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## Sex Ratio and Approximate Date of Fertilization of the Subsocial Spider *Amaurobius ferox* Walckenaer (Araneae: Amaurobiidae)

Kim, Kil Won and Jae Chun Choe\*

Division of Ecoscience, Ewha Womans University, Seoul 120-750, Korea

**ABSTRACT:** Social spiders consistently show highly female-biased sex ratios. However, the sex ratio of subsocial spiders, which have been suggested as an intermediate stage of the evolutionary pathway towards permanent sociality, is generally unknown. We investigated the sex ratio and approximate date of fertilization of the subsocial spider, *Amaurobius ferox* Walckenaer (Araneae: Amaurobiidae). Investigation over 2 years revealed that sex ratio of *A. ferox* was consistent across years and averaged 0.49. By early May (1<sup>st</sup>~10<sup>th</sup>), 66.6% of female *A. ferox* observed in the field had already been inseminated, and by late May (21<sup>st</sup>~31<sup>st</sup>), 95.4% of females had been inseminated. This result suggests that *A. ferox* need a long time or cold temperatures to prepare them for reproduction after the developmental attainment of the adult stage.

**Key words:** *Amaurobius*, Insemination, Sex ratio, Subsocial spider

### INTRODUCTION

Social spiders do not seem to show morphological caste differentiation and reproductive division of labor among members (Buskirk 1981, D'Andréa 1987) even though sociality evolved independently in different families (Kullmann 1972, Krafft 1979, Avilés 1997). Despite the more egalitarian appearance of spider societies in comparison with eusocial insects such as ants or termites, however, some researchers have suggested a caste system based on adult body size and food availability (or prey size) (Rypstra 1993, Ebert 1998). Spiders consistently show a heavily female-biased sex ratio (Vollrath 1986, Frank 1987, Whitehouse and Lubin 2005), which is one of the hallmarks of eusocial insects. A biased sex ratio can influence the evolution of reproductive partitioning (skew): high-skew groups are those in which dominant breeders monopolize a large proportion of the group's reproduction, whereas low-skew groups are those where reproduction is more evenly distributed among group members (Bourke 2001).

There has been hardly any research on sex ratio in subsocial spiders that exhibit maternal brood care beyond the first few developmental instars and an extended phase of tolerance among young within the maternal nest (Buskirk 1981). Subsociality has been suggested as an intermediate stage in the evolutionary pathway towards permanent cooperative sociality (Kullmann 1972, Horel and Krafft 1986). Members of permanent societies live in long-lasting (multigenerational) colonies which typically cooperate in prey capture, nest maintenance, and brood care (Kullmann 1972, Buskirk

1981, D'Andréa 1987, Avilés 1997). Only a single study has been published on the sex ratio of subsocial spider species: Vollrath (1986) reported a sex ratio for the subsocial *Anelosimus jucundus* (Araneae: Theridiidae), a congener of the most studied social spider, *A. eximius*, concluding from a rearing study of two egg sacs that the sex ratio was 1:1.

*Amaurobius ferox* Walckenaer (Araneae: Amaurobiidae) exhibits highly-developed subsocial organization. Maternal caring behavior occurs systematically for a fairly constant interval (Kim and Horel 1998, Kim and Roland 2000). The mother feeds her young with a batch of trophic eggs (Kim and Roland 2000) and with her body (Kim and Horel 1998). This maternal provisioning results in a homogeneous sibling group of the same developmental stage, with little variation of body mass among the siblings (Kim and Roland 2000, Kim et al. 2000). After matrophagy, the young spiders inhabit their natal nest for an average of 3~4 weeks until dispersal (Kim 2000). During this period, the spiderlings exhibit collective predatory activity (Kim et al. 2005ab). In this study, we investigated sex ratio of the subsocial spider, *A. ferox*.

