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Chromosome Study of the Hymenoptera: History, Current State, Perspectives

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Abstract—Two correlated genetic features are characteristic of the order Hymenoptera, i.e., arrhenotoky and haplodiploidy, but multiple transitions to diploid thelytoky also occurred within this group. Karyotypes of approximately two thousand members of the order are recently known. History of the chromosomal study of the Hymenoptera can be provisionally subdivided into four stages, with approximate borders of the 1930s, 1970s and 2000s between them. Although the development of this study can mainly be explained by the technical progress in preparing and analyzing chromosomal preparations, the results obtained with the help of earlier developed methods, also can successfully be used nowadays. In addition to morphometric analysis, a number of differential staining techniques are used to identify particular chromosomes and their segments; these techniques can conditionally be subdivided into two groups, the so-called "traditional" and "modern" ones. First of all, C- and AgNOR-bandings constitute the former methods; these techniques visualize heterochromatic segments and nucleolus organizing regions respectively. Moreover, modern methods are also widely used at present for studying parasitoid karyotypes. These techniques include use of fluorescent dyes (fluorochromes), especially those specifically staining AT- and GC-rich chromosome segments. Fluorescence in situ hybridization (FISH) is a very important method of physical mapping of DNA sequences on chromosomes. Immunocytochemical techniques can be of use to study chemical content and structure of chromosomes; these methods involve use of specific fluorochrome-conjugated antibodies. Nowadays, taxonomic significance of karyotypic study of the order Hymenoptera substantially increases, especially within the framework of the so-called integrative taxonomy, aimed for recognition, delimitation and description of closely related species. Furthermore, a combined use of classical and molecular methods has very good perspectives. Knowledge of hymenopteran phylogeny is necessary for identifying pathways of karyotype evolution of the order, but at least in some cases chromosome characters can be considered as synapomorphies defining different lineages. Karyotypic research also has very important implications for genetic studies of Hymenoptera. The chromosome number equals the number of linkage groups within the genome, but it also can be used as a proxy to the level of genetic recombination, especially in the context of big data approach. In addition, significance of physical mapping of DNA sequences increases in the light of the modern efforts in genome sequencing. FISH is most often used for mapping repetitive sequences, including ribosomal DNA, microsatellites and telomeric segments. Nevertheless, this technique could be useful for mapping unique sequences as well. In the order Hymenoptera, FISH is also successfully used together with chromosome microdissection for identifying particular chromosomes and/or chromosome segments, as well as various chromosomal rearrangements. In addition, chromosomal analysis can reveal the so-called supergenes, i.e., inverted chromosome segments, which accumulate genetic differences. Finally, immunocytochemical techniques can map distribution of various chemical compounds along the chromosomes, including identification of the degree of methylation of the chromosomal DNA.

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INTRODUCTION

The order Hymenoptera is one of the largest, taxonomically complicated and practically important groups of insects. According to certain estimates, the number of potentially recognized species of this order may well exceed one million, mostly at the extent of parasitic forms (Bebber et al., 2014; Forbes et al., 2018). Nevertheless, this group contains not only parasitoids, but also predators, herbivores (including nectar feeders/pollinators), as well as members with different ecology, which play the key role in ecosystems of our planet (LaSalle and Gauld, 1991; Huber, 2017). Three traditional groups are most often recognized within Hymenoptera, i.e., Symphyta (lower Hymenoptera, or horntails and sawflies, which almost exclusively feed on higher plants), Parasitica (parasitoid Hymenoptera, or parasitic wasps, which mostly develop at the expense of various insects and other arthropods), and Aculeata (stinging Hymenoptera, or wasps, bees, ants etc., which are generally represented

by predators and nectar feeders). In turn, the group incorporating both parasitic and stinging Hymenoptera is usually termed Apocrita, or higher Hymenoptera (Gauld and Bolton, 1988). Hymenoptera include a huge number of diverse forms, which are often hardly distinguished externally, and achievements of various biological disciplines must be used to study these forms. Among these disciplines, methods and approaches of modern genetics, including chromosome study (Crozier, 1975), are the most important. This research field, which originated at the end of the 19th century, is currently experiencing a period of rapid development that is partly associated with the use of molecular techniques (Gokhman, 2007, 2019; Cardoso et al., 2018; Cunha et al., 2021). The present work is a brief review of the history, current condition and prospective directions of the chromosome study of the order Hymenoptera.

