Cytogenetic Variability of European Tettigoniinae (Orthoptera, Tettigoniidae): Karyotypes, C- and Ag-NOR-banding

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Karyotypes, C-banding pattern and localization of nucleolus organizer regions (NORs) in 34 European species and subspecies belonging to the subfamily Tettigoniinae are described (the karyotypes of 26 species for the first time). In the males chromosome numbers vary from 2n=33 to 2n=23 and Fundamental Numbers (FN) from 36 to 27. The highest number of chromosomes for this group, 2n=33 (FN=33), was found in Psorodonotus illyricus macedonicus. In species belonging to genera Decticus, Metrioptera, Anterastes, Bucephaloptera, Parapholidoptera, and Eupholidoptera, as well as in Pholidoptera frivaldskyi and Pholidoptera griseoaptera, a karyotype of 2n=30+X0 (FN=31) was found. Ph. macedonica and Ph. aptera aptera are characterized by 2n=28+X0 (FN=31). In species from genera Drymadusa (D. dorsalis limbata) 2n=26+X0: FN=30 and Gampsocleis (G. abbreviata), karyotypes of 2n=22+X0 (FN=36) were found. Ph. macedonica and Ph. aptera aptera as well as G. abbreviata differ in karyotypes from other representatives of these genera. New data confirmed that Robertsonian fusions and tandem fusions played the most important role in the evolution of the chromosome set in Tettigoniinae. Cytogenetic similarities and differences between particular species are discussed.

Key words: Orthoptera, Tettigoniidae, katydids, karyotype, cytotaxonomy, C-banding, NOR.

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The systematics of the subfamily Tettigoniinae, including the former Decticinae, also sometimes considered as the family Tettigoniidae (HELLER et al. 1998), are unsatisfactory at present. This refers to the level of subgenera and genera (e.g. the large genera Metrioptera/Platycleis or Pterolepis/Rha- coeleis), but more importantly also to higher categories. Information concerning which morphological characters – if any – can be used to reliably define groups of related genera and tribes is lacking (e.g. RENTZ & COLESS 1990). Of course, relationships have been established for some groups of genera, but for many they are missing. Careful morphological studies can sometimes improve the situation (see F. & L. WILLEMSE in preparation), but additional information would be highly welcome. Therefore the karyotypes of 26 species of this group, previously undescribed, have been examined in this study. Chromosomal information has been demonstrated to be valuable taxonomically in a large range of vertebrates (FONTANA & RUBINI 1990; SMITH 1990; VOLLETH & HELLER 1994; RUMPLER Y. 2000) and arthropods (BLACKMAN 1980; PETITPIERRE 1997; SHAPOSHNIKOV et al. 1998; WARCHAŁOWSKA-ŚLIWA E. 1998).

### Table 1

General karyotypical features, C-heterochromatin patterns, and NORs in species of Tettigoninae (acro- acrocentric, subacro- subacrocentric, submeta- submetacentric, meta- metacentric, * intraspecific variation of C-heterochromatin)

