

# Females adopt sexual catalepsy to facilitate mating

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## Abstract

Theory predicts that males and females of dioecious species typically engage in an evolutionary sexual conflict over the frequency and choice of mating partner. Female sexual cannibalism, a particularly dramatic illustration of this conflict, is widespread in certain animal taxa including spiders. Nevertheless, females of some funnel weaving spiders that are generally aggressive to conspecifics enter a cataleptic state after male courtship, ensuring the males can mate without risk of attack. In this study, we demonstrated that the physical posture and duration, metabolites, and central neurotransmitters of females of *Aterigena aculeata* in sexual catalepsy closely resemble females in thanatosis but are distinct from those in anesthesia, indicating that the courted females feign death to eliminate the risk of potentially aggressive responses and thereby allow preferred males to mate. Unlike the taxonomically widespread thanatosis, which generally represents a deceptive visual signal that acts against the interest of the receivers, sexual catalepsy of females in the funnel weaving spiders may deliver a sexual-receptive signal to the courting males and thereby benefit both the signal senders and receivers. Therefore, sexual catalepsy in *A. aculeata* may not reflect a conflict but rather a confluence of interest between the sexes.

**Key words:** female catalepsy, funnel weaving spiders, mate choice, mating strategy, sexual behavior.

Males and females of dioecious species, while typically cooperating to facilitate fertilization, nevertheless engage in an evolutionary sexual conflict over the choice and number of mates (Arnqvist and Rowe 2005). Sexual cannibalism, where females consume males at some stage during courtship and mating, is one of the more dramatic manifestations of this conflict (Elgar 2004), and its nature depends, in part, on the timing of sexual cannibalism: sexual cannibalism during mating may allow males to protect their paternity share (Andrade 1996; Fromhage et al. 2005), females to exercise cryptic mate choice (Elgar et al. 2000), or a combination of these effects (Zuk 2016; Tunj et al. 2020). On the other hand, females attempting to capture and cannibalize courting males may be exercising a form of mate choice (Elgar and Nash 1988): mating with males that can avoid capture and cannibalizing males that cannot. While sexual cannibalism appears to provide an unambiguous example of sexual conflict, with females benefiting at the expense of male reproduction, the relative costs and benefits of the behavior to males and females varies both within and between species (Elgar 1992; Elgar and Schneider 2004; Wilder et al. 2009; Schneider and Fromhage 2010; Fisher et al. 2018).

Nevertheless, sexual cannibalism clearly represents, to varying degrees, a mating opportunity cost for males, and diverse

traits are thought to have evolved in response to the risk of sexual cannibalism (Elgar 1992). These include sexual dimorphism (Elgar et al. 1990; Elgar 1991; Fernández-Montraveta et al. 2014; Anderson and Hebets 2016); post copulation thanatosis (Bilde et al. 2006); the capacity to distinguish between females according to risk (Gaskett et al. 2004; Moskalik and Uetz 2011; Xiao et al. 2015; Avigliano et al. 2016); laying silk over the female (Anderson and Hebets 2016; Zhang et al. 2011; but see Trillo et al. 2019); approaching the female only when she is feeding on a prey item (Fromhage and Schneider 2005); sacrificing legs (Neumann and Schneider 2020); and by copulating with moulting females (Uhl et al. 2015).

Sexual catalepsy, where the female adopts a characteristically motionless state, with her legs curled up, at some stage during courtship or copulation has been documented in several funnel weaving spiders (Gering 1953; Singer et al. 2000; Xiao et al. 2015; Liu et al. 2018). Experimental evidence suggests that sexual catalepsy is a male-induced mechanism to reduce the risk of sexual cannibalism (Singer et al. 2000; Xiao et al. 2015). Females may remain motionless and unresponsive for between a few minutes to several hours, during which time the courting male can move the female into a position that allows him to insert his pedipalps, without risk of attack. Sexual catalepsy may be typical of agelenids: we found that

among diverse taxa within the Agelenidae mating occurs only when the female is cataleptic (Galasso 2012; Liu et al. unpublished data).

While sexual catalepsy may reduce the risk to males of sexual cannibalism, it is not clear that it always represents a male strategy in response to sexual conflict. The position adopted by cataleptic females, with her curled legs, closely resembles that adopted by spiders in thanatosis, in which individuals feign death apparently to reduce the risk of attack by predators (Humphreys and Ruxton 2018). Accordingly, cataleptic females may be in a similar state: capable of being active during the mating process but choosing not to be. Spiders are well-known for their sexual cannibalistic behavior (Elgar 1992; Elgar and Schneider 2004; Wilder et al. 2009), but this rarely involves males attacking females (Elgar 1992). Second, it is unlikely that selection favors female responses to male signals, such as pheromones (Singer et al. 2000) or vibrations and physical touching (Xiao et al. 2015) that is contrary to her evolutionary interests (see Peso et al. 2015). Thus, understanding the underlying mechanisms responsible for sexual catalepsy is a useful first step in determining whether sexual catalepsy reflects a conflict or confluence of interest between the sexes.

The funnel weaving spider, *Aterigena aculeata* (Agelenidae), is abundant where it occurs in the Jinggang Mountains, constructing funnel shaped webs near the ground, with the point of the funnel pointing towards the ground. Females of *A. aculeata* are cataleptic for the duration of mating, which typically lasts for a few minutes only. We took 2 approaches to address whether sexual catalepsy in female *A. aculeata* resembles female controlled antipredator thanatosis or is uncontrolled anesthesia. First, we compared the physical positions and duration in those positions of female spiders during sexual catalepsy in the presence of males; thanatosis induced by experimental stimuli; and anesthesia induced by exposure to ether. Second, we compared the dynamics of endogenous metabolites and central neurotransmitters of females under these 3 treatments since metabolites and neurotransmitters have long been documented to play an important role in the regulation of behavior. Investigating the underlying mechanisms of sexual catalepsy provides insights into whether it reflects a female induced behavior, resembling thanatosis, or whether they are induced into an unconscious state.