MAIN GENETIC FEATURES OF HYMENOPTERA

Two initial genetic features are characteristic of the order Hymenoptera. The first one is arrhenotoky, i.e., development of males from unfertilized eggs, apart from females (Heimpel and de Boer, 2008; Gokhman and Kuznetsova, 2018a). In the Hymenoptera, haplodiploidy, or presence of diploid females and haploid males, is usually correlated with arrhenotokous reproduction (Crozier, 1975; van Wilgenburg et al., 2006). However, there is a number of notable exceptions to these rules. Indeed, certain parasitoids were found to carry paternally transmitted genetic factors, which, after entering the fertilized diploid zygote, transform it into a haploid one. In turn, a male develops from the latter (Nur et al., 1988; Werren, 1991; Hunter et al., 1993; van Vugt et al., 2005, 2009). This happens through elimination of the paternal genome or, at least, of its most part. Moreover, several species of ants (Formicidae), in fact, consist exclusively of males, because, on the contrary, the paternal genome of these males, when crossed with females of related species, eliminates the maternal one from the fertilized egg, which again gives rise to a haploid male (Schwander and Keller, 2012). In addition, polyploid, i.e., tri- and tetraploid females (Macy and Whiting, 1969; Leung et al., 2019), as well as diploid (Harpur et al., 2013) and even triploid males, were discovered in a number of hymenopterans. In particular, triploid males were produced by strict inbreeding under laboratory conditions in *Athalia rosae* (Linnaeus) (Tenthredinidae) and *Bombus terrestris* (Linnaeus) (Apidae) (Naito and Suzuki, 1991; Ayabe et al., 2004). In addition, multiple independent transitions to diploid thelytoky, when unfertilized eggs exclusively gave rise to females, occurred within various groups of Hymenoptera (Heimpel and de Boer, 2008; Gokhman and Kuznetsova, 2018a). Either thelytokous or arrhenotokous parthenogenesis is usually characteristic of various species and populations of the Hymenoptera, but the so-called cyclic thelytoky, under which these types of parthenogenesis regularly alternate between different generations, is discovered in many gall wasps of the family Cynipidae (Crozier, 1975). However, thelytoky is absent in a number of gall wasp species, but, on the other hand, it is the family that harbors the only known triploid thelytokous species, *Diplolepis eglanteriae* (Hartig) (Sanderson, 1988). Further modifications of the life cycle related to various combinations of arrhenotokous and thelytokous reproduction, were discovered in certain ants. In particular, workers of some species, like, e.g., *Cataglyphis hispanica* (Emery) and *Wasmannia auropunctata* (Roger), originate from crossings of males and females, which belong to genetically different strains, whereas these females reproduce exclusively via thelytoky (Schwander and Keller, 2012).

STAGES OF THE STUDY OF HYMENOPTERAN CHROMOSOME SETS

First data on hymenopteran chromosomes were obtained 130 years ago, i.e., at the end of the 19th century (Henking, 1892). According to the evidence given in that work, haploid chromosome numbers (n) in *Diplolepis rosae* (Linnaeus) (Cynipidae) and *Lasius niger* (Linnaeus) (Formicidae) were accepted either equal to 9 or close to 10 respectively, although further studies demonstrated that these parameters were estimated correctly only for *D. rosae*, and $n = 15$ was later found in *L. niger* (Crozier, 1975). The history of studying chromosome sets of this order can be conditionally subdivided into four main stages (Gokhman, 2019), which are briefly described below.

First stage (1890s–1920s). During this stage, data on chromosome sets were obtained for about thirty hymenopteran species representing all main groups of the order, i.e., Symphyta, Parasitica and Aculeata (Sanderson, 1932). However, it is noteworthy that these data often did not result from a focused search, but appeared as "by-products" of histological and/or embryological studies. Due to this reason, sectioning technique was used for those studies, which frequently led to wrong estimates of chromosome numbers.

Second stage (1930s–1960s). During this period, the number of hymenopterans with studied chromosome sets increased by an order, reaching about 300 species. A study of parthenogenesis in sawflies of the family Tenthredinidae was published at the beginning of this stage (Sanderson, 1932). In fact, this work summarized all data on hymenopteran chromosomes, which were available that time. On the other hand, concepts on the initial mechanism of sex determination in Hymenoptera were substantially clarified during this period. Indeed, the hypothesis postulating that males of this group, apart from females, developed from unfertilized (hence haploid) eggs, appeared in the middle of the 19th century (see Crozier, 1975).

