Mitotic Karyotype of *Spalangia endius* Walker (Hymenoptera: Pteromalidae), A Pupal Parasitoid of Tephritid Flies (Diptera: Tephritidae) in Thailand

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Summary *Spalangia endius*, a pupal parasitoid of tephritid flies, has a karyotype of $n=4$, the lowest haploid chromosome number ever recorded for a pteromalid. This was determined by examining mitotic cells of the neuroganglia of male pupae. A diploid chromosome number ($2n$)=8 was also determined from the neuroganglia of female pupae. The diploid mitotic karyotype is comprised of 4 metacentric chromosome pairs.

Pteromalid pupal parasitoids (*Spalangia* spp.) are normally found in various dipteran hosts (Clancy 1950, Schmidt and Morgan 1978, Meyer *et al.* 1991, Hogsette *et al.* 1994, Gut and Brunner 1994), *e.g.*, the house fly (*Musca domestica* L.), stable fly (*Stomoxys calcitrans* L.), horn fly (*Haematobia irritans* L.), *Drosophila* spp. and fruit flies (*Rhagoletis* spp., *Bactrocera* spp.). From our observations, *S. endius* is a natural enemy of the tephritid fruit flies, *Bactrocera correcta* Bezzi and *Bactrocera dorsalis* Hendel, which attack various economically important fruits in Thailand (Hardy 1973, White and Elson-Harris 1992). *Bactrocera correcta*, the major host of *S. endius*, often occurs as a mixed infestation with *B. dorsalis*, the major fruit pest in Thailand. There have been attempts to suppress or eradicate *B. dorsalis* in some areas of Thailand using the sterile male technique (Sutantawong 1991). If successful, such control may result in increases in *B. correcta* populations and lead to it becoming an economically important pest in the future. *Spalangia endius* is a potential biological control agent against *B. correcta*.

From our behavioral observations and dissections, *S. endius* can sting both normal fruit fly hosts and those previously parasitized by the braconid parasitoid, *Diachasmimorpha longicaudata* (Kitthawee unpublished data). *Spalangia endius* acts as a primary parasitoid of *B. correcta* and as a hyperparasitoid of *D. longicaudata*. Dissections of exposed puparia have revealed that one *S. endius* larva feeds externally on the body of each host, either the fruit fly or braconid wasp, within the puparium. From our observations, *S. endius* preferred fruit fly hosts rather than fruit fly parasitoids. This agrees with Dresner (1954) who observed that *S. endius* tends to attack the fruit fly host rather than their parasitoids. From our observations, each puparium that showed one emergence hole was associated with one *S. endius* (Kitthawee unpublished data) thus indicating that the *S. endius* parasitoid always emerges at the expense of any other parasitoid. *Spalangia endius* is one of the few pupal parasitoids of fruit flies that may be sufficiently efficient to be useful for suppressing fruit fly populations.

Karyotype investigations of the family Pteromalidae have been conducted for 7 species representing 7 genera (Makino 1951, Gokhman and Quicke 1995). The lack of cytological study of *Spalangia* spp. is surprising in view of the interest in them as a natural enemy of the house fly (Propp and Morgan 1983) and fruit fly (Clancy 1950). Here, we investigate and report the karyotype of the pteromalid, *S. endius*.

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Materials and methods

The pteromalid, *S. endius*, was primarily obtained from fruit fly infested fruit of guava growing in Nakornpathom Province, central Thailand. The culture currently maintained in our laboratory was established by rearing *S. endius* on *B. correcta* pupae. Emerged adults were kept in plastic containers (7×12×4.5 cm) provided with honey.

