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Abstract

Sexually cannibalized males incur a significant fitness cost due to the loss of future mating opportunities and are expected to evolve behaviors to avoid or compensate for such costs. For example, partially cannibalized males may exhibit mate guarding, in which they accompany the female to prevent her from mating with another male. In some species, cannibalized males prolong the duration of copulation. However, little is known about the adaptive significance of the mating behavior of sexually cannibalized males. We hypothesized that mating itself serves a mate guarding function, and that behavioral change caused by cannibalism enhances the mate guarding function. We tested these hypotheses using the polyandrous and sexually cannibalistic praying mantid *Tenodera angustipennis,* with decapitation as a model of sexual cannibalism. We compared latencies to female mating with a rival male among three experimental treatments: unmated treatment, noncannibalistic mating treatment, and cannibalistic mating treatment. Mating itself delayed female remating, revealing its function in mate guarding. Decapitated males exhibited a higher guarding efficiency against rival males via firmer genital coupling. In addition, spermatophore attached to the female genital opening also delayed female remating, revealing an additional function in postmating mate guarding. Although copulation was prolonged due to decapitation, mating by a rival male was not delayed compared to noncannibalistic mating, probably because of weaker postcopulatory guarding. These findings suggest that greater mate guarding by decapitated males during copulation was offset by processes after copulation.

Significance statement

Sexually cannibalized males die and lose the chance for future mating. This means that males that can avoid or compensate for this fitness loss may be favored. We examined this possibility by focusing on the postmating behavior of sexually cannibalized male

mantises. Experimental analysis revealed that cannibalized males grasped the female more firmly during copulation to avoid disruption by other males, and prolonged copulation duration compared with noncannibalized males. These behavioral changes by cannibalized males contributed to delaying female remating with other males to the same extent as noncannibalized males. This suggests that sexually cannibalized males did not fully compensate for the loss of future mating opportunities. Stronger mate guarding via firm genital coupling and prolonged copulation duration in cannibalized males may be offset by weaker postcopulatory guarding such as shorter duration of copulatory plug attachment.

Keywords: mating behavior, mating plug, sperm competition, sexual conflict, *Tenodera angustipennis*

Acknowledgments

We thank H. Miyaji, K. Kamuro, K. Takagi, and Y. Mori for their help in collecting mantids and K. Yoden for his kind support of our field research activity. We also thank G. Holwell and an anonymous reviewer for their constructive comments.

Author contribution

Conceptualization: Hiroto Nishino, Kotaro Morimoto and Yasuoki Takami; Data curation: Hiroto Nishino; Formal Analysis: Hiroto Nishino; Investigation: Hiroto Nishino, Kotaro Morimoto, and Yasuoki Takami; Methodology: Hiroto Nishino and Yasuoki Takami; Project administration: Hiroto Nishino and Yasuoki Takami; Resources: Hiroto Nishino and Kuroda Kazuki; Supervision: Yasuoki Takami; Visualization: Hiroto Nishino; Writing - original draft Hiroto Nishino; Writing - review & editing: Yasuoki Takami, Kuroda Kazuki, and Morimoto Kotaro;

Funding

No funding was received for conducting this study.

Data availability

Data availability The datasets generated during and/or analyzed during the current study are available in the Dyrad repository, https://doi.##

Introduction

Sexual cannibalism, predation of one sex by the opposite sex of the same species before, during or after mating, is a strong agent of sexual conflict commonly found in arthropods, especially in praying mantises and spiders (Elgar 1992; Maxwell 1999). Sexually cannibalistic species are often sexually dimorphic in body size, with females larger than males, making males susceptible to predation (Prete 1992). Sexual cannibalism is more likely to occur when females are undernourished (Lelito and Brown 2006; Barry et al. 2008a), and the behavior can provide a nutritional benefit to females (Barry et al. 2008a). Cannibalized males suffer a loss of fitness because they are deprived of future mating opportunities (Elgar and Schneider 2004). Theory predicts that sexual cannibalism can be adaptive for the male by increasing the number of eggs of the female mate (Buskirk et al. 1984), as confirmed in a spider (Schwartz et al. 2016). However, this indirect increase in male fitness does not necessarily compensate for the cost of lost mating opportunities (Brown and Barry, 2016), suggesting that sexual cannibalism is often not adaptive for males. Therefore, males of sexually cannibalistic species may evolve adaptations to avoid the costs of cannibalism. For example, males are more cautious in their approach, courtship, and mount when approaching hungry females from the front or avoid mating with such females (spiders: Prenter et al. 1994; Moya-Laraño et al. 2004; Fromhage and Schneider 2005; mantids: Gemeno and Claramunt 2006; Lelito and Brown 2006; Barry et al. 2008b, 2010; Maxwell et al. 2010; Scardamaglia et al. 2015; Kadoi et al. 2017; Burke and Holwell 2021), providing evidence for adaptations to avoid the costs of cannibalism in premating processes. In addition, sexually cannibalized males often mate with females that consume them (Roeder 1935, 1967; Andrade 1996). Thus, sexually cannibalized males may be able to compensate for the costs of sexual cannibalism via postmating processes. However, this possibility has been examined only in limited cases (see below).

In species with polyandrous mating systems, males compete for the paternity of the offspring produced by females, i.e., sperm competition (Parker 1970). Sexual selection via sperm competition is expected to favor both offensive and defensive male adaptations (Parker 1984; Simmons 2001). Offensive adaptations include mechanisms for the takeover of mates and displacement of sperm (e.g., Wagge 1979, Takami 2007). Defensive adaptations include mate guarding and copulatory plugs (e.g., Sherman 1983, Dickinson 1995). Males may accompany a female to prevent her from mating with another male (Alcock 1994), and postcopulatory mate guarding is advantageous in species where the sperm of the last mated male is likely to fertilise a greater proportion of a female's eggs (Parker 1970). In addition, males may plug the female genital opening, as in the mutilated pedipalps of male spiders (Miller 2007), thereby hindering remating with other males. These adaptations can also be expected in sexually cannibalistic species, and the behavioral

or physiological effect of sexual cannibalism on the female or the cannibalized male may influence the consequence of mate guarding. In the redback spider *Latrodectus hasselti*, cannibalized males attain higher paternity success by limiting immediate remating by females (Andrade 1996). In the mantid *Pseudomantis albofimbriata*, sexually cannibalized males exhibit prolonged mating (Barry et al. 2011). Although this behavioral change in cannibalized mantid males did not increase competitive fertilization success (i.e., offensive adaptation), it may influence the consequence of sperm competition via mate guarding, as the sperm of the most recently mated male is preferentially used for fertilization (Barry et al. 2011). However, the mating strategy of sexually cannibalized males remains largely unexplored, especially in mantids.