<table>
<thead>
<tr>
<th>Species</th>
<th>Numbers of individuals, collection locality</th>
<th>2n, FN</th>
<th>C-bands</th>
<th>NOR</th>
<th>References other than this paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psorodonotus illyricus macedonicus</td>
<td>4 males, Greece</td>
<td>33, 33 all acro</td>
<td>All paracentromeric thin, M₂, M₃ interstitial, M₂, M₁, most of small bivalents telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decticus verrucivorus</td>
<td>2 males, Poland</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin M₄/₅ interstitial, M₁-M₆, terminal thick</td>
<td>M₄/₅</td>
<td>present paper &amp; WARCHAŁOWSKA-ŚLIWA 1984</td>
</tr>
<tr>
<td>Platycleis (Parnassiana) tenuis</td>
<td>1 male, Greece</td>
<td>31, 31 all acro</td>
<td>very small division, without C-bands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metrioptera roeselii ambitiosa</td>
<td>1 male, 1 female, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, L₁, L₂, M₃, X telomeric thin</td>
<td>M₃/₄</td>
<td>WARCHAŁOWSKA-ŚLIWA 1984</td>
</tr>
<tr>
<td>Metrioptera roeselii roeselii</td>
<td>6 males, Poland</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, L₅*, M₃, M₄, M₅*, X telomeric, L₁, L₂, M₃, M₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metrioptera oblongicollis</td>
<td>2 males, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, L₁, L₂ telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metrioptera brachyptera</td>
<td>5 males, Slovakia</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, L₅, M₁, M₃* interstitial, L₅, M₄ (thick) telomeric, X near centromeric and telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metrioptera bicolor</td>
<td>2 males, Poland</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, L₅*, L₂, M₁, M₄, M₅ telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterastes serbicic</td>
<td>3 males, 1 female, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, X thick centromeric and thin telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bucephaloptera bucephala</td>
<td>1 male, Turkey</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parapholidoptera signata</td>
<td>1 male, Turkey</td>
<td>31, 31 all acro</td>
<td>Two medium size with thick paracentromeric C-band, the rest thin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupholidoptera epirotica</td>
<td>6 males, 1 female, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄ interstitial (subtelomeric) L₁ telomeric heterozygous (two males)</td>
<td>M₃/₄</td>
<td></td>
</tr>
<tr>
<td>Eupholidoptera tauricola</td>
<td>2 males, Turkey</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄ interstitial, L₁* telomeric (both males)</td>
<td></td>
<td></td>
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<tr>
<td>Eupholidoptera smyrnensis</td>
<td>5 males, 1 female, Bulgaria</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄ interstitial, L₁* telomeric (two males)</td>
<td></td>
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<tr>
<td>Eupholidoptera chabrieri garganica</td>
<td>3 males, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄ interstitial L₁* telomeric (one males 20/04)</td>
<td></td>
<td></td>
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<tr>
<td>Eupholidoptera megastyla</td>
<td>4 males, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄* interstitial (in one male (25/03)</td>
<td></td>
<td></td>
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<tr>
<td>Eupholidoptera annulipes</td>
<td>2 males, Turkey</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄ interstitial</td>
<td></td>
<td></td>
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<tr>
<td>Eupholidoptera sp. (ex Andros)</td>
<td>2 males, 2 females, Greece</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄ interstitial (subtelomeric) L₁, L₂ telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupholidoptera prasina</td>
<td>5 males, Turkey</td>
<td>31, 31 all acro</td>
<td>All paracentromeric thin, M₃/₄, M₂ interstitial, L₁, L₂, M₃, M₅ telomeric, X interstitial and telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Location</td>
<td>Chromosome Size</td>
<td>Description</td>
<td></td>
<td></td>
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<tr>
<td>--------------------------</td>
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<tr>
<td>Eupholidoptera anatolica</td>
<td>Turkey</td>
<td>31, 31</td>
<td>All paracentromeric thin, M1,4, M2 interstitial, L1, L2, M3,4, M5,6 telomeric, X interstitial?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupholidoptera mersinensis</td>
<td>Turkey</td>
<td>31, 31</td>
<td>All paracentromeric thin, M1,4,5 interstitial, L1, L2, M3,4, M5,6,7 telomeric, X interstitial?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupholidoptera karabagi</td>
<td>Turkey</td>
<td>31, 31</td>
<td>All paracentromeric thin, X interstitial?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eupholidoptera icariensis</td>
<td>Greece</td>
<td>31, 31</td>
<td>Very small division, without C bands</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pholidoptera frivaldskyi</td>
<td>Greece</td>
<td>31, 31</td>
<td>All paracentromeric thin, M2 interstitial thin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pholidoptera giseoaptera</td>
<td>Poland</td>
<td>31, 31</td>
<td>All paracentromeric thin, M1 interstitial thin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pholidoptera macedonica</td>
<td>Greece</td>
<td>29, 31</td>
<td>All paracentromeric thin, L1 telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pholidoptera aptera aptera</td>
<td>Poland</td>
<td>29, 31</td>
<td>All paracentromeric thin, L1 subsetar, L2-S14, X acro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drymadusa dorsalis limbata</td>
<td>Turkey</td>
<td>27, 30</td>
<td>All paracentromeric thin, M2, S1,2, X subsetar, L1, S1-S13 acro, X subsetar, subacro, M3,4,5, X telomeric in longer arm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pterolepis ferdinandi</td>
<td>Greece</td>
<td>25, 27</td>
<td>All paracentromeric medium size, L1 interstitial + telomeric (in both arms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pterolepis germanicarica</td>
<td>Greece</td>
<td>25, 27</td>
<td>All paracentromeric medium size, L1 interstitial + telomeric (in both arms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pterolepis insularis</td>
<td>Greece</td>
<td>25, 27</td>
<td>All paracentromeric medium size, L1 interstitial + telomeric (in both arms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pterolepis sp. nova</td>
<td>Greece</td>
<td>25, 27</td>
<td>All paracentromeric medium size, M2, M3, M4 interstitial and telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pterolepis edentata</td>
<td>Greece</td>
<td>25, 27</td>
<td>All paracentromeric medium size, L1 interstitial + interstitial (both arms), M23, M2,4,5 thick telomeric, X thin telomeric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gampsocleis abbreviata</td>
<td>Greece</td>
<td>23, 36</td>
<td>All paracentromeric thin (excluding pair S3,9), X, S4 telomeric thin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The great majority of cytotaxonomic analyses have been based on the conventional staining technique. Banding techniques such as C-banding and Ag-NOR-banding allow for a better characterization of tettigonid karyotypes and selectively reveal chromosome regions consisting of constitutive heterochromatin and NOR-sites of the RNA genes. New cytological markers are useful for better insight into the pathways by which the various karyotypes have evolved in Tettigoniidae. Constitutive heterochromatin shows characteristic distribution patterns in karyotypes and contributes to the broad scattering of genome sizes throughout biological taxa (REDI et al. 2001).

