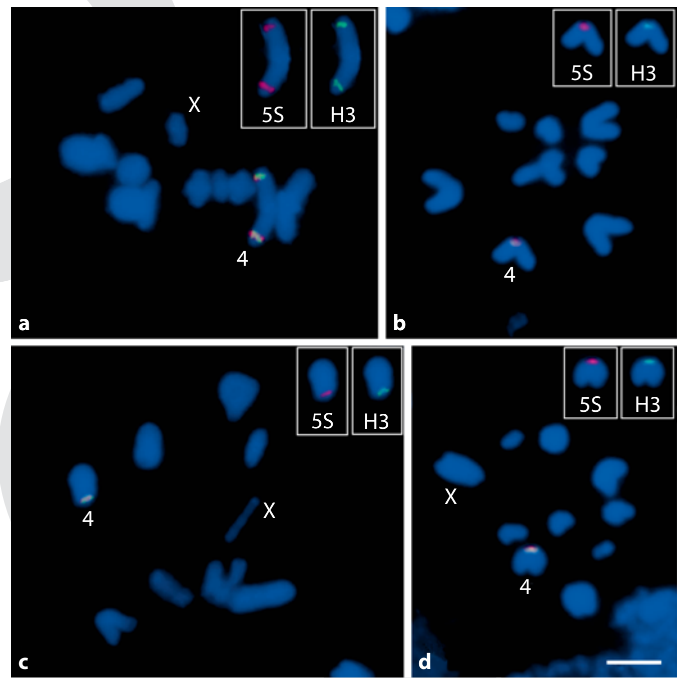

Fig. 2. Double FISH using 5S rRNA (red) and H3 histone (green) genes in metaphase I (**a**, **c**) and metaphase II (**b**, **d**) cells of the species studied in this work. *Tetanorhynchus silvai* (**a**), *Stiphra robusta* (**b**), *Scleratoscopia spinosa* (**c**) and *S. protopeirae* (**d**). Note in the inserts the labeled chromosomes with separated probes. Bar = 5 µm.

Discussion

These first data on the chromosome location of the multigene families in Proscopiidae, an ancient family of grasshoppers, indicate a general conservatism within this group because 18S rDNA is restricted in these 4 species from 3 different genera to one or 2 chromosome pairs, and 5S rRNA and H3 histone genes are colocalized at a single chromosome pair. Although more Proscopiidae species need to be analyzed, this family of grasshoppers seems to show less variation than Acrididae grasshoppers, at least for the 2 classes of rDNA analyzed in this investigation.

The location of 18S rDNA sites in the 7th chromosome pair, in order of decreasing size, observed in the 4 species analyzed in this research indicates that this was probably a consensus location in this group and thus suggests that this might be an ancient placement for these genes. The site located in the 6th pair of *Stiphra robusta* and *T. silvai*, however, might represent a derived pattern. In addition, structural rearrangements, e.g., a small paracentric inversion, might have been involved in changing the 18S

CGR317476.indd 3 13.07.2010 16:20:56

rDNA location toward an interstitial position in *S. robusta*, with no modification in the chromosome morphology. Moreover, the size heteromorphism observed for the 18S rDNA in the 7th chromosome pair in this last species suggests that this DNA seems to be currently subjected to mechanisms changing the amount of repeats between homologous chromosomes. Souza and Moura [2000], studying the same *S. robusta* populations, reported the presence of 3 nucleolar organizer regions, detected by silver nitrate staining that suggests the existence of polymorphism for rDNA site number and location.

The 5S rRNA and H3 histone gene clusters were highly conserved in the 4 Proscopiidae species analyzed and were restricted to a single chromosome pair, i.e., the 4th in order of decreasing size. The chromosomal position of the H3 histone genes is also very conserved in representatives in the Acrididae family, with most species showing a single cluster in the 8th chromosome pair in species with 2n♂ = 23. On the other hand, the H3 histone genes changed position to chromosome 3 as a result of one of the centric fusions that decreased the chromosome number to 2n♂ = 17 in many genera of the Gomphocerinae subfamily [Cabrero et al., 2009]. This conservatism in the number of clusters for H3 histone could be ancient in grasshoppers and there have been no changes occurring since the divergence of the Proscopiidae and Acrididae families, more than 100 million years ago [see Hewitt, 1979]. The presence of a single cluster for histone genes was also reported in 3 mollusk species [Zhang et al., 2007], 35 grasshopper species [Cabrero et al., 2009] and 3 fish species [Pendás et al., 1994]. On the other hand, more than one histone site was observed in 2 mollusk species [Eirín-López et al., 2004; Zhang et al., 2007] and some dipteran species [Hankeln et al., 1993; Schienman et al., 1998; Ranz et al., 2003].