However, it might seem that the existence of diploid males, which was reliably established at least for certain species, directly contradicted this hypothesis. This contradiction was resolved in the paper by Snell (1935), who developed a model of sex determination in Hymenoptera, which we follow up to now, with certain changes and reservations. According to the Snell's hypothesis, this process is associated with the action of certain loci; heterozygosity at least for a single such locus determines female-type development, and organisms, which are either homozygous or hemizygous for all those loci, develop into males. However, acceptance of this model as an initial mechanism of sex determination for the whole order Hymenoptera prolonged for decades (Crozier, 1975). Apparently, it was for this reason that erroneous reports on sex chromosomes in this group appeared even after the publication of Snell's work (Dozortseva, 1936; Dreyfus and Breuer, 1944; Kerr, 1951). These errors were also possible because drawings instead of micrographs were used that time for documenting hymenopteran chromosome sets, which often led to subconscious misinterpretation of the displayed chromosome morphology. As for the chromosome numbers of the members of this order studied at that stage, most of these numbers subsequently turned to be determined correctly. Among a few but notable exceptions, a paper by Whelden and Haskins (1953) on ant chromosomes can be named.

Third stage (1970s–1990s). During this period, the number of hymenopteran species with studied chromosome sets increased to approximately 1200, i.e., about another four times. In the mid-1970s, the thesis by Goodpasture (1974a) appeared one of the important but less known works dedicated to the study of chromosomes of Hymenoptera. In this work, new results of the karyotypic research on about 50 species that belonged to 13 families of Parasitica and Aculeata were reported. Goodpasture also made important conclusions on the general features of morphology and evolution of hymenopteran chromosomes. Regretfully, despite several subsequent publications (Goodpasture, 1974b, 1975a, 1975b; Goodpasture and Grissell, 1975), a considerable amount of these materials remained unknown to a wide range of researchers. Something completely different happened to another fundamental work, which was published almost at the same time, i.e., the first monograph dedicated to the study of hymenopteran chromosomes (Crozier, 1975), which was published in the *Animal Cytogenetics* series. In this monograph, the results of the previous stages of the karyotypic study of Hymenoptera were summarized, and attempts were made to highlight the directions of future research on chromosome sets of this group. In particular, it is noteworthy that Crozier (1975) suggested to ignore the results obtained before the review by Sanderson (1932) was published, and, in some cases, even after that time. Numerous errors, which were subsequently detected among previously obtained data, were an apparent reason for that decision (see above). In addition, information from the former work allowed for stating that parasitic Hymenoptera appeared the least karyotypically studied among the three largest groups of the order. The paucity of the available information and, consequently, an apparent uniformity of chromosome numbers and other features of karyotype structure even led Crozier (1975) to a conclusion suggesting that karyotypic features could be of use in taxonomy only beginning from the family level, apart from Symphyta and Aculeata. This was certainly not true, and it could be easily verified already with works published by Goodpasture (Goodpasture, 1975a; Goodpasture and Grissell, 1975). First of all, the very poor state of knowledge on chromosomes of parasitic Hymenoptera which lasted until the mid-1970s, could be apparently explained by serious technical difficulties in studying parasitoid chromosomes. Nevertheless, a detailed review of karyotypic data on this group, which we published two decades later (Gokhman and Quicke, 1995), showed practical feasibility of overcoming most those difficulties. In addition, Japanese researchers invented a very simple but effective method of obtaining high-quality preparations of sawfly chromosomes, which was described in the survey by Naito (1982). This stage of karyotypic research on Hymenoptera is characterized by a wide array of technical achievements. First, the main technique of the chromosome study changed, i.e., a transition occurred from sectioning first to squashed preparations (Imai and Kubota, 1972), and then to the so-called air-dried ones (Imai et al., 1977, 1988; Palomeque et al., 1987; etc.). This substantially accelerated and simplified the process of making chromosomal preparations, and allowed to reveal previously unknown details of fine structure of hymenopteran chromosomes. Moreover, certain techniques of differential staining, like C-, AgNOR- and G-banding, were used for the karyotypic study of Hymenoptera for the first time (Sumner, 1972; Goodpasture and Bloom, 1975; Imai et al., 1977; Howell and Black, 1980; Burgos et al., 1986; Palomeque et al., 1987; Odierna et al., 1993; Reed, 1993; Lorite et al., 1996). Furthermore, the so-called DNA-specific fluorochromes began to be used for staining chromosome segments in Hymenoptera (Schweizer, 1976; Odierna et al., 1993; Lorite et al., 1997). Finally, results of the studies of chromosome sets became documented exclusively with micrographs, which substantially increased reliability of the obtained data. In fact, foundations of the modern achievements of hymenopteran cytogenetics were therefore laid during this period.

