

Karyotypes and Anatomical Features of the Reproductive Systems of Fulgoroidea (Homoptera, Cicadinea)*

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Data are presented for the first time on karyotypes and some anatomical features of the reproductive system in Auchenorrhyncha [Cicadinea in Soviet terminology—Ed.] from the most primitive fulgoroid family, Tettigometridae. Also investigated were representatives of other primitive families, Delphacidae and Cixiidae, the more evolutionarily advanced Meenoplidae, Derbidae, and Dictyopharidae, and the most advanced, Ricaniidae and Flatidae. The hypothesis is validated that in the superfamily Fulgoroidea the following characters are plesiomorphic: $2n = 30, 29$ (♀, ♂), sex chromosomes type $XX:XO$, 6 seminal (testicular) follicles and tubular, undivided accessory glands in the ♂. In the evolution of Fulgoroidea there is a tendency toward reduction in the number of chromosomes and oligomerization in the number of testis follicles.

Planthoppers (Fulgoroidea) are 1 of 4 recent superfamilies in the suborder Cicadinea, which is comprised of 20 families (Muir, 1930; Metcalf, 1951). This group is interesting in terms of the phylogenetic interrelationships with other superfamilies of the suborder. For a long time it was generally accepted that the Fulgoroidea were the earliest to split off from the main Cicadinea line, and that therefore they were opposed to the 3 other superfamilies — Cercopoidea, Cicadoidea, and Cicadelloidea (Popov, 1980; Shcherbakov, 1984; Savinov, 1987). In his analysis of a large number of morphological characters, Yemel'yanov (1987), on the contrary, demonstrated the derived character states of Fulgoroidea and that their close proximity to Cicadelloidea (Cercopoidea-Cicadoidea) makes them the alternative group of superfamilies. Of interest in connection with this, is the determination of plesiomorphic karyotype characters and features of the internal reproductive system in Fulgoroidea. Their significance in constructing phylogenetic relationships has been demonstrated (Yemel'yanov and Kuznetsova, 1983; Kuznetsova, 1985). Such an analysis may be performed based on concepts concerning the phylogenetic relationships within the superfamily. The Tettigometridae are considered the most primitive family in the evolution of the Fulgoroidea; the next stage is taken by the Delphacidae, after which the subsequent phylogenetic branch is the Cixiidae (Yemel'yanov, 1979, 1987).

The authors investigated the Cicadinea based on the primitive families Tettigometridae, Delphacidae, and Cixiidae, as well as the more advanced families Meenoplidae, Derbidae, and Dictyopharidae, and the most advanced Ricaniidae and Flatidae, in all 20 species in 16 genera (see table). In 11 species we investigated the karyotypes (of these, in only 3 the chromosome mechanism

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Number of chromosomes, mechanisms of sex determination, and reproductive systems characters in the species studied of the Tettigometridae and other fulgoroid families

Family, species	Date, locality No. of spms. examined	Chromosome number, mechanism of sex determination in ♂	Number of follicles per testis	Form of accessory glands	Number of ovarioles per ovary
1	2	3	4	5	6
Tettigometridae <i>Tettigometra eremi</i> Lindh.	VIII.1987 Kirgizia, Ala-Too Range, 3♂s, 5♀s	2n = 26; neo-XY	6	Tubular, elongate, undivided	11-12
<i>T. obliqua</i> Panz.	VIII.1987, Kirgizia, Ala-Too Range, 1♂	2n = 26; neo-XY	6	Same	-
<i>T. varia</i> Fieb.	1982, Asia, 4♂s, 2♀s	2n = 26-28; neo-XY (?)	6	"	7
<i>T. vitellina</i> Fieb.	VIII.1982, Tajikistan, Kir R, Valley. VIII.1987, Kirgizia, Ala-Too Range, 5♂s, 11♀s	-	6	"	6-15
<i>T. costulata</i> Fieb.	Uzbekistan; 20 km N of Takhtakupyr, 5♂s, 2♀s	2n = 28 (?), neo-XY	5, y 1♂ 5-6	"	6
Delphacidae <i>Asiraca clavicornis</i> F.	IX.1986, Kirgizia, Frunze, 6♂s, 2♀s	2n = 29; XO	6	Tubular, very long, undivided	19-20
<i>Copicerus irroratus</i> Swartz.	IX.1986, Cuba, Bejucal, 9♂s, 1♀	2n = 29; XO	6	Same	19-20
<i>Stobaera muiri</i> Kramer	IX.1986, Cuba, Guanabo, 2♂s, 2♀s	2n = 29; XO	-	Compact, bifid	8-9(?)
<i>Metropsis</i> sp.n.	VI.1982, Tajikistan, Turkestan Range, Kusavlisay Gorge, 7♂s	2n = 29; XO	3	-	-
<i>Sogatella furcifera</i> Horvath	VIII.1982, Maritime Terr., Khasan Distr., 1♂	XO	3	-	-
Cixiidae <i>Melanoliarus</i> sp.	XI.1986, Cuba, Guanabo, 1♂	XO	6	-	-
<i>Cixius borusticus</i> Wagn.	VI.1987, Chuvash Aut. Rep. Novocheboksarsk, 1♂	-	4	-	-
<i>Hemitropsis</i> sp.	VII.1987, Kazakhstan, 6♂s	-	4	-	-
Mecynoptilidae <i>Mecynoptilus albosignatus</i> Fieber	VII.1984, Armenia, Khosrov Res., 4♂s	2n = 27; XO	-	-	-