## Materials and Methods

### Collection and maintenance of the funnel weaving spider *A. aculeata*

We maintained control of female mating status by collecting juveniles and subadults of *A. aculeata* from Jinggang Mountains in Jiangxi Province (26°34.32' N, 114°7.86' E) one month prior to the typical breeding season (June–August). The spiders were maintained individually in separate plastic containers (14 cm L × 10 cm W × 5.5 cm H) with small damp cotton balls as a source of drinking water. The plastic containers were housed in a rearing room equipped with a clean air conditioning system. The temperature was 25 ± 2 °C and photo period was 14 L:10 D in the rearing room. Spiders were fed once every 2 days: subadults with house flies (*Musca domestica*), green bottle flies (*Lucillia sericata*) and medium to large sized mealworms *Tenebrio molitor*, and the relatively smaller juveniles with fruit flies *Drosophila melanogaster* and small sized mealworms *T. molitor*.

### Mating assays

We recorded the behavior of mature unmated females and males of the funnel weaving spider *A. aculeata* in a circular, lidded container (20 cm D × 8 cm H) constructed with polymethyl methacrylate, and always cleaned with anhydrous ethanol prior to the assay. After the ethanol had dried, a wet cotton ball was placed within the container to provide moisture. The species is sexually size dimorphic, with females (mean ± standard error of the mean (SEM)): 231.1 ± 11.28 mg weight, 20.94 ± 0.33 mm length (front of head to end of spinner) larger than males (mean ± SEM): 206.8 ± 2.69 mg weight, 16.32 ± 0.09 mm length (total body length). All spiders were fed with 1–2 house flies or mealworms one day prior to the assay, and after the female had completed feeding, she was placed in the observation container, where she constructed a web. The next day, the male was carefully introduced into the observation container, and the behavior of the male and female recorded using a video camera (Logitech Carl Zeiss HD 1080, Logitech Ltd., USA) and event recording software (Video Master, Tianjin Zhongge Technology Co., China). We conducted twelve courtship and mating trials with randomly paired male and female spiders and noted their behavior.

### Thanatosis and anesthesia assays

Mature unmated females were fed with 1–2 house flies or mealworms one day before the thanatosis ( $n = 9$ ) and anesthesia ( $n = 9$ ) assays. Thanatosis was induced by encouraging the female to walk into a 50 ml centrifuge tube, which was then closed and gently shaken. Once the female was motionless, we photographed her physical posture and noted the duration of thanatosis. For the anesthesia treatment, we positioned a 1 cm diameter cotton ball, containing a 1 ml drop of anhydrous ether (AR grade, purity ≥ 99.5, Xilong Science Co., China) at the base of a 50 ml centrifuge tube, which was then sealed for 5 s. We then encouraged the female into the tube, which was sealed for 1 min ensuring the spider was fully anesthetized. We then placed the female in a ventilated area and photographed her physical posture and noted the duration of anesthesia.

### Endogenous metabolites and central neurotransmitters under different treatments

Unmated females were randomly assigned to one of the following treatments: control (individuals kept alone), sexual catalepsy, thanatosis, and anesthesia. Control females were instantly frozen with liquid nitrogen in their usual active state in their plastic container (as above) and frozen female preserved in liquid nitrogen. Sexual catalepsy females were randomly allocated a male and allowed to court and mate in a container (20 cm D × 8 cm H). Liquid nitrogen was poured over the mating pair (ensuring the female was cataleptic) and the pair was separated, and the frozen female preserved in liquid nitrogen. Thanatosis females were walked into a 50 ml centrifuge tube, and thanatosis was induced as above. Liquid nitrogen was then poured over the tube and the frozen female was preserved in liquid nitrogen. Anesthesia females were walked into a 50 ml centrifuge tube, and anesthesia was induced as above. Liquid nitrogen was then poured over the tube and the frozen female was preserved in liquid nitrogen. We obtained 6 females for each treatment.

Female spiders were cut at the pedicel and the cephalothorax (head) was placed in a high throughput tissue grinder

(Shanghai Wanbai Biotechnology Co., China) for 2 min at a frequency of 50 Hz. The cephalothorax tissue powder was then placed in a sample bottle and mixed with 400  $\mu$ L of methanol (AR grade, purity  $\geq$  99.5, Xilong Science Co., China), then centrifuged in a high-speed centrifuge (Eppendorf Centrifuge 5417R, Germany) for 22 min at 4 °C, 13,000 rpm. The supernatant was then blown dry using a nitrogen concentrator, and the sample bottles were stored at -80 °C for subsequent LC-MS/MS and HR-MS/MS analysis.

### Neurotransmitter and metabolomics analyses

The blow-dried supernatant samples were re-dissolved using 50  $\mu$ L of 0.1% methanol, and then centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatant was used for neurotransmitter and metabolomics tests using LC-MS/MS and HR-MS/MS, respectively.

The LC-MS/MS analysis, performed for simultaneous analysis of neurotransmitters, was conducted using an AB SCIEX QTRAP 4500 triple quadrupole mass spectrometer, with both positive and negative ESI ionization modes and MRM scan mode. LC separation was accomplished by a ACQUITY HSS PFP column (2.1  $\times$  100 mm, 1.7  $\mu$ m, Waters USA), equipped with a Waters ACQUITY UPLC I-Class infinite binary pump. Acetonitrile containing 5 mM amine acetate and 0.1% formic acid was used as solvent A, and water containing 5 mM amine acetate and 0.1% formic acid was used as solvent B. The flow rate is 0.2 mL/min, gradient elution starts with 98% B, linearly decreased to 0% B at 8 min, and then increased to 98% B at 2 min. The column temperature was set at 35 °C, the sample room temperature was set at 10 °C and the injection volume is 10  $\mu$ L. The MS parameters were set as follow, ion source temperature was set at 500 °C, curtain gas was set at 30 psi, collision activated dissociation (CAD) gas settings: medium, ion spray voltage was set at 5,500 V and -4,500 V for positive and negative ionization mode, respectively. The ion gas 1 and 2 parameters were set at 50 psi. The data acquisition was performed on AB SCIEX Analyst 1.7.1 software, whereas the data analysis was performed with SCIEX OS-MQ 1.6.1. The ion pair and MS parameters of MRM scan mode were shown in [Table 1](#).