Fourth stage (2000s–present). Up to now, results of the karyotypic research of about two thousand hymenopterans are known. An obvious increase in the proportion of parasitoids among the karyotypically studied species apparently became a substantial feature of this stage. In particular, this can be seen both

from the first monograph, which was specifically dedicated to this topic (Gokhman, 2005), and from the current review (Gokhman, 2022). Moreover, databases and surveys on Symphyta (Westendorff, 2006) and some groups of Aculeata, including bees and ants (Lorite and Palomeque, 2010; Cardoso et al., 2018; Cunha et al., 2021), were created and published. On the other hand, this period is characterized by an intensive use of molecular approaches in the chromosome study. For example, this is expressed in the wide application of the technique of fluorescence in situ hybridization (FISH) (Matsumoto et al., 2002; van Vugt et al., 2005; Gokhman et al., 2014; etc.), which allows to perform the so-called physical mapping of DNA sequences, i.e., to identify their positions on chromosomes. Methods of immunocytochemistry, which reveal chromosomal localization of certain chemical compounds, may appear another potentially important technique within this research field (Bolsheva et al., 2012). Finally, the last, but not least, the rapid development of computerized cladistics should be named, especially of that based on the analysis of molecular data, including those obtained from full genome sequencing. This situation allows to create fairly adequate and reliable phylogenetic reconstructions for an increasing number of hymenopteran groups (see, e.g., Gokhman et al., 2017b), which provides opportunities for a detailed study of the processes of chromosomal evolution within various clades of the order Hymenoptera.

In general, one can conclude that developments in the study of hymenopteran karyotypes were mostly caused by the technical progress in obtaining and analyzing chromosomal preparations. Nevertheless, the results obtained with the help of previously developed methods, are also successfully used nowadays, if they are combined with new theoretical and technical achievements (see below).

IMPLICATIONS OF THE CHROMOSOME STUDY FOR HYMENOPTERAN SYSTEMATICS AND PHYLOGENY

At present, significance of the karyotypic study for taxonomy of the order Hymenoptera is substantially increased. This is especially true within the framework of integrative taxonomy, aimed at recognition, delimitation and description of closely related species (Schlick-Steiner et al., 2010; Gokhman, 2018). In particular, these studies showed that cryptic taxa often hide under the cover of external uniformity of hymenopteran "morphospecies" (see Gokhman, 2009 for review). Perhaps the brightest example of this research can be found in the history of detection and description of *Anisopteromalus quinarius* Gokhman et Baur, a cosmopolitan species of chalcid wasps of the family Pteromalidae, which parasitizes some coleopteran pests of stored products (Baur et al., 2014). Systematists earlier supposed that these parasitoids belonged to *A. calandrae* (Howard), another species with similar distribution and biology. However, our study demonstrated that these species had different chromosome numbers, $n = 5$ and 7 respectively. Moreover, they turned out to be reproductively isolated and substantially different in terms of morphology, lifestyle, behavior and DNA structure (Baur et al., 2014). Thus *A. quinarius* is, in fact, a "good" species, which remained unnoticed by experts due to a number of reasons. On the other hand, chromosomal analysis confirmed that *Lariophagus distinguendus* (Förster), another member of the Pteromalidae, which parasitizes stored-product pests of the order Coleoptera, in actual fact, also represents a complex of two closely related species. Despite different chromosome numbers, $n = 5$ and 6, and notable biological differences, these parasitic wasps are virtually indistinguishable by external characters and can interbreed under laboratory conditions (König et al., 2019). Analogous examples of detecting cryptic species through karyotypic analysis are also known for Symphyta and Aculeata (see Westendorff, 2006 and Seifert, 2009 for reviews). Finally, features of chromosome sets can be an additional argument for classifying members with deviating parameters into different supraspecific taxa. For example, $2n = 38$ was found in the majority of ants of the genus *Acromyrmex*, except for *A. ameliae* De Souza, Soares et Della Lucia with $2n = 36$, but $2n = 22$ was revealed in *A. striatus* (Roger) and related species (Cristiano et al., 2013; de Aguiar et al., 2020; Barros et al., 2021). Further analysis demonstrated that species close to *A. striatus* were characterized by original morphological and molecular features, which allowed for their separation into the newly described genus *Amoimyrmex* (Cristiano et al., 2020).

