Symbiosis (2011) 53:131–137
DOI 10.1007/s13199-011-0116-4

Chromosome numbers are not fixed in Agaonidae (Hymenoptera: Chalcidoidea)

Qing Liu · Xiao hong Ou · Stephen G. Compton · Da rong Yang

Received: 8 February 2011 / Accepted: 22 April 2011 / Published online: 10 May 2011

Abstract Agaonidae (the pollinators of fig trees, *Ficus* spp., Moraceae) are a distinctive family of chalcid wasps with uncertain affinities within the Chalcidoidea. Chromosome numbers have only been described previously for one species (*Blastophaga psenes*, 2n=12, the pollinator of *F. carica*, of subgenus *Ficus*). In this paper, we used a modified technique to analyze the karyology of three *Ceratosolen* species, *Ceratosolen solmsi*, *C. graveli* and *C. emarginatus* which are associated with figs of *Ficus* subgenus *Sycomorus*. Their karyotypes are 2n=10. All the chromosomes are metacentric (NF=20) with little difference in relative lengths, making them hard to distinguish. The variation in chromosome numbers that we have detected suggests that karyology can usefully complement molecular-based studies of the phylogeny of fig wasps.

Keywords *Ceratosolen* · Chromosomes · Fig wasps · Karyology

1 Introduction

Pollinator fig wasps (Hymenoptera, Chalcidoidea, Agaonidae) are the unique pollinators of fig trees (*Ficus* spp., Moraceae). Figs (the inflorescences of fig trees) are formed like a hollow ball, lined by numerous tiny flowers. In order for pollination to take place, female fig wasps must crawl inside the figs, where they then gall the flowers and lay their eggs. Reflecting their specialized biology, fig wasps show peculiar morphological adaptations, extreme host specificity, and life cycles that are tightly synchronized with fig phenology (Compton et al. 2010; Weiblen 2002; Wiebes 1979). The plants are completely dependent upon the wasps for the pollination of their flowers (Wiebes 1979) and the wasps only breed inside the figs, so their interaction is a striking example of an obligate insect–plant mutualism that originated more than 60 million years ago (Herre et al. 2008; Machado et al. 2001; Weiblen 2002; Zhang et al. 2008; Ma et al. 2009).

The taxonomic affiliations of fig wasps within the Chalcidoidea are still uncertain (Cruaud et al. 2009). The family Agaonidae has generally been restricted to the pollinator fig wasps, with non-pollinating fig wasps classified in Eurytomidae, Pteromalidae and Torymidae, but Bouček (1988) downgraded pollinator fig wasps to subfamily rank and included almost all non-pollinating fig wasps in five other subfamilies within an expanded Agaonidae. When Rasplus et al. (1998) re-examined the Agaonidae sensu Bouček using molecular characters they found that it contained several unrelated groups and as a result the current concept of the Agaonidae is once more

Q. Liu · D. r. Yang
Key Laboratory of Tropical Forest Ecology, Xishuangbannan Tropical Botanical Garden, Chinese Academy of Sciences,
Kunming, China

Q. Liu
Graduate University of Chinese Academy of Sciences,
Beijing, China

X. h. Ou
Key Laboratory of Forest Disaster Warning and Control,
Southwest Forestry University,
Kunming, China

S. G. Compton
Faculty of Biological Sciences, University of Leeds,
Leeds LS2 9JT, UK

D. r. Yang (✉)
Xishuangbanna Tropical Botanical Garden,
Chinese Academy of Sciences,
88 Xuefu Road,
Kunming, Yunnan 650223, People's Republic of China
e-mail: yangdr@xtbg.ac.cn

restricted to fig pollinators only, with Boucek's non-pollinating subfamilies re-assigned to various other chalcid families (Cruaud et al. 2009).

