

PDF issue: 2025-06-07

Reproductive phenology and female mating frequency of the praying mantid Tenodera angustipennis in western Japan

Nagata, Yuki ; Nishino, Hiroto ; Kuroda, Kazuki ; Shinohara, Tadashi ; Satomi, Daisuke ; Terada, Karen ; Nishimura, Taira ; Kuroda, Takahiro …

(Citation)

Ecological Entomology, 47(3):423-431

(Issue Date) 2022-06

(Resource Type) journal article

(Version) Accepted Manuscript

(Rights)

This is the peer reviewed version of the following article: [Nagata, Y., Nishino, H., Kuroda, K., Shinohara, T., Satomi, D., Terada, K. et al. (2022) Reproductive phenology and female mating frequency of the praying mantid Tenodera angustipennis in western Japan. Ecological Entomology, 47(3), 423-431], which has been published in final for…

(URL)

https://hdl.handle.net/20.500.14094/90009261



1	
2	Reproductive phenology and female mating frequency of the praying mantid <i>Tenodera</i>
3	angustipennis in western Japan
4	
5	Running title: Reproductive phenology of a praying mantid
6	
7	YUKI NAGATA, ¹ HIROTO NISHINO, ¹ KAZUKI KURODA, ¹ TADASHI SHINOHARA, ¹
8	DAISUKE SATOMI, ¹ KAREN TERADA, ¹ TAIRA NISHIMURA, ¹ TAKAHIRO
9	KURODA, ¹ YOSHITAKA INOUE, ¹ YONGHWAN PARK, ^{1, 2} and YASUOKI TAKAMI ¹
10	
11	¹ Graduate School of Human Development and Environment, Kobe University, Tsurukabuto
12	3-11, Nada, Kobe 657-8501, Japan
13	² Forest Entomology and Pathology Division, National Institute of Forest Science, 57 Hoegi-
14	ro, Dongdaemun-gu, Seoul 02455, Korea
15	
16	Correspondence: Yasuoki Takami, Kobe University, Tsurukabuto 3-11, Nada, Kobe 657-
17	8501, Japan. E-mail: takami@people.kobe-u.ac.jp

20 1. The timing and frequency of female mating are important determinants of male 21 reproductive success. Elucidating reproductive phenology is crucial to understand the 22 evolution of mating behavior and mating systems. 23 2. Mate encounter rate is a key variable for understanding the evolutionary consequences of 24 sexual cannibalism. However, we know remarkably little about female mating frequency in 25 wild populations in mantids, charismatic insects that exhibit sexual cannibalism. 26 3. We examined the reproductive phenology of a wild population of the sexually cannibalistic 27 praying mantid *Tenodera angustipennis*, and paid special attention to female mating 28 frequency. 29 4. Field surveys throughout two reproductive seasons were combined with survival model 30 analysis to estimate the phenology of eclosion, adult sex ratio, female first mating, and 31 oviposition, allowing quantification of time windows for reproductive maturation and female 32 mating. 33 5. Genetic paternity analysis using newly developed microsatellite markers revealed that 34 females mated with two to six males on average before oviposition in the wild. 6. The results provide a comprehensive record of the reproductive phenology and female 35 36 mating frequency in a wild mantid population, and insight into the evolution of male mating 37 behavior under sperm competition and sexual cannibalism. 38 39 Keywords microsatellite, polyandry, paternity, sexual cannibalism, sperm competition, survival analysis 40 41

42 Introduction

43 Sexual selection drives the evolution of male mating traits, and the timing and frequency of 44 female mating are important determinants of male reproductive success (Andersson, 1994). In 45 insects, the length of period between female reproductive maturation and oviposition may 46 determine how many males can mate with a female (i.e., polyandry), and more polyandry can 47 lead to more intensive post-copulatory competition among males, and stronger post-mating 48 sexual selection on male mating traits (Simmons, 2001). Thus, elucidating the reproductive 49 phenology (i.e., timing, duration and seasonality of reproductive events) of organisms is 50 crucial to understand the evolution of mating behavior and mating systems (Shuster & Wade, 51 2003).