Table (continued)

1	2	3	4	5	6
Derbidae <i>Omolichna cubana</i> Fenn.	XI.1986, Cuba, 2♂s	2n = 27 (large bivalent); XO	4	Tubular, long, undivided	
Dictyonharidae <i>Philotheria</i> sp.	VI.1985, Africa, Guinea, Conakry, 1♂	—	6	Tubular, long, undivided	
<i>Rhynchomitra</i> <i>cubana</i> Mel.	XI.1986, Cuba, Esca- leras del Haruco, Tapaste, 1♂	XO	6	—	
<i>Bursinia</i> sp.	1986, Greece, Pelo- ponessus, 1♂	—	4	Compact, divided into 2 indistinct sections	
Ricaniidae <i>Ricania japonica</i> Mel.**	VII.1982, Georgia (Cruzia), Pitsunda, 5♂s	2n = 21; XO	10-12, usually	Compact, bifid	
Flatidae <i>Phantia christophii</i> de Rustecka	VI.1987, Uzbekistan, 20 km ENE Shirykra- bat, 2♂s	2n = 28; neo-XY	6	—	

*Description of karyotypes of *Metropis* sp. n. and *A. clavicornis* is found in our earlier works (Kirillova and Huznetsova, 1986; Kirilova, 1988).

**Number of chromosomes in *R. japonica* was first established by Kurokawa in 1953 (after Halkka, 1959).

of sex determination) and reproductive system (number of testicular follicles and structure of the accessory glands in ♂s and the number of ovarioles in the ♀s). Of 9 species data were obtained only on certain anatomical characters of the reproductive system.

MATERIALS AND METHODS

All data on locality and time of collection, the number of specimens examined, and the results are presented in the table. The method of karyotype preparation was summarized by the authors in previous works (Kuznetsova, 1985; Kirillova and Kunznetsova, 1986). Study of the anatomy of the reproductive system is based on material fixed for karyological analysis (ethanol-acetic acid, 3:1). We investigated the number testicular follicles and ovarioles, and the form of the accessory glands in the ♂ reproductive system. The karyotypes (in metaphase I) were made using a MFN-11 photo attachment. The figure of the reproductive system was made using a RA-4 drawing device.

RESULTS AND DISCUSSION

Karyological features. As a whole, fulgoroid karyological data are known from 145 species in 14 families (Kirillova, 1986). This group is characterized by a rather great diversity in chromosome numbers — from $2n = 20$ to $2n = 38$, with $2n = 26$, 28, and 30 the most frequently encountered. In the superfamily the XX:XO chromosome sex determination type dominates. All other sex

determination mechanisms in the Fulgoroidea should obviously be considered new-XY, resulting from translocation of the X-chromosome (in forms with XO) to one autosome (White, 1973). The only case that is difficult to interpret as neo-XY occurs in the fulgorid, *Limois emelianovi* Osh. (Kuznetsova, 1985). Data on the structure of the karyotypes (size characteristics of the chromosomes) in the superfamily are basically limited to the families Delphacidae (38 spp.) and Dictyopharidae (30 spp.); there are data on only 13 spp. from 12 other families. It should be noted that most often karyotypes break down into 2 structural categories: those with a smooth size progression (symmetrical karyotype), and those with one large bivalent in the set.

