Overall results of the chromosomal study of parasitic wasps of the subfamily Ichneumoninae (Hymenoptera: Ichneumonidae)

V.E. Gokhman

Итоги хромосомного исследования наездников-ихневмонид подсемейства Ichneumoninae (Hymenoptera: Ichneumonidae)

В.Е. Гохман

Botanical Garden, Moscow State University, Moscow 119234, Russia. E-mail: vegokhman@hotmail.com

Abstract. Overall results of the chromosomal study of 94 species of parasitic wasps of the subfamily Ichneumoninae are summarized in the present paper, including new data for the two species, Hoplismenus pica Wesmael (2n = 20) and Ichneumon melanotis Holmgren (2n = 24). The diploid chromosome number in the Ichneumoninae can vary from 2n = 16 to 2n = 34, with a clear mode at 2n = 22. An overview of the main pathways and mechanisms of karyotype evolution within the subfamily and its subordinate taxa is made. Taxonomic implications of chromosomal characters in the Ichneumoninae are outlined.

Key words. Hymenoptera, Ichneumonidae, Ichneumoninae, parasitic wasps, parasitoids, chromosomes, karyotypes, taxonomy.

Introduction

The Ichneumonidae is one of the largest insect families with more than 24 000 described species (Aguiar et al., 2013). In turn, parasitic wasps of the subfamily Ichneumoninae, which exclusively attack butterflies and moths (Lepidoptera), also constitute one of the most speciose groups of the Ichneumonidae (see e.g. Rasnitsyn, 1978). The author of the present paper started a systematic chromosomal study of this subfamily more than thirty years ago. First results of this work were published simultaneously with karyotypic data for a particular member of the Ichneumoninae provided by another research team (Gokhman, 1985; Hedderwick et al., 1985). A few years later, I prepared my PhD thesis on the chromosomal study of more than 60 species of the Ichneumoninae (Gokhman, 1990). Nevertheless, the karyotypic investigation of this subfamily continued during the subsequent years (Gokhman, 1991, 1993, 2001, 2002,
2007; Gokhman, Quicke, 1995; Gokhman, Mikhailenko, 2008). In addition, a monograph on chromosomal analysis of parasitoid Hymenoptera, which contained results of the karyotypic study of more than 90 species of the above-mentioned subfamily, was also published a few years ago (Gokhman, 2009).

The aim of the present paper is therefore an incorporation of the newly obtained results into the existing knowledge of chromosome sets of the Ichneumoninae. Moreover, when my monograph (Gokhman, 2009) had already come out of print, Quicke et al. (2009) published an extensive analysis of the Ichneumonidae that contained the most detailed phylogenetic reconstruction for the family. This work confirmed my earlier conclusions (Gokhman, 1995), at least those concerning reconstruction of phylogenetic relationships of the main groups of the Ichneumoninae. Both these reconstructions, together with the preliminary phylogenetic analysis of the subfamily Ichneumoninae conducted by Hilpert (1992), were used in the present paper for discussion of certain features of karyotype evolution in this group.

The present work is devoted to the anniversary of Dmitri R. Kasparyan, the most prominent Russian expert on Ichneumonidae systematics.

Material and methods

The new karyotypic data given in the present paper were obtained from the study of adult female parasitoids collected by the author at their hibernation sites, i.e. under the bark of fallen trees in a mixed forest in the surroundings of the city of Kaluga (Russia) in April through November 2013. Chromosomal preparations of ovarian tissue were made and analyzed using standard techniques (Gokhman, 2009) with minor modifications. The existing data (Gokhman, 2009) as well as the newly obtained results were used to demonstrate distribution of various groups of the subfamily Ichneumoninae by the chromosome number both at the species and genus levels. In the latter case, calculations were made according to the “genus-karyotype concept” proposed by Crozier (1975) to decrease the effect of uneven study of different taxa. Phylogenetic analysis of chromosomal variation in the Ichneumoninae was based on the reconstructions made by Hilpert (1992), Gokhman (1995), and Quicke et al. (2009).

Results and discussion

Hoplismenus pica Wesmael

2n = 20 (Figs 1a, 1b). This is the first karyotypically studied species of the subtribe Hoplismenina that, in turn, belongs to the tribe Ichneumonini. Nine metaphase plates from a single female were examined. The first pair of submetacentric chromosomes is substantially longer than the remaining ones, which show more or less continuous gradation in length. In addition to the submetacentrics of the first pair, metacentric (the second, fifth, eighth and ninth pairs), submetacentric (the sixth and tenth pairs) and subtelocentric chromosomes (the third, fourth and seventh pairs) also present within the karyotype. The so-called spontaneous C-banding was observed on five metaphase plates which all showed a similar banding pattern. This banding revealed pericentromeric segments of heterochromatin on all chromosomes (Fig. 1b). These segments substantially vary in size between different chromosome pairs; submetacentrics of the first pair carry the largest heterochromatic segment.

