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Cytogenetic variability of the genus *Saga* Charp. (Orthoptera, Tettigoniidae, Saginae): heterochromatin differentiation

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Abstract — Chromosome complements of *Saga hellenica*, *S. natoliae*, and *S. rhodiensis* consist of 2n = 28 + XO in males and 2n = 28 + XX in females (FN = 32 and 34, respectively). Robertsonian fusions and tandem fusion play the most important role in the evolution of the chromosome set in Saginae. The chromosomes of three species of Saginae were analyzed by four different staining methods: C-banding, Ag-NOR, DAPI and CMA_3. The studied species show a uniform karyotype with differences in constitutive heterochromatin content. C-banding patterns plus fluorochrome staining shows differences between paracentomeric, interstitial, and telomeric segments, as well as the heterochromatin associated with NORs.

Key words: cytogenetic, heterochromatin, Orthoptera, *Saga*.

INTRODUCTION

The subfamily Saginae is one of the most ancient of the recent groups within the Tettigonioidea. Its four genera occur in two well separated areas, in Ethiopian Africa and in the western Palaearctic. From the latter region only the genus *Saga* Charpentier with 13 species is known (Kaltenbach 1990). Some of the large, predatory species of this genus are found over quite large areas, by far the most wide-spread being the parthenogenetic species *Saga pedo*.

Up to now, the diploid number, chromosome morphology, and the type of sex determination of Saginae were described for five species of the genus *Saga* – *S. cappadocica* (Matthey 1950), *S. ephippigera* (Matthey 1946; 1948; Goldschmidt 1946), *S. hellenica* (Warchałowska-Śliwa 1998), *S. ornata* (as *S. gracilipes*; Matthey 1946; 1948; Goldschmidt 1946) and *S. pedo* (Matthey 1939; 1941; Goldschmidt 1946). Hitherto, the chromosome banding patterns and heterochromatin distribution in this genus were not described. At the chromosomal level, heterochromatin differentiation resulting in karyotype evolution is a useful tool for the cytotaxonomy of many groups of animals (e.g. Imai *et al.* 1977; Baimai 1998). In Orthoptera one of the most important differences in chromosomes involves changes in the amount and localization of constitutive heterochromatin, a characteristic pattern in the karyotype (Camacho *et al.* 1987; Warchałowska-Śliwa *et al.* 2005). Other cytological markers, beside classical staining such as C-bands and Ag-NOR, connected with fluorescent DNA banding dyes of different specificity, have been performed in order to obtain the nucleotide composition of heterochromatin regions.

The present paper is concerned with a detailed karyotype analyses of three species of the genus *Saga*: *S. hellenica*, *S. natoliae*, and *S. rhodiensis* and a description of constitutive heterochromatin regions using four staining methods: C-banding, Ag-NOR, as well as fluorochrome AT (DAPI) and GC specific staining (CMA_3). It is provided in order to obtain more information on the genome organization of tettigoniid species.

MATERIAL AND METHODS

For the cytogenetic studies, the following adult males and females were collected in Greece, Bulgaria, and Turkey: *Saga hellenica* Kaltenbach,

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1967, CH4514, CH4515, 1 male, 1 female, Greece: Ilia Peloponnes, valley of Erimanthos, 6 km east of Koumanis (37°48'N, 21°47'E), 1-30 VI 1997, coll. K.-G. Heller; 1 male, Greece: Kerkyra, Palaeochorio (=Palies Sinies) below Mt. Pantokrator (39°44'N, 19°52'E), 15 VII 2004, coll. K.-G. Heller; *Saga natoliae* Serville, 1839, 1 male, Bulgaria: Rupite near Petricz (41°25'N, 23°2'E 28 vi 2001, coll. E. Warchałowska-Śliwa, D. Chobanov; *Saga rhodiensis* Salfi, 1929, CH5392, 1 male, Turkey: Antalya, road to Saklikent, above Doyran (36°53'N, 30°29'E), 26 VI 2002, coll. K.-G. Heller. Specimens from Greece and Turkey are deposited in Collectio Heller (CH + reference number), specimens from Bulgaria are stored in the Institute of Systematics and Evolution of Animals PAS (Kraków).

The testes and ovarioles were excised, incubated in a hypotonic solution (0.9% sodium citrate), and then fixed in ethanol:acetic acid (3:1). The fixed material was squashed in 45% acetic acid. Cover slips were removed by the dry ice procedure and then slides were air dried. The C-banding examination was carried out according to SUMNER (1972) with slight modification. The silver staining method for NORs was performed as previously reported (WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1992). In order to reveal the molecular composition of C-heterochromatin, some of slides were stained with chromomycin A_3 (CMA_3) for GC-pairs of nucleotides and 4-6-diamidino-2-phenylindole (DAPI) for AT-specific pairs (SCHWEIZER 1976). Chromosome and fluorochrome labelled slides were analyzed using a Nikon Eclipse 400 light microscope with a CCD DS-U1 camera using the software Lucia Image 5.0. The fixed material is deposited in the Institute of Systematics and Evolution of Animals PAS (Kraków).