Early work on the phylogenetic relationships of pollinator fig wasp genera was based on morphological characters, some of which are potentially labile and subject to directional selection linked to ostiole shape and other host plant characteristics (Cruaud et al. 2009; van Noort and Compton 1996). Ramirez (1978) attempted to resolve Agaonidae phylogeny based on the presence of two characters, mesosternal pollen pockets and coxal corbiculae that are associated with active pollen transfer by female fig wasps. Wiebes (1981) proposed a second phylogenetic hypothesis for the family based on 21 (mainly adult female) morphological characters. He divided the family into two groups (Blastophaginae and the Agaoninae) according to the shape of their mandibular appendage and antennal scape. Subsequent proposed phylogenies (Herre et al. 1996; Jiang et al. 2006; Lopez-Vaamonde et al. 2009; Machado et al. 2001; Weiblen 2001) were based on molecular techniques, but suffered from limited taxon and gene sampling. A recent more exhaustive study by Cruaud et al. (2009) has resulted in a revised phylogeny of the family. They concluded that Agaonidae can be partitioned into four groups: the monophyletic subfamilies Tetrapusiinae (containing the single genus *Tetrapus*) and Kradibiinae (containing *Ceratosolen* and *Kradibia*) and two paraphyletic groups "Agaoninae" and "Blastophaginae". Clearly, despite increasingly extensive taxonomic sampling and sequence data, relationships among the major clades of Agaonidae still remain largely unresolved.

Chromosomal analyses have played an important role in the systematics and species level taxonomy of many insect groups, but their use within the parasitic Hymenoptera remained limited until relatively, largely because of technical difficulties (Gokhman and Quicke 1995; Gokhman 2009). In particular, hymenopteran chromosomes have traditionally been obtained from immature stages (prepupae and pupae) in which dividing cells are common, but it is often difficult to identify the species involved with certainty, unless they are in culture (Gokhman and Quicke 1995). Improved techniques now allow the use of adult female ovaries and male testes, which circumvent some of these problems (Gokhman and Quicke 1995; Imai et al. 1988) and to date the chromosomes of over 420 species of parasitic wasps from 24 families have been recorded (Gokhman 2009). These include nearly 150 species representing 13 families in Chalcidoidea. According to Gokhman and Quicke (1995) the Chalcidoidea may have a plesiomorphic haploid chromosome number of ten. Two main chromosome number types are represented within the Chalcidoidea, from around a modal number of ten chromosomes as shown by the Eurytomidae, Encyrtidae and Mymaridae to five or six in the majority of families (Gokhman and Quicke 1995; Gokhman 2009).

Chromosome numbers have not been widely used to study phylogenetic relationships within the Chalcidoidea, a consequence of the relatively small number of species surveyed and the restricted taxonomic coverage they provide. They have nonetheless informed debate on the relationship between Eupelmidae and Encyrtidae, where the widely accepted hypothesis of a close relationship, based on morphological features, is not supported by karyological data because the two families have haploid numbers of 5–6 and 8–12 respectively (Campbell et al. 2000; Gokhman 2009).

Chromosomal studies of pollinator fig wasps offer particular challenges because of their very small body size (the largest adult females have a body length of about two mm) and because their larvae develop inside figs, where it is often difficult to identify the suitable developmental stage of the wasps and so get appropriate materials for chromosome preparations. Correct identification of the immature stages is also an issue because fig trees can support as many as 30 different species of fig wasps (most of which are parasitoids or gallers, not pollinators), numerous species can be present in a single fig (Hawkins and Compton 1992) and parasitoid or inquiline larvae may be present in galls initiated by the pollinators.

The only previously published study of fig wasp chromosomes is that of Gokhman et al. (2010), who described the karyotype of *Blastophaga psenes* (L.), the pollinator of *Ficus carica* L. Using cells from the cerebral ganglia of prepupae, they showed that diploid females individuals of *B. psenes* have a chromosome set of $2n=12$, and haploid males have $n=6$. This haploid karyotype comprises five large metacentric chromosomes and one smaller sub-telocentric chromosome (Gokhman et al. 2010).