We also examined the timing of reproductive events in this spider. We observed adult female and male *Amaurobius* in the field in late autumn. However, Tahiri et al. (1989) did not observe any reproduction (emergence of the cocoon) in the laboratory for *A. ferox* females ( $n=78$ ) collected in October~December, and of females collected in January~March, only 10% (7/75) reproduced (Tahiri et al. 1989). This result suggests that females collected during these periods might not have been inseminated, or that they might need an additional period of time to produce viable eggs if they

\* Corresponding author; Phone: +82-2-3277-4512, e-mail: jacchoe@ewha.ac.kr

were inseminated. As the majority of females begin to elaborate their cocoons from the end of June, we investigated the proportion of females inseminated in the field in May by verifying the presence of a mating plug on the female's genital plate (Suhm et al. 1996).

## MATERIALS AND METHODS

### Study Species

*Amaurobius ferox*, a common nocturnal European spider, is found in shaded and damp places such as under stones in the forest litter, in old walls and caves, etc. (Locket and Millidge 1951, Cloudsley-Thompson 1957). The spider spins a sticky cribellate web to capture prey and as a protective retreat (Tietjen 1986, Opell 1994). Female body size ranges from 140 to 320 mg with a total body length of 8.5–14 mm, and male body size ranges from 80 to 160 mg with a total body length of 8–12.5 mm (Leech 1971, Lemasle 1977).

The life cycle of *A. ferox* lasts for approximately two years (making it a biennial species), and encompasses two winters (Lemasle 1977, Tahiri et al. 1989). Therefore, individuals of two generations can be simultaneously observed on the ground. Maternal activity begins in early summer, when the females construct a single egg sac (Lemasle 1977). The majority of females begin to elaborate their cocoon from the end of June (Cloudsley-Thompson 1955).

### Collection and Laboratory Rearing for the Sex Ratio Investigation

Sex ratio of *A. ferox* was measured during two years (1995 and 1996). Individuals of the 5<sup>th</sup>–7<sup>th</sup> instar were collected in the spring in the forested area of Nancy, in northeastern France (elevation: 217 m, latitude: 48° 41' N, longitude: 06° 13' E, annual temperature: 9.6 ± 6.3°C, annual precipitation: 74 cm). After collection, the spiders were transferred to a room maintained at 20 ± 2°C and lit by fluorescent lights (approx. 100 lux, photoperiod of 12/12 h), and kept individually in custom-designed transparent plastic boxes (L: 90 mm, W: 160 mm, H: 75 mm) (Kim 2001). The spiders were fed 20 (± 2) mg cricket larvae (*Gryllus dimaculatus*). Mortality during laboratory rearing was less than 5%.

We raised young individuals in the laboratory to observe sex ratio for the following two reasons: First, sex ratio of adult spiders in the field are normally characterized by small numbers of males (Schaefer 1987). For example, Austin (1984) found a sex ratio of 1:2.3 in *Clubiona robusta*, and Huhta (1965) observed an average sex ratio of 1:3.5 in *Finnish foresta*. The surplus of females could be partly explained by the fact that males have a shorter life span and usually die very soon after mating (Schaefer 1987). Adult male spiders in most species also range more widely in search of fe-

males, which might also make it harder to locate males than females. Moreover housing of adult *A. ferox* in the lab precludes the chance for close or sustained observations in the field. Second, there is a possibility of differential mortality of the sexes during the juvenile period (Clutton-Brock 1985), which would be overlooked in an embryonic analysis.

### Estimation of the Fertilization Period

We began to observe female *Amaurobius* in the field from the first day of May. (The majority of females begin to elaborate their cocoon from the end of June.) We determined whether insemination had occurred by verifying the presence of mating plugs, which originate from the male palpal organ and indicate that copulation has occurred, on the genital plates of the females (Suhm et al. 1996). We divided the observation period (the month of May) into three segments: the first decade (ten days) of the month, the middle ten days of the month, and the last ten days. We calculated the proportion of females observed in the field that had been inseminated during each period.