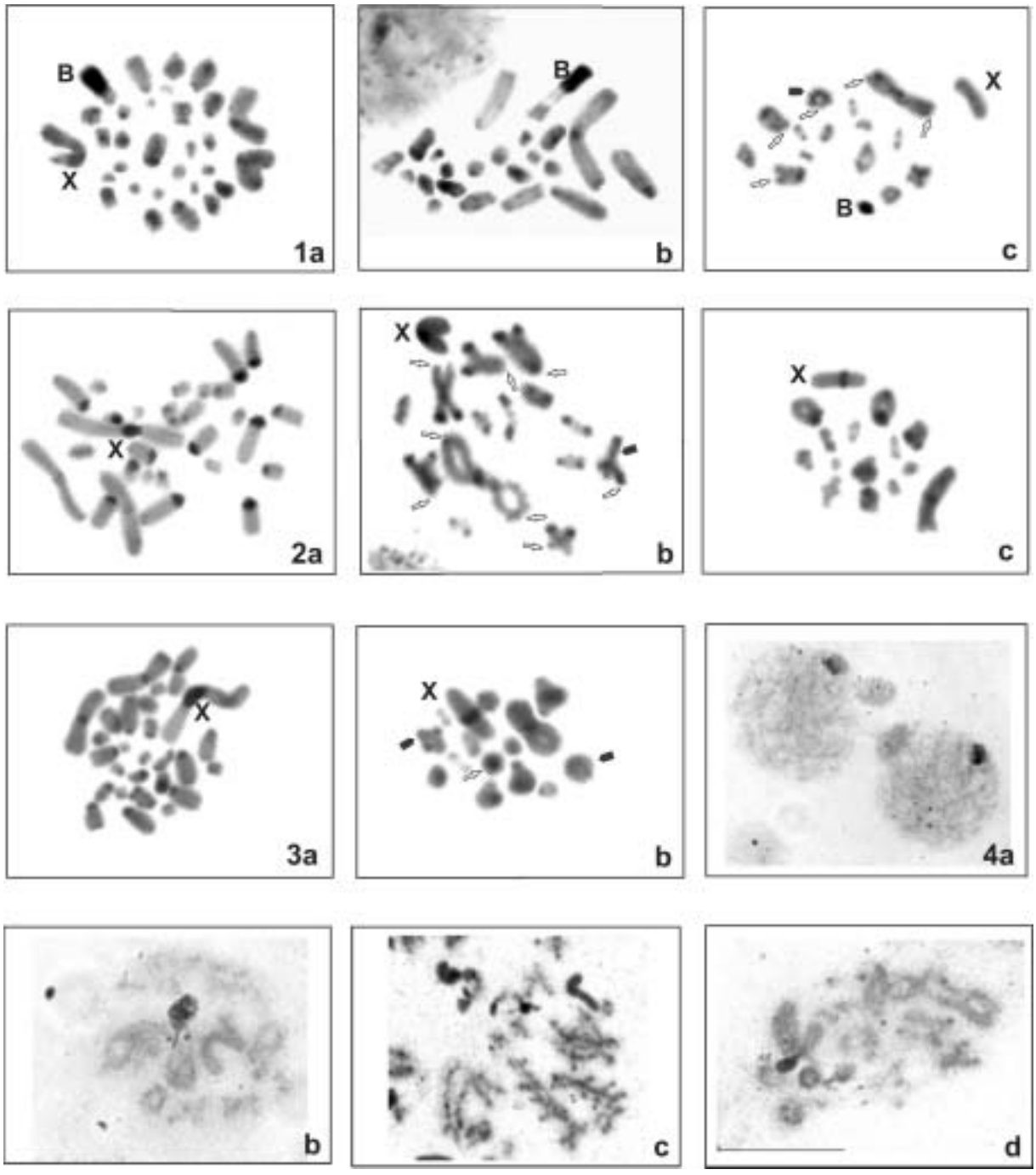
RESULTS

Saga hellenica, S. natoliae, and *S. rhodiensis* are characterized by 2n♂ = 29 and 2n♀ = 30 (FN = 32 and 34, respectively). Autosomes can be divided into three size groups: one large metacentric (L_1), seven medium (M_2-M_8), and six small acrocentric pairs (S_9-S_{14}). The submetacentric X chromosome is the largest in the set. All species show the XO♂ and XX♀ type of chromosome sex determination. In two males of *S. hellenica* a single, mitotically unstable B chromosome was detected. This supernumerary chromosome was a large element, smaller only than the first metacentric pair. At mitotic metaphase, the B chromosome consists of a short segment of euchromatin in the paracentromeric region and a heterochromatic segment twice as longer as the former. The proportion of euchromatin and heterochromatin depends on condensation of this chromosome in particular stages of division (Fig. 1a-c).