Females of the sexually cannibalistic praying mantid *Tenodera angustipennis* mate multiple times in the field, resulting in multiple paternity. Multiple males can be attracted by, and mount a female in the field (Nagata et al. 2022). Microsatellite genotyping revealed that 97% (28/29) of clutches obtained from wild females or oothecae included multiple paternity, with 6.34 ± 3.29 fathers (Nagata et al. 2022), suggesting strong sperm competition and a possible benefit of mate guarding in this species. Although quantitative evaluation is difficult due to small sample sizes, cannibalistic mating occurs in the field (2 of 5 mating pairs detected in two years field censuses, Nagata et al. 2022) and in the laboratory (2 of 9 mating pairs, especially by hungry females, Kadoi et al. 2017). Consuming male mates may increase female fecundity and the fitness of cannibalised males. However, it may be insufficient to compensate for the loss of future male reproductive opportunities as in a congeneric species (Brown and Barry 2016). This is also suggested by male mate choice in this species: males of this species avoid mating with hungry females, showing adaptation to sexual cannibalism via a premating process (Kadoi et al. 2017). However, it is unclear in this species whether sexual cannibalism leads to changes in male behavior such as prolonged copulation, and whether copulation functions in mate guarding to prevent females from remating with rival males. In addition, male insemination success via mate guarding depends on the dynamics of sperm transfer. In mantids, sperm is indirectly transferred to the female via a spermatophore, which is formed *in copula* and attached to the female genital opening (e.g., Holwell 2007). Thus, understanding the dynamics of sperm transfer is pivotal to evaluate the efficiency of mate guarding behaviors. In this study, we tested the hypotheses that (1) mating itself functions as a mate guarding behavior, and (2) sexually cannibalized males change their mating behaviors, reinforcing mate guarding. We conducted a behavioral experiment to compare the efficiency of mate guarding among unmated males, males experiencing noncannibalistic mating and males experiencing cannibalistic mating (decapitated). We also analyzed the dynamics of sperm transfer by fixing mating pairs at various stages of mating. Based on our results, we discuss the adaptive

significance of male mate guarding behavior in sexually cannibalistic species.

Materials and methods

Organism preparation

A total of 11 nymphs, 71 females, and 96 males of *T. angustipennis* were collected from rice fields and surrounding grasslands in Kobe, Japan (34°50'N, 135°09'E) from the end of August to the end of September in 2021 and 2022. Adults raised from nymphs and those collected from the fields were used in this experiment. It is generally difficult to determine whether field-collected adults had mated or not. However, most adults were unlikely to have mated because the frequency of mated females starts to increase from early September and reaches 50% at the end of September in this field (Nagata et al. 2022).

The collected nymphs and adults were kept individually in 860 ml plastic cups (10 cm in diameter and 10 cm in height) topped with gauze under constant conditions in an incubator (25°C, 16L8D). Nymphs and males were fed two crickets (*Acheta domesticus*, 0.262 ± 0.0564 g, $N = 10$), and females were fed three crickets every 3 days. They were supplied water with a sprayer until use in the experiment. Final instar nymphs were allowed to eclose in tall cups (10 cm in diameter and 20 cm in height). To control variation in hunger status, experimental males and females were fed crickets to satiety on the day before the experiment. Experimental individuals were about 1 month old or older after eclosion, corresponding to the reproductive phenology in the wild (the median time from eclosion to female first mating is 26 days, Nagata et al. 2022).

Mating experiment

The hypothesis that mating itself serves a mate guarding function predicts that a rival male cannot easily takeover a female during mating with a defensive male (i.e., when their copulatory organs are joined), resulting in longer latencies to remating than those for females that are not mating. Additionally, the hypothesis that change in mating behavior by a sexually cannibalized male enhances the effect of mate guarding predicts that the frequency of takeover by a rival male is lower, and the time to female remating is longer, for a cannibalized defensive male than for defensive males in noncannibalistic mating.

To evaluate these predictions, a mating experiment was designed using one female, one defensive male, and one rival male in a clear plastic box (37 cm length × 45 cm width × 18 cm height). Gauze was attached inside the lid. The space in the box was divided 2:1 by a clear acrylic plate with many holes (4 mm diameter, 4 cm intervals), allowing mantids in one area to detect mantids in the other area by visual and olfactory cues. The wide and narrow spaces were used as the mating arena and display space, respectively. Three

treatments were conducted: (1) no mating (control), (2) noncannibalistic mating, and (3) cannibalistic mating (Fig. 1). In the control treatment, a female and a defensive male were introduced separately into the mating arena and display space, respectively; after 51 min, a rival male was introduced 10–20 cm behind the female. This latency to the introduction of the rival male allowed the acclimation of the female and the defensive male to the experimental arena and was the average time from the introduction of a female and a defensive male to mating in the first 5 cases of noncannibalistic and cannibalistic mating treatments. In the noncannibalistic mating treatment, a female and a defensive male were introduced into the mating arena and allowed to mate, and the display space was kept empty. After mating was confirmed, a rival male was gently introduced 10–20 cm behind the mating pair. In the cannibalistic mating treatment, a female and a defensive male were introduced into the mating arena and allowed to mate. After mating was confirmed, the defensive male was decapitated by fine scissors to imitate sexual cannibalism, and then a rival male was introduced 10–20 cm behind the mating pair. Sexually cannibalized males are frequently decapitated by the female (Roeder 1967, p. 164; Rubenstein and Alcock 2019, p. 119), and decapitation or incapacitation of the brain (Matsumoto and Sakai 1999) or the subesophageal ganglion (Roeder 1935, 1967) promotes, rather than hinders, male mating activity. Note that the same number of mantids were introduced into the box for all treatments; thereby, the perceived risk of cannibalism (the number of individuals) and other cues that depend on individual density were kept constant (i.e., one male and one female were within in the sight of the focal rival male).

Mating experiments were performed at 25 ± 2°C in the laboratory with fluorescent ceiling light. Individuals were randomly assigned to the three treatments. Due to limited sample sizes, males were used up to twice in a defensive and rival role (once in each role) with an interval of at least one day, except for decapitated defensive males in the cannibalistic mating treatment. The repeated use of males may influence their behavior via the previous experience of the other role. Thus, the presence or absence of previous use of defensive and rival males were included in the following analyses. A total of 59 females and 76 males were used to construct the unmated treatment (N = 15), noncannibalistic mating treatment (N = 20), and cannibalistic mating treatment (N = 18). Mating behavior was recorded every 5 s with a video camera (JVC Everio GZ-RX130) for at most 72 h (4320 min) from the introduction of the rival male. To evaluate the effects of physical contact between defensive and rival males, we recorded whether the rival male touched the mating pair, whether the mating was disrupted by this rival male's behavior, and whether the defensive male touched the female or rival male after the mating finished. The time

from the introduction of the rival male to the start of female mating with the rival male was also recorded to evaluate the ability of the defensive male to prevent female remating (1 min increments). The copulation durations of defensive males in the noncannibalistic and cannibalistic mating treatments were also recorded to confirm prolonged copulation by decapitation. In addition, attachment of a spermatophore to the tip of the female abdomen was evaluated at the end of rival male's mating, in which we only recorded whether it was present or absent. The duration of spermatophore attachment was measured as the time from the end of copulation to detachment of the spermatophore. During all trials, we observed and recorded behaviors. Thus, it was not possible to record data blind. All experimental individuals were frozen at -20°C after the experiment.

Dynamics of sperm transfer

For further understanding of the effect of disruption of copulation by rival males on insemination success of defensive males, we performed the additional analysis of the dynamics of sperm transfer. We were interested in the timing of sperm transfer into the spermatheca. If sperm transfer occurs earlier or later than disruption of copulation by rival males, mate guarding behaviors may be less or more important for defensive males, respectively. The number of sperm transferred to the spermatheca and that remained within the spermatophore were measured at various stages of mating using unmated adults that eclosed from nymphs in the laboratory in 2012. Pairs were allowed to mate in the mating arena, and then frozen by liquid nitrogen at the following four stages: (1) in copulation between 60 and 120 min after the start of noncannibalistic mating (N= 11), (2) at the end of noncannibalistic mating with a spermatophore still attached to the female (N= 6), (3) when the spermatophore detached from the female after noncannibalistic mating (N= 4), and (4) when the spermatophore detached after cannibalistic mating (N= 5). We did not control occurrence of sexual cannibalism, and naturally cannibalized cases were assigned to the stage 4. Detachment of spermatophores was checked by eye. Time elapsed was measured to the nearest 1 min from the onset of mating to fixation in (1), the duration of copulation in (2), and the total duration of copulation and spermatophore attachment in (3) and (4). Spermatophores were collected by removal from the female abdomen in (1) and (2), and detached spermatophores remaining in the mating arena were also collected in (3) and (4). Frozen females were dissected to remove the spermatheca under a binocular microscope (EZ4HD; Leica, Wetzlar, Germany).