Phylogenetic knowledge is very important for detecting pathways of karyotype evolution of a given group (Cristiano et al., 2013; Afonso Neto et al., 2022; etc.). In particular, this can be clearly seen in an example of the chromosome sets of the very same *Lariophagus distinguendus* complex, in which species with $n = 5$ has a long metacentric chromosome, and an acrocentric and a shorter metacentric in the karyotype with $n = 6$ correspond to its arms (Gokhman et al., 2019). Since $n = 5$ is the most frequent chromosome number in the family Pteromalidae, this could create an impression that we were dealing with chromosomal fission, and the species with $n = 6$ was derived. However, molecular analysis showed that the lineage with $n = 5$ originated within the *L. distinguendus* complex with an initial $n = 6$, and a chromosomal fusion, not fission, therefore occurred (König et al., 2019). Based on the results of full genome sequencing, phylogenetic analysis also allowed to determine the order of pericentric inversions in some chalcid wasps of the genus *Aphelinus* (Aphelinidae). For example, it was detected that a rearrangement of this kind in the second chromosome was shared by the two sister species of the varipes group with $n = 4$, *A. hordei* Kurdjumov and

A. kurdjumovi Mercet, and another inversion turned the same metacentric chromosome into an acrocentric in the latter species (Gokhman et al., 2017b).

In addition to identifying pathways of chromosomal evolution using phylogenetic reconstructions based on foreign characters (Cristiano et al., 2013; Micolino et al., 2019; Travenzoli et al., 2019a; Afonso Neto et al., 2022), in a number of cases, some karyotypic features can also be considered as synapomorphies that mark various clades. The phylogenetic tree of certain wasps of the family Eurytomidae, which parasitize flies that belong to Tephritidae, can be an appropriate example (Gokhman and Mikhailenko, 2008). For instance, $n = 10$ is characteristic of most members of the genus *Eurytoma*, but the chromosome number in all species that attack tephritid flies is substantially lower due to consecutive chromosomal fusions, i.e., $n = 7$, 6 and 5 in *E. robusta* Mayr, *E. serratulae* (Fabricius) and *E. compressa* (Fabricius) respectively. The first of these species is the only examined member of the robusta group, and two others – of the compressa (=tibialis) group. These parasitoids are therefore separated from other studied species of *Eurytoma* not only by the karyotype structure, but also by the combination of morphological and biological features. Many of those characters, including chromosomal fusions, are synapomorphies, which define the topology of the obtained tree (Gokhman and Mikhailenko, 2008).

PLACE OF CHROMOSOMAL ANALYSIS IN THE GENETIC STUDY OF HYMENOPTERA

The chromosome number is known to define the number of linkage groups within a given genome. Direct estimates of this parameter are particularly important at present, when full genome sequencing of hymenopterans became a fairly ordinary procedure. Under these circumstances, an independent control of the degree of completeness of assembling the studied DNA sequences at the chromosome level becomes quite significant, especially if these chromosomes are of smaller size (see, e.g., Wittmeyer et al., 2022). In addition, the chromosome number can be used as a proxy for the rate of genetic recombination, especially in the context of big data. For example, recent studies showed that an average variation of chromosome numbers in social Hymenoptera, i.e., variance of recombination rates in this group, was about three times more than that in the solitary ones (Ross et al., 2015).

Up to now, a combination of various techniques was used for the karyotypic study of Hymenoptera. These methods are specifically designated for identifying particular chromosomes and their segments (Gokhman, 2005). Morphometric analysis is obviously the most accessible technique among those based on the usual (routine) chromosome staining (see, e.g., Gebiola et al., 2012). In particular, this study allows for calculating relative lengths and centromere indexes of each chromosome. It is also noteworthy that the use of morphometric analysis for identifying possible karyotypic rearrangements is the most effective for the low-numbered chromosome sets, which is a predominant characteristic of certain groups of parasitic wasps (Gokhman et al., 2017b; König et al., 2019).