C-banding patterns - C-heterochromatin content and its distribution are shown in Table 1 and Figs 1-3. Paracentromeric C-bands are present in all autosomes and in the X chromosome of three examined species. These bands in most of chromosomes of *S. hellenica* are restricted to the centromeric region (thin C-bands), with the exception of two medium pairs (M_2, M_3) (Fig. 1a-c). On the other hand, in *S. natoliae* and *S. rhodiensis* C-bands occupy the region next to the centromere (thick C-bands) in most of autosomes and in the X chromosome. It is worth mentioning that the X chromosome differs in the amount of paracentromeric C-bands, in *S. rhodiensis* this band is larger than that of *S. natoliae* (Figs 2a-c, 3a,b). The $M_{8/9}$ chromosome in *S. natoliae* and *S. rhodiensis* possesses a paracentromeric band varying in size (Figs 2a-c, 3a,b). The distribution of interstitial and telomeric C-bands are found to vary among species (Table 1). When interstitial C-bands are present, they are always located in medium pairs, whereas telomeric ones are found in large and medium sized chromosomes (Figs 1-3).

Table 1 — A comparison of the results obtained in the heterochromatin of three species of *Saga*.

species	C-banding	NOR	bright DAPI -	CMA_3
S. hellenica	Paracentromeric: L_1, M_4-S_{14}, X thin; M_2 and M_3 thick M_6 interstitial L_1, M_2, M_3 M_6 telomeric	M_3	L_1, M_2, M_6, B	L_1, M_2, M_3
S. natoliae	Paracentromeric: L_1, S_{11}-S_{14} thin; M_2-S_{10}, X thick M_6 interstitial L_1, M_2 – M_8 telomeric	$M_{8/9}$	$M_{8/9}$ X	$M_{8/9}$, X
S. rhodiensis	Paracentromeric: most of autosomes and X thick M_5, M_6 interstitial L_1, M_2 – M_5 telomeric	M_6	$M_{8/9}$, X	M_6, X



Figs 1-4 — C-banded karyotypes of males. **Fig. 1**. *Saga hellenica*. Mitotic metaphase (a), (b – not complete), and diakinesis (c) showing the B chromosome (B) with different proportion of euchromatic / heterochromatic parts. Arrowhead indicates interstitial, whereas arrows telomeric C-bands. The X chromosome (X) with thin paracentromeric C-bands. **Fig 2.** *S. natoliae*. C-banded mitotic chromosomes (a), diakinesis (b), and metaphase I (c) showing large blocks of heterochromatin in paracentromeric regions in most of the chromosomes, and thin C-bands of interstitial (arrowhead) and distal regions (arrows). Note the X chromosome with a thick paracentromeric C-block. **Fig. 3**. *S. rhodiensis*. Mitotic metaphase (a) and metaphase I (b), X chromosome with a thick paracentromeric C-block is larger than that of *S. natoliae*. Arrowheads indicate interstitial C-bands in two medium pairs. Bivalent heterozygous for size of paracentromeric C-bands (arrows). **Fig. 4.** Silver-stained early prophase (a) and diplotene cells of.S. *hellenica* (b), *S. natoliae* (c), and *S. rhodiensis* (d) showing one active NOR with a different location.