A spermatophore or spermatheca was put in a 1.5 ml experimental tube with 100 µl of distilled water, incised with forceps, mixed using a vortex mixer (2,000 rpm, 60 s), and subsequently suspended in 900 µl of distilled water (total 1,000 µl). The sample (0.1 µl) was put on a glass slide and dried, and sperm were counted for 15 replicates per female using a

microscope (Olympus CX41, Tokyo, Japan; 100× magnification). If the concentration of sperm was too high, 100 µl of suspended samples was re-suspended with 200 or 300 µl of distilled water. The average of 15 replicates was then used to estimate the total number of sperm in the spermatophore or spermatheca. These sperm counts were highly repeatable within samples (spermatheca: $R^2 = 0.896$, $F_{26, 404} = 125.7$, $P < 0.0001$; spermatophore: $R^2 = 0.744$, $F_{26, 404} = 43.2$, $P < 0.0001$).

Statistical analysis

Since the body size and condition of experimental individuals may influence copulation duration and other mating traits (Prokop and Václav 2005), we measured the length of the pronotum using a digital caliper to the nearest 0.01 mm as a measure of body size and body weight to the nearest 0.1 mg just before the experiment. Body condition was calculated as body weight$^{1/3}$/pronotum length. The cubic-root transformation was used for adjustment of the dimension of body weight to the pronotum length (Lawrence 1992, Prokop and Václav 2005, 2008). The length of the pronotum and body condition of the female, defensive male, and rival male (see below) were compared among treatments and years by general linear models. The length of the pronotum and body condition of the defensive male differed significantly among treatments, and the length of the pronotum of the rival male differed significantly between years (Table S1). Despite significant differences in male size and condition between treatments and years, these variations were largely overlapped across treatments and years. Hence, to control these possible biases, we included the length of male pronotum, male body condition, and year as covariates in the following analyses. Year was not significant throughout the analyses ($P > 0.05$) and excluded from final models.

Prior to hypothesis testing, the assumption that the sexually cannibalized male prolongs copulation was checked. The duration of copulation and spermatophore attachment were compared between the noncannibalistic mating and cannibalistic mating treatments by parametric survival models. One of these durations or sum of these durations was the response variable, and the treatment, pronotum length, body condition, and the previous use of the defensive and rival males were included as explanatory variables. Models with Weibull, log-normal, Fréchet, exponential, and loglogistic distributions were compared, and the most appropriate distribution was chosen based on the Akaike information criterion corrected for small sample sizes (AICc).

The frequencies of male physical contacts, mating disruption, and spermatophore formation were compared between noncannibalistic and cannibalistic

treatments by generalized linear models (GLMs) with a binomial distribution and a logit link function. The latency to copulation by the rival male was compared among unmated, noncannibalistic mating, and cannibalistic mating treatments by parametric survival models. The treatment, pronotum length, body condition, and the previous use of the defensive and rival males were included as explanatory variables. If a focal event did not occur in the observation period, the event was treated as right-censored in survival models. Analyses were performed using JMP ver. 14 (SAS Institute 2018). The Bonferroni method was used for multiple comparisons, unless otherwise indicated.

Results

Mating experiment

Our results confirmed the presumption that the copulation duration is longer for sexually cannibalized males than for noncannibalized males. The copulation duration for defensive males was significantly longer in the cannibalistic mating treatment (median [95% confidence interval] for males without a previous use, 389 [305, 497] min, N = 18) than in the noncannibalistic mating treatment (200 [153, 263] min, N = 20, $P < 0.001$) (Table 1, Fig. 2). Even after excluding cases of disrupted copulation, the copulation duration of defensive males was still longer in the cannibalistic mating treatment (440 [366, 530] min, N = 14) than in the noncannibalistic mating treatment (254 [200, 323] min, N = 16) ($P < 0.001$). By contrast, the spermatophore attachment time was significantly longer in the noncannibalistic mating treatment (122 [61, 240] min, N = 18) than in the cannibalistic mating treatment (30 [13, 69] min, N = 16) ($P = 0.017$), which was also longer when the rival male was larger and had a previous experience, and was shorter when the defensive male was larger (Table 1). This result unchanged when removing the cases without spermatophore attachment (i.e., time = 0) (noncannibalistic: 268 [197, 365] min, N = 9; cannibalistic: 85 [55, 131] min, N = 13; $P = 0.0006$). The effects of male sizes could be due to differences between experimental years (Table S1), and note that male size variation were accounted for in these analyses. Difference of the sum of the copulation duration and spermatophore attachment duration was not statistically significant between the cannibalistic mating treatment (467 [310, 702] min) and the noncannibalistic mating treatment (355 [239, 526] min) (Table 1, Fig. 2b).

The analysis of frequencies of male physical contacts and mating disruption revealed lower takeover rate in cannibalistic matings, consistent with the hypothesis that sexually cannibalized males change mating behavior and reinforce mate guarding. The rival male contacted the mating pair (by pouncing on the pair, in most cases) slightly more frequently in the cannibalistic (67% [12/18]) mating treatment than in the noncannibalistic (50% [10/20]) and mating ($P = 0.041$) (Fig. 3a). Larger defensive males were contacted

more frequently and larger and better-conditioned rival males contacted the pair less frequently (Table S2a). Immediately after this contact, the disruption of genital coupling occurred significantly more frequently in the noncannibalistic mating treatment (60% [6/10]) than in the cannibalistic mating treatment (17% [2/12]) (Fig. 3b, Table S2b). The frequency of spermatophore formation was slightly higher in the cannibalistic (94% [15/16]) than in the noncannibalistic (72% [13/18]) mating treatments, but this was not statistically different (GLM, $P = 0.77$) (Table S3). All cases of spermatophore formation failure (N = 6) occurred after direct contact by the rival male. In addition, we observed that rival males could not mate with females with attached spermatophores (0% [0/29], excluding four cases in which spermatophore detachment could not be determined). We did not observe spermatophore removal by the rival male if defensive males completed mating. However, the female expelled the spermatophore from the abdominal terminalia or removed it using her legs (cf. Holwell 2007).

The latency to copulation by rival males differed significantly among the three treatments (unmated, 121 [68, 215] min, N = 15; noncannibalistic mating, 2071 [1051, 4083] min, N = 20; cannibalistic mating, 1426 [750, 2713] min, N = 18; Table 2, Fig. 4a). Rival males with previous use showed significantly shorter latencies to copulation (Table 2). As predicted by the hypothesis that mating itself functions in mate guarding, the latency to copulation by the rival male was significantly longer in the noncannibalistic and cannibalistic mating treatments than in the unmated treatment (Fig. 4a, Table S4a, b). These differences among treatment were significant after correction for multiple comparisons (i.e., a 5% significance level for three comparisons of $P = 0.0167$, Table S4). However, contrary to the hypothesis that sexually cannibalized males change mating behavior and reinforce mate guarding, the latency to copulation did not differ between the noncannibalistic and cannibalistic mating treatments (Fig. 4a, Table S4c).

