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Distribution of the Major rDNA Loci among Four Hemipteran Species of the Family Tingidae (Heteroptera, Cimicomorpha)

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Male karyotypes of four lace bug species, *Agramma fallax* (Horváth, 1906), *Copium teucrii* (Host, 1788), *Galeatus sinuatus* (Herrich-Schaeffer, 1838) and *Dictyla humuli* (Fabricius, 1794), were analyzed for the first time using both conventional chromosome staining and FISH with 18S rDNA and telomeric $(TTAGG)_n$ probes. All species show the same karyotype 2n = 12 + XY but differ in the number and distribution of major rDNA loci. No TTAGG signals were detected.

Key words: Karyotype, FISH, rDNA, $(TTAGG)_n$, lace bugs, Heteroptera.

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In recent years, the methods of molecular cytogenetics have become firmly established in the practice of cytogenetic studies of insects. Fluorescence *in situ* hybridization (FISH) allows determining the position of genes and DNA sequences directly on cytological preparations. This technique is especially important for insects with holokinetic chromosomes, including true bugs (Heteroptera), since the absence of centromeres in these chromosomes hampers distinguishing between individual elements within karyotypes.

One of the most-used FISH markers in bug cytogenetics is the localization of clusters of ribosomal genes (rDNA) in karyotypes. Data on the rDNA site distribution are currently available for more than a hundred Heteroptera species from 12 families of the infraorders Nepomorpha, Cimicomorpha and Pentatomomorpha (PANZERA *et al.* 2012, 2015; PITA *et al*. 2013; POGGIO *et al*. 2014; GOLUB *et al.* 2015, 2016; BARDELLA *et al*. 2016; ANGUS *et al*. 2017; SALANITRO *et al*. 2017; for other references see GROZEVA *et al*. 2015).

Among the 37 Tingidae species studied cytologically so far, almost all show 12 autosomes in a diploid complement; the sex chromosome systems are either of an X(0) or an XY type (UESHIMA 1979). The only exception is *Acalypta parvula* (Fallén, 1807) having, according to different authors, 12 or 10 autosomes in different populations (GOLUB *et al*. 2015). So far, a total of 10 species and 7 genera have been studied by FISH with an 18S rDNA probe (GOLUB *et al.* 2015, 2016). The major rDNA loci, at a rate of only one or two per diploid complement, occur either on sex chromosomes or on one autosomal pair. Within the chromosome, ribosomal clusters are located subterminally or interstitially. In some cases, these differences occur between closely related species sharing the same karyotype. Examples can be found in the genera *Tingis* Fabricius, 1803 and *Dictyla* Stål, 1874 (GOLUB *et al.* 2015, 2016).

Here, four lace bug species, namely, *Agramma fallax* (Horváth, 1906), *Copium teucrii* (Host, 1788), *Galeatus sinuatus* (Herrich-Schaeffer, 1838) and *Dictyla humuli* (Fabricius, 1794), were studied for the first time using both conventional chromosome staining and FISH with an 18S rDNA probe. In addition, the species were checked

against the $(TTAGG)_n$ telomeric sequence. This sequence is of widespread occurrence in different insect groups (SAHARA *et al*. 1999; FRYDRYCHOVÁ *et al*. 2004; VITKOVÁ *et al*. 2005); however it has not been found in any of the lace bug species studied so far in this respect (GOLUB *et al.* 2015). The aim of our study was to enrich chromosome markers and gain a deeper understanding of the cytogenetics of the lace bug family Tingidae.

Material and Methods

The material studied is presented in Table 1.

Chromosomal preparations were obtained from young males fixed in a mixture of 96% ethanol and glacial acetic acid (3:1). Permanent slides were made using a dry ice quick-freezing technique (CONGER & FAIRCHILD 1953). Chromosome preparations were stained using a Feulgen-Giemsa method by GROZEVA and NOKKALA (1996) to study the chromosome number and karyotype structure. FISH with 18S rDNA and $(TTAGG)_n$ telomeric probes was carried out according to GROZEVA *et al.* (2015) with some modifications described in GOLUB *et al.* (2015, 2016). As a positive control for the $(TTAGG)_n$ telomeric probe, several species of Psylloidea (Hemiptera, Homoptera) known to be $(TTAGG)_n$ -positive (unpublished data) were used. Chromosome slides were analyzed under a Leica DM 6000 B microscope. Images were taken with a Leica DFC 345 FX camera using Leica Application Suite 3.7 software with an Image Overlay module.