![Figure 1](image-url). Karyograms of parasitic wasps of the subfamily Ichneumoninae: Hoplismenus pica (a, b) and Ichneumon melanotis (c). a, c – routine chromosomal staining; b – spontaneous C-banding. Scale bar – 10 μm.
Ichneumon melanotis Holmgren

2n = 24 (Fig. 1c). Only the chromosome number determined in a single specimen from the Moscow Province (Gokhman, 1990) was previously known for this species. Six metaphase plates from four females were studied in the present paper. All chromosomes gradually decrease in size within the chromosome set. As in the previous species, metacentric (the first, third, fourth and ninth pairs), submetacentric (the second and sixth to eighth pairs) and subtelo-centric chromosomes (the fifth and tenth to twelfth pairs) present within the karyotype.

Data on chromosome sets of 94 species of the subfamily Ichneumoninae are currently available. Since the number of described species of this group can roughly be estimated as one-fourth of an analogous value for the family Ichneumonidae in general (Rasnitsyn, 1978), the amount of the Ichneumoninae with known chromosome sets therefore constitutes approximately 1.5 % of the species number for the subfamily. If this estimate is correct, then the Ichneumoninae must be considered one of the best karyotypically studied groups of parasitic wasps. Members of the subfamily Ichneumoninae with known chromosome sets belong to the tribes Phaegogenini and Ichneumonini, and those classified under the latter group further belong to the subtribes Barichneumonina, Protichneumonina, Craticheumonina, Hoplismenina, Ichneumonina, Amblytelina, and Eurypalina (see Hilpert, 1992). The subdivision of the Phaegogenini into subtribes is also proposed (Diller, 1981), but phylogenetic relationships of these groups, apart from those of the Ichneumonini, are unknown. Moreover, most differences between the subtribes of the Phaegogenini are likely to be based on reductions (see Diller, 1981), and therefore monophyly of these groups seems doubtful.

The chromosome number substantially varies within the Ichneumoninae; this parameter can differ more than twice between certain species, i.e. from 2n = 16 in both Patroclioides dubitatorius (Sulzer) [= P. chalybeatus (Gravenhorst)] and Ctenichneumon funereus (Geoffroy) to 2n = 34 in Virgichneumon digrammus (Gravenhorst) as well as in Chasniolas motatorius (Fabricius) (Gokhman, 1985, 1990, 1993, 2007). Interestingly, both the highest and the lowest 2n values in the Ichneumoninae were found not only within the tribe Ichneumonini, but also within its largest subtribe, Ichneumonina (Gokhman, 1993). The modal chromosome number of the Ichneumoninae is 2n = 22; it is the most frequent both at the genus and species levels (Figs 2a, 2b). In general, chromosome numbers are relatively stable within most genera of the Ichneumoninae, although these numbers can be substantially diverse in certain groups such as Virgichneumon Heinrich and Craticheumon Thomson (see below).

Chromosomes of many Ichneumoninae more or less gradually decrease in size within a given karyotype, except for a few members of the subtribe Barichneumonina, e.g. Vulgichneumon saturatorius (Linnaeus) with 2n = 18, which diploid set contains seven pairs of large bi-armed chromosomes and two pairs of small acrocentrics (Gokhman, 1990). Meta-, submeta-, and subtelo-centric chromosomes usually predominate within karyotypes of the Ichneumoninae, although chromosome sets of a few members of the subtribes Barichneumonina and Craticheumonina contain considerable amounts of acrocentrics. Karyotypes of the subfamily Ichneumoninae are therefore relatively symmetrical (see: Gokhman, 2009).

Chromosome sets of the overwhelming majority of the Ichneumoninae were studied using routine chromosome staining. Only karyotypes of the three members of the genus Dirophanes Förster were examined with the help of differential staining, i.e. C-banding (Gokhman, 1997). This study demonstrated that chromosome sets of closely related species of the Ichneumoninae with the same 2n value can strongly differ in the size and localization of heterochromatic segments and, on the contrary, karyotypes of members of the same genus with different chromosome numbers can be similar in this respect. Nevertheless, the data obtained (including these published in the present paper) suggest that most species of the subfamily Ichneumoninae have relatively small pericentric (sometimes also telomeric and intercalary) segments of heterochromatin.