In the noncannibalistic mating treatment, we observed that 40% (8/20) of defensive males mated with the female again or pounced on a rival male that tried to mount the female. However, the presence or absence of these behaviors did not affect the latency to copulation by rival males (Table S5). Even after excluding these eight cases from the noncannibalistic mating treatment, the latency to copulation by the rival male did not differ between the noncannibalistic (325 [108, 975] min, N = 12) and cannibalistic (771 [373, 1595] min, N = 18) mating treatments (Fig. 4b, Table S4d).

Dynamics of sperm transfer

We observed an increase in the number of sperm transferred to the spermatheca

over time after copulation started and eventual stabilization after 400 min (Fig. 5a). When copulation was interrupted within 120 min from the onset of copulation, we did not detect sperm transfer to the spermatheca in 9 of 11 cases (stage 1, N= 11, copulation duration: 95 ± 8.9 min, number of sperm transferred: 175,333 ± 125,995). When copulation finished and spermatophores were still attached, we observed a gradual increase in the number of sperm transferred as the copulation duration increased (stage 2, N= 6, copulation duration: 180 ± 27 min, number of sperm transferred: 471,992 ± 169,670). The number of sperm transferred further increased when spermatophores detached (stage 3, N= 4, copulation duration: 204 ± 43 min, spermatophore attachment duration: 94 ± 26 min, number of sperm transferred: 851,333 ± 290,600). Copulation duration and the number of sperm transferred were greatest in cannibalistic matings (stage 4, N= 5, copulation duration: 368 ± 45 min, spermatophore attachment duration: 145 ± 38 min, number of sperm transferred: 1,711,400 ± 73,576).

We observed the greatest sperm remaining within the spermatophore during the initial 120 min, after which the quantity decreased (Fig. 5b). Spermatophores were not attached to the female abdomen in 5 of 11 cases in stage 1, as indicated by zero values in Fig. 5b. Once the spermatophore was attached, the number of sperm transferred increased (stage 1, N= 11, number of remaining sperm: 163,090 ± 65,528). When copulation was complete, most sperm remained within the spermatophore (stage 2, N= 6, sperm: 221,444 ± 70,207). When the spermatophore was detached from the female, most of the spermatophores were virtually emptied (stage 3, N= 4, 18,167 ± 15,817; stage 4, N= 5, 32,533 ± 24,803). Collectively, these results indicated that males allocate more sperm to the spermatophore during longer copulations and most sperm were transferred to the female during postcopulatory spermatophore attachment.

Discussion

Sexual cannibalism is fatal to the male but may induce adaptive responses in postmating behavior. Our experiment revealed how genital coupling and prolonged copulation in response to decapitation (mimicking sexual cannibalism) serve a mate guarding function in the praying mantid *T. angustipennis*. Our results supported our first hypothesis that mating by the defensive male itself has a mate guarding function. As expected, the latency to mating by rival males was significantly longer in the noncannibalistic mating and cannibalistic mating treatments than in the unmated treatment. Since 70% (14/20) and 90% (16/18) of the defensive males in the noncannibalistic and cannibalistic mating treatments, respectively, completed copulation after the introduction of the rival male, rival males were not free to mate with a female while the copulatory organs of the female and defensive male were physically coupled. Additionally, we observed that rival

males could not remove the spermatophore attached to the female abdominal terminalia and could not mate while the spermatophore was attached after defensive males completed mating. These results suggest that a mature spermatophore functions as a mating plug, and that the spermatophore attachment time contributes to the mate guarding duration. These findings confirm the mate guarding functions of copulation (i.e., genital coupling) as well as the plug-like spermatophore of defensive males.

Second, we evaluated the hypothesis that sexually cannibalized males change mating behavior and reinforce mate guarding. The lower mating disruption rate in the cannibalistic mating treatment than in the noncannibalistic mating treatment suggest that physical coupling of male and female genitalia may be stronger in the former. This process would be expected to facilitate the attachment of the plug-like spermatophore and improve insemination success. This process may depend on variation in male genital morphology (Holwell et al. 2010). In contrast, however, the frequencies of spermatophore formation did not differ between noncannibalistic and cannibalistic mating treatments. This may be because defensive males had already started to form an immature spermatophore prior to being contacted by rival males. Note that we did not discriminate immature and mature spermatophores in the mating experiment. As shown in the dynamics of sperm transfer (Fig. 5), most sperm were transferred after copulation finished and during postcopulatory spermatophore attachment, suggesting that interruption of spermatophore formation by the rival male decreases insemination success of defensive males. Thus, sexually cannibalized males are more likely to construct mature spermatophores via firmer genital coupling and attain higher insemination success than noncannibalized males.

We also confirmed another aspect of behavioral change in response to decapitation: the copulation duration was significantly longer in the cannibalistic mating treatment (i.e., after the decapitation of defensive males) than in the noncannibalistic mating treatment. Two processes may explain this difference in copulation duration: (1) prolonged copulation was physiologically induced by decapitation or (2) prematurely terminated copulation was more frequent in the noncannibalistic mating treatment. Even after removing the effects of the second process, the difference in copulation duration was retained (see Results), suggesting that the first process was important. Prolonged copulation after decapitation was consistent with results of studies of sexual cannibalism in the redback spider (Andrade 1996) and a mantid (Barry et al. 2011). These results are also relevant to male control of copulation duration in mantids (Holwell 2008). Females of sexually cannibalistic mantids frequently eat the male forebody (including the head) first, and the nerve cells in the head constitute the center of inhibitory control in insects (Roeder 1967). In male mantids, when the suboesophageal ganglion leading to the brain is broken, mating behavior is released from inhibition (Roeder 1935, 1967). This process may result in prolonged mating and firmer

genital coupling by decapitated males.

Contrary to our second hypothesis, the latency to mating by the rival male was not longer in the cannibalistic mating treatment than in the noncannibalistic mating treatment, although the copulation duration was prolonged and genital coupling was strengthened in response to decapitation. This can be explained by shorter spermatophore attachment duration in the cannibalistic mating treatment, as shown in qualitative differences in the decreases in the proportion of females not yet mated by the rival male (Fig. 4), and in the proportion of females in mating and a spermatophore attached (Fig. 3b), between the noncannibalistic and cannibalistic mating treatments. Combining these curves, males in the cannibalistic mating treatment performed stronger mate guarding (probably via firmer genital coupling during prolonged copulation) than males in the noncannibalistic mating treatments. However, the former males showed weaker postcopulatory mate guarding than the latter males due to shorter attachment of a mating plug (i.e., spermatophore). Thus, enhanced mate guarding via strengthened genital coupling and prolonged copulation by cannibalized males was offset by processes after mating finished.

In this study, we observed active defensive behaviors by intact defensive males that had finished mating, such as pouncing on the rival male or immediate remating with the female. To our knowledge, this is the first report of such behaviors by male praying mantids. These behaviors may prevent females from mating with the rival male in the noncannibalistic mating treatment. However, a difference in the latency to copulation by the rival male was not detected between cases with and without such defensive behaviors, possibly due to the small sample sizes. In the field, a female can be mounted by multiple males (Nagata et al. 2022), one of which males may exhibit this active defensive behavior. The defensive function of this behavior is expected to be effective when population density and female mating frequency are high as observed in this species (Nagata et al. 2022), and when defensive males do not move away from the female immediately after mating. To test this possibility, studies in which the population density is manipulated over a larger space are needed.