52 Sexual cannibalism is the predation of potential or actual mating partners in one sex 53 (mostly male) by the other sex (female) during mating. As a result, females may gain 54 nutritional benefit (Birkhead et al., 1988; Barry et al., 2008; Welke & Schneider, 2012; 55 Brown & Barry, 2016; c.f. Maxwell, 2000), but males may suffer large fitness costs due to a 56 loss of future mating opportunities. Thus, sexual cannibalism can be a strong agent of sexual 57 conflict (Elgar 1992; Elgar & Schneider 2004; Schneider 2014). Theory predicts that mate 58 encounter rate is a key variable for understanding the evolutionary consequences of sexual 59 cannibalism (Buskirk et al., 1984; Barry & Kokko, 2010). Thus, determining the mate 60 encounter rate may provide insight into the evolution of sexual cannibalism. Most males of 61 the sexually cannibalistic red back spider can mate with only one female due to high male 62 mortality when moving between sedentary females (Andrade, 1996). Given the opportunity of 63 male's additional mating is limited, fitness loss in cannibalized males diminishes, resulting in 64 the resolution of sexual conflict and the evolution of male suicidal behavior facilitating sexual 65 cannibalism (Andrade, 1996). By contrast, large fitness costs to the male due to sexual 66 cannibalism may lead to counter-adaptation in the male, in which selection favors male

67 mating traits avoiding cannibalism (Moya-Larano et al., 2004; Fromhage & Schneider, 2005; 68 Gemeno & Claramunt, 2006; Lelito & Brown, 2006; Barry et al., 2009; Scardamaglia et al., 69 2015; Kadoi et al., 2017). Mantid males are known to encounter multiple females in 70 experimental (Inoue & Matsura, 1983) and wild (Maxwell, 1998; Christensen & Brown, 71 2018) populations. Fitness gain by cannibalized males through increased offspring by 72 nutritional contribution is smaller than fitness loss by losing future mating (Brown & Barry, 73 2016). These results suggest that sexual cannibalism in mantids is not an adaptive suicidal 74 behavior but a result of sexual conflict. 75 Unlike male mate encounter rate, less is known about female mate encounter rate or 76 female mating frequency in sexually cannibalistic species, especially mantids. Because 77 females of *Pseudomantis albofimbriata* stop emitting pheromones attracting males after the 78 first mating, females are believed to be monogamous or engaging in very low levels of 79 polyandry in the wild (Barry et al., 2011). However, in a laboratory setting where males can 80 locate females visually, females can mate with multiple males (Barry et al., 2011). In a semi-81 natural setting, females of the praying mantid *Tenodera angustipennis* mated with an average 82 of 1.8 (up to 4) males (Inoue & Matsura, 1983). Genetic analysis of paternity in a clutch 83 indicated that Ciulfina klassi females mated with one to four males in the wild, while 84 congeneric C. rentzi females were monogamous (Umbers et al., 2011). Similarly, 2 of 18 85 *Tenodera aridifolia* females were estimated to mate with two males (Watanabe *et al.*, 2011). 86 Although these studies suggest that female multiple mating is possible, we still know 87 remarkably little about female mating frequency in wild mantid populations. 88 Herein, we investigated the reproductive phenology of a wild population of the 89 sexually cannibalistic *T. angustipennis*, paying special attention to female mating frequency. 90 Based on the previous record of encounter rate between the male and female (Inoue &

91 Matsura, 1983), we hypothesized that this species has a polyandrous mating system. Field

92 surveys were conducted throughout two reproductive seasons to characterize the phenology of 93 eclosion, adult sex ratio, female first mating, and oviposition. Survival model analysis was 94 performed to estimate the median times of reproductive events, from which we estimated time 95 windows available for female reproductive maturation and multiple mating. Additionally, we 96 constructed novel microsatellite markers for this species, and estimated female mating 97 frequency based on wild-caught females and wild-collected oothecae. We discuss the mode of 98 female multiple mating in the wild, the possibility of sperm competition, and implication to 99 the evolution of sexual cannibalism.

100

101 Materials and methods

102 *Field surveys*

103 Tenodera angustipennis is widely distributed throughout south and east Asia, 104 including the Japanese Archipelago. This species is univoltine, and common and inhabits 105 open environments, such as grassland, cultivated fields, and riverbeds. Our research area was rice fields and surrounding grasslands in Kobe, Japan (34°49'38.0"N, 135°09'23.9"E, about 106 107 1500 m²), where Canadian goldenrod Solidago canadensis and Japanese pampas grass 108 Miscanthus sinensis are the dominant vegetation, in addition to cultivated rice Oryza sativa. 109 Field surveys were conducted for 2 or 3 h in the morning (8:30 to 10:30 or 11:30) 110 from mid-August to the end of October over 2 years (2017, Aug. 17-Oct. 18; 2018, Aug. 111 18-Oct. 24) at approximately 2 week intervals. These periods encompassed the onset of adult 112 emergence to the end of reproduction. In every survey, two to five collectors searched around 113 the area for 2 to 3 h and collected nymphs, adults, and oothecae. The number of collected 114 nymphs, male adults, female adults, and oothecae was recorded. We also recorded the number 115 of mating pairs with or without sexual cannibalism. These surveys were performed along 116 predetermined routes, but the discovery rate of mantids and oothecae seemed to depend on

collecting effort (i.e., the number and composition of collectors and survey time) because they
are cryptic (Fig. 1). This effect was corrected in the analysis (see below). Collected mantises
were transferred to the laboratory for analysis of mating status (see below) and other
behavioral studies (to be reported elsewhere). Thus, we removed collected individuals from
the wild population. This effect was assumed to be negligible because the study population
seems to be sufficiently large relative to our sample sizes.