There are about 800 species of fig trees currently recognized, an increasing number of which have two or more host specific species recorded from them, but less than 400 species of pollinators have been described so far (the Universal Chalcidoidea Database http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/). *Ceratosolen* and *Blastophaga* represent two major branches of fig wasp evolution. *Blastophaga* species (Subfamily "Blastophaginae") all pollinate fig trees of Subgenus *Ficus,* though the genus as currently recognized includes several unrelated species and is clearly polyphyletic (Cruaud et al. 2009; Lopez-Vaamonde et al. 2009). *Ceratosolen* (Subfamily Kradibiinae) is the largest genus within Agaonidae, with over 130 described species (Wiebes 1963, 1994). They pollinate fig trees of Subgenus *Sycomorus* and are unique in that they are associated with both monoecious and dioecious plant hosts.

Here we describe features of the chromosomes of *Ceratosolen* species reared from four *Ficus* species and compare them with the chromosomes of *B. psenes* in order to determine whether chromosome number is of potential value for studies of the phylogeny of pollinator fig wasps.

2 Materials and methods

2.1 Study site and species

This study was carried out in the Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences, Yunnan, China, located at 21°41″ N, 101°25″ E, at an altitude of approximately 600 m.

Living pollinator fig wasp larvae, prepupae and adults were collected from single male trees of *Ficus hispida* Linnaeus f., *F. semicordata* Buchanan-Hamilton ex Smith, *F. auriculata* Loureiro, and *F. oligodon* Miquel between April 2009 and March 2010 (Table 1). All four fig species are dioecious and belong to *Ficus* Subgenus *Sycomorus*. *Ficus hispida* (Subsection *Sycocarpus*, Section *Sycocarpus*) is pollinated by *Ceratosolen solmsi* Mayr (Subgenus *ceratosolen*) (Wiebes 1994). *F. semicordata* (Section *Hemicardia*) is pollinated by *Ceratosolen gravelyi* Grandi (Subgenus *ceratosolen*) (Wiebes 1994). Although usually considered as separate species, *F. auriculata* and *F. oligodon* (Section *Sycomorus*, Section *Neomorphe*) are considered by Berg and Corner (2005) to represent a single species. The two taxa are ecologically and morphologically distinct in China, where they have been described as two ecotypes of the same *Ficus* species (personal communication). They are both pollinated by *Ceratosolen emarginatus* Mayr (Subgenus *ceratosolen*) (Wiebes 1994).

To ensure that only pollinator larvae were present in the figs, branches bearing young, unpollinated figs were enclosed in fine mesh nylon bags. This prevented pollinators and non-pollinators gaining access. When the enclosed figs reached the receptive stage, three or four pollinator foundresses (obtained from other trees nearby) were introduced into 30 or more figs. The bags were then replaced around the figs until the fig wasp offspring were ready to be sampled.

2.2 Chromosome preparations (immature and adult tissues)

After the pollinated figs had been developing for about 10 or 20 days they were removed from the trees. Galled ovules containing developing fig wasp larvae and prepupae were opened and their cerebral ganglia were dissected out under a ZEISS Discovery V12 microscope. The chromosomes were visualized using a modified version of the technique developed by Ling (1984): (1) Dissection in Ringer's solution on a culture dish (2) Transfer of the organs to fresh tissue culture fluid (85% Ringer's solution and 15% calf blood serum) with 0.07 mg/ml colchicine in another culture dish and left for 4 h at 28°C in an incubator (3) Transfer of the organs to 1% hypotonic sodium citrate solution for 30–50 min at 28°C in an incubator. (4) Transfer of the materials to freshly prepared fixative (methanol: glacial acetic acid in the ratio 3:1) for 1–3 h at 4°C in a refrigerator (5) Transfer of the materials onto a pre-cleaned plain slide with two drops of 60% glacial acetic acid, and after 1 h, maceration of the organs as quickly as possible using dissecting needles to spread the cells and cell-mass (6) After drying for at least 1 day, stain the preparations using freshly prepared Giemsa solution (3% in M/15 Sorensen's pH 6.8 phosphate buffer: Na_2HP0_4 4.75 g/ KH_2P0_4 4.5 g/d.w. 1,000 ml) for 30 min at room temperature. Each slide was then rinsed with a small amount of running tap-water and allowed to dry naturally. Digital images of the chromosomes were obtained using an OLYMPUS BX51 optical microscope, and treated using Photoshop 8.0 image processing software. These methods were modified from those of Ling (1984) in that calf blood serum was used to culture the cerebral ganglia of the fig wasps after dissection as this promotes cell growth and division, and that we also simplified the process of fixation, using Carnoy's fluid to replace the three different fixatives used previously.

