Folia biologica (Kraków), vol. **56** (2008), No 3-4
doi:10.3409/fb.56_3-4.209-212

Karyotypic Diversity in the Subfamily Eurytominae (Hymenoptera: Eurytomidae)*

Vladimir E. GOKHMAN and Andrey P. MIKHAILENKO

Accepted April 22, 2008

GOKHMAN V. E., MIKHAILENKO A. P. 2008. Karyotypic diversity in the subfamily Eurytominae (Hymenoptera: Eurytomidae). Folia biol. (Kraków) **56**: 209-212.

New data on the karyotype structure of five species of the subfamily Eurytominae are described, including the following species: *Eurytoma rosae* (2n=20), *E. robusta* (n=7, 2n=14), *E. serratulae* (n=6, 2n=12), *E. compressa* (2n=10) and *Sycophila biguttata* (2n=18). 2n=10, 12 and 14 are reported for the first time for the Eurytominae, in which only 2n=20 and 18 have been previously found. The taxonomic and phylogenetic implications of the new chromosomal information are discussed. Karyotypes containing ten subtelo/acrocentric chromosomes which gradually decrease in size can be considered as initial for the Eurytominae. *Eurytoma robusta* + (*E. serratulae* + *E. compressa*) form a particular clade where all branches are well-supported by morphological, biological and chromosomal apomorphies.

Key words: Chromosomes, karyotype, Hymenoptera, Eurytomidae, Eurytominae.

Vladimir E. GOKHMAN, Andrey P. MIKHAILENKO, Botanical Garden, Moscow State University, Moscow 119991, Russia.
E-mail: gokhman@bg.msu.ru.

The family Eurytomidae is a medium-sized group of the superfamily Chalcidoidea containing about 90 genera and more than 1400 species worldwide (NOYES 2004). However, chromosomes of only five members of the largest subfamily Eurytominae, belonging to the genera *Bephratelloides*, *Eurytoma* and *Sycophila*, have been previously examined (GOODPASTURE 1974; GOKHMAN & QUICKE 1995; GOMES *et al*. 1996; GOKHMAN 2005a, b). These studies revealed apparently low variation in chromosome number: 2n=20 in all but one investigated species (2n=18 was found only in *Sycophila biguttata*). Eurytomidae are therefore considered as a comparatively "high-numbered" chalcid family together with Mymaridae and Encyrtidae (n=8-12), as opposed to the "low-numbered" ones; n=2-7 in almost all other Chalcidoidea, except for the family Aphelinidae, which harbours forms with both high and low chromosome numbers (GOKHMAN 2005a, 2006). Nevertheless, we have recently examined karyotypes of several eurytomine species with 2n=10, 12, 14, 18 and 20. The results of this study are given below.

Material and Methods

Adult females of parasitic wasps were reared from galls of Cynipidae (Hymenoptera) and Tephritidae (Diptera) and flower heads of wild Rosaceae and Asteraceae collected in the Moscow Area (Ozhigovo, 60 km SW Moscow) by the senior author, as well as in the Krasnodar Prov. (near Krasnodar) by V.V. Kostjukov and in the Stavropol Prov. of Russia (Prietokskiy, 150 km SE of Stavropol) by E. N. Yegorenkova in 2006-2007. Chromosome preparations were obtained from ovaries according to the standard technique for studying chromosomes in adult parasitic wasps (GOKHMAN & QUICKE 1995). Cell divisions were studied and photographed using the optic microscope Zeiss Axioskop 40 FL fitted with the digital camera AxioCam MRc. To obtain karyograms, the resulting images were processed with the image analysis program AxioVision version 3.1 and Adobe Photoshop version 6.0. Mitotic chromosomes were classified into four groups (metacentric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A)) according to LEVAN *et al*. (1964) and IMAI *et al*. (1977); meiotic chromo-

*The study was partially supported by grant no. 07-04-00326 from the Russian Foundation for Basic Research.

somes were classified according to the monograph of DARLINGTON (1965). Arm numbers (NF) were also calculated. Parasitic wasps were identified by the senior author, most of the identifications were confirmed by M.D. Zerova. The taxonomic position of the studied species is generally given according to NOYES (2004), but species groups of the genus *Eurytoma* are given according to BUGBEE (1967) and ZEROVA (1995, 2007). Voucher specimens are deposited in the Zoological Museum, Moscow State University, Moscow, Russia.