Data on C-banding patterns in mitotic and meiotic cells, and information on the nucleolus organizer region (NOR), are limited in the European Tettigoniinae (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a; WARCHAŁOWSKA-ŚLIWA et al. 1992, 1994). Here a further study on the karyotypes of 34 taxa of the subfamily is provided in order to obtain more information on the genome organization of these orthopteran species.

Material and Methods

Adult males and females were collected in Greece, Turkey, Poland, and Slovakia for the cytogenetic study (see Appendix for taxonomic data and specific localities). Specimens from Greece and Turkey are deposited in Collectio Heller (CH + reference number), the others – in the Institute of Systematics and Evolution of Animals PAS (Kraków).

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. Coverslips were removed by the dry ice procedure and then preparations 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). The fixed material is deposited in the Institute of Systematics and Evolution of Animals PAS (Kraków).

Results

The chromosome number (2n), morphology of chromosomes (Fundamental Number = FN), the C-banding patterns, and NOR locations in the species studied are reported in Table 1. The diploid chromosome number of 26 species representing 10 genera was investigated for the first time.

Most of the European species of Tettigoniinae analyzed in this paper in the genera Decticus, Platycleis, Metrioptera, Anterastes, Bucephaloptera, Paraholodiopetera, Eupholodiopetera, and some species of Pholidoptera, have 2nσ=31 (FN=31; only acrocentric chromosomes) (Fig. 1). However, in Pholidoptera macedonica, the chromosome number is reduced to 2nσ=29 (FN=31) with one submetacentric pair (L1), similar to Ph. aptera (Fig. 2) described earlier (WARCHAŁOWSKA-ŚLIWA 1988).

The highest number of chromosomes for this group, 2nσ=33, was found in Psorodonotus illyricus macedonicus (Fig. 3).

In Drymadusa dorsalis limbata the complement is reduced to 2nσ=27, FN=30 with a meta/submetacentric L1 pair and the submeta/subacrocentric X chromosome (Fig. 4).