Material from adult males and females was obtained by collecting figs when they were in male phase (characterized by wasp offspring coming out from their galls). The figs were taken back to the laboratory, where they were placed in tightly closed muslin bags (20 cm× 15 cm). After the wasps emerged from the figs their chromosomes were prepared according to the technique developed by Gokhman and Quicke (1995).

Table 1 Collection dates and sample sizes of *Ceratosolen* species collected at Xishuangbanna Tropical Botanical Garden

Species	Dates and locations of collection	Host *Ficus*	Nos. of karyotyped individuals
Ceratosolen (*C.*) *solmsi*	April 2009, XTBG	*F. hispida*	20
Ceratosolen (*C.*) *gravelyi*	April 2009, XTBG	*F. semicordata*	10
Ceratosolen (*C.*) *emarginatus*	March 2010, XTBG	*F. auriculata*	15
		F. oligodon	20

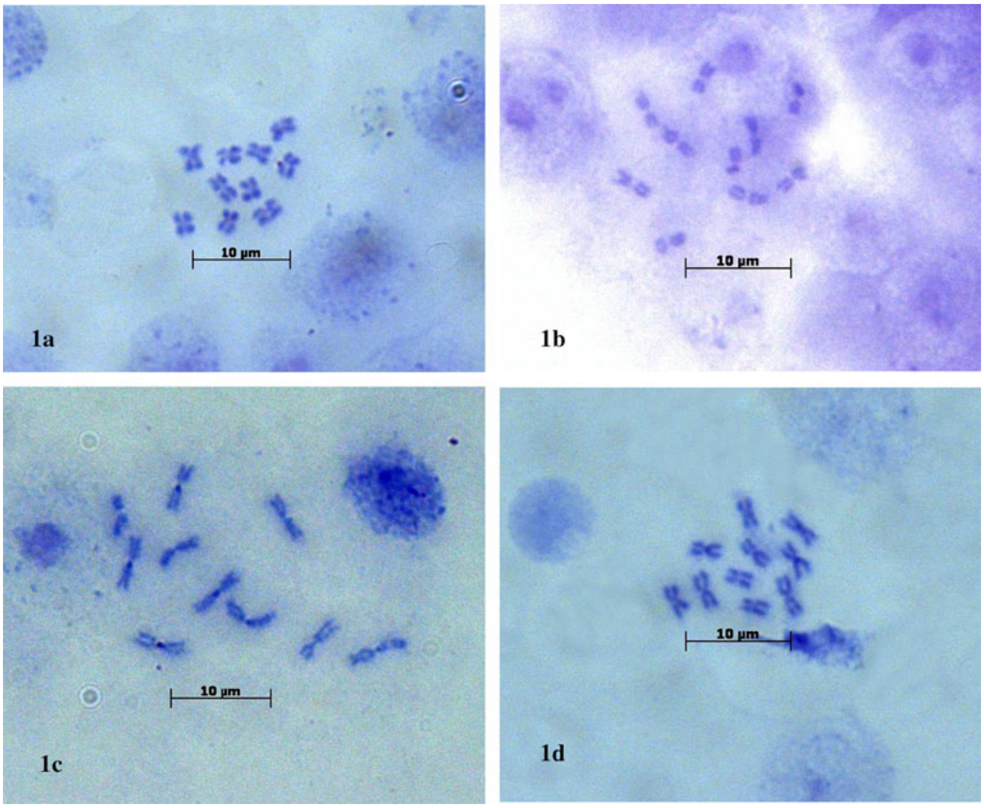

Fig. 1 Mitotic metaphase chromosomes of three *Ceratosolen* species. **a** *Ceratosolen solmsi*, 2n=10. **b** *Ceratosolen gravelyi*, 2n=10. **c** *Ceratosolen emarginatus* (from *Ficus auriculata*), 2n=10. **d** *Ceratosolen emarginatus* (from *F. oligodon*), 2n=10

Morphometric values such as centromeric index (CI) and relative length (RL) were calculated on at least five metaphase plates for each species. Fundamental number (NF) was assessed as the total number of arms in a diploid complement. Karyotypes were constructed following the criteria of Levan et al. (1964), with the chromosomes classified by their centromere positions and relative sizes.

3 Results

The gonads from adult male and female fig wasps displayed no cell divisions and the following descriptions of chromosomes are based only on material from the cerebral ganglia of immatures.