123

124 *Female mating status*

125 Field-collected adult females (N = 32 in 2017 and N = 61 in 2018) were allowed to 126 oviposit in the laboratory to collect oothecae, which were used for genetic analysis of 127 paternity. Females were housed individually in a plastic jar (13 cm diameter \times 10 cm high) 128 topped with cotton mesh, and fed two crickets (*Acheta domesticus*, 0.262 ± 0.0562 g, N = 52) 129 and sprayed with water three times per week. These were placed in incubators maintained at 130 25 °C with a 16 h light:8 h dark photoperiod. After oviposition, females were frozen at -131 20 °C. Oothecae were individually stored in a small plastic cup (7 cm diameter \times 3.5 cm 132 high), kept under the same conditions as adults, and then moved to 5 °C from November to 133 the following March. From the start of April, oothecae were kept at room temperature, and 134 hatched nymphs were collected and stored in 99% ethanol. Field-collected oothecae were 135 treated in the same manner.

To investigate female mating status, spermatheca of field-caught females were
dissected after oviposition. Spermatheca were removed from the female abdomen under a
stereo microscope (Leica EZ4HD) using fine forceps. Spermatheca were placed in a 1.5 mL
experimental tube with 200 µL particle-free water, incised by fine forceps, and vortexed.
After adding 800 µL of water and gently mixing, individual ejaculates stored in the

141 spermatheca were finally suspended in 1000 μ L water. Ten 1 μ L aliquots were placed on a

142 glass slide, dried, and examined for the presence of sperm using a stereo microscope.

143

144 *Genetic analysis of female mating frequency*

145 Eight polymorphic microsatellite markers were newly developed for *T. angustipennis* 146 (Table S1). Prior to this, we tested primers developed for other species, but one locus for the 147 congeneric T. aridifolia (Watanabe et al., 2011) was monomorphic, and none of the nine loci 148 for Ciulfina rentzi (Attard et al., 2009) were amplified in T. angustipennis. Since 149 polyneopteran insects including mantids are notorious for their large genomes (Koshikawa et 150 al., 2008), we focused on RNA sequences as a target for microsatellite identification (e.g., Du 151 et al., 2015; Wang et al., 2012; Zhang et al., 2012; Wang et al., 2015). Total RNA was 152 extracted from the head of an adult female using an RNeasy Mini Kit (Qiagen), and processed 153 using a TruSeq Stranded mRNA LT Sample Prep Kit (Illumina) for library construction. 154 Paired-end sequencing $(2 \times 101 \text{ bases})$ was performed using NovaSeq 6000 (Illumina), 155 resulting in 74,045 contigs (average 702.47 bases, total 52,014,029 bases) after de novo 156 assembly by Trinity (Grabherr et al., 2011). Microsatellites with dinucleotide to 157 hexanucleotide repeats were searched and PCR primer sequences were determined using 158 Msatcommander (Faircloth, 2018). As a result, 69 primer pairs were identified, and primers 159 were synthesized for 52 randomly selected loci and checked for amplification and 160 polymorphism based on 36 T. angustipennis individuals. Finally, eight loci were chosen with 161 respect to polymorphism and amplified length. Observed and expected heterozygosity were 162 computed, and Hardy-Weinberg equilibrium was tested based on 999 random 163 pseudoreplicates using GenAlEx 6.5 (Peakall & Smouse, 2012) (Table S1). Cross-species 164 amplification was also checked for T. aridifolia (N = 17), revealing that three of eight loci

165 (Tang25872, Tang26190 and Tang30982) were amplified and polymorphic. No loci were 166 amplified for *Hierodula patellifera* (N = 4) or *Statilia maculata* (N = 6).