Results

Eurytoma rosae Nees (Fig. 1). 2n=20 (2M + 6SM + 12ST); NF=40. All chromosomes are bi-armed (although most of them are either submeta- or subtelocentrics) and gradually decrease in size within the karyotype.

E. robusta Mayr (Figs 2, 6). n=7, 2n=14 (8M + 4SM + 2ST); NF=28. As in the other species studied in this paper, all chromosomes of *E. robusta* are bi-armed. However, three pairs of metacentrics are obviously longer than the other chromosomes. There are seven bivalents in meiosis; each of the largest three carries two chiasmata, the other four bear single ones.

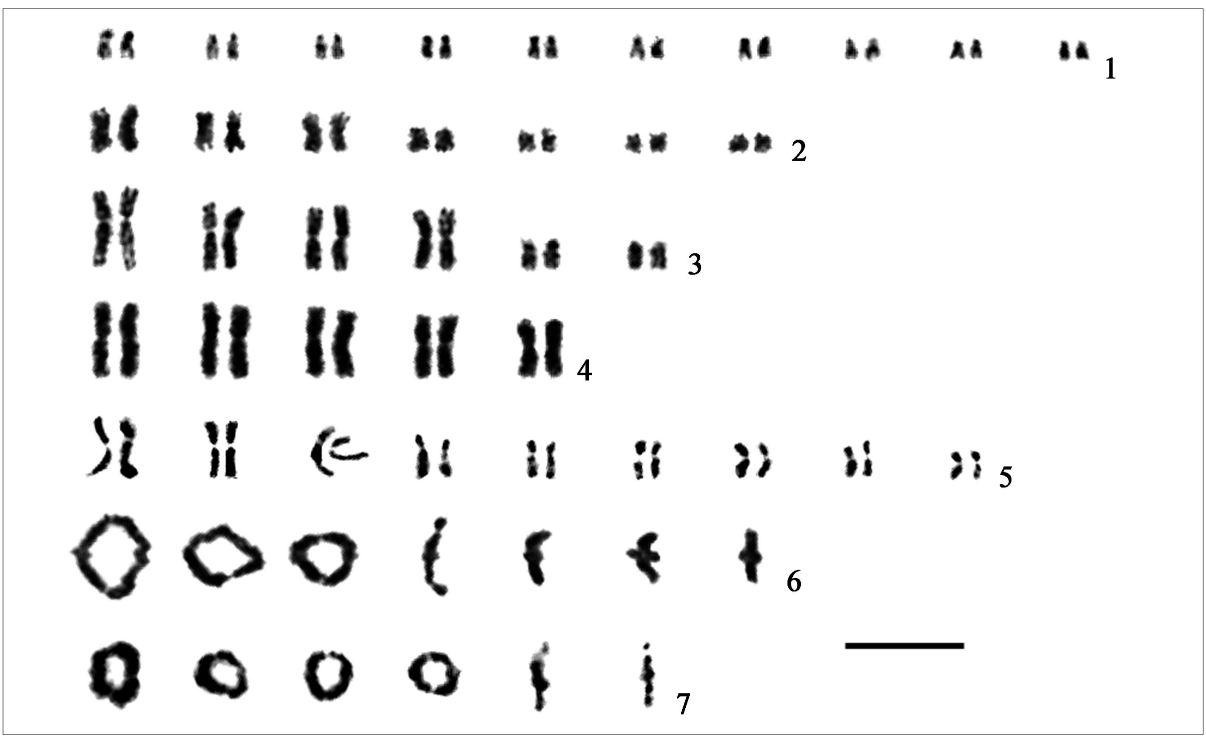
E. serratulae (Fabricius) (Figs 3, 7). n=6, 2n=12 (8M + 2SM + 2ST); NF=24. Four pairs of metacentrics are obviously longer than the other chromosomes (submeta- and subtelocentrics). There are six bivalents in meiosis; each of the largest four bears two chiasmata, the other two carry single ones.

E. compressa (Fabricius) (= *tibialis* Boheman) (Fig. 4). 2n=10 (10M); NF=20. Five pairs of large metacentric chromosomes present in the karyotype.

Sycophila biguttata (Swederus) (Fig. 5). 2n=18 (4M + 14SM); NF=36. All chromosomes gradually decrease in size; two of the largest ones are metacentrics.