Attempts to derive the plesiomorphic characters of the karyotype for all fulgorids have already been made (Kuznetsova, 1987). The hypothesized plesiomorphic number of chromosomes in this group is $2n = 30, 29$ (♀, ♂); the sex determination mechanism is XX:XO; the karyotype is of symmetrical type. To date, however, data have been lacking on the primitive, ancestral fulgoroid family Tettigometridae. All species investigated in this article have $2n = 26-28$ (Fig. 1a-c; Table). In *Tettigometra varia* and *T. costulata* the number of chromosomes could not be accurately determined. It equals 26 or 28 in the diploid set; however, for *T. costulata* it is most likely $2n = 28$. The mechanism of sex determination in all cases we determined to be neo-XX;neo-XY. In all species in metaphase I a univalent chromosome is lacking, which is always present in species of the XO-type mechanism. We also did not find heteromorphic bivalents. The former condition made it possible to rule out the XO-type, the latter provided grounds for believing that the studied species have the neo-XY mechanism in which the sex chromosomes are not always heteromorphic (White, 1973).

As already noted, the neo-XY mechanism is always secondary and occurs as a result of merging of one autosome and sex chromosome in the original karyotype with the XO mechanism (White, 1973; Kuznetsova, 1985). A second homologue of the merged autosome transforms into a Y-chromosome. The merger results in reduction in the number of chromosomes by one pair. Cases of reversal of neo-XY aberration to the initial XO system are unknown not only in Cicadinea, but also in other insects (White, 1973). Thus, the presence of neo-XY type sex chromosomes indicates the secondary, derived nature of such a karyotype and, at the same time, the directional nature of the change in the number chromosomes (from a larger to a smaller number) in the evolution of related species. In this connection we suggest that Karyotypes of all *Tettigometra* species we studied are derived. The plesiomorphic karyotype for tettigometrids apparently includes 30 chromosomes with an XX:XO type mechanism. It was previously demonstrated that such a karyotype was plesiomorphic for another primitive fulgorid family, Delphacidae (Kirillova, 1988), as well as for Dictyopharidae (Kuznetsova, 1985). Thus, this confirms the hypothesis (Kuznetsova, 1987) that a $2n = 30, 29$ (♀, ♂) karyotype with XX:XO sex chromosomes is plesiomorphic for all the Fulgoroidea.

It may be that the karyotype of *T. costulata* with $2n = 28$ and neo-XY is the first stage in transformation of the primitive karyotype with $2n = 30$ and XO-type mechanism, and that the other karyotypes of *Tettigometra* species are the result of the next evolutionary step. It follows that these species are advanced relative to *T. costulata* based on the karyotype. However, morphologically (Yemel'yanov, 1980), and including data we obtained on the anatomy of the reproductive system, (below), *T. costulata* is the most advanced species in the genus. The apparent contradiction can be explained as a result of the mosaic nature of evolution.

In connection with this we should discuss the possible causes for the appearance of secondary karyotypes in the most primitive fulgoroid family, Tettigometridae. It is known that the Tettigometridae possess, besides primitive features, many characters of specialization: secondarily non-hopping larvae, sclerotization of the elytra, change in venation of the hind-wings, and myrmecophily (Yemel'yanov, 1987); apparently, the karyotypic characters correlate with this. A similar