3.1 *Ceratosolen solmsi*

The diploid chromosome number of female *Ceratosolen solmsi* is 2n=10. All chromosomes are metacentric (M), the arm number (NF) in this species is therefore 20 (Fig. 1a). The mitotic karyotype consists of five pairs of chromosomes, and each pair has a similar chromosome morphology (Fig. 2a). The chromosomes vary progressively in length, but with two pairs noticeably larger than the others (Fig. 2a; Table 2).

3.2 *Ceratosolen gravelyi*

Ceratosolen gravelyi females have a diploid chromosome number of 2n=10 and NF=20 (Fig. 1b). The karyotype consists of five pairs of metacentric chromosomes, all with similar morphology (Fig. 2b). The chromosomes vary progressively in length (Fig. 2b; Table 3).

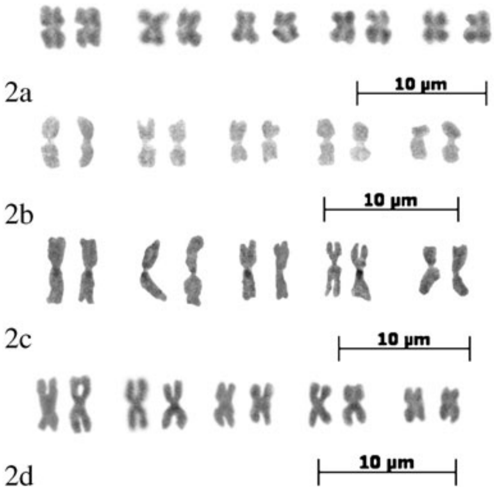

Fig. 2 Karyotypes of three *Ceratosolen* species. **a** *Ceratosolen solmsi*, 2n=10. **b** *Ceratosolen gravelyi*, 2n=10. **c** *Ceratosolen emarginatus* (*Ficus auriculata*), 2n=10. **d** *Ceratosolen emarginatus* (*F. oligodon*), 2n=10

Table 2 Parameters of chromosomes of *Ceratosolen solmsi* based on measurements of five mitotic metaphase cells (means ± S.D.)

Chromosome pair	Relative length (RL)	Centromere index (CI)
1	11.89±0.95	47.75±2.29
2	10.87±0.40	47.70±1.27
3	9.82±0.46	47.91±2.15
4	9.24±0.20	44.39±3.07
5	8.18±0.87	44.56±2.23

3.3 *Ceratosolen emarginatus* (from *F. Auriculata*)

Ceratosolen emarginatus females from *F. auriculata* have a diploid chromosome complement of 2n=10 and NF=20 (Fig. 1c). The haploid karyotype comprises two pairs of larger metacentric chromosomes and three pairs of smaller metacentric chromosomes (Fig. 2c; Table 4).