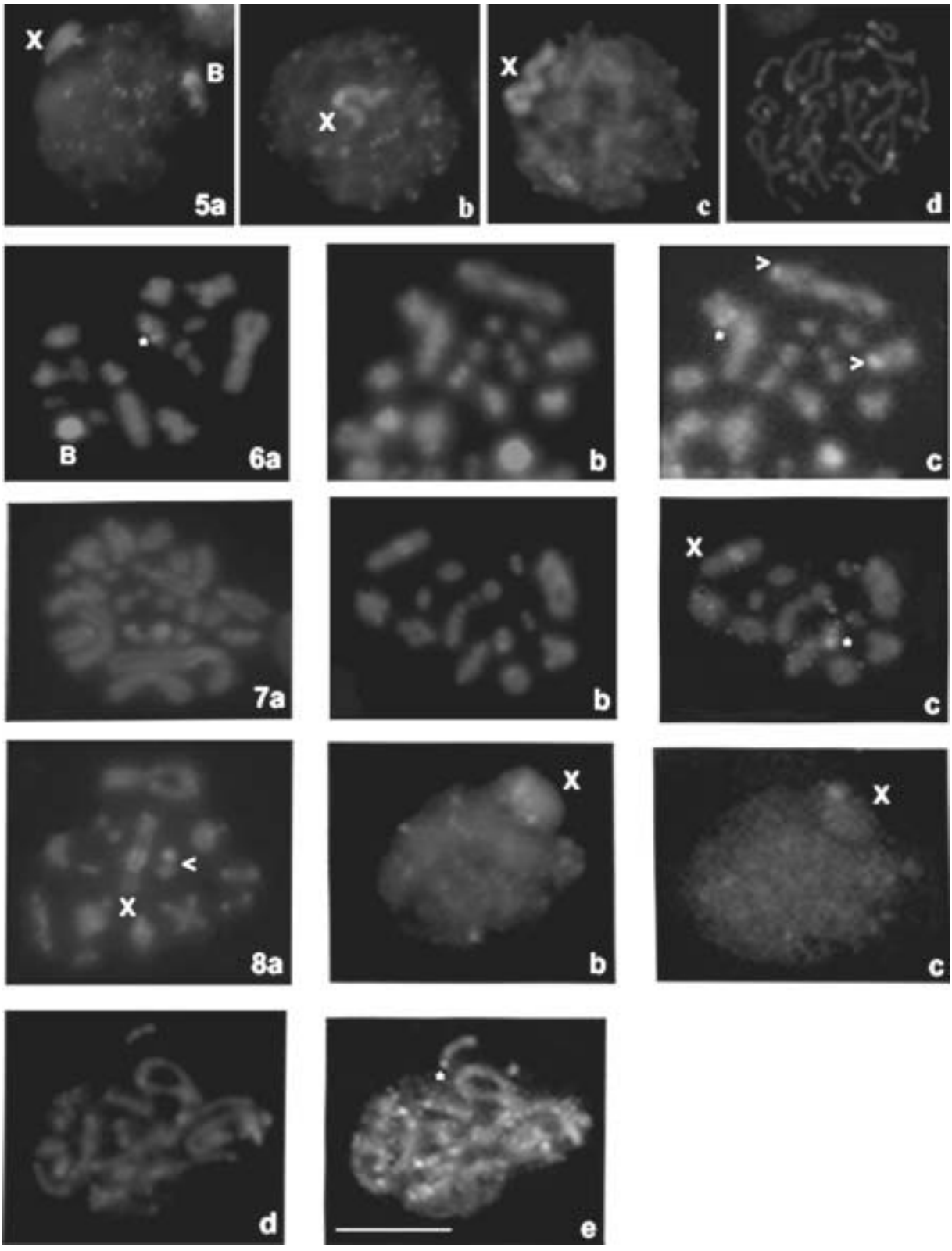
Ag-NORs - At meiotic prophase a single active NOR located on the medium sized bivalents were observed in three species (Table 1) (Fig. 4a-d). In *S. hellenica* the nucleolar remnants appear to be associated with paracentromeric or telomeric regions of the M_3 bivalent. The two other species showed one active NOR located on bivalents $M_{8/}$ S_9 (paracentromeric region) and on M_6 (telomeric region), respectively.

Fluorochrome banding patterns - In all species the paracentromeric, interstitial, and telomeric C-bands in most of the bivalents show weak positive signals after DAPI (Fig. 5a-c). Signals are better resolved in mitotic prophases because differences in fluorescence intensity are associated with the condensation of chromosomes (Fig. 5d). Bright DAPI signals in M_6 of *S. hellenica* are visible in the interstitial region, this band is clearly associated with C-bands (Fig. 6a). Also, the B chromosome present in this species, consisting mostly of heterochromatin, reveals a bright signal after DAPI staining (compare Fig. 1a-c and Fig. 6a,b). The X chromosome in *S. natoliae* and *S. rhodiensis* is marked by bright fluorescence, connected with paracentromeric C-bands. It is clearly visible in mitotic metaphase, meiotic prophase, and metaphase I stage. (Figs 7, 8). $M_{8/9}$ in *S. natoliae* and *S. rhodiensis* is heterozygous for the size of the supernumerary heterochromatin and is an AT-enriched region, as revealed by the bright bands obtained with DAPI (Figs 7a,b; 8a,b). CMA_3 staining produces positive fluorescence in the areas associated with the nucleolar organizer regions in three studied species (Table 1). In *S. hellenica* and *S. rhodiensis* positive NOR-associated areas were found on bivalents M_3 and M_6, whereas this region appears negative after DAPI staining (Figs 6b,c and 8b-e). In contrast, the NOR-associated heterochromatin of the $M_8/_9$ bivalent in *S. natoliae* is formed by two proximate regions of different structure: one band positive after DAPI and the second positive after CMA_3 (Fig. 7b,c). Two bivalents, L_1 and M_2 of *S. hellenica,* reveal distal/interstitial CMA_3 bands (Fig. 6c). CMA_3 staining of the X chromosome condensation stage in *S. natoliae* and *S. rhodiensis* produced a bright signal in the paracentromere region. At this stage bright fluorescence in the same site is observed after DAPI staining (Figs 7b,c and 8b,c). However, in early meiotic prophase, a CMA_3 positive signal is seen on one small part of the centromeric region. (Fig. 8b,c)