We used the HR-MS/MS method for the metabolomics analysis, using a Q Exactive Plus Qrbitrap HRMS system

(Thermo Fisher, USA), equipped with U3000 UHPLC system and a heated electrospray ionization (HESI) ion source. The chromatographic separation was performed on a Waters ACQUITY UPLC BEH Amide column (1.7 m, 2.1  $\times$  150 mm) with mobile phases of water containing 0.1% formic acid and 10 mM ammonium acetate (solvent A) or acetonitrile containing 0.1% formic acid (solvent B). The gradient profile started with 0% A and was held for 5 min, then linearly increased over 1 min to 25% A where it was held for 9 min, then linearly increased over 1 min to 50% A where it was maintained for 9 min. The gradient was then decreased to 0% A within 1 min and a 1 min equilibration time was incorporated between runs. The flow rate was 0.3 mL/min. MS data collection was performed with a Full MS-ddMS2 scan in both positive and negative ionization mode in the range of m/z 100–1,000. MS parameters were set as follow: sheath gas flow was set at 40 arb, auxiliary gas flow rate was set at 15 arb, capillary temperature was set at 350 °C, Aux gas heater temperature was set at 350 °C, spray voltage was set at  $\pm$  3.5 kv for positive and negative ionization mode, respectively. The resolution of MS is 70,000, and the resolution of MS/MS is 17,500. The metabolomic data analysis workflow and identification of endogenous metabolites were performed by Compound discoverer 3.2 software local database, the statistics analysis was performed on MetaboAnalyst 5.0.

### Data analysis

The variation of the duration of catalepsy, thanatosis, and anesthesia are described as mean  $\pm$  SE, and analyzed using one-way ANOVA. The duration data of the females were Log10 transformed for the ANOVA analysis. P-values were obtained after Bonferroni correction. The endogenous metabolites of female spiders under normal conditions, sexual catalepsy, thanatosis, and aesthesia were quantitatively measured using the MetaboAnalyst 5.0 platform, and the main components analyzed using Principal Components Analysis (PCA) and Hierarchical Clustering Analysis (HCA). The Euclidean distance was used for the clustering analysis. We compared the neurotransmitter levels of all groups using one-way ANOVA, followed by post-hoc tests. P-values were obtained after Bonferroni correction. All data analyses were conducted using SPSS 19.0, and plotting was completed using Origin 2018.

## Results

### Behaviors associated with courtship and mating

We conducted a total of twelve pairs of mating experiments, of which 9 pairs successfully mated and 3 pairs did not. The behavior leading to mating in *A. aculeata* is detailed in [Table 2](#). In brief, males commenced courting the female shortly after they were placed into her container. Initially, the male slowly approached the female, vibrating her web. As the male got close to the female and touched her with one of his legs, she entered a state of catalepsy, with her legs curled up, and mating commenced. The duration of mating, during which time the male physically grasps the female with his legs and repeatedly inserts his pedipalp into her genital opening, was short with an average of 2 min. The males of the three pairs that did not successfully mate, exhibited typical courtship behavior, and no contact with females, but the females did not respond and none of them fell into a cataleptic state. Cannibalistic behavior did not occur among the unmated pairs during the

**Table 1** The ion pair and MS parameters of MRM scan mode

Name	Q1	Q3	DP	CE
Serotonin	177	160	2	17
Dopamine	154.1	137	6	14
Tyramine	138	121.1	35	14
Octopamine	154	136	2	11
Histamine	112.1	95.1	50	25
Noradrenaline	170	152.1	5	10
Acetylcholine	146.1	87.1	6	18
$\gamma$ -Aminobutyric	104	87	40	14
Glutamic acid	148	84.1	65	22
Glycine	76.1	30.1	60	21
Aspartic acid	134.1	88	60	17

Note: Q1: quadrupole 1, Q3: quadrupole 3, DP: declustering potential, CE: collision energy.

**Table 2 Description of sexual behaviors of the funnel weaving spider *Aterigena aculeata***

No.	Stage and Duration (min)	Definition	Behavioral Description	
			Male	Female
1	Male adaptation 3.88 ± 0.63	The moment when the male was placed on the female's web to when he makes his first move on the web.	Remained motionless, staying in the female's container.	Remained quiescent (motionless).
2	Male courting 3.30 ± 1.12	The moment when the male spider makes the first move to when he first touches the female.	Repeatedly vibrated the female's web with his pedipalps. At the end of this stage, he walked or rushed to the female.	Remained quiescent (motionless) for most of the time. Sometimes moved slightly.
3	Mating 2.20 ± 0.33	The moment when the male first inserts his pedipalp to when the pedipalp is withdrawn and is released from the female's genital opening.	Inserted either his left or right pedipalp into the female's genital opening, and then inserted the other palp. Each pedipalp was used once only.	Entered a cataleptic state with all legs curled up once the male touched her body (any part) with his leg(s) and remained in catalepsy during copulation.
4	Female recovery 0.22 ± 0.07	The moment when the male releases the female's body to when the female resumed activity.	Withdrew his pedipalp and released the cataleptic female and left.	Most females resumed activity as soon as the male released her. A few needed dozens of seconds to start moving

3 h observation period. If the tested female ignored the courting male, he eventually stopped courting, and either remained motionless on the web or walked off the web.

We determined whether sexual catalepsy in females of *A. aculeata* resembles female controlled thanatosis or an uncontrolled unconscious state by first comparing the behavior of females under the four treatments: normal state; sexual catalepsy in the presence of males; thanatosis induced by experimental stimuli; and anesthesia induced by exposure to ether. Unmated females in the absence of males typically extended their first and second pairs of legs in the forward direction, whereas the third and fourth pairs of legs stretched towards the back. The spiders were alert and any slight external stimulation induce a behavioral reaction (Figure 1A). Mating female spiders were in a state of catalepsy, with their legs curled up and were unresponsive to the behavior of the male (Figure 1B). Anesthetized females had their legs stretched slackly around the body (with occasional spasms) and were unresponsive to stimulations from the external environment (Figure 1C). Females in thanatosis had similar characteristics to those under sexual catalepsy, with their legs curled up (Figure 1D). The duration of sexual catalepsy and thanatosis in females was short, lasting around 2 min, whereas it took around 85 min for females to recover following exposure to an anesthetic (Figure 1E). Statistics showed that the difference in duration among the three groups was significant ( $F = 39.751$ ,  $df = 2$ ,  $P < 0.001$ , Figure 1E). Results of Post-hoc test showed that differences between sexual catalepsy and anesthesia group ( $P < 0.001$ ), thanatosis and anesthesia group ( $P < 0.001$ ) were significant while that between sexual catalepsy and thanatosis group was not significant ( $P = 0.153$ ). Thus, the physical posture of females in sexual catalepsy closely resembles that of females in externally stimulated thanatosis, and the duration of these behaviors was very similar, contrasting with both the posture and duration of females subject to externally induced anesthesia.