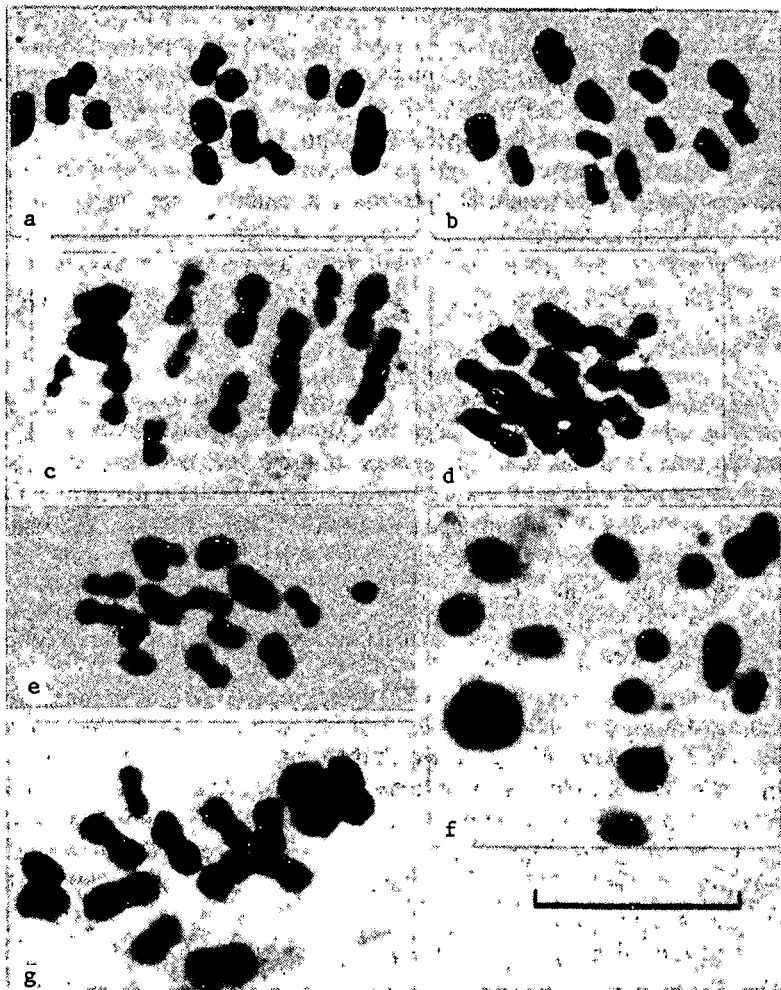


Fig. 1. Karyotypes of some fulgoroid families (in metaphase I): a-c) Tettigometridae (*Tettigometra eremi*, $n = 13$, neo-XY; *T. obliqua*, $n = 13$, neo-XY; *T. costulata*, $n = 14$, neo-XY); d) Derbidae (*Omolicna cubana*, $n = 14$, XO); e) Meenoplidae (*Meenoplus albosingatus*, $n = 14$, XO); f) Faltidae (*Phantia christophii*, $n = 14$, neo-XY) g) Dictyopharidae (*Rhyrchomitra cubana*, $n = 14$, XO). Arrows indicate sex chromosomes. Magnification: ob) $\times 100$ oc. $\times 7$.

change in karyotypes in association with specialization was recorded earlier, for representatives of the most advanced tribe Almanini in the family Dictyopharidae, which is adapted to arid conditions (Kuznetsova, 1985).

Data we obtained on the karyotypes of representatives of the more advanced fulgoroid families Delphacidae, Meenoplidae, Derbidae, Dictyopharidae, and Flatidae (Fig. 1d-g; Table) — basically agree well with previously available data on these families (Kirillova, 1986). In *Omolicna cubana* (Derbidae) $2n = 28$ in the ♀ and the sex determination mechanism is XX:XO. In the karyotype there is a pair of large chromosomes (Fig. 1d). Considering the trend toward fusion of autosomes and the appearance of a large bivalent that is observed as well in other fulgoroid families, it can be assumed that the *O. cubana* karyotype is secondary. The derived nature of the karyotype in

Phantia christophii (Flatidae), which also has $2n = 28$, is confirmed by the presence in it of a neo-XY sex determination mechanism (Fig. 1f). As a whole, there are few data on the Derbidae and flatidae (Kirillova, 1986) and they do not allow us at this point conclude that their karyotypes are plesiomorphic. It may be, however, that in these families, as in Fulgoroidea as a whole, that a karyotype with $2n = 30, 29$ (φ , σ) and an XX:XO mechanism is plesiomorphic.

Reproductive system characters. The phylogenetic and taxonomic significance of the number of testicular follicles in Cicadinea was demonstrated by Yemel'yanov and Kuznetsova (1983), in which article it was suggested that the number of ovarioles are also taxonomically diagnostic. In some groups of Cicadinea (Delphacidea) the form of the σ accessory glands has phylogenetic and taxonomic significance (Kirillova, 1989).

The number of testicular follicles in fulgoroids ranges between 3 and 20 per testis, while the number of ovarioles is between 3 and 30 per ovary (Yemel'yanov and Kuznetsova, 1983; Kaplin, 1985). In the evolution of the individual families are of the Fulgoroidea as a whole, a tendency is observed toward oligomerization in the number of testicular follicles. In contrast to the follicles, the number of ovarioles often shows intraspecific variation. There are as yet few data on the structure of the accessory glands of the σ reproductive system in fulgoroids. Structurally, the accessory glands in σ fulgoroids vary; more often they are tubular, sometimes bifid, and the extent of variation differs (Ivanov, 1928; Kirillova, 1989. According to Kirillova, (1989), in the evolution of the Delphacidae, there is a tendency for the accessory glands to shorten and divide.

In the almost all Tettigometrid species we investigated there are 6 testicular follicles per testis. The exception is *T. costulata* (Fig. 2), in which there are 5 per testis (although in one individual there were 6 and 5 follicles in the different testes). The data correlate well with the view that the subgenus *Stirometra*, of which the only member is *T. costulata* is highly derived (Yemel'yanov, 1980). It should be noted that primitive delphacid members of the subfamily Asiracinae '*Asiraca clavicornis* and *Copicerus irroratus*' have 6 testicular follicles (Kirillova, 1989). Data obtained on the Tettigometridae confirm the previous hypothesis that the primitive number of testicular follicles is 6 for the Fulgoroidea as a whole (Yemel'yanov and Kuznetsova, 1983).¹ Thus, the tendency noted for the Fulgoroidea toward oligomerization in the number of testicular follicles is manifest also within individual families. This is 6 and 5 in the Tettigometridae, 6 and 3 in Delphacidae, and 6 and 4 in Cixiidae and Dictyopharidae.