Sexual cannibalism results in a male fitness cost (i.e., the loss of future mating opportunities) (Elgar and Schneider 2004). Mantid males evolved mate choice behaviors in premating processes as a counter-adaptation decreasing the risk of sexual cannibalism (Gemeno and Claramunt 2006; Lelito and Brown 2006; Barry et al. 2008b, 2010; Maxwell et al. 2010; Scardamaglia et al. 2015; Kadoi et al. 2017; Burke and Holwell 2021), thereby some males can avoid the cost of sexual cannibalism. However, the other males are cannibalized by the female in the process of mating. We focused on these cases to seek evidence for counter-adaptation that compensates for the cost of sexual cannibalism in postmating processes. We hypothesized that change in mating behavior by sexually

cannibalized males is adaptive and compensates for this cost. Although the genital coupling was strengthened and copulation duration was prolonged in response to sexual cannibalism (i.e., decapitation) which potentially contribute to mate guarding, the realized efficiency of mate guarding, with respect to latency to mating by rival males, did not differ from that of males that were not sexually cannibalized. This result indicated that behavioral change by sexually cannibalized males is insufficient to compensate for the loss of future mating opportunities. However, in other words, the cannibalized males may achieve the same level of mate guarding in their last mating by changing mating behaviors. In addition, our finding of active postmating defensive behaviors by intact males also suggests another cost of sexual cannibalism: cannibalized males lose the opportunities for checking and interfering with rival males. This cost of sexual cannibalism has rarely been noted. Collectively, the results of this study provide novel insight into the various costs of sexual cannibalism and a counteradaptation by the cannibalized male.

Declarations

Ethical approval

The behavioral studies (including collecting, raising individuals and mating experiments) complied with the legal requirements of Japan. All applicable international, national, and/or institutional guidelines for the use of animals were followed.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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Table 1　Analyses of the effect of decapitation on copulation duration, spermatophore attachment duration, and their sum by parametric survival models. Significant effect ($P < 0.05$) was shown in boldface

	Copulation duration (distribution=Weibull)				Spermatophore attachment duration (distribution=exponential)				Copulation + spermatophore attachment duration (distribution=Weibull)			
	Coefficient ± s.e.	d.f.	Likelihood χ^2	*P*	Coefficient ± s.e.	d.f.	Likelihood χ^2	*P*	Coefficient ± s.e.	d.f.	Likelihood χ^2	*P*
Decapitation(yes/no)	0.33 ± 0.081	1	12.79	**<0.001**	-0.69 ± 0.27	1	5.72	**0.017**	-0.14 ± 0.13	1	1.12	0.29
Previous use of defensive male (yes)	0.060 ± 0.082	1	0.53	0.47	0.11 ± 0.23	1	0.24	0.63	-0.08 ± 0.13	1	0.35	0.56
Previous use of rival male (yes)	-0.14 ± 0.10	1	1.60	0.21	0.71 ± 0.38	1	3.91	**0.048**	0.01 ± 0.20	1	0.00	0.97
Size of defensive male	-0.030 ± 0.088	1	0.12	0.73	-0.75 ± 0.38	1	4.17	**0.041**	-0.06 ± 0.14	1	0.18	0.67
Condition of defensive male	50.84 ± 58.18	1	0.74	0.39	87.39 ± 155.52	1	0.32	0.57	105.21 ± 83.92	1	1.55	0.21
Size of rival male	-0.026 ± 0.082	1	0.10	0.75	0.95 ± 0.39	1	6.37	**0.012**	0.11 ± 0.15	1	0.52	0.47
Condition of rival male	11.18 ± 57.56	1	0.04	0.84	249.29 ± 170.76	1	2.43	0.12	61.58 ± 88.08	1	0.52	0.47

Table 2　Parametric survival models of the latency to copulation by rival males in three experimental treatments (distribution = exponential). Significant effect ($P < 0.05$) was shown in boldface

	Coefficient±s.e.	d.f.	Likelihood χ^2	P
Treatment		2	44.48	**<0.001**
Previous use of defensive male (yes)	-0.20±0.17	1	1.39	0.24
Previous use of rival male (yes)	-0.50±0.19	1	5.97	**0.015**
Size of defensive male	0.097±0.17	1	0.33	0.56
Condition of defensive male	-58.55±129.51	1	0.21	0.65
Size of rival male	0.057±0.19	1	0.09	0.77
Condition of rival male	232.64±134.31	1	3.37	0.066

Table S1　General linear models for the size and condition of the female, defensive male, and rival male in various treatments and years. Significant effect ($P < 0.05$) was shown in boldface. Mean ± s.d. were shown for each treatment, and different letters indicate significant difference in post-hoc tests (Tukey-Kramer HSD test, $P < 0.05$)

	Coefficient ± s.e.	d.f.	*F*	*P*	No mating	Noncannibalistic mating	Cannibalistic mating
Size of female							
Treatment		2,47	0.74	0.48	25.45 ± 1.51[a]	25.35 ± 1.51[a]	24.77 ± 2.17[a]
Year (2021/2022)	0.015±0.26	1,47	0.00	0.95			
Condition of female							
Treatment		2,47	0.17	0.84	0.0563 ± 0.0028[a]	0.0565 ± 0.0022[a]	0.0561 ± 0.0021[a]
Year (2021/2022)	0.00±0.00	1,47	3.27	0.077			
Size of defensive male							
Treatment		2,47	3.96	**0.025**	23.55 ± 1.17[ab]	23.82 ± 1.23[a]	22.75 ± 1.21[b]
Year (2021/2022)	-0.30±0.17	1,47	2.93	0.093			
Condition of defensive male							
Treatment		2,47	4.77	**0.013**	0.0412 ± 0.0013[a]	0.0397 ± 0.0016[b]	0.0410 ± 0.0018[a]
Year (2021/2022)	0.00±0.00	1,47	1.01	0.32			
Size of rival male							
Treatment		2,47	1.04	0.36	23.70 ± 1.07[a]	23.16 ± 1.26[a]	23.33 ± 1.34[a]
Year (2021/2022)	-0.46±0.17	1,47	7.19	**0.010**			
Condition of rival male							
Treatment		2,47	2.55	0.089	0.0414 ± 0.0013[a]	0.0404 ± 0.0014[a]	0.0404 ± 0.0016[a]
Year (2021/2022)	0.00±0.00	1,47	2.19	0.15			

Table S2　Generalized linear models for (a) the proportion of the rival male contacted the mating pair and (b) the proportion of the disruption of genital coupling after contact by rival male. Significant effect ($P < 0.05$) was shown in boldface

	Coefficient±s.e.	d.f.	Likelihood χ^2	P
(a) Proportion of rival male contacted mating pair				
Treatment (noncannibalistic/cannibalistic)	-1.14±0.63	1	4.19	**0.041**
Previous use of defensive male (yes)	-0.87±0.49	1	3.66	0.056
Previous use of rival male (yes)	0.48±0.75	1	0.42	0.52
Size of defensive male	1.57±0.70	1	6.73	**0.0095**
Condition of defensive male	174.23±371.44	1	0.23	0.63
Size of rival male	-1.50±0.72	1	6.43	**0.011**
Condition of rival male	-927.33±476.72	1	5.60	**0.018**
(b) Proportion of disruption of genital coupling after contact by rival male				
Treatment(noncannibalistic/cannibalistic)	2.56±1.24	1	8.91	**0.0028**
Previous use of defensive male (yes)	0.04±0.72	1	0.00	0.95
Previous use of rival male (yes)	0.01±1.18	1	0.00	0.99
Size of defensive male	-0.65±1.09	1	0.39	0.53
Condition of defensive male	843.13±582.67	1	2.43	0.12
Size of rival male	0.59±1.02	1	0.37	0.55
Condition of rival male	-386.98±749.03	1	0.27	0.60