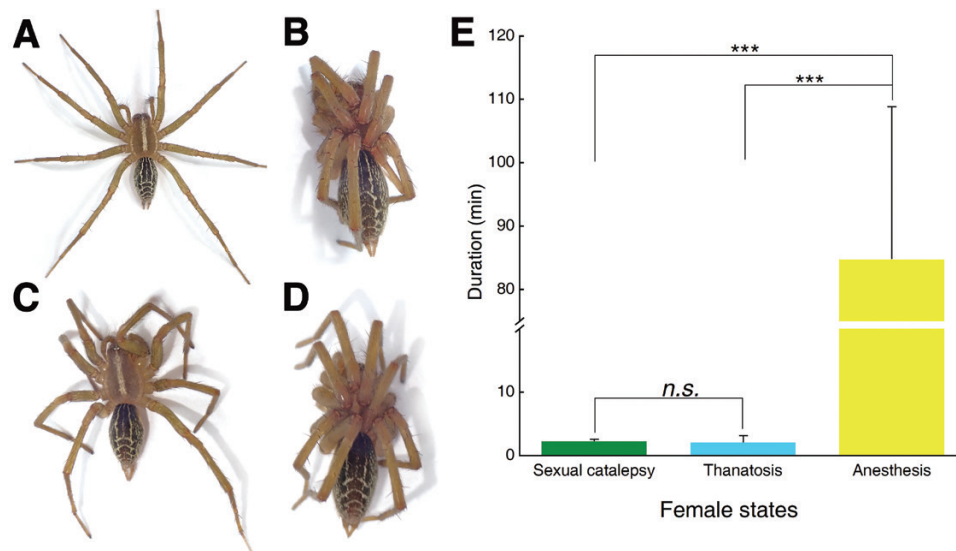
### Metabolomics of female spiders undergoing sexual catalepsy, anesthesia, and thanatosis

We investigated endogenous metabolites of females when they entered sexual catalepsy, thanatosis, and anesthesia, and control females. This analysis revealed strikingly similar patterns: the metabolic processes of female spiders in catalepsy

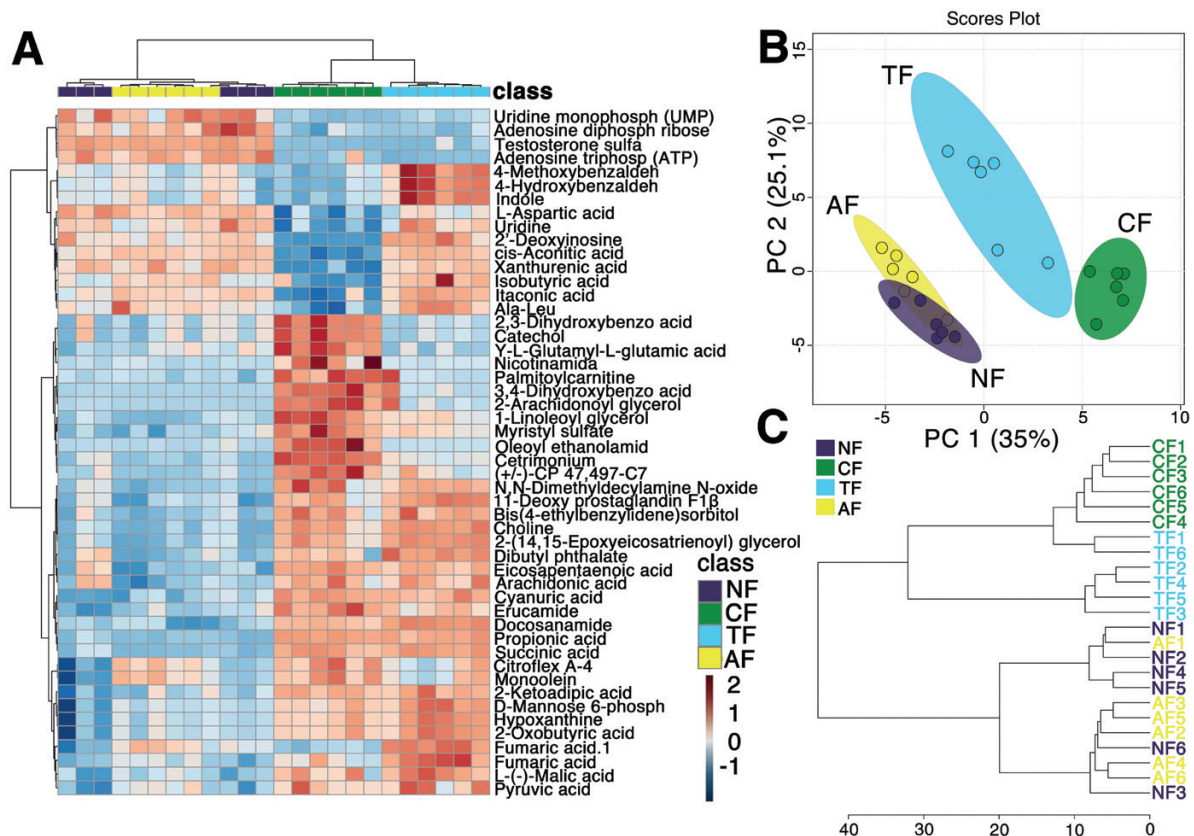
were very similar to those in externally stimulated thanatosis, and unambiguously differ from the females from the control and anesthesia treatments. Among the endogenous metabolites detected from all females that were sampled, 259 metabolites displayed significant differences in the level of contents among the four groups. Fifty metabolites with the greatest difference were selected for the hierarchical cluster analysis heat map (Figure 2A). We can see from the heat map that females from the control group share many metabolites with anesthetic females. Cataleptic and thanatoid females similarly shared metabolites, and the levels of these metabolites typically increased in these treatments. Principal components analysis of all detected metabolites of female spiders under normal conditions, sexual catalepsy, thanatosis, and anesthesia revealed clustering of individuals within the same treatment group. In particular, spiders from the control and anesthetic treatments partially overlap, whereas spiders from the sexual catalepsy and thanatosis treatments were closer to one another, despite being mutually exclusive to spiders from the other treatment groups (Figure 2B). Cluster analysis of the metabolites confirmed that spiders from the four treatment groups were clearly separated into 2 major branches: one comprising the thanatosis and sexual catalepsy treatments, and the other comprising the anesthesia and control treatments (Figure 2C).