The accumulated information on the karyotype structure of closely related species and forms of the Ichneumoninae (see Gokhman, 2009) suggests that the following rearrangements contributed to the evolution of chromosome sets in this group: deletions and duplications of the constitutive heterochromatin, inversions, translocations, tandem fusions and centric fissions. However, a thorough karyotypic study of the Ichneumoninae using modern techniques of chromosomal analysis would be needed to reveal the impact of each of these rearrangements in more detail. Nevertheless, a decrease in the chromosome number in the Ichneumoninae is likely to have occurred through chromosomal fusions (mostly tandem ones), whereas an increase in this parameter usually took place via the origin of aneuploids and the subsequent
restoration of even chromosome numbers. In addition, the increase in the chromosome number sometimes also occurred through centric fissions accompanied by the tandem growth of the constitutive heterochromatin and emergence of the so-called pseudoacrocentric chromosomes, e.g. in *Chasmias motatorius* (Gokhman, 1985). In other words, mechanisms of the chromosome number evolution in the Ichneumonidae were strongly asymmetrical (Gokhman, 2009).

According to the most detailed phylogenetic reconstruction of the family Ichneumonidae based on an extensive dataset which contained both morphological and molecular information (Quicke *et al*., 2009), the tribe Phaeogenini, along with Alomyini s.str. with completely unknown karyotypes, is the least advanced group of the subfamily Ichneumoninae. Since \(2n = 22\) was found in the overwhelming majority of the genera and species of the Phaeogenini, it can be considered as the modal chromosome number of this group. The same \(2n\) value is characteristic of many other Ichneumoninae, although substantially higher variation in this parameter is observed within the tribe Ichneumonini. This value, i.e. \(2n = 22\), together

![Figure 2. Karyotype distribution of parasitic wasps of the subfamily Ichneumoninae by the chromosome number [according to Gokhman (2009), updated] at the species (a) and genus levels (b).]
with the karyotypic predominance of bi-armed chromosomes that more or less gradually decrease in size, must represent the ancestral character state for the whole subfamily (Gokhman, 1990). If this is true, then an independent decrease in the chromosome number through tandem fusions that occurred in a number of genera of the tribe Phaeogenini was the main trend of the chromosome number evolution within this group. On the contrary, a considerable increase in 2n value is characteristic of the majority of the Ichneumoni, despite the decrease in the chromosome number within certain subtribes and genera (as well as groups of genera) of this tribe (Gokhman, 1990). An increase in the proportion of acrocentrics within a given chromosome set, i.e. karyotypic dissymmetrization, can also be observed in some Ichneumoni that mostly belong to the less advanced subtribes, such as Barichneumonina and Cratichneumonina (Gokhman, 2009).

The above-mentioned differences between trends of karyotype evolution in certain larger taxa of the Ichneumoninae could be explained from the viewpoint of the general mode of phylogenetic change in these groups (Gokhman, 1995, 2009). According to this hypothesis, evolution of the tribe Phaeogenini occurred in a relatively narrow adaptive zone, which might be considered ancestral for the subfamily Ichneumoninae in general. After the emergence of the group, more advanced Ichneumoninae entered the new, relatively broad and free zone, in which the intensive adaptive radiation of the Ichneumonini took place. The enormous species diversity of this tribe that includes large numbers of similar-looking members has therefore resulted from this radiation.

As can be seen from the phylogenetic reconstruction of the karyotypically studied groups of the Ichneumoninae proposed by the author (Fig. 3), the data obtained do not allow for detection of any specific trend of karyotype evolution within these groups. In addition, all species of the tribe Phaeogenini that do not belong to the largest and most diverse subtribe Phaeogenina, i.e. members of the genus Heterischmus Wesmael from the corresponding subtribe as well as a few genera of the subtribe Dicaelotolina (Diller, 1981) have the same chromosome number, 2n = 22. Moreover, their karyotype structure is comparable to that of other species of the Phaeogenini. As far as the tribe Ichneumonini is concerned, both a substantial increase in the chromosome number in a few members of the subtribes Barichneumonina, Cratichneumonina, and especially Ichneumonina, as well as a certain decrease in the chromosome number in some other taxa can be observed. The latter groups include the subtribes Hoplismenina and Eurylabina, which both contain the only studied species. Together with the parallel pathways of karyotype evolution in many of these taxa (e.g. chromosomal fusions in the two latter subtribes), this lack of evolutionary specificity could be explained by the possible polyphyly of the above-mentioned groups. Indeed, Quicke et al. (2009) who conducted a detailed morphological and molecular analysis of the Ichneumonidae have not found any support for monophyly of the majority of currently accepted tribes and subtribes of the