DISCUSSION

Most species of the Palaearctic tettigoniids of subfamilies Tettigoniinae and Phaneropterinae have 2n = 31♂ % chromosomes, all of them acrocentric (FN = 31). This karyotype was suggested as ancestral for most species of Tettigoniidae (e.g. HEWITT 1979; WARCHAŁOWSKA-ŚLIWA 1998; WARCHAŁOWSKA-SLIWA *et al*. 2005). According to an earlier study some species of the genus *Saga* are also characterized by this karyotype (2n = 31♂), i.e. *S. cappadocica* (MATTHEY 1950), and *S. ornata* (MATTHEY 1946). The three species studied here differ in chromosome number from this ancestral pattern. They have one large metacentric autosome and a submetacentric X chromosome. This karyotype may be formed as a result of one Robertsonian translocation and a pericentric inversion in the X chromosome, and a reduced chromosome number of 2n = 29♂. These characteristics may be considered as synapomorphies indicating a close phylogenetic relationship. This assumption, however, is not supported by the morphological similarity used by KALTENBACH (1967) to define several subgroups within the genus *Saga*. According to his ideas, the six species studied

Figs. 5- 8 — Fluorochrome banding. **Fig. 5**. Early meiotic prophase of S. *hellenica* (a), *S. natoliae* (b), *S. rhodiensis* (c), and early mitotic metaphase of *S. natoliae* (d) stained with DAPI. Paracentromeric heterochromatin (a-d), part of the B chromosome (a), and paracentromeric C-blocks of the X chromosome (a, b, c) differ in the amount of heterochromatin and appear bright (X and B indicated). **Fig. 6**. Diakinesis of *S. hellenica* after DAPI (a, b) and CMA_3 staining (c). Note that the DAPI bright signals in M_6 are visible in the interstitial region associated with interstitial C-bands (a) (star).The areas associated with the NOR showed a negative segment on bivalents M_3 with DAPI (b), whereas this region appears positive with CMA_3 (c) (star). Two bivalents, L_1 and M_2 possess distal/interstitial CMA_3 bands (c) (with ">"). **Fig. 7.** Mitotic metaphase (a) and diakinesis (b, c) of *S. natoliae.* The heterochromatin associated with the NOR in pair $M_{8/9}$ shows a weak response after DAPI (a, b) and a strong signal with CMA_3 (c) (star). Note the presence of a bright fluorescent paracentromeric band in the X chromosome (b, c). **Fig. 8**. DAPI and CMA_3 of *S. rhodiensis*. Diakinesis after DAPI staining (a). One small bivalent ($M_{8/9}$) heterozygous for size of supernumerary heterochromatin (with "*S. natoliae* (compare with Fig. 7b). In early meiotic prophase, the X chromosome shows a positive signal after DAPI (b) and CMA_3 staining (c) at different sites in the paracentromeric region. Prometaphase (d, e). Note that the NOR is associated with a bright fluorescent band (e) (star).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karyologically should be arranged into three groups: A) *S. natoliae*, *S. rhodiensis*, *S. ephippigera*, B) *S. hellenica*, *S. cappadocica*, C) *S. ornata*. On the other hand, the karyological data coincide quite well with the song pattern of these species. In *Saga,* as in nearly all tettigoniids, acoustic communication is an essential part of the mating system. *S. natoliae*, *S. rhodiensis*, and *S. hellenica* possess a song consisting of uninterrupted sequences of syllables with a duration of a few seconds (HELLER 1988), while *S. cappadocica* and *S. ephippigera* present distinctly different patterns. A close relationship of the first three species is also supported by their strictly allopatric distribution (see KALTENBACH 1967; WILLEMSE 1984) around the Aegean sea. The tetraploid, parthenogenetic species *Saga pedo* (4n = 68, n = 17; MATTHEY 1948; GOLDSCHMIDT 1946) is assumed to have evolved between the Black and Caspian seas (KALTENBACH 1967). Its ancestor may be related to *S. ephippigera*, the species with the highest chromosome number (one male possessed a supernumerary pair of chromosomes - 2n = 33) of all sexually reproducing species and the only *Saga* species occurring directly south of the Caucasian region.