Table S3　Generalized linear model for the proportion spermatophore attachment

Spermatophore attachment (yes/no)	Coefficient±s.e.	d.f.	Likelihood χ^2	P
Treatment (noncannibalistic/cannibalistic)	-0.24±0.82	1	0.08	0.77
Previous use of defensive male (yes)	-0.71±0.76	1	0.97	0.32
Previous use of rival male (yes)	-8.97±11876.91	1	0.19	0.66
Size of defensive male	-1.60±1.07	1	3.14	0.077
Condition of defensive male	419.68±629.64	1	0.45	0.50
Size of rival male	1.25±0.98	1	2.21	0.14
Condition of rival male	857.60±706.35	1	1.81	0.18

Table S4　Parametric survival models for pairwise comparisons of the latency to copulation by rival male between treatments

	Coefficient±s.e.	d.f.	Likelihood χ^2	*P*
(a) Cannibalistic mating vs. unmated (distribution = exponential)				
Treatment(cannibalistic/unmated)	1.22±0.19	1	33.41	**<0.001**
Previous use of defensive male (yes)	-0.08±0.24	1	0.12	0.734
Previous use of rival male (yes)	-0.58±0.21	1	6.33	**0.01**
Size of defensive male	0.09±0.19	1	0.23	0.63
Condition of defensive male	15.06±149.45	1	0.01	0.92
Size of rival male	-0.29±0.23	1	1.51	0.22
Condition of rival male	158.02±160.11	1	1.04	0.31
(b) Noncannibalistic mating vs. unmated (distribution = Weibull)				
Treatment(noncannibalistic/unmated)	1.50±0.34	1	15.94	**<0.001**
Previous use of defensive male (yes)	-0.11±0.33	1	0.11	0.73
Previous use of rival male (yes)	-0.60±0.37	1	2.19	0.14
Size of defensive male	0.00±0.33	1	0.00	0.99
Condition of defensive male	-10.44±259.84	1	0.00	0.97
Size of rival male	0.23±0.38	1	0.35	0.55
Condition of rival male	84.28±260.99	1	0.10	0.75
(c) Noncannibalistic mating vs. cannibalistic mating (distribution = exponential)				
Treatment(noncannibalistic/cannibalistic)	0.13±0.23	1	0.31	0.58
Previous use of defensive male (yes)	-0.24±0.20	1	1.45	0.23
Previous use of rival male (yes)	-0.26±0.26	1	0.92	0.33
Size of defensive male	0.13±0.25	1	0.27	0.60
Condition of defensive male	-142.81±166.54	1	0.77	0.38
Size of rival male	0.29±0.24	1	1.60	0.21
Condition of rival male	362.40±177.51	1	5.24	**0.02**
(c) Noncannibalistic mating（excluding defensive males showing active defense behavior） vs. cannibalistic mating (distribution = exponential)				
Treatment(noncannibalistic/cannibalistic)	-0.43±0.29	1	2.27	0.13
Previous use of defensive male (yes)	0.55±0.33	1	2.78	0.096
Previous use of rival male (yes)	-0.11±0.33	1	0.11	0.74
Size of defensive male	-0.06±0.24	1	0.06	0.80
Condition of defensive male	-66.68±153.95	1	0.19	0.66
Size of rival male	-0.07±0.26	1	0.09	0.77
Condition of rival male	552.71±177.35	1	12.43	**<0.001**

Table S5　Parametric survival model for the effect of active defense behavior by the defensive male on the latency to copulation by the rival male in the noncannibalistic mating treatment (distribution = exponential)

	Coefficient±s.e.	d.f.	Likelihood χ^2	P
Active defense behavior (yes/no)	0.50±0.45	1	1.38	0.24
Previous use of defensive male (yes)	-0.04±0.43	1	0.01	0.93
Previous use of rival male (yes)	-0.18±0.73	1	0.06	0.81
Size of defensive male	0.94±0.56	1	2.61	0.11
Condition of defensive male	503.50±294.67	1	2.53	0.11
Size of rival male	-0.04±0.43	1	0.01	0.92
Condition of rival male	-132.67±282.80	1	0.21	0.64

Figure legends

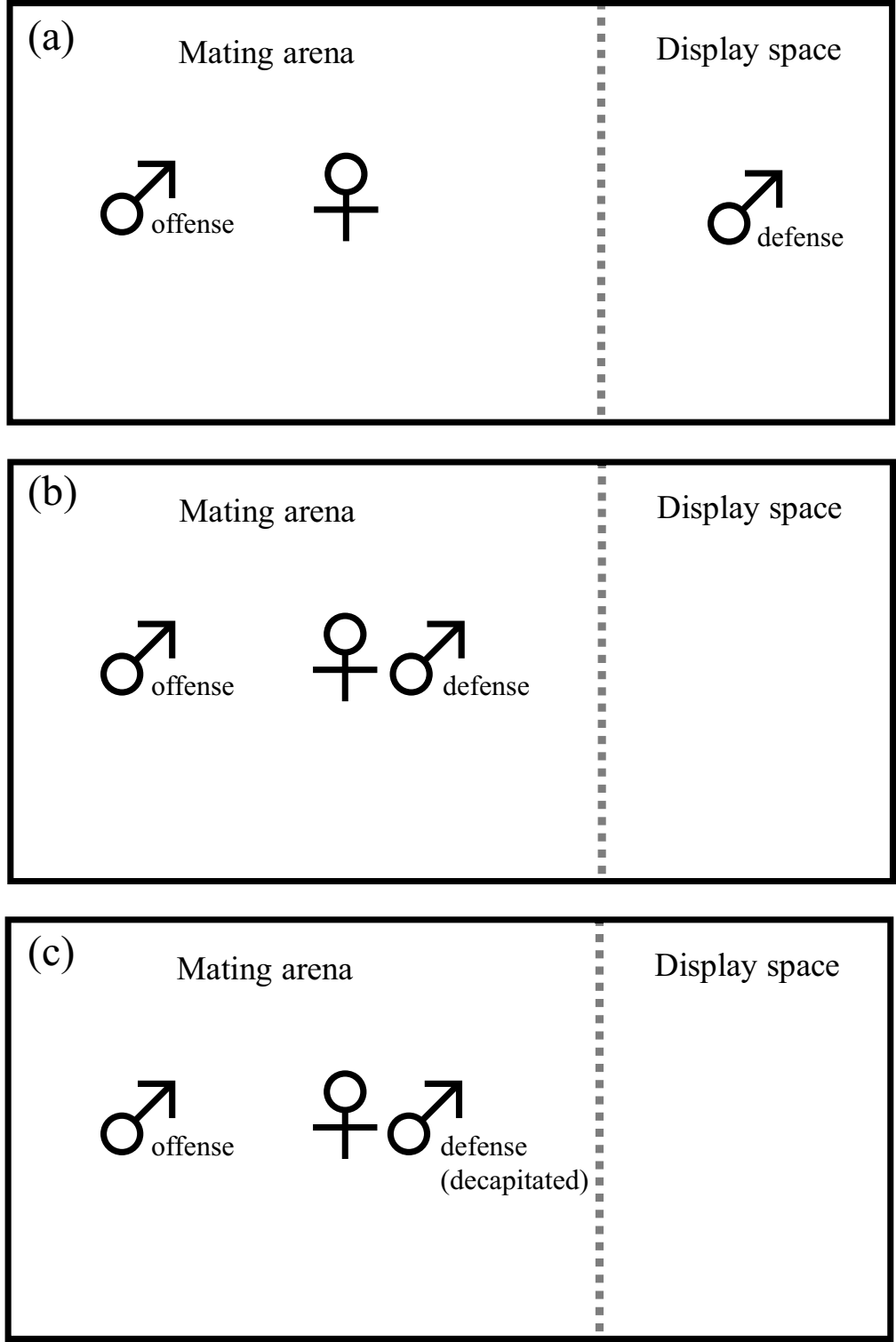
Fig. 1 Schematic diagram of the experimental treatment. (a) Unmated, (b) noncannibalistic mating, and (c) cannibalistic mating