Discussion

Although this study confirms that n=10 is the modal chromosome number in the subfamily Eurytominae (GOKHMAN 2005a), lower n values (specifically, n=9, 7, 6 and 5) were also detected (see Table 1). It is more difficult, however, to determine the initial karyotype structure for the studied group. Since karyotypes containing ten (or eleven) subtelo/acrocentric chromosomes which gradually decrease in size are found not only in the Eurytominae (especially in the *rosae* species group of *Eurytoma*), but also in the other "high-numbered" chalcids, i.e. in the Aphelinidae and Encyrtidae, as well as in some Cynipoidea (GOKHMAN 2005a), chromosomal sets of this kind can be considered as initial at least for the Eurytominae, and perhaps also for the whole family. If this is true, all other karyotypes of the Eurytomidae which have n=10 and contain bi-armed chromo-



Figs 1-7. Chromosomes of Eurytominae. Mitotic karyograms: 1) *Eurytoma rosae*, 2) *E. robusta*, 3) *E. serratulae*, 4) *E. compressa*, 5) *Sycophila biguttata*. Meiotic (diplotene) karyograms: 6) *Eurytoma robusta*, 7) *E. serratulae*. Bar = 10 μm.

Table 1

Chromosome numbers in the family Eurytomidae (values given in brackets are extrapolated from the known ones)

Species	n	2n	References
Genus *Bephratelloides*			
B. pomorum (Fabricius)	10	20	GOMES *et al*. 1996
Genus *Eurytoma*			
gigantea species group			
E. californica Ashmead	10	?20	GOODPASTURE 1974
rosae species group			
E. brunniventris Ratzeburg	(10)	20	GOKHMAN 2005a
E. rosae Nees	(10)	20	present paper
phragmiticola species group			
E. flavimana Boheman	(10)	20	GOKHMAN 2005b
robusta species group			
E. robusta Mayr	7	14	present paper
tibialis species group			
E. serratulae (Fabricius)	6	12	present paper
E. compressa (Fabricius)	(5)	10	present paper
Genus *Sycophila*			
S. biguttata (Swederus)	(9)	18	GOKHMAN & QUICKE 1995; present paper

Table 2

List of apomorphic character states for the clade "*Eurytoma robusta* + (*E. serratulae* + *E. compressa*)"

Clade	Apomorphic character states
Eurytoma robusta + (*E. serratulae* + *E. compressa*)	karyotype with not less than three large M; parasitism on Tephritidae in flower heads of Asteraceae
E. serratulae + *E. compressa*	karyotype with not less than four large M; presence of lateral mesepisternal teeth in front of middle coxae; endoparasitism
E. robusta	presence of mesosternal keel
E. compressa	karyotype with five large M
E. serratulae	parasitism in tephritid galls on Asteraceae

somes (*Bephratelloides pomorum*, *Eurytoma flavimana* and probably also *E. californica*) are derived from the initial ones either by inversions or, more probably, by the tandem growth of the constitutive heterochromatin on the shorter chromosome arms (GOKHMAN 2005a). Similar processes could have also taken place in the genus *Sycophila*, but at least one chromosome fusion occurred in *S. biguttata* (see below).

Nevertheless, the karyotype with ten subtelo/acrocentrics is considered as initial at least for all studied species of the genus *Eurytoma* with the possible exception of *E. flavimana* and *E. californica*. Moreover, chromosomal evolution in certain clades of this group can be tracked in detail. Specifically, karyotypic features of *Eurytoma compressa*, *E. serratulae* and *E. robusta* clearly indicate that they all belong to a particular lineage characterized by the accumulation of chromosomal fusions (similar to Robertsonian fusions). Furthermore, this clade is also supported by a few morphological and biological characters (see Table 2). This is especially true for *E. compressa* and *E. serratulae* which both belong to the *tibialis* species group and are very closely related (ZEROVA 1995). Karyotypic structure of the studied *Eurytoma* species therefore suggests that the lineage "*E. robusta* + (*E. serratulae* + *E. compressa*)" is marked at least by three chromosomal fusions, the clade "*E. serratulae* + *E. compressa*" – by another rearrangement of the same kind, and the fusion process is completed in the latter species. This result does not seem surprising since *Eurytoma* species, parasitizing gall-forming Hymenoptera and Diptera on herbaceous plants (and similar hosts), are considered the most advanced in the genus (ZEROVA 2007). Unidirectional chromosomal fusions and the corresponding decrease in chromosome number (see GOKHMAN 2005a, 2006) therefore allow the determination of the polarity of certain phylogenetic changes in the genus *Eurytoma*.