![Phylogenetic tree of karyotypically studied tribes and subtribes of the subfamily Ichneumoninae](image)

Figure 3. Phylogenetic tree of karyotypically studied tribes and subtribes of the subfamily Ichneumoninae [according to Hilpert (1992), Gokhman (1995) and Quicke et al. (2009)] with shown variation ranges of the haploid chromosome number. The most frequent chromosome numbers of certain groups are given in brackets.
subfamily Ichneumoninae. However, groups of genera with lower chromosome numbers (which must be treated as synapomorphies in this particular case) can be listed within subordinate taxa of the Ichneumonini (Gokhman, 2009). The corresponding examples include the genera *Patrocloides* Heinrich with \(2n = 16\) and *Pseudoamblytelys* Heinrich with \(2n = 18\) from the subtribe Ichneumonina; although the genus *Ctenichneumon* Thomson, which also has \(2n = 16\), formally belongs to the subtribe Amblytelyina, it might be considered here as well. Species of the genus *Dirophanes* with \(2n = 20\) and 18 also fit the similar pattern.

As far as taxonomic aspects of chromosomal variation in the Ichneumoninae are concerned, the karyotypic diversity of this group more or less corresponds to the morphological one, at least at first sight. A more detailed investigation shows that chromosome sets of the tribe Phaeogenini are fairly uniform (as noted above), and some genera of the Ichneumonini from the less advanced subtribes Barichneumonina and Craticheumonina are the most variable, perhaps due to the so-called rule of archaic diversity (Mamkaev, 1968). Indeed, all karyotypically studied species of the former subtribe have different chromosome numbers (Gokhman, 1990). For example, *Baranisosobas ridibundus* (Gravenhorst) has \(2n = 24\), and *Vulgichneumon saturatorius* has \(2n = 18\). Moreover, in both studied species of the genus *Virgichneumon*, i.e. *V. faunus* (Gravenhorst) and *V. digrammus*, \(2n = 22\) and 34 respectively. The diversity of chromosome numbers in the genus *Craticheumon* is also impressive. For example, two studied *Craticheumon* species, i.e. *C. culex* (Müller) [= *C. fabricator* (Fabricius)] and *C. viator* (Scopoli), have \(2n = 24\) and 28 respectively. The two other species also fit this pattern, even if *C. sicarius* (Gravenhorst) with \(2n = 22\) might in fact be included into the genus *Sycania* Cameron, and *C. rufifrons* (Gravenhorst) which has \(2n = 26\) perhaps also should be included into a separate genus (A.P. Rasnitsyn, personal communication). At the same time, 20 out of 29 studied members of the largest genus *Ichneumon* Linnaeus have \(2n = 24\), although species with \(2n = 26\) (for example, *I. inquinatus* Wesmael), 22 (*I. sarcitorius* Linnaeus, etc.) and even 20 (*I. submarginatus* Gravenhorst) can also be found within this group (Gokhman, 1990, 1993).

Nevertheless, taxonomic use of chromosomal characters in the Ichneumoninae is the most effective at the species level, especially if a morphological study is unable to detect or reliably separate closely related species. In particular, two species of the tribe Phaeogenini, *Tycherus australogeminus* Gokhman and *Aethecerus ranini* Gokhman which both have \(2n = 22\), were detected and described using karyotypic data for the first time in parasitoid Hymenoptera (Gokhman, 2009). Moreover, this information was incorporated into the general descriptions of these taxa as diagnostic features that distinguished them from closely related *T. ischiomelinus* (Gravenhorst) and *Ae. dispar* Wesmael which have \(2n = 18\) and 24 respectively (Gokhman, 1991). In addition, forms with \(2n = 24\) and 26 were detected within each of the two morphological species, *Ichneumon extensorius* Linnaeus and *I. suspiciousus* Wesmael. These forms have an apparent species status, but are virtually indistinguishable by their external morphology (Gokhman, 1993).

In conclusion, data obtained from the chromosomal analysis of parasitic wasps of the subfamily Ichneumoninae during the last 30 years, allowed not only to describe common features of chromosome sets and to outline the main pathways of karyotype evolution in this group, but also to detect previously unknown species-level forms. Some of these forms were later described as new species according to the routine taxonomic procedure (Gokhman, 2009). We can therefore hope that chromosomal analysis will successfully be used to explore many other subfamilies of the Ichneumonidae, together with other less studied parasitoid groups.

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References