Results

Cytogenetic analyses were carried out on 37 male specimens of *Agramma fallax*, *Copium teucrii*, *Galeatus sinuatus* and *Dictyla humuli*. As seen in Figure 1a-h representing nuclei at diakinesis/metaphase I transition (a, e, f, g, h) and those at metaphase I (b-d), all four species exhibited the same chromosome number of 2n = 14 constituted by 6 autosomal pairs plus the XY sex chromosomes in males. In each karyotype, the bivalents are of similar size and indistinguishable from each other; the sex chromosomes differ slightly in size. At diakinesis, X and Y chromosomes are separated; however they form a pseudo-bivalent at MI. The MI plates, when observed from the pole, are radial with the XY-pseudo-bivalent lying at the center of the ring formed by autosome bivalents (Fig. 1d).

FISH with the 18S rDNA probe mapped major ribosomal clusters on both sex chromosomes (X and Y) in *A. fallax* (Fig. 1b), with hybridization signal much more intense (multiple) in one of them. In the remaining three species, the ribosomal signals were located on one autosomal bivalent (Fig. 1d, f, h). As seen in Figure 1f and h (diakinesis), the signals are positioned interstitially on homologues in *G. sinuatus* and *D. humuli.* The $(TTAGG)_n$ telomeric probe did not produce fluorescent signals in any of the studied species.

Discussion

All data on *A. fallax*, *C. teucrii*, *G. sinuatus* and *D. humuli* were obtained here for the first time. Moreover, the chromosome information on *G. sinuatus* is the first for the genus *Galeatus* Curtis, 1833 in general. In the literature, there are data on karyotype (2n = 12 + XY) of *Dictyla convergens* (Herrich-Schaeffer, 1835) erroneously identified as *Monanthia humuli* (non Fabricius, 1794) by SOUTHWOOD & LESTON (1959) and as *D. humuli* by UESHIMA (1979) (see DRAKE & RUHOFF 1960 for nomenclature of these taxa).

All the species studied in the present paper have karyotype 2n = 14 (12 + XY); the autosomes are of similar size while the sex chromosomes differ slightly in size. The longer chromosome is suggested to be the X and the smaller one the Y. Such karyotype structure is most characteristic of the family Tingidae, being found in the overwhelming majority of studied species (UESHIMA 1979; GROZEVA & NOKKALA 2001; GOLUB *et al.* 2015, 2016). Despite maintaining the same karyotypes (2n = 12 + XY while occasionally 2n = 12 + X) and similar karyotype structure, the lace bug species

Table 1

Material used for chromosome analysis

Species	Number of males/Giemsa cells /FISH cells examined	Host plant, date and locality of collection
Agramma fallax (Horváth, 1906)	10/112/50	*Juncus* sp., August, 2016, Voronezh region, Russia
Copium teucrii (Host, 1788)	8/62/69	*Teucrium polium* L., August, 2016, Voronezh region, Russia
Galeatus sinuatus (Herrich-Schaeffer, 1838)	3/48/31	*Salvia* sp., July, 2016, Voronezh region, Russia
Dictyla humuli (Fabricius, 1794)	16/101/56	*Symphytum officinale* L., June, July 2016, Teberda Nature Reserve, North Caucasus, Russia

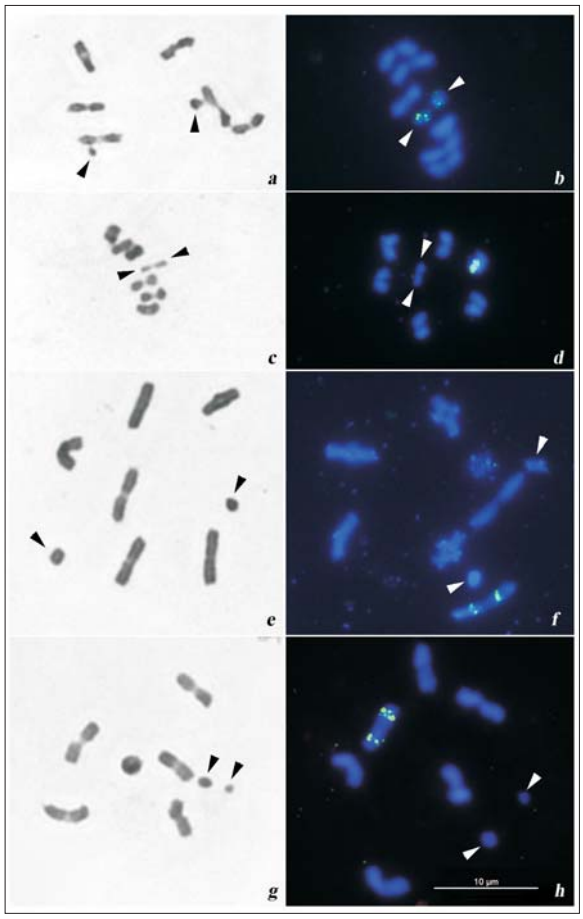

Fig. 1 a-h. Meiotic karyotypes after Giemsa staining (left column) and FISH with 18S rDNA probe (right column; green signals). a, b – *Agramma fallax*, diakinesis/MI transition (a) and MI (b); c, d – *Copium teucrii*, MI, side view (c) and polar view (d); e, f – *Galeatus sinuatus*, diakinesis/MI transition; g, h – *Dictyla humuli*, diakinesis/MI transition. Arrowheads show sex chromosomes lying separately during diakinesis/MI transition or forming a pseudo-bivalent at MI. Bar = 10 µm.

