Chromosomal analysis: an effective research tool in phylogenetics and taxonomy of parasitoid Hymenoptera

Хромосомный анализ – эффективный инструмент исследований по филогенетике и таксономии паразитических перепончатокрылых (Hymenoptera)

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Key words: Hymenoptera, parasitic wasps, parasitoids, chromosomes, karyotypes, phylogenetics, taxonomy.
Ключевые слова: Hymenoptera, паразитические перепончатокрылые, паразитоиды, хромосомы, кариотипы, филогенетика, таксономия.

Abstract. A brief review of phylogenetic and taxonomic implications of chromosomal analysis of parasitoid Hymenoptera is given. Although karyotypic research of parasitoids is generally represented by studies using pre-existing phylogenetic reconstructions of certain taxa, accumulating evidence can suggest some chromosomal synapomorphies that define a number of clades. As far as taxonomic aspects of the chromosomal analysis of parasitoid Hymenoptera are concerned, this analysis is most effective at the species level.

Резюме. Сделан краткий обзор использования хромосомного анализа паразитических перепончатокрылых для целей филогении и таксономии. Хотя исследования кариотипов паразитоидов в основном представлены работами, использующими уже существующие филогенетические реконструкции тех или иных таксонов, накапливающиеся данные позволяют предположить некоторые хромосомные синапоморфии, определяющие ряд филогенетических ветвей. Что же касается таксономических аспектов хромосомного анализа паразитических перепончатокрылых, то этот анализ является наиболее эффективным на видовом уровне.

Parasitoid Hymenoptera are one of the most abundant, taxonomically complicated and economically important insect groups [Heraty, 2009]. However, many problems of their phylogeny and taxonomy remain unresolved [Quicke, 1997]. Currently, chromosomal analysis of parasitoid Hymenoptera is a quickly developing research field [Fusu, 2008; Gokhman, 2009; Van Vugt et al., 2009; Bolsheva et al., 2012; Gebiola et al., 2012; Carabajal Paladino et al., 2013; Gadau et al., 2015]. The present paper reviews recent advancements in karyotypic study of this group and its application to phylogenetics and taxonomy of parasitoid Hymenoptera.

The author dedicates this paper to the memory of the late Prof. G.M. Dlussky, an outstanding researcher of insects of the order Hymenoptera.

Phylogenetic implications of karyotypic study of parasitoid Hymenoptera

Phylogenetic research is very important for analysis of karyotype evolution of living organisms because using this kind of analysis is the only reliable way to identify karyotypic changes that have an independent evolutionary history (see, for example, Ross et al. [2015]). In the process of this research, certain phylogenetic presumptions, i.e. general assumptions about relationships between different taxa, are routinely used. In the end of the 1980s, Rasnitsyn and Dlussky [1988] published the first version of these presumptions, which were later revised [Rasnitsyn, 1996, 2006] and adapted to phylogenetic analysis of chromosomal data [Gokhman, 2009]. However, during previous years karyotypic research of parasitoid Hymenoptera was generally represented by studies of chromosomal rearrangements using pre-existing phylogenetic reconstructions of certain groups, for example, of the superfamilies Ichneumonoidea and Chalcidoidea [Gokhman, 2009, 2013]. This is generally true for many lower taxa as well, e.g. for some species groups of the genus *Aphelinus* Dalman, 1820 (Aphelinidae) (author's unpublished data).

Nevertheless, accumulating evidence can suggest some chromosomal synapomorphies that define a number of clades, e.g. those within the genus *Eurytoma* Illiger, 1807 (Eurytomidae) [Gokhman, Mikhailenko, 2008]. Most members of this group have $n = 10$, although certain species, i.e. *Eu. robusta* Mayr, 1878 (*robusta* species group), *Eu. serratulae* (Fabricius, 1798) and *Eu. compressa* (Fabricius, 1794) (both latter species belong to *tibialis* species group) have $n = 7$, 6 and 5 respectively. Karyotype structure of the studied *Eurytoma* species therefore suggests that the lineage *Eu. robusta* + (*Eu. serratulae* + *Eu. compressa*) is marked by at least three chromosomal fusions, the clade *Eu. serratulae* + *Eu. compressa* is marked