3.4 *Ceratosolen emarginatus* (from *F. oligodon*)

Female *Ceratosolen emarginatus* from *F. oligodon* have chromosomes that could not be distinguished from individuals reared from *F. auriculata* (Figs. 1d and 2d; Table 5).

4 Discussion

Although karyological features such as chromosome number and morphology can be useful for distinguishing between closely related species (Gokhman 2009), there were only small differences between the three *Ceratosolen* species we investigated. When it comes to *Ceratosolen emarginatus*, which is recorded from both *F. auriculata* and *F. oligodon*, the diploid chromosome number, morphology and relative lengths of chromosome are highly consistent across individuals reared from both host plants, and there are no evident genetic differentiation at present.

Gokhman (2009) suggested that a successful karyotaxonomic study has two prerequisites: the presence of tissues with relatively high numbers of mitoses and/or meiosis and that the species being studied should be reliably identified. By introducing identified female fig wasps into figs that were protected from oviposition by other species we both obtained larval and prepupal material of a known age and ensured their identity. Our modified version of the methods developed by Ling (1984) for other insects was effective for the processing of chromosomes of immatures of *Ceratosolen* species, but we failed to obtain suitable material from the gonads of adults using the methods of Gokhman and Quicke (1995). This may reflect the very brief period of mating and oviposition among pollinator fig wasps. Mating is completed within a few hours and females emerge with fully-formed eggs, do not feed, and have a maximum adult lifespan of around 48 h. In contrast, numerous cell divisions and metaphase chromosomes were visible in the preparations from cerebral ganglia of both later instar larvae and prepupae. The larvae have small heads and are hard to dissect from their galls, so prepupae were found to be the best stage for fig wasp chromosome preparations.

Table 3 Parameters of chromosomes of *Ceratosolen gravelyi* based on measurements of five mitotic metaphase cells (means ± S.D.)

Chromosome pair	Relative length (RL)	Centromere index (CI)
1	11.43±0.54	46.89±2.34
2	10.37±0.22	47.35±2.21
3	10.00±0.37	45.69±1.94
4	9.49±0.26	44.02±1 .60
5	8.71±0.70	48.14±1.48

Table 4 Parameters of chromosomes of *Ceratosolen emarginatus* (from *Ficus auriculata*) based on measurements of five mitotic metaphase cells (means ± S.D.)

Chromosome pair	Relative length (RL)	Centromere index (CI)
1	11.37±0.62	46.48±2.21
2	10.60±0.29	47.18±0.52
3	9.72±0.33	46.19±3.08
4	9.44±0.50	45.84±2.70
5	8.87±0.48	47.36±2.67

The three *Ceratosolen* species, *C. solmsi*, *C. gravelyi* and *C. emarginatus*, have similar karyotypes with a diploid chromosome number of 2n=10. All the chromosomes were metacentric, with little difference in relative length, making it difficult to pair homologues on the basis of morphometrics. This is in contrast to the chromosome numbers and morphology of *B. psenes*, which has a diploid chromosome number 2n=12, with five pairs of large metacentrics and one pair of smaller sub-telocentrics (Gokhman et al. 2010). Clearly chromosome numbers are not fixed in Agaonidae and they may prove to be of phylogenetic value in the future, once additional genera are investigated.

Table 5 Parameters of chromosomes of *Ceratosolen emarginatus* (from *F. oligodon*) based on measurements of six mitotic metaphase cells (means ± S.D.)

