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Chromosomes of *Eupristina verticillata* Waterston, 1921 and an overview of known karyotypes of chalcid wasps of the family Agaonidae (Hymenoptera)

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Abstract

The karyotype of *Eupristina verticillata* Waterston, 1921 (Agaonidae) from Italy was studied for the first time using chromosome morphometrics. The present study showed that this species has n = 6 and 2n = 12, with five larger metacentrics and a smaller acrocentric chromosome in the haploid set. A brief overview of known karyotypes of chalcid wasps of the Agaonidae is given; certain features of karyotype evolution of this family are discussed.

Keywords

Chalcidoidea, Agaonidae, chromosomes, karyotypes

Introduction

Chalcidoidea are one of the most speciose, taxonomically complicated and economically important groups of parasitoid Hymenoptera (Godfray 1994, Quicke 1997). With its exceptionally high morphological and ecological diversity, this superfamily includes about 23 thousand known species (Huber 2017), but karyotypes of less than

200 members of this group are studied up to now (Gokhman 2013). For example, in the family Agaonidae, a highly specialized tropical/subtropical group of Chalcidoidea exclusively associated with fruits of the plant genus *Ficus*, chromosomes of only six species were previously studied (Gokhman et al. 2010, Liu et al. 2011, Chen et al. 2018). We have recently examined chromosomes of another member of this family, *Eupristina verticillata* Waterston, 1921 collected in Italy about 30 years after its introduction (Lo Verde et al. 1991, 2007). In addition, we briefly overview the current state of knowledge of the karyotypic diversity of Agaonidae.

Material and methods

Origin of the material studied

Green syconia of *Ficus microcarpa* Linnaeus, 1782 (Moraceae) containing immature stages of *E. verticillata* were collected by the senior author in July 2018 in the Villa Comunale (city park) of Napoli, Italy (40°49'58.4"N 14°14'3"E). Immediately after collection, the syconia were dissected in the lab, and wasp prepupae were extracted from the galls.

Preparation of chromosomes

Chromosomal preparations were obtained from cerebral ganglia of prepupae of *E. verticillata* generally following the protocol developed by Imai et al. (1988) with certain modifications. Ganglia were extracted from insects dissected in 0.5% hypotonic sodium citrate solution containing 0.005% colchicine. The extracted ganglia were then transferred to a fresh portion of the hypotonic solution and incubated for 30 min at room temperature. The material was transferred onto a pre-cleaned microscope slide using a Pasteur pipette and then gently flushed with Fixative I (glacial acetic acid: absolute ethanol: distilled water 3:3:4). The tissues were disrupted using dissecting needles in an additional drop of Fixative I. Another drop of Fixative II (glacial acetic acid: absolute ethanol 1:1) was applied to the center of the area, and the more aqueous phase was blotted off the edges of the slide. The slides were then dried for approximately half an hour and stored at room temperature. Chromosome preparations were stained with freshly prepared 3% Giemsa solution in 0.05M Sorensen's phosphate buffer (Na_2HPO_4 + KH_2PO_4, pH 6.8) for a few hours.

Image acquisition and analysis

Metaphase plates were analyzed under a Zeiss Axioskop 40 FL epifluorescence microscope (Carl Zeiss, Göttingen, Germany). Images of chromosomes were taken with Zeiss AxioCam MRc digital camera using Zeiss AxioVision software version

3.1. To prepare illustrations, the resulting images were arranged and enhanced with Adobe Photoshop 8.0. KaryoType version 2.0 software was also used for taking measurements from four diploid metaphase plates of *E. verticillata* having well-defined chromosome morphology. For each chromosome of the haploid set, we report both its relative length (100 × length of the chromosome divided by total length of the set) and centromeric index (100 × length of the shorter arm divided by total length of the chromosome). The chromosomes were classified into metacentrics (M), subtelocentrics (ST) and acrocentrics (A) following guidelines provided by Levan et al. (1964).