Five species of the genus Pterolepis are characterized by 2nσ=25. This is a chromosomal set with one extremely long metacentric L1 (FN=27) (Fig. 5a,b,c). The lowest chromosome number in this group was found in Gampsocleis abbreviata 2nσ=23 (FN=36) with L1 submetacentric, five pairs metacentric, and five pairs acrocentric. The metacentric X chromosome is the largest element in the karyotype as in G. glabra (WARCHAŁOWSKA-ŚLIWA et al. 1992).

All analyzed species show the X0σ and XX♀ type of sex chromosome determination.

Table 1 shows the C-banding patterns of 34 species, and Figures 1-13 give some examples of the results. Most of the species of Tettigoniinae have paracentromeric C-bands in the vicinity of the centromeric regions. In most cases, the paracentromeric C-bands are restricted to the centromeric region (thin C-bands), e.g. in the whole autosomal complement and the X chromosome of Metrioptera roeselii (Figs 1, 6), Eupholodiopetera mersiensis (Fig. 7), and Pholidoptera aptera (Fig. 2). In other cases, C-bands occupy the region next to the centromere (thick C-bands) as in the two medium-sized pairs of Paraholodiopetera signata, and in all chromosomes of Pterolepis sp.n.

In 22 species of Tettigoniinae, shown in Table 1, interstitial C-bands on one or more chromosomes are present. Interstitial C-bands are located for instance near the paracentromeric region of the X chromosome in Eupholodiopetera karabagi (Fig. 8), in the interstitial region of pairs M2, M3 in Psorodonotus illyricus macedonicus (Fig. 3), in the pair M3/4 of Decticus verrucivorus (Fig. 9), in the pairs M5/6 and M5 of Eupholodiopetera anatolica (Fig. 10), in the interstitial parts of both arms in pair L1 of Pterolepis Ferdinandi as well as in pairs M2-M4 of Pterolepis insularis (Fig. 5a), and of Pterolepis sp. n. Only in Metrioptera brachyptera (in one indi-
Individual variation in M4 due to the presence or absence of interstitial C-bands is observed (Fig. 11). When telomeric C-bands are present, they are located in chromosomes of different sizes and in most cases they are thin (Table 1, Fig. 6). However, in three pairs of autosomes (M3-M6) of Decticus verrucivorus and in M2, M3 of Metrioptera brachyptera, these bands are thick (Figs 9, 11). It is also worth mentioning that sometimes size variation of telomeric C-bands of two homologous chromosomes may be present, e.g. in two pairs (L2, M5) of Metrioptera roeselii, or in some individuals in four species of Eupholidoptera (Fig. 12).

To sum up information connected with heterochromatin, the C-banding technique revealed that most of the examined species with 2n=31 in males show very small (thin) segments of constitutive heterochromatin in all autosomes and in X chromosomes (excluding terminal C-bands in Decticus verrucivorus). However, in some species of the genera Pterolepis and in Drymadusa dorsalis these segments are larger (thick). Differences in the amount of heterochromatin are clearly visible in the interphase nuclei and in the early prophase as e.g. in the genera Psorodonotus, Metrioptera, Pholidoptera, Decticus, Drymadusa, and Pterolepis (Figs 13a-f).

A B chromosome was detected in one male of Pterolepis ferdinandi. It was a acrocentric small element in the complement (Fig 5b). The location of chromosome NOR’s was shown in only 9 of the species studied. For other species the NOR was not revealed owing to the absence of diplotenes in the studied cells. Eight species, namely Decticus verrucivorus, Metrioptera roeselii, Euphidoptera epirotica, E. mersinensis, E. karabagi, Pholidoptera griseoaptera, and Ph. aptera each showed a single active NOR located on