Chromosome pair	Relative length (RL)	Centromere index (CI)
1	11.67±0.44	46.96±1.88
2	11.07±0.47	45.41±2.99
3	9.65±0.29	45.81±1.70
4	9.02±0.33	44.35±2.13
5	8.58±0.33	44.83±2.27

Gokhman and Quicke (1995) observed that the vast majority of Chalcidoidea have small numbers of chromosomes, with values ranging from 3 to 7, but with most having $n=5$ or $n=6$. The families Agaonidae, Pteromalidae, Torymidae and Ormyridae may form a common clade (Campbell et al. 2000; Gibson et al. 1999). The karyotype structure found in *B. psenes* (five large metacentrics and a smaller sub-telocentric/acrocentric) is characteristic of many Torymidae and was also recorded in Ormyridae (Gokhman 2009; Gokhman et al. 2010). Conversely, the karyotype we recorded for *Ceratosolen* species, with only five metacentric chromosomes, has been recorded in a minority of Torymidae, in Ormyridae (where *Ormyrus* species can have five or six chromosomes) and most species of Pteromalidae (Gokhman 2009). According to Gokhman et al. (2010) the karyotype structure of *B. psenes* and others is likely to represent the plesiomorphic situation and the chromosome complements of five have then originated through chromosomal fusion from an ancestral karyotype with an additional subtelocentric/acrocentric chromosome (Gokhman 2009; Gokhman et al. 2010). This suggests that the karyotype of *Ceratosolen* species might be derived from that of *B. psenes*, a conclusion that is not consistent with the phylogenetic relationship between *B. psenes* and *Ceratosolen* species described by Cruaud et al. (2009). Their phylogeny suggests that *B. psenes* and *Ceratosolen* are not closely related within the Agaonidae, but does not resolve which group may be representative of the chromosomal ground plan within the family. The Neotropical genus *Tetrapus* is usually regarded as the sister group to all other agaonids and clearly its chromosome count would be of particular interest.

Further research, covering a wide range of pollinator fig wasp genera, will be needed to establish patterns in the evolution of the agaonid karyotype, and whether they are of phylogenetic significance. Chromosome banding techniques, such as C-banding, G-banding, silver staining of nucleolar organizer regions (NOR) and other molecular methods (FISH), may also prove useful for phylogenetic studies of this interesting group.

Acknowledgements This work was funded by the Chinese Natural Science Foundation (30970403, 30970439) and also supported by grants from the Key Laboratory of Forest Disaster Warning and Control Program of Southwest Forestry University (ZK10B302). We express our sincere gratitude to V.E. Gokhman (Botanical Garden, Moscow State University) and Wen-hui Nie (Kunming Institute of Zoology, Chinese Academy of Science) for their good suggestions on the experiment and Rodrigo A. S. Pereira for commenting on an earlier version of the manuscript. We thank Sun Fuping for his help on image processing. We also thank Cao Yong-qiang, Yang Pei, Li Zong-bo, Ma Wen-juan, Xi Yi-cui, Miao Bai-ge and Wang Gang for their assistance.

References

Berg CC, Corner EJH (2005) Moraceae-*Ficus*. *Flora* Malesiana, Ser, I, 17⁄2, Leiden

Bouček Z (1988) Australasian Chalcidoidea (Hymenoptera): a biosystematic revision of genera of fourteen families, with a reclassification of species. CAB International, Wallingford

Campbell B, Heraty J, Rasplus JY, Cha K, Steffen-Campbell J, Babcock C (2000) Molecular systematics of the Chalcidoidea using 28S-D2 rDNA. In: Austin AD, Dowton M (eds) Hymenoptera: evolution, biodiversity and biological control. CSIRO, Collingwood, 59pp

Compton SG, Ball AD, Collinson ME, Hayes P, Rasnitsyn AP, Ross AJ (2010) Ancient fig wasps indicate at least 34 million years of stasis in their mutualism with fig trees. Biol Lett 6:838–842

Cruaud A, Jabbour-Zahab R, Genson G, Cruaud C, Couloux A, Kjellberg F, van Noort S, Rasplus JY (2009) Laying the foundations for a new classification of Agaonidae (Hymenoptera: Chalcidoidea), a multilocus phylogenetic approach. Cladistics 25:1–29

Gibson GAP, Heraty JM, Woolley JB (1999) Phylogenetics and classification of Chalcidoidea and Mymarommatoidea—a review of current concepts (Hymenoptera, Apocrita). Zool Scr 28:87–124