by another rearrangement of the same kind, and the fusion process is completed in the latter species. However, recent analysis using FISH with 18S rDNA probe showed that this pattern is more complicated [Gokhman et al., 2014a]. Specifically, both *Eu. robusta* and *Eu. serratulae* have a single rDNA cluster whereas *Eu. compressa* shows two sites of this kind in the haploid set. Furthermore, rDNA clusters are localized on the largest metacentric in the first species and on a medium-sized metacentric in the second species, whereas both the largest and the medium-sized metacentric chromosomes carry these sites in the remaining one. Karyotypes of *Eu. robusta* and *Eu. serratulae* might therefore have resulted from parallel independent losses of the different rDNA clusters.

Telomeres are multifunctional terminal structures of linear eukaryotic chromosomes. In many insects, including most aculeate Hymenoptera, telomeric DNA is usually composed of lengthy stretches of a pentanucleotide sequence motif, $(TTAGG)_n$ [Frydrychová et al., 2004]. However, we were recently able for the first time to show loss of the TTAGG repeat in the superfamilies Ichneumonoidea, Cynipoidea and Chalcidoidea, i.e. in the most prolific clades of parasitoid Hymenoptera, and perhaps in the latter group in general [Gokhman et al., 2014a]. Together with detection of the apparently parallel loss of the above-mentioned repeat in the single studied member of the superfamily Vespoidea [Menezes et al., 2013], our results suggest that telomere structure can be much more variable among different hymenopteran taxa than it was supposed just a few years ago.

To conclude, the phenomenon of the so-called karyotypic orthoselection, i.e. presence of common evolutionary traits that are manifested in different lineages and caused by canalizing selection [White, 1969, 1973], should also be mentioned. Karyotypic orthoselection leads to the parallel development of similar character states and therefore hampers phylogenetic studies. For example, independent chromosomal fusions within karyotypes of different taxa of the superfamily Chalcidoidea produced similar chromosome sets with lower n values and prevalence of bi-armed chromosomes [Gokhman, 2013]. Again, this situation shows importance of phylogenetic analysis for studying karyotype evolution in parasitoid Hymenoptera [Gokhman, 2009].

Taxonomic aspects of chromosomal analysis of parasitoid Hymenoptera

As far as taxonomic implications of the chromosomal analysis of parasitoid Hymenoptera are concerned, this analysis is most effective at the species level [Gokhman, 2009]. For example, closely related species of parasitoids can differ in their chromosome numbers and other karyotypic features. Specifically, in the genus *Leptopilina* Förster, 1869 (Figitidae), both *L. heterotoma* (Förster, 1862) and *L. victoriae* Nordlander, 1980 have n = 10, whereas *L. boulardi* (Barbotin, Carton et Kelner-Pillault, 1979) from another species group has n = 9 [Gokhman et al., 2011]. Moreover, haploid karyotype of *L. boulardi* contains a very large metacentric that lacks from chromosome sets of the two other species.

A number of similar cases can be found within the superfamily Chalcidoidea. For example, karyotypes of four species of the genus *Metaphycus* Mercet, 1917 (Encyrtidae) were studied a few years ago [Gokhman, 2010]. Among them, most closely related species, *M. flavus* (Howard, 1881) and *M. luteolus* (Timberlake, 1916) both have n = 10, whereas *M. angustifrons* Compere, 1957 and *M. stanleyi* Compere, 1940 have n = 9 and 5 respectively. Analogously, three species of the genus *Anastatus* Motschulsky, 1859 (Eupelmidae), namely, *A. lichtensteini* (Ruschka, 1921), *A. catalonicus* Bolivar y Pieltain, 1935 and *A. bifasciatus* (Geoffroy, 1785) all have n = 5, but another examined member of this genus, *A. ruficaudus* Ferrière, has n = 10 [Fusu, 2008]. Within the vast and taxonomically complicated genus *Chrysocharis* Förster, 1856 (Eulophidae), *Chrysocharis* sp. aff. *albipes* (Ashmead, 1904) has $2n$ = 12, whilst two other studied members of this genus, *Ch. laomedon* (Walker, 1839) and *Chrysocharis* sp. aff. *laomedon*, both have $2n$ = 10 due to chromosomal fusion [Gokhman et al., 2014b].