As for the structure of the accessory glands in fulgoroids, apparently the tubular form with each gland undivided is plesiomorphic. We found accessory glands with a similar structure in all investigated σ Tettigometrids (Fig. 2), in *Philotheria* sp., of the primitive subfamily Dictyopharinae (Dictyopharidae), and also as was demonstrated by Kirillova (1989), in primitive delphacids of the subfamilies Asiracinae, Kelisiinae, and Stenocraninae.

The number of ovarioles in the Fulgoroidea and other Cicadinea varies within the species. In 6 specimens of *T. vitellina* the number of ovarioles per ovary was, respectively, 6-6, 10-10, 11-11, 12-12, 15-15, 14-14. Such variability limits the use of this character for taxonomy. It cannot be ruled out, however, that it may have phylogenetic significance. For example, oligomerization in the number of ovarioles is observed in the Dictyopharidae from 6 in the primitive Dictyopharinae to 4 in the more advanced Almanini (Orgériinae) (Ivanov, 1928; Kaplin, 1985). It is possible that a similar evolutionary trend is also characteristic of other Fulgoroid families. According to our data, there are 2 delphacid species of the primitive subfamily Asiracinae with 19-20 ovarioles per ovary, whereas *Stobaera muiri* of the advanced subfamily Delphacinae has 8-9(?). Also in the family

¹At that time the number of testicular follicles (6) was known only in *Tettigometra oblique* Panz. (Ivanov, 1928).

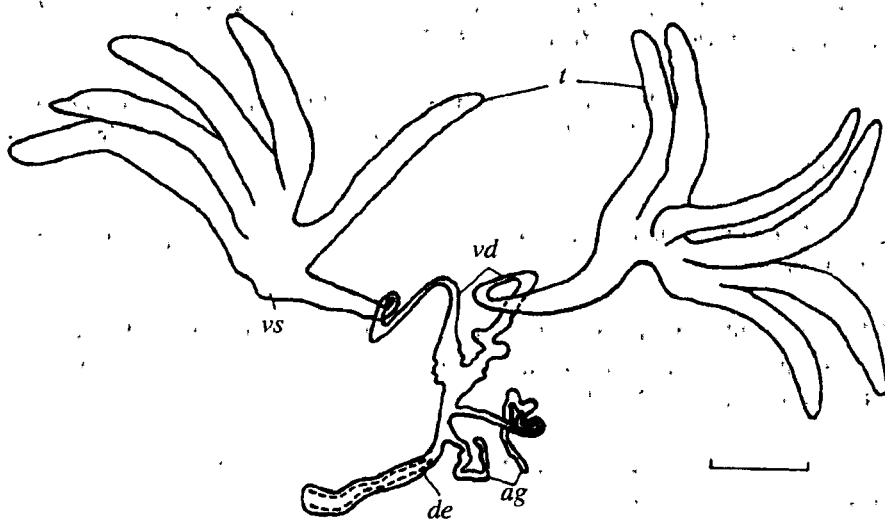


Fig. 2. Structure of ♂ reproductive system in *Tettigometra costulata*: t) testes; vd) vasa deferentia; vs) seminal vesicles; ag) accessory glands; de) ejaculatory duct. Scale: 0.4 mm.

Tettigometridae; *T. eremi* has 11-12 ovarioles and the more advanced *T. costulata* apparently has 6 (Table).

Thus, the obtained data on the most primitive family, Tettigometridae, and on certain other fulgoroid families, make it possible to postulate the plesiomorphic karyotype, anatomical features of the reproductive system (number of testicular follicles, and structure of the accessory glands), as well as the evolutionary trends of the changes in all these characters in the superfamily. We believe that in the Tettigometridae and Fulgoroidae as a whole, the following are plesiomorphic characters: 1) $2n = 30, 29$ (♀, ♂); karyotype with chromosomes gradually declining in size; sex-determining mechanism $XX:XO$; 2) 6 testicular follicles per testis; 3) tubular, undivided accessory glands in ♂.

In the evolution of the Tettigometridae and Fulgoroidea as a whole, there is observed: 1) reduction in the number of chromosomes by fusion of autosomes to form one large chromosome pair or by fusion of one autosome to the sex chromosome to result in the neo-XY mechanism; 2) oligomerization in the number of testicular follicles; 3) and possibly, oligomerization in the number of ovarioles.

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