Moreover, techniques of differential staining of hymenopteran karyotypes are tentatively subdivided into two groups, i.e., "classic," or "traditional," and "modern" ones. First of all, among the "classic" techniques, are the so-called C- and AgNOR-bandings, which visualize heterochromatic blocks of chromosomes and nucleolus organizer regions (NORs) respectively (Palomeque et al., 1987; Reed, 1993; Gebiola et al., 2012; Piccoli et al., 2018; Menezes et al., 2019; etc.). In addition, multiple attempts were undertaken to use the so-called G-banding, which is usually produced through the treatment of chromosomal preparations with proteolytic enzymes, mostly with trypsin, for studying hymenopteran chromosomes (Odierna et al., 1993; Lorite et al., 1996). Nevertheless, this banding presents a particular problem in insects, including Hymenoptera, although it allows to identify certain elements within a given karyotype. However, apart from vertebrates, detection of homologous chromosomes appears impossible, even between related species. Finally, treatment of chromosomes of a particular ant species with restriction endonucleases (Lorite et al., 1999) allowed to obtain the results which were closer either to C- or to G-banding, depending on the enzyme.

In addition to the traditional methods, modern ones are widely used at present for studying hymenopteran karyotypes. In particular, these techniques include the use of fluorochromes that specifically stain DNA, and primarily the chromosome segments enriched with AT and GC base pairs. For example, propidium iodide binds to DNA of any composition, whereas chromomycin A_3 (CMA_3) and 4',6-diamidino-2-phenylindole (DAPI) stain GC and AT base pairs respectively (Schweizer, 1976). In practice, since DNA of hymenopteran chromosomes is mostly represented by the AT-enriched fraction, these chromosomes are more or less fully stained with DAPI, often except for narrow segments with an increased content of GC pairs. In turn, these segments, which are stained with CMA_3, usually represent NORs (Gokhman et al., 2016; Tavares and Teixeira, 2022), but CMA_3-positive segments are not always associated with nucleolus organizers (Menezes et al., 2011). In particular, these segments can occupy terminal positions on many, if not all, chromosomes of a given set (Gokhman et al., 2017a; Menezes et al., 2019; Barbosa et al., 2021).

Fluorescence in situ hybridization (FISH) is the most important means of physical mapping of DNA

sequences, which allows for identifying their positions on chromosomes. From technical point of view, this method is based on hybridization of a fluorochrome-conjugated DNA probe, which is applied to a chromosomal preparation, with a particular strain of DNA within a certain chromosome (Speicher and Carter, 2005). At present, FISH is more frequently used in the study of hymenopteran chromosomes to reveal repetitive sequences, i.e., primarily, loci of ribosomal DNA (rDNA), as well as segments with higher concentrations of microsatellites and transposons (Lorite et al., 2012; Gokhman et al., 2014; Piccoli et al., 2018; Menezes et al., 2019, 2021; Micolino et al., 2019; Travenzoli et al., 2019b; Pereira et al., 2021a, 2021b; Tavares and Teixeira, 2022; etc.). For example, haploid chromosome sets of the majority of Hymenoptera carry one or two rDNA sites, but the number of these sites usually increases in high-numbered karyotypes, reaching 4, 6 and 15 in certain members of Symphyta, Parasitica and Aculeata respectively (Matsumoto et al., 2002; Paladino et al., 2013; Menezes et al., 2021).