The presence of a single site for 5S rDNA observed in the 4 Proscopiidae species analyzed here is relatively common in eukaryotes and has been reported in vertebrates and some mollusks [Mandrioli, 2000; Sola et al., 2000; Martins and Galetti, 2001; López-Piñón et al., 2005; Insua et al., 2006; Huang et al., 2007]. In grasshoppers, the 5S rDNA sequences have been previously mapped in only 2 species, *Eyprepocnemis plorans* from 3 populations [Cabrero et al., 2003] and *Rammathocerus brasiliensis* [Loreto et al., 2008]. Both species showed several clusters of 5S rDNA. Therefore, in grasshoppers, the 5S rRNA multigene family appeared to show a more dynamic evolution compared to H3 histone genes. But the Proscopiidae species analyzed here also showed a conservative pattern for the 5S rRNA genes, which might result from their ancient association with the histone genes and their submission to the same constraints that have conserved the chromosomal location of histone genes in grasshoppers in general. Although the 5S rDNA can present high evolutionary dynamics in some genomes, this statement seems to not be applicable in the Proscopiidae family because the 5S rRNA genes are restricted to a single chromosome locus.

The association of 5S rRNA and H3 histone genes has previously been reported in 4 other invertebrate species. In insects, Cabral-de-Mello et al. [2010] described, for the first time, the association of 5S rRNA and H3 genes in the beetle *Dichotomius geminatus*. Likewise, this association has also been reported in 2 crustacean species and one mussel species [Drouin and Moniz de Sá, 1995; Barzotti et al., 2000; Eirín-López et al., 2004]. In the Proscopiidae family, the H3 histone and 5S rRNA genes form a conspicuous cluster, with both multigene families apparently interspersed among each other. However, additional studies by means of fiber-FISH, Southern blot and DNA sequencing are necessary to clarify this statement. Available evidence on the interspersed arrangement of 5S rRNA and histone genes suggests the possibility that it may represent the ancestral condition for arthropods, considering that this arrangement occurs in beetles, grasshoppers and crustaceans. Alternatively, bearing in mind that the 5S rDNA shows the capability to move within the genome, the 5S rDNA repeats could have invaded the histone clusters several times during the evolution of these groups.

The absence of an association among major and 5S rRNA genes is a widespread condition among eukaryotes. The 5S and the major rRNA genes are organized in a linked configuration in fungi but in distinct genomic arrays in higher eukaryotes [Drouin and Moniz de Sá, 1995]. According to Martins and Galetti [2001], the separation of 18S and 5S rRNA genes in distinct genomic arrays could represent a functional advantage, considering that the 18S rRNA is transcribed by the RNA polymerase I and 5S rRNA by the RNA polymerase III. In contrast, the specific association of 5S rRNA and H3 histone genes cannot be explained by an advantage in the co-transcription process since these sequences are also transcribed by different RNA polymerases, RNA polymerase III and II, respectively.

Considering that Proscopiidae is an ancient group of grasshoppers, the condition of association of 5S rDNA and H3 histone genes can represent a basal condition before the diversification of grasshoppers. Further chromosomal studies using these sequences in other representa-

CGR317476.indd 4 13.07.2010 16:20:57

tives of Proscopiidae family and in other grasshopper families, such as Acrididae and Romaleidae, are necessary. Although the molecular organization of the association of 5S and H3 genes is still to be elucidated, our data reinforce the previous findings concerning the possible association of these genes and contribute to understanding the dynamics of multigene families in invertebrate genomes.

Acknowledgements

The authors are grateful to Dr. Juan Pedro M. Camacho for his valuable suggestions and critical review of this manuscript. The study was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Barzotti R, Pelliccia F, Bucciarelli E, Rocchi A: Organization, nucleotide sequence, and chromosomal mapping of a tandemly repeated unit containing the four core histone genes and a 5S rRNA gene in an isopod crustacean species. Genome 43:341–345 (2000).

Cabral-de-Mello DC, Moura RC, Martins C: Chromosomal mapping of repetitive DNAs in the beetle *Dichotomius geminatus* provides the first evidence for an association of 5S rRNA and histone H3 genes in insects, and repetitive DNA similarity between the B chromosome and A complement. Heredity 104:393–400 (2010).

Cabrero J, Camacho JPM: Location and expression of ribosomal RNA genes in grasshoppers: Abundance of silent and cryptic loci. Chromosome Res 16:595–607 (2008).

Cabrero J, Bugrov A, Warchalowska-Sliwa E, López-León MD, Perfectti F, Camacho JPM: Comparative FISH analysis in five species of Eyprepocnemidine grasshoppers. Heredity 90:377–381 (2003).

Cabrero J, López-León MD, Teruel M, Camacho JPM: Chromosome mapping of H3 and H4 histone gene clusters in 35 species of acridid grasshoppers. Chromosome Res 17:397–404 (2009).

Caradonna F, Bellavia D, Clemente AM, Sisino G, Barbieri R: Chromosomal location and molecular characterization of three different 5S ribosomal DNA clusters in the sea urchin *Paracentrotus lividus*. Genome 50:867–870 (2007).

Charlesworth B, Sniegowski P, Stephan W: The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371:215–220 (1994).

Descamps M: Révision des Eumastacoidea aux échelons des familles et des sous-familles (genitalia, répartition, phylogénie). Acrida 2: 161–298 (1973).