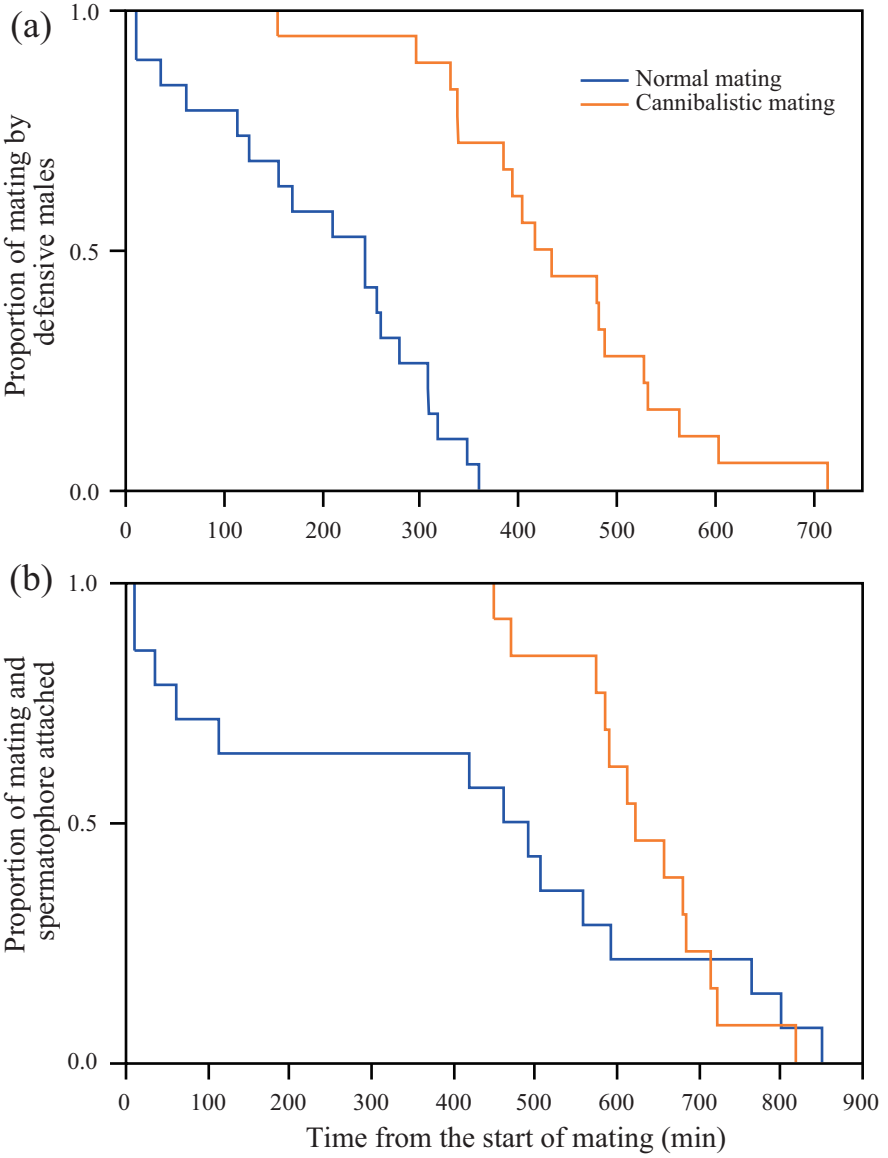
Fig. 2 Effect of male decapitation on (a) copulation duration and (b) copulation duration + spermatophore attachment duration. Blue and orange lines indicate the noncannibalistic and cannibalistic mating groups, respectively

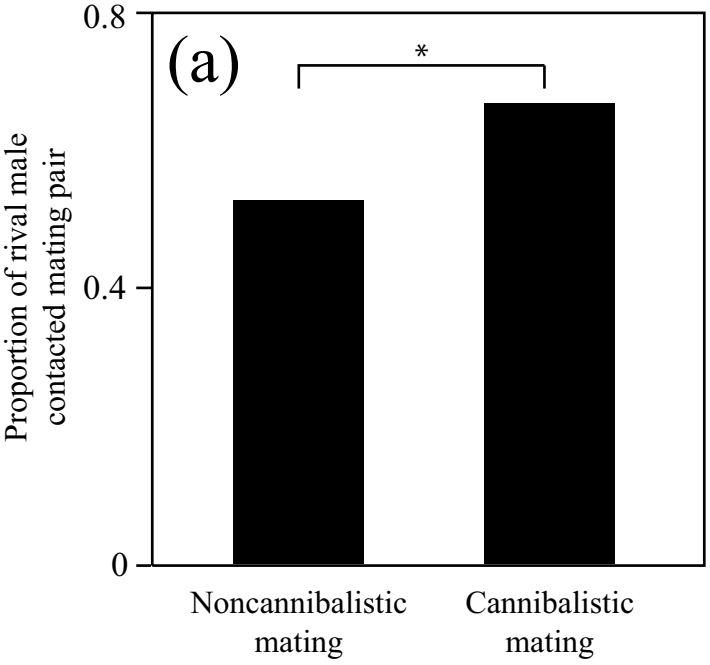
Fig. 3 Comparisons of (a) the proportion of the rival male contacted the mating pair and (b) the proportion of the disruption of genital coupling after contact by rival male between noncannibalistic and cannibalistic mating treatments. Full details of statistical analyses are shown in Table S2. *$P < 0.05$, ** $P < 0.01$

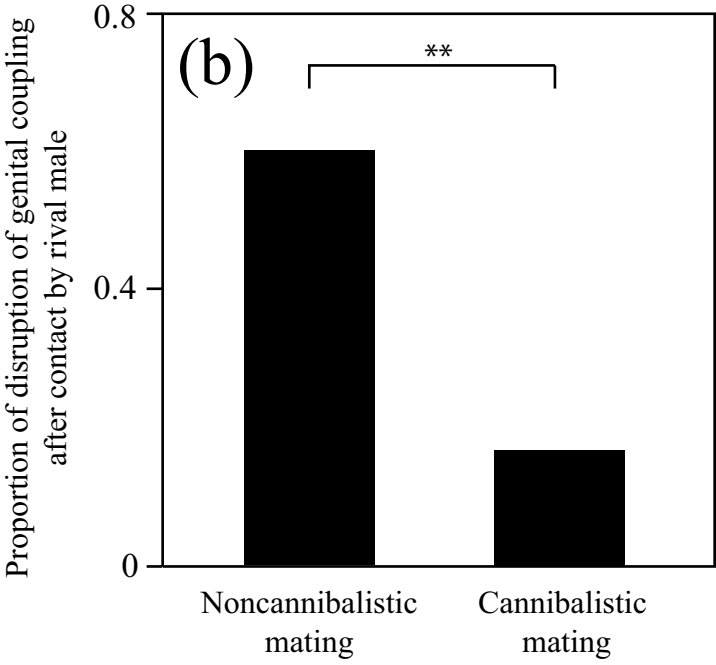
Fig. 4 Comparison of mate guarding by defensive males among three experimental groups: unmated (black), noncannibalistic mating (blue), and cannibalistic mating (orange). Results for (a) all samples and (b) the subset samples excluding defensive males showing active defense behavior from the noncannibalistic mating treatment. *$P < 0.05$, ns $P > 0.05$