The diversity of telomere structure in Hymenoptera apparently deserves to be mentioned separately. Just 10–15 years ago, experts believed that the TTAGG telomeric repeat, which is present in many groups of insects, is also characteristic of all Hymenoptera. However, subsequent analysis demonstrated that only a few members of the families Formicidae and Apidae were studied in this respect (Meyne et al., 1995; Frydrychová et al., 2004; Micolino et al., 2019, 2020; de Castro et al., 2020; etc.), and attempts to detect this repeat in other Apocrita failed up to a certain point (Gokhman et al., 2014; Menezes et al., 2017). Nevertheless, the TTAGG repetitive sequence was subsequently discovered within telomeres of the lower Hymenoptera, i.e., sawflies of the families Tenthredinidae, Cephidae and Orussidae. It was first done using FISH (Gokhman and Kuznetsova, 2018b), and then with the help of bioinformatic techniques (Zhou et al., 2022). The TTAGG telomeric repeat is therefore apparently initial for this order (Gokhman and Kuznetsova, 2018b), but it has been recently found that this motif can be substituted with very different repeats within Apocrita. These motifs can vary from the poly-T mononucleotide sequence of several hundred base pairs, which was revealed in a particular parasitic wasp of the family Ichneumonidae, up to, e.g., TTATTGGG, TTGCGTCTGGG and TTAGGTTGGGG telomeric repeats. With some variations, the latter sequences are characteristic of the studied members of chalcid and vespoid wasps as well as bumblebees of the genus *Bombus* respectively (Dalla Benetta et al., 2020; Lukhtanov, 2022). Moreover, according to bioinformatic data, the TTAGG repeat is present in a number of other Aculeata, in addition to ants and certain bees. Taken together, this information testifies in favor of the unprecedented diversity of telomeric sequences in Hymenoptera (Lukhtanov, 2022).

In addition, the FISH technique was used for mapping "supergenes" (inverted chromosome segments which accumulate genetic differences), as well as unique genes, in certain Hymenoptera (Matsumoto et al., 2002; Wang et al., 2013; Thompson and Jiggins, 2014). On the other hand, a combination of this method with the so-called chromosome microdissection, i.e., isolation of the nucleoprotein material from particular chromosomes or their specific regions with subsequent extraction of DNA, its amplification, and conjugation of the resulting probes with fluorochromes (Speicher and Carter, 2005), turned very promising. The use of these FISH probes allows to perform the so-called chromosome painting, which gives an opportunity to identify particular chromosomes and their specific segments, and also provides information on DNA sequences, which are characteristic of certain regions (Rütten et al., 2004; Fernandes et al., 2011; Martins et al., 2013; Lopes et al., 2014; Gokhman et al., 2019).

Finally, methods of immunochemistry can be used for studying the chemical composition and structure of hymenopteran chromosomes. These techniques imply use of specific antibodies conjugated with fluorochromes. As far as I know, the only work has been recently performed with this technique on chromosomes of Hymenoptera (Bolsheva et al., 2012). In this paper, antibodies against 5-methylcytosine were used, and this allowed for identifying the degree of DNA methylation along the chromosomes.

PERSPECTIVES OF THE CHROMOSOME STUDY OF HYMENOPTERA

All above-mentioned information shows that the study of hymenopteran chromosome sets currently continues to undergo rapid development. In this situation, identification of the most promising directions of developing these studies appears a fairly hard task, even for the immediate future. Nevertheless, at least some of these directions can be more or less reliably outlined. As for the expected serious breakthroughs in terms of research on particular groups, they apparently include parasitoid Hymenoptera, since the proportion of their studied species is quite low, especially if compared with many Aculeata and Symphyta (Gokhman, 2022). Beyond any doubt, modern techniques, like FISH and methods of immunochemistry, will become increasingly more widespread. Judging from certain indications, a combination of the chromosome study as such, with that of the genome size, can appear fruitful (Gokhman et al., 2017b; Moura et al., 2020). Hopefully, studies of hymenopteran meiosis, including those using advanced techniques, will also become fairly productive. Finally, it is noteworthy that the use of techniques of the chromosome study for the purposes of systematics and evolutionary genetics is the most effective if coupled with phylogenetic analysis, which is based on molecular characters, often com-

bined with morphological ones. This combination allows for obtaining very interesting and important results, especially at the level of related species and forms (see, e.g., Gokhman, 2018).

CONCLUSIONS

In 130 years, which elapsed from the beginning of the cytogenetic study of Hymenoptera, karyotypic data on about two thousand members of the order were obtained. This value constitutes a smaller part of the general number of described hymenopteran species, but, nevertheless, the mentioned results show substantial implications and perspectives of the chromosome study both for taxonomy and genetic research on Hymenoptera. It is important to understand that not only data on routine chromosome staining, but those obtained with the help of more advanced techniques, primarily staining with DNA-specific fluorochromes and fluorescence in situ hybridization (FISH), can be successfully used for these purposes. Further progress of the chromosome study of Hymenoptera apparently includes studies of new taxa and use of molecular techniques.

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COMPLIANCE TO ETHICAL STANDARDS

The present paper does not contain studies on warm-blooded animals as research objects.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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