Gokhman VE (2009) Karyotypes of parasitic Hymenoptera. Springer Science + Business Media B.V., Dordrecht. XIII

Gokhman VE, Quicke DLJ (1995) The last twenty years of parasitic Hymenoptera karyology: an update and phylogenetic implications. J Hymenopt Res 4:41–63

Gokhman VE, Mikhailenko AP, Fursov VN (2010) Chromosomes of *Blastophaga psenes* (Hymenoptera: Agaonidae). J Hymenopt Res 19:187–188

Hawkins BA, Compton SG (1992) African fig wasp communities: vacant niches and latitudinal gradients in species richness. J Anim Ecol 61:361–372

Herre EA, Machado CA, Bermingham E, Nason JD, Windsor DM, McCafferty S, van Houten W, Bachmann K (1996) Molecular phylogenies of figs and their pollinator wasps. J Biogeogr 23:521–530

Herre EA, Jandér KC, Machado CA (2008) Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. Annu Rev Ecol Evol Syst 39:439–458

Imai HT, Taylor RW, Crosland MWJ, Crozier RH (1988) Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interation hypothesis. Jpn J Genet 63:159–185

Jiang ZF, Huang DW, Zhu CD, Zheng WQ (2006) New insights into the phylogeny of fig pollinators using Bayesian analyses. Mol Phylogenet Evol 38:306–315

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220

Ling FY (1984) Studies on the karyotypes of seven species of flies. Zool Res (Suppl.) 5:51–56 (In Chinese with English abstract)

Lopez-Vaamonde C, Wikström N, Kjer KM, Weiblen G, Rasplus JY, Machado CA, Cook JM (2009) Molecular dating and biogeography of fig-pollinating wasps. Mol Phylogenet Evol 52:715–726

Ma WJ, Peng YQ, Yang DR, Guan JM (2009) Coevolution of reproductive characteristics in three dioecious fig species and their pollinator wasps. Symbiosis 49:87–94

Machado CA, Jousselin E, Kjellberg F, Compton SG, Herre EA (2001) Phylogenetic relationships, historical biogeography, and character evolution of fig-pollinating wasps. Proc R Soc Lond B 268:685–694

Ramirez WB (1978) Evolution of mechanisms to carry pollen in Agaonidae (Hymenoptera, Chalcidoidea). Tijdschr Entomol 121:279–293

Rasplus JY, Kerdelhué C, Le Clainche I, Mondor G (1998) Molecular phylogeny of fig wasps (Hymenoptera). Agaonidae are not monophyletic. Compte Rendu de l'Académie des Sciences de Paris 321:517–527

van Noort S, Compton SG (1996) Convergent evolution of Agaoninae and Sycoecinae (Agaonidae, Chalcidoidea) head shape in response to the constraints of host fig morphology. J Biogeogr 23:415–424

Weiblen GD (2001) Phylogenetic relationships of fig wasps pollinating functionally dioecious *Ficus* based on mitochondrial DNA sequences and morphology. Syst Biol 50:243–267

Weiblen GD (2002) How to be a fig wasp. Annu Rev Entomol 47:299–330

Wiebes JT (1963) Taxonomy and host preferences of Indo-Australian fig wasps of the genus *Ceratosolen* (Agaonidae). Tijdschr Entomol 106:1–112

Wiebes JT (1979) Coevolution of figs and their insect pollinators. Annu Rev Ecol Syst 10:1–12

Wiebes JT (1981) The phylogeny of the Agaonidae (Hymenoptera, Chalcidoidea). Neth J Zool 32:395–411

Wiebes JT (1994) The Indo-Australian Agaoninae (pollinators of figs). North-Holland, Amsterdam, p 208

Zhang FP, Peng YQ, Guan JM, Yang DR (2008) A species of fig tree and three unrelated fig wasp pollinators. Evol Ecol Res 10:611–620