### Neurotransmitter contents of females undergoing sexual catalepsy, anesthesia, and thanatosis

Finally, we asked whether central neurotransmitters, some of which play an essential role in regulating spider behavior (Jones et al. 2011; Hebets et al. 2015) differ qualitatively and quantitatively in females of *A. aculeata* across the control, cataleptic, thanatoid, and anesthetic treatments. Eleven neurotransmitters that are commonly present in animals were detected, including 6 biogenic amines, 1 choline, and 4 amino acids (Figure 3A). Adrenaline was not detected in any of the samples. ANOVA revealed that except for serotonin ( $F = 0.055$ ,  $df = 3$ ,  $P = 0.983$ ) and glutamate ( $F = 0.110$ ,  $df = 3$ ,  $P = 0.953$ ), levels of other 9 neurotransmitters were significantly different among the four groups (DA:  $F = 4.468$ ,  $df = 3$ ,  $P < 0.05$ ; TA:  $F = 5.904$ ,  $df = 3$ ,  $P < 0.01$ ; OA:  $F = 4.847$ ,  $df = 3$ ,  $P < 0.05$ ; HA:  $F = 4.292$ ,  $df = 3$ ,  $P < 0.05$ ; NA:  $F = 5.583$ ,  $df = 3$ ,  $P < 0.01$ ; ACH:  $F = 16.932$ ,  $df = 3$ ,  $P < 0.001$ ; GABA:



**Figure 1** States and duration of cataleptic, thanatoid and anesthetic females of the funnel weaving spider *Aterigena aculeata*, dorsal view. (A) an individual female, in resting state, (B) a cataleptic female just finished mating, with her legs still curled up, (C) an anesthetic female with her all legs stretched slackly, (D) a thanatoid female with all her legs tightly curled up. (E) duration of cataleptic, thanatoid, and anesthetic females. \* ( $P < 0.05$ ); \*\*\* ( $P < 0.001$ ); *n.s.* no significant difference ( $P > 0.05$ ).



**Figure 2** The analysis of endogenous metabolites of the female groups under normal, cataleptic, thanatoid and anesthetic states in the funnel weaving spider *Aterigena aculeata*. (A) hierarchical cluster analysis heat map, (B) principal components analysis scores plot, (C) dendrogram, NF: Normal female; CF: Cataleptic female; TF: Thanatoid female; and AF: Anesthetic female.

$F = 13.131$ ,  $df = 3$ ,  $P < 0.001$ ; Gly:  $F = 3.501$ ,  $df = 3$ ,  $P < 0.05$  and Asp:  $F = 4.085$ ,  $df = 3$ ,  $P < 0.05$ ). Compared with females under normal, levels of tyramine ( $P < 0.05$ ), octopamine ( $P < 0.05$ ), histamine ( $P < 0.05$ ),  $\gamma$ -Aminobutyric acid ( $P < 0.001$ ), and aspartic acid ( $P < 0.05$ ) varied significantly

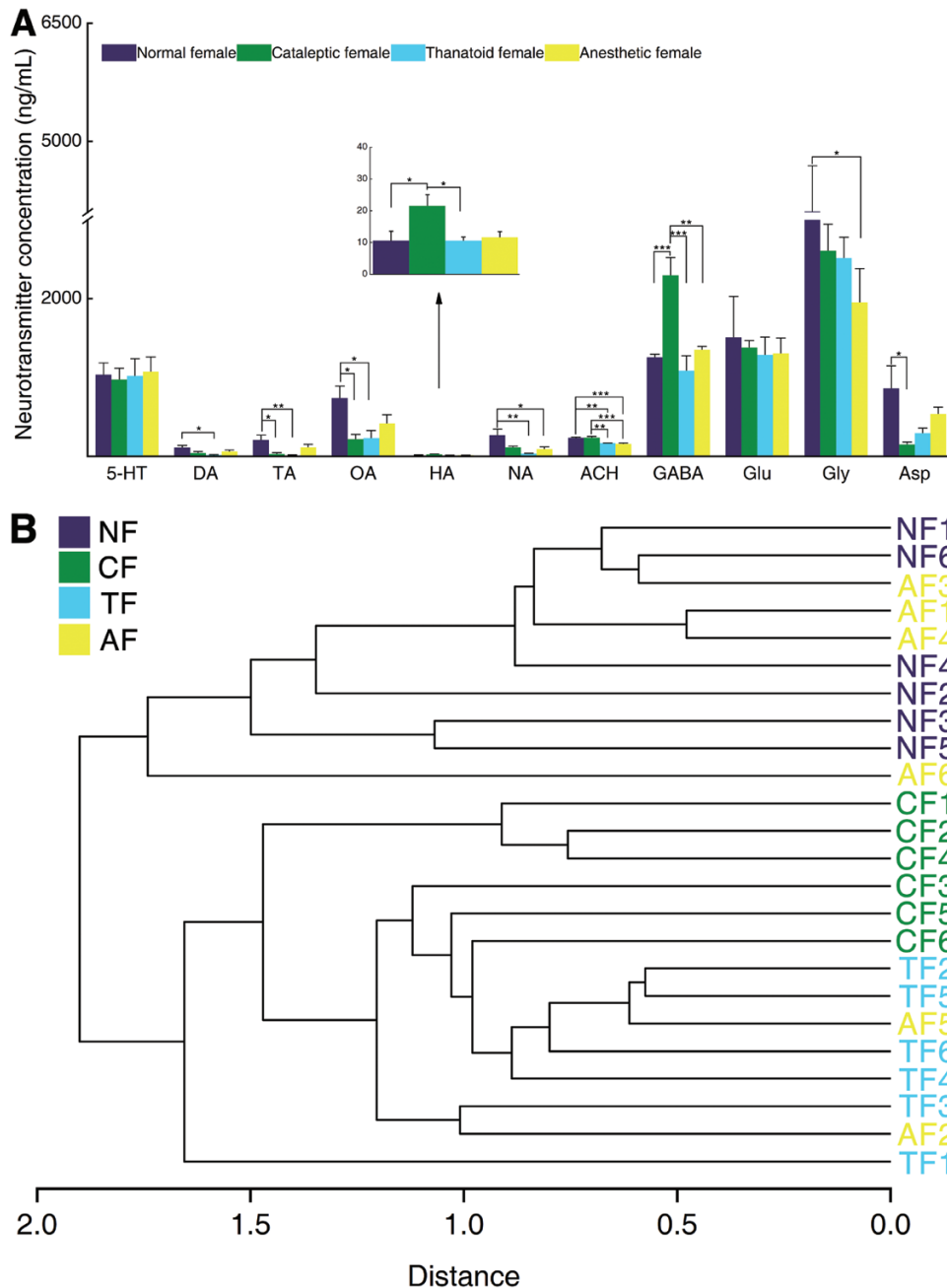
when females entered a cataleptic state (Figure 3A). There were 5 neurotransmitters significantly decreased in level when females were induced into thanatoid state (Figure 3A), including dopamine ( $P < 0.05$ ), tyramine ( $P < 0.01$ ), octopamine ( $P < 0.05$ ), noradrenaline ( $P < 0.01$ ), and acetylcholine