Figs 1-5. C-banded karyotypes of males. Fig. 1. Metrioptera roeselii and Fig. 2. Pholidoptera aptera aptera – 2n=31, FN=31 and 2n=29, FN=31 respectively, metaphase I, both species with very thin paracentromeric C-bands. Fig. 3. Psorodonotus illyricus macedonicus – 2n=33, FN=33, metaphase I, arrows indicate interstitial C-bands. Fig. 4. Drymadusa dorsalis limbata – 2n=27, FN=30, metaphase I, Figs 5a, b. (a) Pterolepis insularis and (b) Pterolepis ferdinandi – 2n=25, FN=27, metaphase I with interstitial C-bands (arrows), B (supernumerary chromosome), Fig. 5c. Pterolepis edentata, mitotic metaphase with extremely long metacentric pair L1. Bar = 10 μm.
Figs 6-13. Fig. 6. *Metrioptera roeselii*, mitotic metaphase I, arrows indicate telomeric C-bands. Fig. 7. *Eupholidoptera smyrnenesis*, metaphase I. Fig. 8. *Eupholidoptera karabagi*, meiotic prophase, the X chromosome with interstitial C-band (arrow). Fig. 9. *Decticus verrucivorus*, mitotic metaphase, arrows indicate telomeric C-bands. Fig. 10. *Eupholidoptera anatolica*, mitotic with interstitial C-bands. Fig. 11. *Metrioptera brachyptera*, diakinesis, \( \bigtriangledown \) indicates variability of interstitial heterochromatin, arrows indicate telomeric C-bands, thick in autosomes and thin in the X chromosome. Fig. 12. *Eupholidoptera chabrieri garganica*, metaphase I, \( \bigtriangledown \) indicates variability in telomeric C-band. Figs 13a-f. Nuclei with heterochromatin: (a) *Metrioptera roeselii*, (b) *Pholidoptera aptera aptera*, (c) *Psorodonotus illyricus macedonicus*, (d) *Decticus verrucivorus*, (e) *Drynadaus dorsalis limbata*, (f) *Pterolepis insularis*. Bar = 10 \( \mu \)m.
the medium-sized bivalents (M<sub>3:4</sub> and M<sub>4:5</sub>), and in Gampsocleis abbreviata on the M<sub>6</sub> bivalent. Active NORs were detected distally in D. verrucivorus, M. roeselii, G. abbreviata, and in a near distal position in Eupholidoptera epirotica, whereas they are located interstitially in Eupholidoptera mersinensis, E. karabagi, Pholidoptera frivaldsky, P. griseoaptera, and Gampsocleis. Pholidoptera aptera aptera showed one NOR located on L<sub>1</sub> probably near a distal position. (Figs 14a-f).

**Discussion**

Most species of the Palearctic Tettigoniinae have been found to have 2n=31 with only acrocentric chromosomes (FN=31) (see review WARCHALOWSKA-SLIWA 1998). The same is true of the species sampled in this study in the genera Anterastes, Bucephaloptera, Decticus, Eupholidoptera, Parapholidoptera, Platycleis, and Metrioptera. For these species, karyology gives no clues as to their systematic relationships. There are, however, some species which differ from this basic pattern and which have to be discussed separately. Only one of the examined species has a chromosome number higher than the modal number of this subfamily. Psorodonotus illyricus macedonicus, like its congeneric species P. specularis Fischer de Waldheim, 1839 (WARCHALOWSKA-SLIWA 1998), shows 2n=33?. One of the possible mechanisms producing this unusually high number rests in the relatively complicated chromosomal reorganization that could be connected with chromosome aneuploidy. Taxonomically this character can in the future be used to clarify the problematic relationships between Psorodonotus and the genus Uvarovina (Peltastes) (see STOROZHENKO 2004).

Nine species have chromosome numbers lower than the modal number of the subfamily. They may have their origin in independent Robertsonian fusions of acrocentric chromosomes. Interestingly, the simplest case, the fusion of two pairs of chromosomes resulting in 2n=29, was only found here in the closely related Pholidoptera aptera and P. macedonica, but not in other species of this genus (see WARCHALOWSKA-SLIWA 1998 for review). This character could help the taxonomy if it is used to define the morphologically difficult P. aptera group (for characteristics of the song pattern see HELLER 1988).