Recently an extensive phylogenetic study of the Eurytominae based on morphological characters has been performed (LOTFALIZADEH *et al*. 2007). This study confirms that the genus *Eurytoma* is not monophyletic (see also CHEN *et al*. 2004). However, the *rosae* group *sensu* LOTFALIZADEH *et al*. (2007) (which includes *Eurytoma brunniventris* and *E. compressa*) is monophyletic in the main analysis of this genus (see Cladogram 6). Nevertheless, the authors also placed *E. robusta* on this cladogram in a separate species group (*robusta*) which is fairly distant from their *rosae* group. On the other hand, LOTFALIZADEH *et al*. (2007) define the well-supported clade "*Eurytoma robusta* + (*E. brunniventris* + *E. compressa*)" in their preliminary analysis (Cladogram 2).

The obtained results also demonstrate that independent multiple decreases in chromosome number, characteristic of many groups of parasitic wasps including Chalcidoidea, e.g. Aphelinidae (GOKHMAN 2005a, 2006), take place in the Eurytomidae as well. Moreover, these rearrangements occur at least in two lineages of the subfamily Eurytominae, namely, in the genus *Sycophila* and in a certain clade of *Eurytoma*. Syn- and autapomorphic chromosomal fusions can be therefore potentially used in phylogenetic studies of the Eurytominae (especially within the genus *Eurytoma*), in which modern studies still fail to resolve relationships of many groups or lead to ambiguous results (CHEN *et al*. 2004; LOTFALIZADEH *et al*. 2007).

Acknowledgements

The authors are very grateful to Dr. V. V. KOSTJUKOV (All-Russian Institute for Biological Plant Protection, Krasnodar, Russia) and E. N. YEGORENKOVA (Ulyanovsk Teachers' Training Institute, Ulyanovsk, Russia) for collecting insect galls as well as to Prof. M. D. ZEROVA (Institute of Zoology, National Academy of Sciences, Kiev, Ukraine) for checking identifications of parasitic wasps.

References

BUGBEE R. E. 1967. Revision of chalcid wasps of genus *Eurytoma* in America north of Mexico. Proc. US Nat. Museum **118**: 433-552.

CHEN Y., XIAO H., FU J., HUANG D.-W. 2004. A molecular phylogeny of eurytomid wasps inferred from DNA sequence data of 28S, 18S, 16S and COI genes. Molecular Phylogenetics and Evolution **31**: 300-307.

DARLINGTON C. D. 1965. Cytology. J. & A. Churchill Ltd., London.

GOKHMAN V. E. 2005a. Karyotypes of parasitic Hymenoptera. KMK Scientific Press Ltd., Moscow. (In Russian).

GOKHMAN V. E. 2005b. New chromosome records for the superfamily Chalcidoidea (Hymenoptera). Cytologia **70**: 239-241.

GOKHMAN V. E. 2006. Karyotypes of parasitic Hymenoptera: Diversity, evolution and taxonomic significance. Insect Science **13**: 237-241.

GOKHMAN V. E., QUICKE D. L. J. 1995. The last twenty years of parasitic Hymenoptera karyology: an update and phylogenetic implications. J. Hymenoptera Research **4**: 41-63.

GOMES L. F., PEREIRA M. J. B., POMPOLO S. G., CAMPOS L. A. O. 1996. Determination of the karyotype of *Bephratelloides pomorum* (Hymenoptera: Eurytomidae). Brazilian Journal of Genetics **19**(3): 127 (abstract A.144).

GOODPASTURE C. 1974. Cytological data and classification of the Hymenoptera. Unpublished PhD Thesis. University of California, Davis.

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

LEVAN A., FREDGA K., SANDBERG A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201-220.

LOTFALIZADEH H., DELVARE G., RASPLUS J.-Y. 2007. Phylogenetic analysis of Eurytominae (Chalcidoidea: Eurytomidae) based on morphological characters. Zool. J. of the Linnean Society **151**: 441-510.

NOYES J. S. 2004. Universal Chalcidoidea Database: http://www.nhm.ac.uk/research-curation/projects/chalcidoids/.

ZEROVA M. D. 1995. Parasitic Hymenoptera – Eurytominae and Eudecatominae of Palaearctics. Naukova Dumka, Kiev. (In Russian).

ZEROVA M. D. 2007. On the subdivision of Palearctic species of the genus *Eurytoma* (Hymenoptera, Eurytomidae) into species groups. (In: Studies on Hymenopterous Insects. A. P. Rasnitsyn, V. E. Gokhman eds. KMK Scientific Press Ltd., Moscow): 28-37. (In Russian).