( $P < 0.01$ ). Content of noradrenaline ( $P < 0.05$ ), acetylcholine ( $P < 0.001$ ), and glycine ( $P < 0.05$ ) decreased when females were in anesthesia (Figure 3A). Cluster analysis of the levels of the 11 neurotransmitters revealed that within a distance between 1.74 and 1.90, female spiders from the four treatment groups were gathered into 2 main branches (Figure 3B). One branch contains females from the control and anesthetic treatment groups only, whereas the other branch contains all females from the cataleptic and thanatoid treatment groups.

This indicated that the overall change in neurotransmitter levels of the females from the sexual cataleptic and thanatoid treatments was highly similar, but distinct from the levels of anesthetic females.

### Discussion

We showed that sexual catalepsy in the funnel weaving spider *A. aculeata* has a remarkable resemblance to thanatosis, consistent with the interpretation that the onset, and duration of this behavior is under female control. Copulation occurs only when the female enters a cataleptic state, in which her legs are partially curled up and she remains unresponsive to any movement by her male mate. The physical posture of female sexual catalepsy closely resembles that of females in externally stimulated thanatosis (Figure 1A–D), and the duration of these behaviors is very similar (Figure 1E). This contrasts markedly with both the posture and duration of females



**Figure 3** The content (A) and cluster analysis (B) of central neurotransmitters in females under normal, cataleptic, thanatoid, and anesthetic states in the funnel weaving spider *Aterigena aculeata*. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Neurotransmitters are 5-HT: Serotonin; DA: Dopamine; TA: Tyramine; OA: Octopamine; HA: Histamine; NA: Noradrenaline; ACH: Acetylcholine; GABA:  $\gamma$ -Aminobutyric acid; Glu: Glutamate; Gly: Glycine; and Asp: Aspartic acid. The data are  $\log_{10}$  transformed for the cluster analysis. The error bars are SE.

with externally induced anesthesia (Figure 1). Analyses of metabolomics revealed strikingly similar patterns: the metabolic processes of female spiders in catalepsy are very similar to those in thanatosis, and unambiguously differs from the females from the control and anesthesia treatments (Figure 2). Further, analysis of 11 common neurotransmitters in the central nervous system revealed that the neurotransmitter levels of cataleptic females were similar to that of thanatoid females, but distinct from the levels of anesthetic females (Figure 3). Combined, these data suggest that female catalepsy during mating in *A. aculeata* is broadly equivalent to thanatosis.

As females in many spiders are sexually cannibalistic and thus pose significant risks to courting mates, it might seem more reasonable that males induce female sexual catalepsy in the funnel weaving spiders. Becker et al (2005) showed that females of the funnel weaving spider *Agelenopsis aperta* can be induced into a quiescent state (the authors referred to “catalepsy” as quiescence) when exposed to volatiles of the male homogenate in a short distance, implying that the male was able to induce female catalepsy. Such an explanation assumes that the volatiles of the male homogenate include those that might be produced by an intact male. Perhaps males of the funnel weaving spider *A. aculeata* similarly produce a signal during courting that causes the female to enter catalepsy. However, our data indicated that female catalepsy is a state of thanatosis, which is similar to the self-controlled thanatosis triggered by external stimuli.

The metabolomics of spiders from the control and anesthetic treatments partially overlap but are clearly distinct from the cataleptic and thanatosis females (Figure 2B). Perhaps the metabolic process of the anesthetized spiders ceased following exposure to ether, and thus the metabolomics remained similar to that of the control females. Metabolites and neurotransmitters of females under sexual catalepsy were similar to those of females under thanatosis excited by external stimuli (Figure 2), suggesting that female sexual catalepsy in *A. aculeata* involves elements of metabolic and neurological pathways of thanatosis excited by external stimuli.