Results and discussion

The diploid karyotype of *E. verticillata* contains the largest pair of metacentric chromosomes, two pairs of shorter metacentrics, two other pairs of considerably smaller metacentric chromosomes, and the shortest pair of acrocentrics (Fig. 1; Table 1). On the other hand, its haploid karyotype contains six chromosomes which sizes and shapes are similar to those of the diploid chromosome set (not shown here); the chromosome number in this species is therefore n = 6 and 2n = 12.

The karyotype of *E. verticillata* is structurally similar to that of the only known member of the family Agaonidae with the same chromosome number (Table 2), *Blastophaga psenes* (Gokhman et al. 2010), although chromosome sets of these two species differ in some details. Specifically, diploid karyotypes of both *E. verticillata* and *B. psenes* contain five pairs of metacentrics, but the smallest pairs within their chromosome sets are acrocentric and subtelocentric respectively. In addition, the lengths of most metacentric chromosomes differ substantially in the former species compared to *B. psenes* (see Gokhman et al. 2010). Moreover, the smallest pair of chromosomes is much shorter than the preceding one in the latter species, in contrast to *E. verticillata*. These two chalcid wasps belong to different subfamilies of Agaonidae, Blastophaginae and Agaoninae respectively (Cruaud et al. 2010), and the similar karyotype structure of these species may be considered a plesiomorphic feature of the family in general (Gokhman et al. 2010). Furthermore, chromosome sets of all other studied members of Agaonidae, i.e. two species of the same genus *Eupristina* Saunders, 1882, *E. altissima* and *Eupristina* sp. with n = 5, as well as three members of the genus *Ceratosolen* Mayr, 1885 from the subfamily Kradibiinae, *C. solmsi*, *C. gravelyi*, and *C. emarginatus* with the same chromosome number (Table 2), contain only large metacentrics of similar size (Liu et al. 2011, Chen et al. 2018). Analogously to the situation observed in a few other chalcid families, e.g. Eulophidae, Torymidae and Ormyridae, results of the present study apparently confirm that the karyotypes with n = 5 resulted from the fusion between the smallest acrocentric/subtelocentric chromosome and a particular metacentric in an ancestral chromosome set with n = 6 (Gokhman 2013). If this is true, lower chromosome numbers of corresponding species could represent their putative synapomorphies within certain genera.

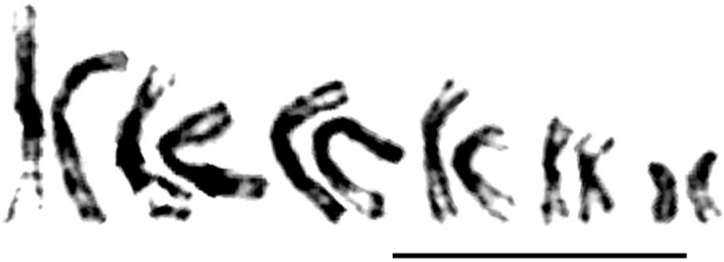

Figure 1. Diploid karyotype of *E. verticillata*. Scale bar: 10 µm.

Table 1. Relative lengths (RL) and centromeric indices (CI) of chromosomes of *E. verticillata* (mean ± SD).

Chr. no.	RL	CI
1	23.30 ± 1.35	42.38 ± 3.97
2	21.00 ± 0.77	44.53 ± 3.15
3	20.64 ± 0.76	43.11 ± 5.28
4	15.63 ± 0.31	46.72 ± 2.71
5	11.51 ± 0.78	46.21 ± 2.82
6	7.92 ± 0.46	0

Table 2. Characteristics of diploid karyotypes of studied species of Agaonidae.

Species	2n	Chromosome formula	Reference
Blastophaga psenes (Linnaeus, 1758)	12	10M + 2ST	Gokhman et al. 2010
Ceratosolen emarginatus Mayr, 1916	10	10M	Liu et al. 2011
C. gravelyi Grandi, 1916	10	10M	Liu et al. 2011
C. solmsi (Mayr, 1885)	10	10M	Liu et al. 2011
Eupristina altissima Balakrishnan & Abdurahiman, 1981	10	10M	Chen et al. 2018
E. verticillata Waterston, 1921	12	10M + 2A	Present study
Eupristina sp.	10	10M	Chen et al. 2018

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