An interesting feature of the *S. hellenica* karyotype is the occurrence of a supernumerary chromosome in two males. The large B chromosome, acrocentric and almost heterochromatic, was mitotically and meiotically unstable. The occurrence of B chromosomes has been previously noted in a few species of tettigoniids (e.g. BATTAGLIA 1964; JOHN & LEVIS 1968; WHITE 1973; HEWITT 1979; CAMACHO *et al.* 1981; WARCHAŁOWSKA-ŚLIWA *et al.* 1992). Different hypotheses have been proposed to explain the origin of the B chromosomes in natural populations of Orthoptera, and various mechanisms of B chromosome formation have been suggested. They could have been derived from autosomes through their fragmentation, degradation or DNA amplification in paracentromeric regions, or from polysomic autosomes (JONES & REES 1982; PATTON 1977). However, an explanation for the origin of the B chromosome in *S. hellenica* at the moment is unfeasible and requires further detailed cytogenetic studies.

In Tettigoniidae heterochromatin analyses using the C-banding technique and NOR Ag-staining were used in comparative studies of species belonging to the same genus (e.g. WARCHAŁOWSKA-ŚLIWA *et al.* 1994; 1995; 2000; 2005; WARCHAŁOWSKA-ŚLIWA & HELLER 1998). There are many examples in Orthoptera of species from the same genus with very similar karyotypes that differ in the amount of heterochromatin**.** Distribution of interstitial and telomeric C-bands in autosomes and the X chromosomes are usually found to vary among species of one genus. Many authors consider that C-banding patterns may change quickly as the result of a high degree of variation of constitutive heterochromatin (MORESCALCHI 1977; CABRERO & CAMACHO 1986; SANTOS *et al.* 1983). Thus, in the opinion of some investigators, analysis of C-heterochromatin does not provide a clear relationship of taxonomic proximity (e.g. SANTOS *et al.* 1983). On the other hand, qualitative and quantitative variation for C-bands were observed in some tettigoniid species of genera *Metrioptera, Eupholidoptera* (WARCHAŁOWSKA-ŚLIWA *et al.* 2005), and *Montana* (WARCHAŁOWSKA-ŚLIWA *et al.* 1994). Considerable C-band differences between pamphagid species of the genus *Eumigus* paralleled differences in penis morphology (CABRERO *et al.* 1985). When C-bands are compared between species of *Saga*, discrete differences between three species were found, revealing that *S. natoliae* and *S. rhodiensis* possess a more similar C-banding pattern and amount of heterochromatin than *S. hellenica*, confirming the close relationship of the former two species (KALTENBACH 1967).

The Ag-staining of the NOR is one of the methods used for demonstrating the position of the gene complex at 18S and 28S ribosomal DNA in the chromosome set (GOODPASTURE & BLOOM 1975). When chromosome NORs are compared among species of the same genus they are usually more stable than C-bands and usually located on the same bivalent (CAMACHO *et al.* 1987, WARCHAŁOWSKA-ŚLIWA *et al.* 1992, 2005). However, the three species of the genus *Saga* described in this paper are characterized by possessing NORs in different chromosomes, thus making it a good marker to analyse relationships between species of this group.

Three species of the genus *Saga* revealed discrete differences not only in the amount of heterochromatin in their genomes, but also in the number of heterochromatin bands and their location. In these species C-banding patterns and fluorochrome staining showed differences between paracentomeric, interstitial, and telomeric segments, as well as the heterochromatin associated with the NORs (Table 1). The use of fluorescent DNA-banding dyes refines the characterization of heterochromatin regions in terms of their relative enrichment with AT- or GC- base pairs (SCHWEIZER 1976). At least some categories of constitutive heterochromatin can be distinguished in the genus *Saga* in this way. The paracentro-