Thanatosis is taxonomically widespread and generally occurs when animals are exposed to threats from natural enemies, representing a deceptive “signal” that acts against the interest of their natural enemy receivers. Thanatosis is thought to minimize the risk of any further attack by the predator from which there is little chance of escape (Humphreys and Ruxton 2018): thanatosis following capture may result in the predator releasing their grip on the no-longer struggling prey, offering an opportunity to escape. Thanatosis that occurs before capture may make the immobile prey less easily detected, or a lack of movement may mean the predator no longer recognizes the individual as a prey item (Humphreys and Ruxton 2018). Thanatosis can also occur within a mating context: for example, it allows males of the nursery web spider *Pisaura mirabilis* to escape post-copulatory attack by their sexually cannibalistic partner (Bilde et al. 2006; Hansen et al. 2008), and female dragonflies *Aeshna juncea* (Khelifa 2017) and robber flies *Efferia varipes* (Dennis and Lavigne 1976) enter thanatosis to avoid unwanted “harassment” from courting males. In our study, however, female spiders in *A. aculeata* fell into sexual catalepsy, physiologically equivalent to thanatosis, after the male commenced courtship and followed with successful copulation (Table 2). This suggests that females exercise sexual catalepsy when they are ready for mating with the courting male: males mated with females

only when they displayed sexual catalepsy. Accordingly, sexual catalepsy in female *A. aculeata* may represent a signal of sexual receptivity to the male, benefitting both the female and receiving male.

Behaviors arising from sexual conflict are widespread in animals (Arnqvist and Rowe 2005), including sexual coercion by males, who physically force females into unwanted copulation in insects, isopods, fish, reptiles, and mammals (Sparkes et al. 1996; Watson-Capps 2009; Rossi et al. 2010; Takahashi and Watanabe 2010; Tsurui-Sato et al. 2019; Iglesias-Carrasco et al. 2020; Moldowan et al. 2020; Smit et al. 2022), and sexual cannibalism by females, who attack and consume males at some stage during courtship or mating and documented in many arthropods including mantises, spiders, crickets, amphipods, copepods, and some gastropods (Elgar 2004; Burke and Holwell 2021). Although female sexual catalepsy might initially seem to reflect a case of male-controlled sexual conflict, our experiments suggest that it is under female control and benefits both females and males. Perhaps sexual catalepsy allows females to exercise some choice in mating partner: initially, females remain potentially aggressive to courting males, remaining that way to non-preferred males, and adopting sexual catalepsy to males that are able to touch the female. Females adopting catalepsy allow preferred males to mate safely and quickly. Female sexual catalepsy in the funnel weaving spider *A. aculeata* may thus represent an unusual example of a confluence rather than conflict of interest between the sexes.

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## Author Contributions

Y.H.X. and M.A.E. designed experiments. J.H.L., K.K.L., Y.T. and W.H.W. conducted the experiments. X.X. and J.H. Liang assisted experiments. J.H.L. wrote the original manuscript, Y.H.X. and M.A.E. revised the manuscript.

## Competing Interest Statement

The authors declare no competing interests.

## References

- Anderson AG, Hebets EA, 2016. Benefits of size dimorphism and copulatory silk wrapping in the sexually cannibalistic nursery web spider *Pisaurina mira*. *Biol Lett* 12:201509571–201509575.
- Andrade MCB, 1996. Sexual selection for male sacrifice in the Australian redback spider. *Science* 271:70–72.
- Arnqvist G, Rowe L, 2005. *Sexual Conflict*. New Jersey: Princeton University Press, 1–20.
- Avigliano E, Scardamaglia RC, Gabelli FM, Pompilio L, 2016. Males choose to keep their heads: Preference for lower risk females in a praying mantid. *Behav Processes* 129:80–85.
- Becker E, Riechert S, Singer F, 2005. Male induction of female quiescence/catalepsy during courtship in the spider *Agelenopsis aperta*. *Behavior* 142:57–70.