For Drymadusa dorsalis limbata a Robertsonian fusion and an additional tandem fusion followed by an inversion, resulting in 2n<sup>σ</sup>=27 (FN=30) (one metacentric pair and submeta/subacrocentric X chromosome originated by pericentric inversion) have to be assumed. A very similar karyotype is known from Bergiola montana (WARCHALOWSKA-SLIWA & BUGROV 1996a), another genus of the
Drymadusini. Several other species (from the genera Atlanticus, Anatalanticus, Tadzhikia, Ana-
drymadusina, Ceraceocerius, Paratlastanticus) from this tribe have karyotypes with a reduced number of chromosomes (2n = 25-29) (WARCHAŁOWSKA-
ŚLIWA & BUGROV 1996a).

The five species of the genus Pterolepis (formerly Rhacocleis, HELLER et al. 1998) showed a karyotype with 2n = 25 (FN=27). In this case, the first pair L1 occurs probably as a result of one cen-
tric fusion between two autosomal pairs and two other pairs as a result of tandem fusions. From chromosome data, nothing can be said about the phylogenet-
ic relationships of this genus. How-
ever, in no species of the Platycleidini s.str. such low chromosome numbers are found, whereas they occur quite frequently in genera character-
ized morphologically by two spines at the proster-
num, e.g. Drymadusini, Pterolepis/Rhacocleis, Gampsocleis.

Within the genus Gampsocleis, however, spe-
cies with very different karyotypes were found. Several species (see WARCHAŁOWSKA-ŚLIWA 1998 for review) show chromosomes which do not differ in number nor in shape from the typical ones of the subfamily (2n =31, FN=31). On the other hand, two species, G. glabra and G. abbrevi-
ata, have only 23 chromosomes with FN=26. Since these two species are the most western re-
presentatives of the genus, a common origin of this highly derived karyotype can be assumed and the reduction in chromosome number happened cer-
tainly independently from that in other genera mentioned above.

An interesting feature of the Pterolepis ferdin-
dandi karyotype is the sporadic occurrence of B chromosomes. According to many authors (e.g. BATTAGLIA 1964; JOHN & LEVIS 1968; WHITE 1973; HEWITT 1979; CAMACHO et al. 1981) the existence of B chromosomes, previously noted in few species of tettigonoids, indicates that these elements are rare in this group and often unstable (WARCHAŁOWSKA-ŚLIWA et al. 1992).

In tettigonoids (similar to other Orthoptera) the C-band ing technique is used, among others, in comparative studies of populations, species and genera of the subfamilies Bradyporinae (NAVAS CASTILLO et al. 1986; WARCHAŁOWSKA-ŚLIWA & BUGROV 1996b, 1998) and Pantheporopterae (WARCHAŁOWSKA-ŚLIWA & HELLER 1998; WAR-
ferent genera are the result of the heterochromatin differentiation as is reflected by the existence of intraspecific variation for many species (SANTOS et al. 1983; CABRERO & CAMACHO 1986a; VISERAS et al. 1991). However, C-banding patterns of Euro-
pean Tettigoninae have been described only in a few genera: Gampsocleis (WARCHAŁOWSKA-
ŚLIWA et al. 1992), Montana (WARCHAŁOW-
SKA-ŚLIWA et al. 1994), Tettigonia (WARCHAŁOW-
SKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1995), Anadrymadusa, as well as in some genera of Dry-
madusini from Asia (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a). In most of these genera, and in most described in the present paper, thin paracent-
tromeric C-bands were uniformly present in all autosomes and in the X chromosome. Exception-
ally, in Parapholidoptera (on three medium-sized pairs), Gampsocleis (on one small-sized pair) and the earlier described (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a) Tadzhikia (three small pairs), these C-blocks occupy the region next to the centromere (thick C-blocks). However, in Pterolepis all chromosomes are characterized by medium-
sized paracentromeric C-bands, while the metacentric X chromosomes in the latter genus have a thick (double) block in this region. Similarly to the above mentioned species, most genera and species of Pantheporopterae (Poecililimon, Isophya, Metaplastes, Polysarcus, and Andreinimon) possess thick para-
centromeric C-bands on one or on almost all chro-