During cytological work, we found that the pupal stage provided the best chromosome preparation. This stage was dissected from the host pupae in a colchicine-hypotonic solution (0.01% colchicine in 0.50% sodium citrate solution). Metaphase chromosomes were processed according to the following method based on Kitthawee *et al.* (1999). Brains of pupae were treated with demecolcine solution (Sigma) for 10 min and returned to fresh colchicine-hypotonic solution for 15 min. Tissues were fixed in 3:1 absolute ethanol:glacial acetic acid for 2–3 min, covered with a cover slip and then squashed. Slides were frozen for 2–3 min and the cover slips were removed before drying, staining and squashing in 2% lacto-aceto-orcein for 3 min. Slides were viewed under a compound microscope at 100×. Well spread metaphase chromosomes were photographed with Kodak Technical Pan Film for further analysis. At least 30 well spread cells were scored for chromosome number. The mitotic karyotypes were determined and analysed based on the centrometric position as described by Levan *et al.* (1964). The karyogram was arranged in a diploid set from the largest to the smallest chromosome.

Results

Karyotype data of *S. endius* are summarized in Table 1. The chromosome number of the haploid male is $n=4$ (Fig. 1a) and of the diploid female is $2n=8$ (Fig. 1b). Their mitotic metaphase karyotypes are uniform in that all chromosomes are metacentric in shape. A karyogram (Fig. 2) shows the metacentric chromosomes.

Table 1. Relative length, centrometric index, and type of each mitotic chromosome of *S. endius*

Chromosome pair	Relative length Mean±S.E.	Centrometric index Mean±S.E.	Type
1	14.91±0.09	0.583±0.002	Metacentric
2	12.71±0.09	0.565±0.003	Metacentric
3	11.73±0.09	0.539±0.003	Metacentric
4	10.66±0.15	0.519±0.004	Metacentric

Fig. 1. Photomicrograph(s) of metaphase chromosomes of *S. endius*. a) haploid male chromosome $n=4$. b) diploid female chromosome $2n=8$. The bar indicates 1 μ m.

Fig. 2. Diploid mitotic karyotype of *S. endius* showing size and shape.

Discussion

Chromosome numbers of hymenopteran insects are known for nearly 150 species representing 20 families. However, Makino (1951) only listed one species, in the genus *Pteromalus*, for the family Pteromalidae and it had a haploid chromosome number $n=5$. Since Makino, there have been other reports. Crozier (1975) updated the list and included the chromosome number of another pteromalid, *Nasonia vitripennis* ($n=5$). Most recently, the list was updated by Gokhman and Quicke (1995) who listed 6 species from 6 genera in the family Pteromalidae. The haploid chromosome numbers of these latter species were all $n=5$ except for a species in the genus *Anisopteromalus* which had the highest haploid number of 7.

Haploid chromosome numbers in the family Pteromalidae have been reported ranging from 5 to 7, as noted above. The haploid chromosome number of $n=5$ is the most common and is considered as the lowest number in the Pteromalidae (Makino 1951, Crozier 1975, Gokhman and Quicke 1995). Here, we found that *S. endius* has a diploid chromosome number of $2n=8$ and a haploid chromosome number of $n=4$ which are the lowest diploid and haploid chromosome numbers so far recorded for species in the family Pteromalidae. The results presented here are the first published documentation of chromosome numbers of a species in the subfamily Spalanginae of the family Pteromalidae. The diploid metaphase karyotype revealed 4 large metacentric chromosome pairs. These lower-numbered karyotypes have larger chromosomes than high-numbered karyotypes and their investigation may determine useful taxonomic characters in this parasitoid group.

Mitotic chromosomes of *Spalangia* spp. are difficult to prepare unlike those of other hymenopteran parasitoids because they are tiny parasitoids and the neuroganglionic cells are difficult to locate. It is also difficult to determine the stages of these insects that are most suitable for chromosome preparation. However, the results presented here indicate that *S. endius* is suitable for future cytological study. Such study may prove useful for identifying sibling species in the *S. endius* complex (Legner 1983) and may indicate a possible mechanism of chromosomal evolution within the genus *Spalangia*. It is also hoped that this knowledge will aid in the development of this group as biological-control agents of fruit flies.

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