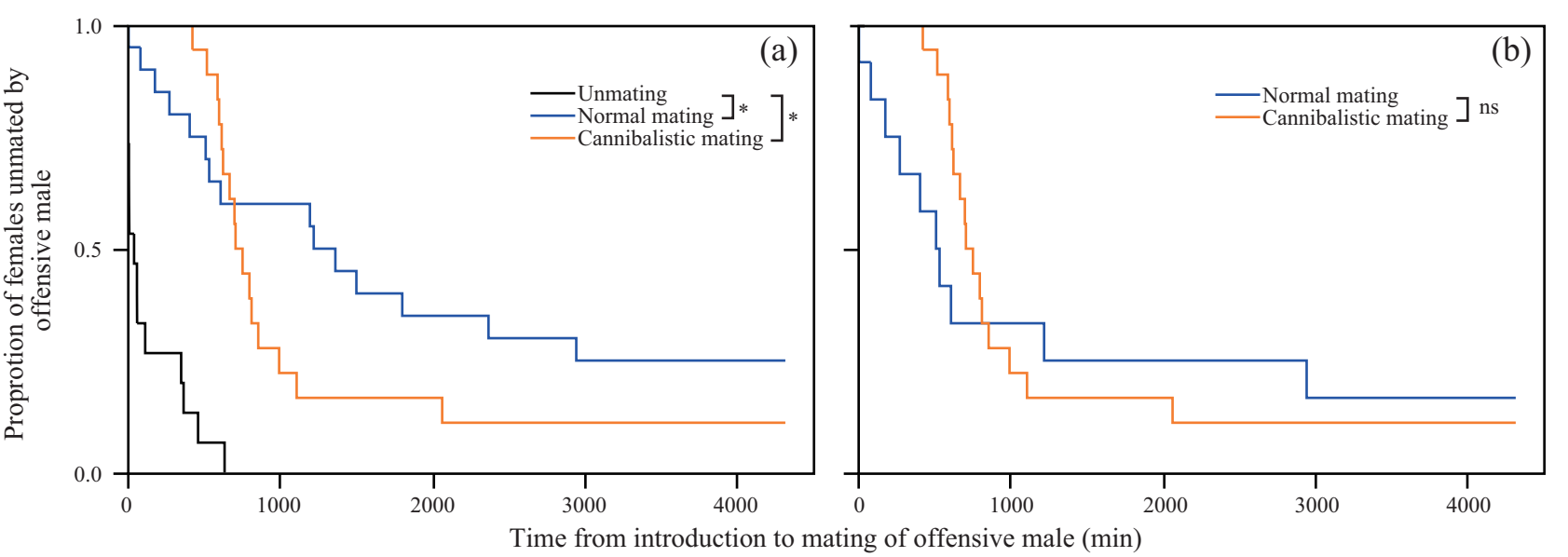
Fig. 5 The dynamics of sperm transfer in *Tenodera angustipennis*. (a) Temporal change in the number of sperm transferred into the spermatheca and (b) that remained within the spermatophore. Orange points refer to noncannibalistic copulation terminated before 120 min; green plots refer to noncannibalistic copulation finished with a spermatophore still attached to the female; blue points refer to noncannibalistic copulation with a spermatophore detached; and red points refer to cannibalistic copulation with a spermatophore detached

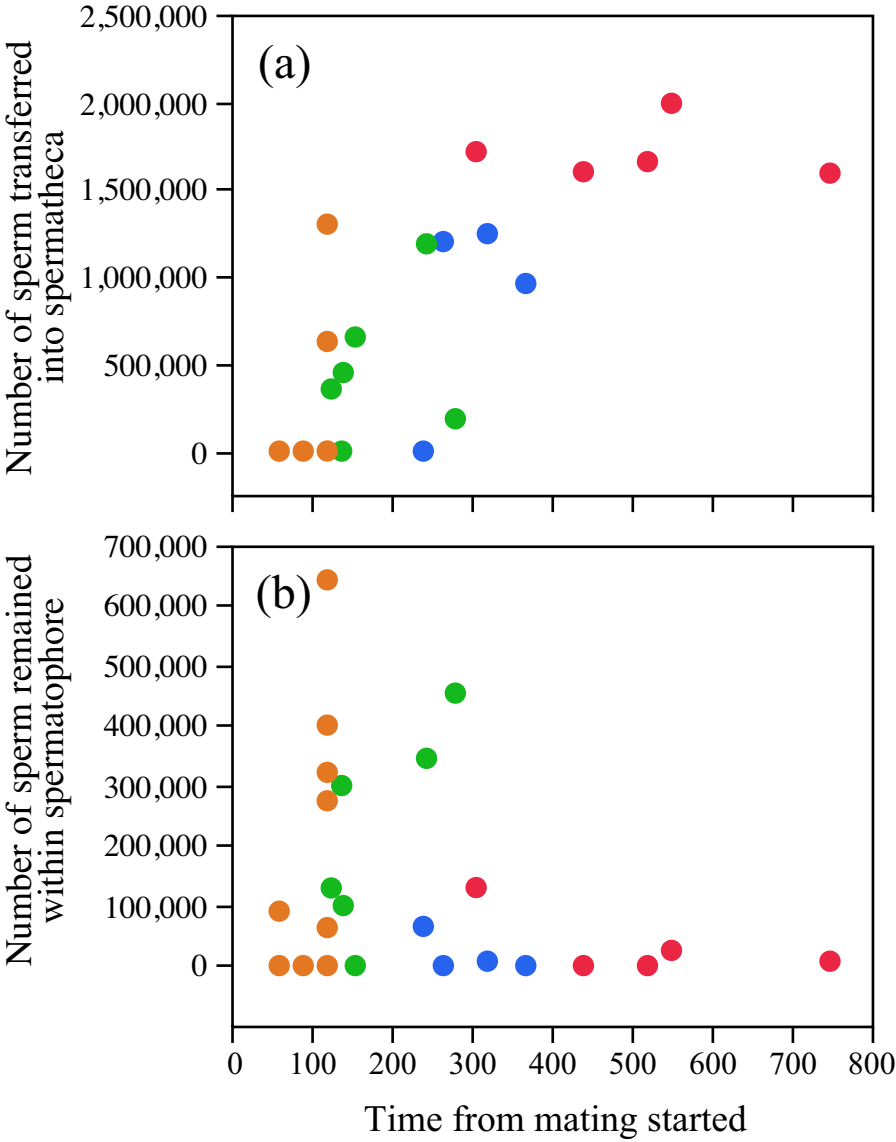

(a)
Mating arena
Display space
offense
defense
(b)
Mating arena
Display space
offense
defense
(c)
Mating arena
Display space
offense
defense
(decapitated)


(a)
1.0
0.5
0.0
Proportion of mating by defensive males
Normal mating
Cannibalistic mating
0
100
200
300
400
500
600
700
(b)
1.0
0.5
0.0
Proportion of mating and spermatophore attached
0
100
200
300
400
500
600
700
800
900
Time from the start of mating (min)


(a)
*
0.8
0.4
0
Proportion of rival male contacted mating pair
Noncannibalistic mating
Cannibalistic mating


(b)
**
0.8
0.4
0
Proportion of disruption of genital coupling after contact by rival male
Noncannibalistic mating
Cannibalistic mating


(a)
(b)
Unmating
Normal mating
Cannibalistic mating
*
*
ns
1.0
0.5
0.0
0
1000
2000
3000
4000
Proprotion of females unmated by offensive male
Time from introduction to mating of offensive male (min)


(a)
Number of sperm transferred into spermatheca
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
(b)
Number of sperm remained within spermatophore
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0
0
100
200
300
400
500
600
700
800
Time from mating started