## RESULTS AND DISCUSSION

### Sex Ratio

The mean sex ratio was 0.49 with little variation between years (Table 1).

Social spiders show a highly female-biased sex ratio (Avilés et al. 2000), e.g. an overall proportion of males of 0.28 in the social spider *Diaea socialis* (Araneae: Thomisidae) (Rowell and Main 1992). Researchers have also found a primary female bias in embryos of social species, e.g. 0.08 in *Anelosimus eximius* and 0.09 in *A. domingo*, while more even sex ratio was observed in non-social species in this genus (Avilés and Maddison 1991).

Sociality in spiders is thought to have evolved via the subsocial pathway by a prolongation of an early tolerance phase without dispersal (Kullmann 1972, Krafft 1979, Avilés 1997). However, *A. ferox* did not show a female-biased sex ratio. (Several additional species of the genus *Amaurobius* are also considered to be subsocial (Ex., *A. fenestralis* and *A. similes*; Kaston 1965, Lemasle 1977, Tahiri et al. 1989)).

Sex ratio of *A. ferox* was similar to that reported in the subsocial

Table 1. Sex ratio of *Amaurobius ferox*

Year	Number of spiders	Female	Male	Sex ratio
1995	131	56	75	0.57
1996	198	108	90	0.45
Total	326	164	165	0.49

*Anelosimus jucundus* (a congener of the social spider, *A. eximius*) (Vollrath 1986). In the case of eusocial insects where a reproductive division of labor occurs, individual interest in the group is focused around reproduction. But, for spiderlings in a subsocial group, groups are foraging societies whereby individuals benefit from collective predation on the maternal web (Kim et al. 2005a,b). In temporary foraging groups, foraging efficiency should be more important than reproductive efficiency. Therefore, the sex ratio of subsocial spiders might not be related to their reproductive efficiency. To provide more reasonable explanations for the sex ratio of subsocial groups, further investigation in more subsocial species is needed.

#### Estimation of the Fertilization Period

During the first decade of the study period (the 1<sup>st</sup>–10<sup>th</sup> of May), we observed 12 adult females, of which eight had mating plugs on their genital plates. In the middle decade of May, the proportion of inseminated females did not still reach 100%; we recorded 21 inseminated individuals among the 30 observed. By the end of May, most females were inseminated, only one out of 22 females observed did not have a mating plug (Table 2).

From this result, we estimate that 33.4% of *Amaurobius* females were inseminated from May 11<sup>th</sup>–31<sup>st</sup>, while the remaining 66.6% of the females inseminated before May 10<sup>th</sup>. Copulation of *A. ferox* was frequent during the month of May. However, we do not know how long their mating period continues.

*A. ferox* is a stenochronous spider which reproduces during a definite season of the year, mostly in spring and summer. Immature spiders of stenochronous species hibernate as instars (Schaefer 1987). However, we observed adult spiders of *Amaurobius* in the field in autumn. As the spiders reached the adult stage, it seems possible that females and males meet and copulate. Moreover adult males do not build expanded cribellate web to capture prey, but rather wander seeking females. It is possible that males cohabit within females' retreats.

The results of this study and the observations of Tahiri et al. (1989) suggest that *A. ferox* need a long period or a period of cold temperatures to prepare them for reproduction after the develop-

mental attainment of the adult stage. A period of winter may affect the physiological state of the adult female, preparing her to accept male copulation attempts.

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Table 2. Proportion of *Amaurobius ferox* females inseminated in the field during May

Day in May	Number of females verified	Inseminated	Not inseminated	Proportion of inseminated females
1 <sup>st</sup> –10 <sup>th</sup>	12	8	4	66.6%
11 <sup>th</sup> –20 <sup>th</sup>	30	21	8	70.0%
21 <sup>st</sup> –31 <sup>st</sup>	22	21	1	95.4%

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