Recently, an extensive karyotypic study of the genus *Pnigalio* Schrank, 1802 (Eulophidae) in which all examined species appeared to have the same chromosome number, $2n$ = 12, showed general concordance between species affinities based on morphological and karyotypic characters [Gebiola et al., 2012]. Nevertheless, two other phenomena were detected. The first case represents morphologically distinct species without reciprocal differences in chromosomal characters, like *P. vidanoi* Navone, 1999 and certain taxa of the *P. soemius* (Walker, 1839) complex. The second instance includes morphologically similar species that strongly differ in their karyotype structure, i.e. *P. agraules* (Walker, 1839) and *P. mediterraneus* Ferrière et Delucchi, 1957.

Several groups containing closely related species that differ in their karyotype structure can be found within the family Pteromalidae. Among these groups, apparent correlation between the degree of morphological and chromosomal similarity is also observed. Specifically, only subtle differences in both external morphology and karyotype structure were found between three species of the genus *Nasonia* Ashmead, 1904, i.e. *N. vitripennis* (Walker, 1836), *N. longicornis* Darling, 1990 and *N. giraulti* Darling, 1990 all having n = 5 [Gokhman, Westendorff, 2000]. On the other hand, *Anisopteromalus quinarius* Gokhman et Baur, 2014, a new cosmopolitan species that has been recently differentiated from the apparently well-known *A. calandrae* (Howard, 1881), obviously differs from the latter species in morphological, molecular, ecological and karyotypic features, with these species having n = 5 and 7 respectively [Baur et al., 2014]. Moreover, another biologically similar parasitoid, *Lariophagus distinguendus* (Förster, 1841), also appeared to harbour two closely related species [König et al., 2015] which have different chromosome numbers, n = 5 and 6 (author's unpublished data). In addition, morphometric analysis of their karyotypes suggests that they differ by two consecutive chromosomal rearrangements, i.e. by a centric fission and a pericentric inversion. However, these species, which can occasionally hybridize, are apparently closer to each other in terms of morphology and karyotype structure than those

belonging to the genus *Anisopteromalus* Ruschka, 1912. Nevertheless, apart from the members of the latter genus, both *Lariophagus* Crawford, 1909 species are already described, and in this case only species status of a particular name should be restored from synonyms (H. Baur, personal communication).

Future perspectives

Future progress of karyotypic research will undoubtedly lead to new discoveries of cryptic species of parasitoid Hymenoptera. In addition, the above-mentioned examples show that chromosome studies will further facilitate species diagnostics in this group. Moreover, FISH and other advanced cytogenetic techniques will be successfully used in phylogenetic analysis of parasitoids [Gokhman et al., 2014a]. The accumulated evidence also suggests that a combination of chromosomal analysis and genome size estimates can be very useful for reconstructing genome evolution of parasitoid Hymenoptera ([Gokhman et al., 2011] and author's unpublished data).

Acknowledgements

I am very grateful to Keith R. Hopper (USDA-ARS and University of Delaware, Newark, Delaware, USA) and Johannes L.M. Steidle (Institut für Zoologie, Universität Hohenheim, Stuttgart, Germany) for allowing me to study laboratory stocks of parasitoids maintained at their respective institutions, as well as to Hannes Baur (Abteilung Wirbellose Tiere, Naturhistorisches Museum der Burgergemeinde Bern, Bern, Switzerland) for providing useful taxonomic information.

The present study was partly supported by a research grant No. 15-04-07709 from the Russian Foundation for Basic Research.

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