The C-banding patterns and distribution of inter-
stitial and telomeric C-bands in autosomes and the X chromosomes are usually found to vary among genera and between species of one genus. For in-
stance, qualitative and quantitative variation of C-
bands was observed in Metrioptera and Eup-
holiophila (present paper), as well as in Montana (WARCHAŁOWSKA-ŚLIWA et al. 1994). Only Pholido-
ptera frivaldski and P. griseoaptera as well as Pterolepis ferdinandi and P. germanica, possess the same C-banding patterns similar to Anadry-
madusa picta and A. robusta (WARCHAŁOWSKA-
ŚLIWA & BUGROV 1996a).

Intraspecific polymorphism mainly connected with different numbers of additional heterochro-
matin blocks (indicated in Table 1 as *) was de-
scribed in a few species as in earlier studies of repre-
sentatives of the genus Gampsocleis (WARCHA-

The silver Ag-staining of the NORs is one of meth-
ods used for demonstrating the position of the gene complex at 18S and 28S ribosomal DNA in the chromosome set (GOODPASTURE & BLOOM 1975). In mammals and plants NOR studies are pro-
vided on mitotic divisions, however, in Orthop-
tera these regions may be stained only in meiotic cells. The location of NORs in six genera of tettigo-
niids is presented in Table 1. The distribution of
NORs often characterizes species within one genus, but sometimes shows variation even between specimens of the same species (WARCHAŁOWSKA-SLIWA & MARYAŃSKA-NADACHOWSKA 1995). In some Tettigoninae the NOR occurs in the large bivalents as in the genus *Tettigonia* and in representatives of Drymadusini (WARCHAŁOWSKA-SLIWA & MARYAŃSKA-NADACHOWSKA 1995; WARCHAŁOWSKA-SLIWA & BUGROV 1996a, respectively), or in the middle-sized bivalents, e.g. in *Decticus*, *Eupholidoptera*, *Metrioptera* (the present paper) as well as in *Gampsocleis* and *Montana* (the present paper and WARCHAŁOWSKA-SLIWA et al. 1992, 1994). In species from the genera *Eupholidoptera*, *Pholidoptera* (only with 2n=31), or *Gampsocleis* (the present paper and WARCHAŁOWSKA-SLIWA et al. 1992), the NORs are located in most cases probably on the same bivalent (at least according to size).

The heterochromatin associated with NORs sometimes shows a positive C-banding response. In ten species described in this paper there are NORs which correspond to a C-band in the equivalent position (Table 1). On the other hand, NORs in *Gampsocleis abbreviata* do not show correspondence with C-bands; the nature of heterochromatin associated with these NORs requires further investigation using other banding techniques (CMA3 and FISH, e.g. SOUZA et al. 2003). The present study demonstrates a high degree of conservatism of the NOR location pattern, agreeing with observations on the genera *Montana* and *Gampsocleis* (WARCHAŁOWSKA-SLIWA et al. 1992, 1994). Thus, NORs are useful chromosome markers for interspecific comparison in the Tettigoninae, like in the acridid subfamilies Oedipodinae (VISERAS et al. 1991) and Gomphocerinae (CAMACHO & CABRERO 1986a,b).

In summary, the present results show some similarities and differences between karyotypes, C-banding patterns, and NOR patterns within European species of Tettigoniae. It is worth stressing that modification of morphology of chromosomes caused a decrease in chromosome number in the genus *Pterolepis* and in some species of *Drymadusa*, *Gampsocleis* and *Pholidoptera*. The further employment of molecular techniques will probably provide additional information on the evolution of the genome in e.g. *Pterolepis*, *Gampsocleis*, *Pholidoptera*, and Drymadusini.

**References**


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