Drouin G, Moniz de Sá M: The concerted evolution of 5S ribosomal genes linked to the repeated units of other multigene families. Mol Biol Evol 12:481–493 (1995).

Eirín-López JM, Ruiz MF, González-Tizón AM, Martínez A, Sánchez L, Méndez J: Molecular evolutionary characterization of the mussel *Mytilus* histone multigene family: First record of a tandemly repeated unit of five histone genes containing an H1 subtype with 'orphon' features. J Mol Evol 58:131–144 (2004).

Hankeln T, Keyl HG, Ross R, Schmidt ER: Evolution of histone gene loci in chironomid midges. Genome 36:852–862 (1993).

Hewitt GM: Grasshoppers and Crickets (Gebrüder-Borntraeger, Berlin 1979).

Huang X, Hu J, Hu X, Zhang C, Zhang L, et al: Cytogenetic characterization of the bay scallop, *Argopecten irradians irradians*, by multiple staining techniques and fluorescence in situ hybridization. Genes Genet Syst 82:257–263 (2007).

Insua A, López-Piñón MJ, Freire R, Méndez J: Karyotype and chromosomal location of 18S–28S and 5S ribosomal DNA in the scallops *Pecten maximus* and *Mimachlamys varia* (Bivalvia: Pectinidae). Genetica 126: 291–301 (2006).

Liana A: Matériaux pour la connaissance des Proscopiidae (Orthoptera). Mitteilungen Hamburg Zoolog Museum Inst 77:229–260 (1980).

López-Piñón MJ, Insua A, Méndez J: Chromosome analysis and mapping of ribosomal genes by one- and two-color fluorescent in situ hybridization in *Hinnites distortus* (Bivalvia: Petinidae). J Hered 96:1–7 (2005).

Loreto V, Cabrero J, López-León MD, Camacho JP, Souza MJ: Possible autosomal origin of macho B chromosomes in two grasshopper species. Chromosome Res 16: 233–241 (2008).

Mandrioli M, Colomba MS, Vitturi R: Chromosomal analysis of repeated DNAs in the rainbow wrasse *Coris julis* (Pisces Labridae). Genetica 108:191–195 (2000).

Martins C, Galetti PM Jr: Two rDNA arrays in Neotropical fish species: is it a general rule for fishes? Genetica 111:439–446 (2001).

Matt S, Flook PQ, Rowel CHF: A partial molecular phylogeny of the Eumastacoidea *s. lat.* (Orthoptera, Caelifera). J Orthop Res 17:43–55 (2008).

Mesa A, Ferreira A: Have the Australian Morambinae and the neotropical Proscopiidae evolved from a common ancestor? A cytological point of view (Orthoptera, Caelifera, Eumastacoidea). Acrida 10:205–217 (1981).

Moura RC, Souza MJ, Tashiro T: Cytogenetics characterization of the genera *Scleratoscopia* and *Tetanorhyncus* (Orthoptera, Proscopiidae). Cytologia 61:169–178 (1996).

Otte D, Eades DC, Naskrecki P: Orthoptera Species File Online (Version 2.1). http://osf2.orthoptera.org (2003).

Pendás AM, Morán P, García-Vázquez E: Organization and chromosomal location of the major histone cluster in brown trout, Atlantic salmon and rainbow trout. Chromosoma 103:147–152 (1994).

Ranz JM, González J, Casals F, Ruiz A: Low occurrence of gene transposition events during the evolution of the genus *Drosophila*. Evolution 57:1325–1335 (2003).

Schienman JE, Lozovskaya ER, Strausbaugh LD: *Drosophila virilis* has atypical kinds and arrangements of histone repeats. Chromosoma 107:529–539 (1998).

Sola L, De Innocentiis S, Gornung E, Papalia S, Rossi AR, et al: Cytogenetic analysis of *Epinephelus marginatus* (Pisces: Serranidae), with the chromosome localization of the 18S and 5S rRNA genes and of the (TTAGGG)n telomeric sequence. Marine Biol 137:47–51 (2000).

Souza MJ, Moura RC: Karyotypic characterization and constitutive heterochromatin in the grasshopper *Stiphra robusta* (Orthoptera, Proscopiidae). Cytobios 101:137–144 (2000).

Vitturi R, Colomba M, Mandrioli M, Pirrone AM: rDNA (18S–28S and 5S) co-localization and linkage between ribosomal genes and (TTAGGG)n telomeric sequence in the earthworm *Octodrilus complanatus* (Annelida: Oligochaeta: Lumbricidae) revealed by single- and double-colour FISH. J Hered 93: 279–282 (2002).

Wang Y, Guo X: Chromosomal rearrangement in Pectinidae revealed by rRNA loci and implications for bivalve evolution. Biol Bull 207:247–256 (2004).

Zhang L, Bao Z, Wang S, Huang X, Hu J: Chromosome rearrangements in Pectinidae (Bivalvia: Pteriomorphia) implied based on chromosomal localization of histone H3 gene in four scallops. Genetica 130:193–198 (2007).

CGR317476.indd 5 13.07.2010 16:20:57