- Bilde T, Tuni C, Elsayed R, Pekár S, Toft S, 2006. Death feigning in the face of sexual cannibalism. *Biol Lett* 2:23–25.
- Burke NW, Holwell GI, 2021. Male coercion and female injury in a sexually cannibalistic mantis. *Biol Lett* 17:20200811.
- Dennis DS, Lavigne RJ, 1976. Ethology of *Efferia varipes* with comments on species coexistence (Diptera: Asilidae). *J Kans Entomol Soc* 49:48–62.
- Elgar MA, 1991. Sexual cannibalism, size dimorphism, and courtship behavior in orb-weaving spiders (Araneidae). *Evolution* 45:444–448.
- Elgar MA, 1992. Sexual cannibalism in spiders and other invertebrates. In: Elgar MA, Crespi BJ, editors. *Cannibalism: Ecology and Evolution among Diverse Taxa*. Oxford: Oxford University Press, 129–156.
- Elgar MA, 2004. Polyandry, sperm competition and sexual conflict. In: Bolhuis JJ, Giraldeau LA, editors. *The Behavior of Animals: Mechanisms, Function and Evolution*. London: Blackwells, 272–293.
- Elgar MA, Ghaffar N, Read AF, 1990. Sexual dimorphism in leg length among orb-weaving spiders: A possible role for sexual cannibalism. *J Zool* 222:455–470.
- Elgar MA, Nash DR, 1988. Sexual cannibalism in the garden spider *Araneus diadematus*. *Anim Behav* 36:1511–1517.
- Elgar MA, Schneider JM, 2004. The evolutionary significance of sexual cannibalism. *Adv Study Behav* 34:135–163.
- Elgar MA, Schneider JM, Herberstein ME, 2000. Females control paternity in a sexually cannibalistic spider. *Proc Royal Soc B* 267:2439–2443.
- Fernández-Montraveta C, González JM, Cuadrado M, 2014. Male vulnerability explains the occurrence of sexual cannibalism in a moderately sexually dimorphic wolf spider. *Behav Processes* 105:53–59.
- Fisher AM, Cornell SJ, Holwell GI, Price TAR, 2018. Sexual cannibalism and population viability. *Ecol Evol* 8:6663–6670.
- Fromhage L, Elgar MA, Schneider JM, 2005. Faithful without care: The evolution of monogyny. *Evolution* 59:1400–1405.
- Fromhage L, Schneider JM, 2005. Safer sex with feeding females: Sexual conflict in a cannibalistic spider. *Behav Ecol* 16:377–382.
- Galasso AB, 2012. *Comparative analysis of courtship in Agelenopsis funnel-web spiders (Araneae, Agelenidae) with an emphasis on potential isolating mechanisms*. Knoxville: The University of Tennessee. [https://trace.tennessee.edu/utk\\_graddiss/1377/](https://trace.tennessee.edu/utk_graddiss/1377/)
- Gaskett AC, Herberstein ME, Downes BJ, Elgar MA, 2004. Changes in male mate choice in a sexually cannibalistic orb-web spider (Araneae: Araneidae). *Behavior* 141:1197–1210.
- Gering RL, 1953. Structure and function of the genitalia in some American agelenid spiders. *Smithsonian Miscellaneous Collections* 121:1–91.
- Hansen LS, Gonzales SF, Toft S, Bilde T, 2008. Thanatosis as an adaptive male mating strategy in the nuptial gift-giving spider *Pisaura mirabilis*. *Behav Ecol* 19:546–551.
- Hebets EA, Hansen M, Jones TC, Wilgers DJ, 2015. Octopamine levels relate to male mating tactic expression in the wolf spider *Rabidosia punctulata*. *Anim Behav* 100:136–142.
- Humphreys RK, Ruxton GD, 2018. A review of thanatosis (death feigning) as an anti-predator behavior. *Behav Ecol Sociobiol (Print)* 72:22–38.
- Iglesias-Carrasco M, Vincent A, Head ML, 2020. Disentangling the costs of mating and harassment across different environments. *Anim Behav* 165:79–88.
- Jones TC, Akoury TS, Hauser CK, Neblett MF, Linville BJ et al., 2011. Octopamine and serotonin have opposite effects on antipredator behavior in the orb-weaving spider *Larinioides cornutus*. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 197:819–825.
- Khelifa R, 2017. Spatiotemporal pattern of phenology across geographic gradients in insects *Dissertation*, University of Zurich, Faculty of Science, 86–90.
- Liu JH, Xiao YH, Chen ZW, Liu KK, Xu X, 2018. Comparative study on sexual behavior of three funnel-web spiders presenting catalepsy during copulation. *Acta Arachnologica Sinica* 27:44–50.
- Moldowan PD, Brooks RJ, Litzgus JD, 2020. Sex, shells, and weaponry: Coercive reproductive tactics in the painted turtle *Chrysemys picta*. *Behav Ecol Sociobiol (Print)* 74:1–14.
- Moskalik B, Uetz GW, 2011. Experience with chemotactile cues indicating female feeding history impacts male courtship investment in the wolf spider *Schizocosa ocreata*. *Behav Ecol Sociobiol (Print)* 65:2175–2181.
- Neumann R, Schneider JM, 2020. Males sacrifice their legs to pacify aggressive females in a sexually cannibalistic spider. *Anim Behav* 159:59–67.
- Peso M, Elgar MA, Barron AB, 2015. Pheromonal control: reconciling physiological mechanism with signalling theory. *Biol Rev* 90:542–559.
- Rossi BH, Nonacs P, Pitts-Singer TL, 2010. Sexual harassment by males reduces female fecundity in alfalfa leaf cutting bee, *Megachile rotundata*. *Anim Behav* 79:165–171.
- Schneider JM, Fromhage L, 2010. Monogynous mating strategies in spiders. In: Kappeler P, editor. *Animal Behavior: Evolution and Mechanisms*. Berlinatesrevides: Springer-Verlag, 441–464.
- Singer F, Riechert SE, Xu H, Morris AW, Becker E et al., 2000. Analysis of courtship success in the funnel-web spider *Agelenopsis aperta*. *Behavior* 137:93–117.
- Smit N, Baniel A, Roura-Torres B, Amblard-Rambert P, Charpentier MJ et al., 2022. Sexual coercion in a natural mandrill population. *Peer Community Journal* 2:1–17.
- Sparkes TC, Keogh DP, Pary RA, 1996. Energetic costs of mate guarding behavior in male stream-dwelling isopods. *Oecologia* 106:166–171.
- Takahashi Y, Watanabe M, 2010. Female reproductive success is affected by selective male harassment in damselfly *Ischnura senegalensis*. *Anim Behav* 79:211–216.
- Trillo MC, Laborda A, Francescoli G, Aisenberg A, 2019. Fifty shades of silk: sexual behavior and bridal veil deposition in the spider *Ctenus longipes*. *Acta Ethol* 22:47–56.
- Tsurui-Sato K, Fujimoto S, Deki O, Suzuki T, Tatsuta H et al., 2019. Reproductive interference in live-bearing fish: the male guppy is a potential biological agent for eradicating invasive mosquitofish. *Sci Rep* 9:1–9.
- Tuni C, Schneider J, Uhl G, Herberstein ME, 2020. Sperm competition when transfer is dangerous. *Philos Trans R Soc Lond, B, Biol Sci* 375:1–7.
- Uhl G, Zimmer SM, Renner D, Schneider JM, 2015. Exploiting a moment of weakness: Male spiders escape sexual cannibalism by copulating with moulting females. *Sci Rep* 5:1–7.
- Watson-Capps JJ, 2009. Evolution of sexual coercion with respect to sexual selection and sexual conflict theory. In: Muller MN, Wrangham RW, editors. *Sexual Coercion in Primate and Humans: An Evolutionary Perspectives on Male Aggression against Females*. Cambridge: Harvard University Press, 23–41.
- Wilder SM, Rypstra AL, Elgar MA, 2009. The importance of ecological and phylogenetic conditions for the occurrence and frequency of sexual cannibalism. *Annu Rev Ecol Evol Syst* 40:21–39.
- Xiao YH, Zunic-Kosi A, Zhang LW, Prentice TR, Mcelfresh JS et al., 2015. Male adaptations to minimize sexual cannibalism during reproduction in the funnel-web spider *Hololena curta*. *Insect Sci* 22:840–852.
- Zhang SC, Kuntner M, Li DQ, 2011. Mate binding: Male adaptation to sexual conflict in the golden orb-web spider (Nephilidae: *Nephila pilipes*). *Anim Behav* 82:1299–1304.
- Zuk M, 2016. Mates with benefits: when and how sexual cannibalism is adaptive. *Curr Biol* 26:R1230–R1232.