

Comparison of Brood Productions in the Cold-Treated Pairing vs. Not Cold-Treated Pairing in a Stenochronous Spider

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ABSTRACT: To understand whether experience of cold season in reproductive behaviors in the adults of *Amaurobius ferox*, the paired adults of a female and a male were exposed under 'cold-treated environment' and 'not cold-treated environment', respectively. I investigated effects of the cold treatment on the brood production of *A. ferox*. In not cold treatment in which male-female pairs were formed in October at a temperature of 20°C ($\pm 2^\circ\text{C}$) and continuously kept under not cold-treated environment, only 3 of 50 pairs successfully reproduced (reproduction was defined as the emergence of spiderlings). In cold treatment where individuals were kept in cold conditions for 3 months prior to pair formation, 57 out of 60 couples succeeded in reproducing. Females which did not experience the low temperature displayed strong aggressiveness toward males. This behavioral inhibition might be the primary barrier to copulation of *A. ferox* that decrease following a period of low temperature. The reproductive inhibition might help the females to allocate the maximum amount of energy in a given environment to reaching the adult stage and to delay reproduction in unfavorable wintering conditions.

Key words: *Amaurobius*, Life-cycle polymorphism, Mating behavior, Stenochronous spider

INTRODUCTION

Life-cycle polymorphism including multiple alternatives in the timing and duration of substages in the life-cycle of one species, has been reported in terrestrial arthropods (Tauber et al. 1986, Schaefer 1987, Framenau and Elgar 2005, Danks 2007). The diversity in life-cycle patterns is prominent in species distributed over a broad range of environmental conditions (Danks 1994, 2007), but less observed within single populations (Waldbauer 1978). Most individuals even within polymorphic populations overwinter in a species-specific stage, which plays an important role in synchronizing the life cycle within a population (Framenau and Elgar 2005).

Spider populations fall into three types of life cycle: eurychronous, stenochronous and diplochronous (Schaefer 1977, 1987). This excludes some species such as tarantulas (Mygalomorphae) with a lifetime of more than 3 years. Eurychronous spiders do not reproduce at a definite period and overwinter in different stages, in which phases are controlled only by temperature variations; there is no state of diapause, while developmental time may be short or long (Marc et al. 1999). Some Linyphiidae, Clubionidae (e.g. *Clubiona phragmitis*) and Tetragnathidae (e.g. *Meta menzei*) have this type of biological cycle. Stenochronous species show precise reproductive and dispersing periods. Species referred as diplochronous by

Schaefer (1987) were in fact spring-summer stenochronous spiders that undergo two mating periods during a year, in spring and autumn (Danks 1994, 2007). If the diplochronous adults are able to mate in autumn, the females do not lay their eggs before spring; adults overwinter in this case.

In the Holarctic region, most spiders are stenochronous with spring-summer reproduction, and over-winter as immature instars (Schaefer 1987). The penultimate instar generally avoids precocious maturation in autumn. *Pardosa* spiders (Lycosidae) were found not to overwinter in the adult stage in the Holarctic region (Kiss and Samu 2002). Sdae adults in the genus *Pardosa* seem to be unable to overwinter, regulation of autumnal development in immature individuals is important to avoid untimely maturation before winter, especially in geographical areas with unpredictable warm periods in autumn (Kiss and Samu 2002).

The black lace weaver spider, *Amaurobius ferox* (Amaurobiidae), is known as a stenochronous spider which spends winter as immature instars, becomes adults during spring, and reproduces in summer (Lemasle 1977, Tahiri et al. 1989). The life cycle of *A. ferox* lasts approximately 2 years (i.e., a biennial species), and individuals of two generations are simultaneously observed on the ground. However, I have observed adult *Amaurobius* in the field in autumn. Therefore, it appears possible that males and females meet and copulate in autumn.

Tahiri et al. (1989) observed no reproduction (emergence of

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spiderlings) of *A. ferox* females ($n = 78$) collected in nature during the months of Oct~Dec from a forested area in northeastern France, suggesting that the females were not yet inseminated in that period. However, Tahiri et al. (1989) did not verify the presence of a mating plug on the females which is an evidence of copulation in *Amaurobius* species (Suhm et al. 1996). Among females collected during the months of Jan~Mar, nearly 10% (7/75) reproduced in the laboratory (Tahiri et al. 1989), and Kim and Choe (2007) observed that 66.6% *A. ferox* females collected at the same area in early May (May 1st~10th) were inseminated. These results suggest that fertilization of *A. ferox* occurs mostly in spring and summer, and *A. ferox* might need a winter period to prepare them to copulate in spite of the developmental attainment of the adult stage in the autumn.