meric, interstitial, and telomeric C-bands (differing in the amount of heterochromatin between species) displayed A-T specific DAPI fluorescence in most of the autosomes, while the G-C specific CMA_3 produced a differential negative response in the form of non-staining parts. On the other hand, the use of fluorochrome banding showed the same heterogeneity among similar C-banding regions. The heterochromatin associated with NORs seems be different between species of *Saga*. In the two species *S. hellenica* and S. *rhodiensis,* this heterochromatin contains G-C base pairs, while that associated with the NOR in pair $M_{8/9}$ in *S. natoliae* shows a weak response after DAPI and a strong signal with CMA_3. The CMA_3 staining produced positive fluorescence in the telomeric region of chromosomes L_1, M_2 in *S. hellenica*, but this heterochromatin is probably not connected with NORs. The results obtained for the X chromosomes of *S. natoliae* and *S. rhodiensis* are particularly interesting. The paracentromeric heterochromatin shows an equal, but different in size, response after DAPI and CMA_3 marked by C-banding. These results demonstrate the existence of variable levels of interspersed A-T and G-C base pairs in different regions of the chromosomes of these species. Different categories of heterochromatin regions have also been found in some grasshoppers (e.g. BELLA & SORRANO 1993; RODRIQUEZ INIGO *et al*. 1993).

In summary, the techniques applied in the present study show some similarities and differences between constitutive heterochromatin within three species of the genus *Saga*. These techniques show that taxonomically closely related species with the same chromosome number do not display identical C-bands pattern due to accumulation of some rearrangements since divergence from their common ancestor. Karyotypic differences between *S. hellenica, S. natoliae*, and *S. rhodiensis* show that chromosomal divergence occurred during their speciation. Further studies of the other species of this genus are necessary for a better understanding of the karyotype evolution of this group, also the employment of molecular techniques will provide additional information on the evolution of this genome.

REFERENCES

BAIMAI V., 1998 — *Heterochromatin accumulation and karyotypic evolution in some dipteran insects.* Zoological Studies, 37: 75-88.

BATTAGLIA E., 1964 — *Cytogenetics of B-chromosomes*. Caryologia**,** 17: 245-299.

BELLA J. L. and SERRANO L., 1993 — *Heterochromatin heterogeneity and rapid divergence of the sex chromosomes in Chorthippus parallelus parallelus and C. p. erythropus*. Genome, 36: 542-547.

CABRERO J., CAMACHO J. P. M. and PASCUAL F., 1985 — *Cytotaxonomic studies on pamphagids genus Eumigus. Detection of two chromosomal races in E. monticola (Rambur) (Insecta, Orthoptera)*. Caryologia, 38: 1-12.

CABRERO J. and CAMACHO J. P. M., 1986 — *Cytogenetics studies in gomphocerine grasshopper. I. Comparative analysis of chromosome C-banding pattern*. Heredity, 56: 365-372.

CAMACHO J. P. M., OROZCO J. C. and PASCUAL F., 1981 — *Chromosomal rearrangements and karyotypic evolution in Decticinae (Orthoptera: Tettigonioidea)*. Cytologia, 46: 209-215.

CAMACHO J. P. M., PASCUAL F., BELA J. and CABRERO J., 1987 — *Cytological markers for analyzing evolutionary relationship between related species of orthopteroids*. In: Baccetti B. (Ed.) "Evolutionary Biology of Orthopteroid Insects", p.148-156., Ellis Horwood Limited.

GOLDSCHMIDT E., 1946 — *Polyploidy and parthenogenesis in the genus Saga*. Nature,158: 587-588.

GOODPASTURE C. and BLOOM S. E., 1975 — *Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining.* Chromosoma, 53: 37-50.

HELLER K.-G., 1988 — *Bioakustik der europäischen Laubheuschrecken*. Verlag Josef Margraf, Weikersheim, 1–358.

HEWITT G. M., 1979 — *Grasshoppers and crickets. Animal Cytogenetics, 3. Insecta I. Orthoptera*. Borntraeger, Berlin, Stuttgart.

IMAI H. T., CROZIER R. H. and TAYLOR R. W., 1977 — *Karyotype evolution in Australian ants*. Chromosoma, 59: 341-393.

JOHN B. and LEVIS K. R., 1968 — *The chromosome complement.* Protoplasmatologia. Vol. 6. Springer, Vienna, New York.

JONES B. and REES H., 1982 — *B chromosomes*. Academic press, London.

KALTENBACH A., 1967 — *Unterlagen für eine Monographie der Saginae I. Superrevision der Gattung Saga Charpentier (Saltatoria: Tettigoniidae)*. Beitr. Entomol., 17: 3-107.

KALTENBACH A. P., 1990 — *The predatory Saginae*. In: W. J. Bailey, D. C. F. Rentz (Eds.), "The Tettigoniidae, Biology, Systematics and Evolution",,p. 280—302,Springer Verlag, Hong Kong.

MATTHEY R., 1939 — *La formule chromosomiale de la sauterelle.parthenogenetique Saga serrata Fabr. et de l'Ephippigera vitium*. C. R. Soc. Biol. Paris, 132: 369-370.