differ in major rDNA loci number and location, with differences even between congeneric species (GOLUB *et al.* 2015, 2016). Here, we added evidence for the genus *Agramma* Stephens, 1829 in which *A. fallax* shows rDNA clusters on both sex chromosomes (present study), whereas *A. femorale* Thomson, 1871 on the X chromosome only (GOLUB *et al.* 2015). In the three remaining species, namely, *Copium teucrii*, *Galeatus sinuatus* and *Dictyla humuli*, rDNA clusters were observed on an autosomal bivalent. Because of the similar size of the bivalents, it was impossible to distinguish whether the rDNA bivalent is the same or not in different species. In contrast to the genera *Copium* Thunberg, 1822 and *Galeatus*, which have never been studied by FISH, *D. humuli* is the third species of the genus studied in respect of rDNA. Based on our earlier data, *D. echii* (Schrank, 1782) has major rDNA clusters on both sex chromosomes (GOLUB *et al.* 2015) while *D. rotundata* (Herrich-Schaeffer, 1835) on an autosomal bivalent (GOLUB *et al.* 2016) as does *D. humuli*. On the basis of present knowledge, we can conclude that there are at least three rDNA distribution patterns in Tingidae: on one sex chromosome, on both sex chromosomes and on one autosomal pair. This variation was observed among species belonging to the same genus as in *Agramma* and *Dictyla* and also in *Tingis* (GOLUB *et al.* 2016). The variation in rDNA location between closely related species should reflect, to some degree, genome rearrangements involved in their divergence.

The chromosomal location of rDNA clusters can also be different in different lace bug species, the clusters being either at the sub-terminal or interstitial position on a chromosome. With autosomes, the interstitial location seems to be dominant (GOLUB *et al.* 2016; present paper), whereas sex chromosomes appear more variable in this respect. For example, the X chromosome shows multiple clusters in *Agramma fallax* (present study) and *A. femorale* (GOLUB *et al.* 2015), while only one interstitial cluster in *Tingis crispata* (Herrich-Schaeffer, 1838) (GOLUB *et al.* 2015) and one terminal cluster in *Dictyla echii* (GOLUB *et al.* 2016). Whether the rDNA loci tend to be located terminally or interstitially on chromosomes of lace bugs (shortly discussed in GOLUB *et al.* 2016) remains an open question for more in-depth studies.

The variation of ribosomal patterns between closely related species with the same chromosome number is also known in other genera of true bugs, e.g., *Deraeocoris* Kirschbaum, 1856 of the family Miridae (GROZEVA *et al*. 2011) as well as *Rhodnius* Stål, 1859 and *Triatoma* Laporte, 1882 of the family Reduviidae (BARDELLA *et al*. 2010; PANZERA *et al.* 2012, 2015; PITA *et al*. 2013). This fact clearly reflects that holokinetic chromosomes undergo important genomic reorganizations without altering the number of autosomes.

In common with previously studied lace bug species (GOLUB *et al*. 2015), no species from the present study revealed the presence of the $(TTAGG)_n$ telomeric sequence. It may be suggested that this group lacks the canonical "insect" telomeric motif considered to be ancestral for Insecta and Arthropoda as a whole (SAHARA *et al.* 1999). It is worthy of note that quite recently there was an opinion that the $(TTAGG)_n$ motif is lacking from the Heteroptera in general (FRYDRYCHOVÁ *et al*. 2004; GROZEVA *et al*. 2011). However, more recently this sequence was detected in several unrelated Heteroptera taxa such as Coleorrhyncha (KUZNETSOVA *et al.* 2015) as well as the families Belostomatidae (KUZNETSOVA *et al.* 2012; CHIRINO *et al.* 2017) and Nepidae (ANGUS *et al*. 2017) from one of the basal Infraorders Nepomorpha. Moreover, the assumed ancestral insect $(TTAGG)_n$ repeat was just

revealed in the evolutionarily young family Reduviidae, the subfamily Triatominae (PITA *et al.* 2016) from the evolutionarily advanced infraorder Cimicomorpha to which Tingidae belong as well.

In summary, we should note that more work is needed to elucidate the organization of telomeres in different true bug groups including the lace bug family Tingidae.

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