I hypothesized that the cold condition influences reproductive behaviors in the adults of *A. ferox*. I manipulated environments experienced by adult individuals to determine the effects of a simulated 'cold condition' and 'no cold condition' on the spiders' brood production.

MATERIALS AND METHOD

Study Species

Amaurobius ferox Walckenaer (Araneae, Amaurobiidae) is a common nocturnal European spider. They are found in shaded and damp places such as under stones in forest litter, in old walls, and in caves (Locket and Millidge 1951-1953, Cloudsley-Thompson 1955), which precludes close or sustained observations in the field. The spider spins a sticky cribellate web to capture prey and to use 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).

Maternal activity begins in early summer when the females construct a single egg sac (Lemasle 1977, Kim and Horel 1998). The majority of females begin to elaborate their cocoon from the end of June to July (Cloudsley-Thompson 1955). In October, both juvenile spiders of the year and adult spiders born in the previous year are observed.

Experimental Design

I collected *A. ferox* individuals during late summer and autumn under stones in forest litter, in holes in old walls, and in other sheltered places in the forested area of Nancy, France (Northeast France, N 48° 41', E 6° 13', elevation: 217 m, annual temperature: 9.6 ± 6.3 °C, annual precipitation: 74 cm). On the day of collection the spiders were individually installed in glass terraria (20 × 12 × 20

cm) containing a block of plaster on one side and a mixture of sand and charcoal (70 mm in depth) on the other (Gundermann et al. 1993). One corner of the terrarium was roofed over and darkened by covering the outside surface with black paper to promote web installation. The paper was then removed for observation.

The spiders were fed with 20 (± 2) mg cricket larvae (*Gryllus dimaculatus*) and maintained at a temperature of 20°C (± 2 °C) with a 12L/12D cycle. Fluorescent lights provided approximately 100 lux. All of the spiders became adult in October (determined by the chitinization of the genital plate of females and male apophysis).

I conducted two different treatments on the experimental groups: 'Not Cold Treatment' - I put an adult female and male together in October at a temperature of 20°C (± 2 °C) ($n = 50$ pairs).

'Cold Treatment' - I kept females and males individually at 6°C (± 1 °C) for three months starting in October, and then males and females were placed together in January ($n = 60$ pairs).

For not cold treatment, a male was deposited on the web of a female that had already been settled for 5 days in the terrarium (20 × 12 × 20 cm). For cold treatment, adults were moved individually into plastic boxes (10.0 × 7.0 × 2.5 cm) and kept in a refrigerator for 3 months (Oct~Jan). The lid of the box was made with a grilled window for aeration, and a piece of cotton placed in a corner of the box was humidified twice a week. The spiders were maintained at a temperature of 6°C (± 1 °C) and a 0/24 hour light-dark cycle. During winter the individuals stay in the dark under the ground. The box was exchanged with a clean one every two weeks because of fungus growing. No food was provided. No mortality was observed during this period. In January the spiders were taken out of the refrigerator and placed in the terraria (20 × 12 × 20 cm). A male was deposited on the web of each female 5 days after she settled on the web. After installation in the terraria, the spiders were fed with 20 (± 2) mg cricket larvae and maintained at a temperature of 20°C (± 2 °C) with a 12L/12D cycle. Fluorescent lights provided approximately 100 lux.

Observations

I observed male behaviors on the female's web and verified females' insemination by the presence of mating plugs (Suhm et al. 1996). Reproductive activities of each female were recorded until emergence of spiderlings from the cocoon.

RESULTS

The experimental manipulation revealed significantly different brood productions between the treatments, pairing without cold condition and pairing with a passage of the cold condition (Fisher's exact test for the number of reproductions in the treatments: $p <$

0.0001). In not cold treatment 3 out of 50 pairs reproduced. I observed males trying to approach the females, but the females showed aggressive responses, raising their first pair of legs. Ninety four % of the pairs (47 out of 50) did not copulate, while the females displayed no tolerance toward the males. Nevertheless, males stayed on the females' webs for a long time (1~3 months). No male was killed by his partner.

In cold treatment where the individuals had been kept in the cold for 3 months prior to pair formation, 57 out of 60 pairs (95%) produced the offspring. I observed the mating plugs on the females' abdomens in an average of 3.8 ± 2.7 days after the placement of the males on the females' webs. An average of 38.3 ± 6.0 days later, the fertilized females built normal-looking cocoons.