MATTHEY R., 1941 — *Etude biologique et cytologique de Saga pedo Pallas (Orthoptera- Tettigoniidae)*. Rev. Suisse Zool., 48: 91-102.

MATTHEY R., 1946 — *Demonstration du caractere geographique de la parthenogenese de Saga pedo Pallas et de la polyploidie, par comparaison avec les especies bisexuees S. ephippigera Fisch. et S. gracilipes Uvar*. Experientia, 2: 260-261.

MATTHEY R., 1948 — *A propos de la polyploidie de Saga pedo Pallas (Orthoptera- Tettigoniidae)*. Experientia, 4: 26-27.

MORESCALCHI A., 1977 — *Phylogenetic aspects of karyological evidence*. In: M. K. Hecht, P. C. Goody., B. M. Hecht (Eds), "Major Patterns of Vertebrate Evolution", Plenum Press Corporation, New York.

PATTON J. L., 1977 — *B chromosome systems in the pocket mouse, Perognathus baileyi: meiosis and C-bands studies*. Chromosoma, 60: 1-14.

RODRIGUEZ INIGO E., BELLA J. L. and GARCIA DE LA VEGA C., 1993 — *Heterochromatin differentation between two species of the genus Dociostaurus (Orthoptera: Acrididae)*. Heredity, 70: 458-465.

SANTOS J. R., ARANA P. and GIRALZEZ R., 1983 — *Chromosome C-banding patterns in Spanish Acridoidea*. Genetica, 61: 65-74.

SHWEIZER D., 1976 — *Reverse fluorescent chromosome banding with chromomycin and DAPI*. Chromosoma, 58: 307-324.

SUMNER S. G., 1972 — *A simple technique for demonstrating centromere heterochromatin*. Exp. Cell Res., 75: 304-306.

WARCHAŁOWSKA-ŚLIWA E., 1998 — *Karyotype characteristics of katydid orthopterans (Ensifera, Tettigoniidae) and remarks on their evolution at different taxonomic levels*. Folia biol. (Kraków), 46: 143-176.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A.G. and MARYAŃSKA-NADACHOWSKA A., 1995 — *Karyotypes of three species of the genera Poecilimon Fisch. and Isophya Br.-W. (Orthoptera, Tettigonioidea, Phaneropterinae) from the North Caucasus*. Caryologia, 48: 27-34.

WARCHAŁOWSKA-ŚLIWA E. and HELLER K.-G., 1998 — *C-banding patterns of some species of Phaneropterinae (Orthoptera, Tettigoniidae) of Europe*. Folia biol. (Kraków), 46: 177-181.

WARCHAŁOWSKA-ŚLIWA E., HELLER K.-G., MARYAŃSKA-NADACHOWSKA A. and LEHMANN A., 2000 — *Chromosome evolution in the genus Poecilimon (Orthoptera, Tettigonioidea, Phaneropteridae)*. Folia biol. (Kraków), 48: 127-136.

WARCHAŁOWSKA-ŚLIWA E. and MARYAŃSKA-NADACHOWSKA A., 1992 — *Karyotypes, C-bands, NORs location in spermatogenesis of Isophya brevipennis Brunner (Orthoptera: Phaneropteridae)*. Caryologia, 45: 83-89.

WARCHAŁOWSKA-ŚLIWA E., MARYAŃSKA-NADACHOWSKA A. and BUGROV A.G., 1992 — *Karyotypes, C-heterochromatin, and NOR in three species of the genus Gampsocleis Fieb. (Orthoptera: Tettigonioidea: Decticinae)*. Folia biol. (Kraków), 40: 119-127.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G. and MARYAŃSKA-NADACHOWSKA A., 1994 — *Karyotypes, C-banding pattern, and NORs of the genus Montana Zeuner 1941 (Orthoptera, Tettigonioidea, Decticinae)*. Folia biol. (Kraków), 42: 89-94.

WARCHAŁOWSKA-ŚLIWA E., HELLER K-G. and MARYAŃSKA-NADACHOWSKA A., 2005 —*Cytogenetic variability of european Tettigoniinae (Orthoptera, Tettigoniidae): Karyotypes, C-and Ag-NOR-banding*. Folia biol. (Kraków), 53: 161-171

WHITE M. J. D., 1973 — *Animal Cytology and Evolution*, 3rd ed. Cambridge Univ. Press, London.

WILLEMSE F., 1984 — *Catalogue of the Orthoptera of Greece. Fauna Graeciae* 1: i–xii, p.1–275; Athens (Hellenic Zoological Society).

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