Mating behavior of *A. ferox* was observed in cold treatment. When the male entered into the female's web, it advanced slowly toward the female staying in its retreat. The male caused web vibration using slow alternate movement of its pedipalps. The female responded to this courtship signal by shifting its position toward the male, and they turned face to face. Then, the male turned its body to face in the opposite direction. When the male was almost in contact with the female, it made rapid movements of its pedipalps and shivered its abdomen. Sometimes the female advanced toward the male and made menacing movements, and the male moved back, but stayed on the female's web. Then, it approached the female again. The male seemed to become more and more stimulated and its vibratory movement became faster. Eventually, the female made a pendular movement while suspend under the web before standing still. When the female tolerated the male (i.e., when she did not react to the contact of the male), the male introduced one of the pedipalps into the female's genital opening. No sexual cannibalism was observed during copulation, and attacks by the female against the male were rare. After the copulation, the male remained on the female's web for an average of 10.9 ± 14.9 days. During this period the female did not show any aggressive behavior toward the male on their webs. After leaving the webs of the females, the males were found on the ground or up against the lid of the terrarium.

DISCUSSION

The results suggest that the experimentally formed 'autumn-pairing' individuals (without the passage of cold period) involved mechanisms inhibiting reproduction of *Amaurobius ferox*. Only 6% of the pairs produced spiderlings in not cold treatment, while 95% succeeded in reproducing in cold treatment where the individuals had been treated with simulated cold conditions. This result agrees with the previous observations (Tahiri et al. 1989, Kim and Choe

2007) in which fertilization of *A. ferox* occurred mostly in spring and summer.

I must point out that the experiment could not rule out possibilities of spiders' physiological maturation to be needed to reproduce after developmental attainment of the adult stage. However, three females among the autumn-pairs in the not cold treatment produced young, which suggests certain flexibilities of reproduction. It appears that a cold period is dispensable for reproduction in *A. ferox*. The species might have diplochronous traits that undergo two mating periods during a year, in spring and autumn. However, the proportion of reproducing individuals in the non-overwintering *Amaurobius* was much fewer than in the overwintering individuals.

The first barrier preventing copulation of *A. ferox* seemed to be female aggressiveness toward males, and this barrier likely disappeared after the period of low temperature. In both of the two treatments, the males were interested in the females and approached them. However, the females in not cold treatment did not permit the males' approaches. The experimental cold period might play an important role in altering the physiological state of the adult female to make it receptive to the male's approach. Seasonal conditions such as the cold winter induce animals to change the physiological state, which might in turn provoke behavioral changes.

A. ferox is different from *Pardosa* spiders in the timing of maturation. *Pardosa* species do not overwinter in the adult stage in the Holarctic region (Kiss and Samu 2002). Therefore, the penultimate instars avoid precocious maturation in autumn (Kiss and Samu 2002). In the wolf spider, this strategy might contribute to higher synchrony of developmental stages in the overwintering and spring populations. For *Amaurobius*, however, individuals are able to attain the adult stage in autumn. Behavioral inhibition of reproduction may help the females to allocate the maximum amount of energy in a given environment to reaching the adult stage and to delay reproduction in unfavorable conditions.

Miyashita (1969) observed a strong inhibition of molting behavior in the penultimate instars of the wolf spider, *Pardosa astrigera* at the beginning of the overwintering season. The inhibition was persistent even in warm conditions with long day lengths. Schaefer (1987) has shown that penultimate instars of the wolf spider *Pirata piraticus* overwinter in diapause, while the younger juvenile stages overwinter in quiescence. A similar pattern occurs in the spider *Pholcus crypticolens* (Pholcidae), in which a strong developmental delay under a short photoperiod resulted from prolongation of the last instar (Miyashita 1990). Apparently the phenomenon is not the case of *A. ferox*, as the diapause entails a basic conversion in the hormonal status of the spider as an obligatory part of the life cycle (Kiss and Samu 2002).

Framenau and Elgar (2005) reported in a wolf spider that the

life-cycle polymorphism influences the individual body-size, survival rate, and offspring survival. Brood production in autumn maturing *A. ferox* would be a constraint on the offspring survival of the overwintering stages. This aspect needs further studies.

Studies on the relationships between environmental conditions, physiological constraints and behavioral responses are important as non-beneficial responses will be costly in a quickly changing environment. The question whether differences in temperature regimes will affect the mating behavior, is a very interesting one, and this area certainly requires additional researches.

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