167 A total of 29 oothecae (7 from 27 oviposited by field-caught females in 2017, 3 of 12 168 field-collected oothecae in 2017, 19 from 59 oviposited by field-caught females in 2018, and 169 0 of 11 field-collected oothecae in 2018) hatched successfully and were subjected to genetic 170 analysis of paternity based on eight microsatellite loci. We observed relatively low hatching 171 rates, possibly due to the harsh overwintering conditions. Note that unmated females of T. 172 angustipennis oviposit unfertilized eggs. Total DNA was extracted from ovipositing females and offspring hatched from oothecae (N = 5 to 31, mean \pm s.d. = 22.9 \pm 8.8) using a Wizard 173 174 Genomic DNA Purification Kit (Promega). Multiplex PCR amplification of the eight loci was 175 performed using fluorescently-labeled primers and a Multiplex PCR Assay Kit (Takara) with 176 the annealing temperature of 60 °C. Amplified products were analyzed using an ABI3130x1 177 genetic analyzer and GeneMapper software (Thermo Fisher Scientific).

178 The hypothesis that *T. angustipennis* has a polyandrous mating system predicts 179 multiple paternity in a clutch. To examine this prediction, the paternity of offspring was 180 estimated based on microsatellite genotypes of the mother and her offspring (for field-caught 181 females) and those of offspring only (for field-collected oothecae). First, we calculated the 182 number of fathers based on allele count; the maximum number of alleles among the eight loci 183 in a clutch was divided by 2 (for diploid), 1 was subtracted (for the mother), and the number 184 was rounded up to an integer. This approach is simple but it underestimates when the number 185 of loci is small and allelic diversity is low. In addition, we also estimated the paternity of 186 offspring using a likelihood method with COLONY ver. 2 (Jones & Wang, 2010). The rates 187 of allelic dropout and other genotyping errors were both set to 0.01. Females and males were 188 presumed polygamous and monogamous, respectively. Full-likelihood analyses were

performed with a 'long' run and allele frequency updating deactivated. The estimated numberof fathers for each clutch was recorded as the number of males mated with the female.

191

192 *Statistical analysis*

193 Since both the collecting effort per day (collection time and the number of collectors)
194 and the collecting ability of individual collectors varied, we attempted to correct these factors
195 to obtain the relative number of collected mantises per unit collecting effort. For the 2017
196 data, we simply corrected the number of mantises collected per day by dividing by collection
197 time and the number of collectors.

198 Variation in collecting ability among collectors could not be corrected because we did not 199 record the number of collected mantises for each collector in 2017. However, in 2018, we 200 recorded the number of mantises collected by each collector, from which we estimated the 201 variation in collecting ability among collectors. We constructed a generalized linear model 202 (GLM, log link and Poisson distribution) with the number of collected mantises per collector 203 per day as the objective variable, and the ID of collectors, the total number of collectors, and 204 the collection time each day as the explanatory variables. The log-transformed total number of 205 collected mantises per day was also included as an offset term. As a result, significant 206 variation in collecting ability was detected (Table S2). The coefficients estimated for 207 individual collectors provided a measure of collecting ability on a log-transformed scale (i.e., 208 GLM with log link). Thus, we estimated the collecting effort per day as the sum of 209 antilogarithms of coefficients of individual collectors participating, and multiplied by the 210 collection time. Next, the number of mantises per day was divided by the collecting effort. 211 Since these estimates provide the relative number of collected mantises per unit collecting 212 effort, we can compare these among days in 2018.

213 We asked how long period is available for female multiple mating. In addition, we 214 assessed how long period is required for female reproductive maturation, which is relevant to 215 female nutritional requirement and tendency of practicing sexual cannibalism. To estimate the 216 lengths of these periods, we estimated the phenology of eclosion, female first mating, and 217 oviposition in the wild, by constructing parametric survival models. In the model of eclosion, 218 captures of a nymph provided right censored data for the timing of eclosion, while those of an 219 adult provided left censored data. Similarly, in the model of female first mating, captures of 220 an unmated female provided right censored data for the timing of female first mating, while 221 those of a mated female provided left censored data. In the model of oviposition, collection of 222 oothecae provided interval data spanning from the day of the previous survey to the day of 223 collection, during which oothecae were assumed to be oviposited. We included the year as an 224 explanatory variable to examine variation among years. We also included sex as an 225 explanatory variable in the model of eclosion to examine sex difference (i.e., the possibility of 226 protandry). We compared assumptions of Weibull, exponential, Gaussian, logistic, log-227 normal, and log-logistic distributions, and the optimal distribution was selected by consulting 228 the Akaike information criterion (AIC). Based on these models, we estimated the 2.5%, 25%, 229 50%, 75%, and 97.5% quantiles (i.e., days) of the events occurring. These analyses were 230 performed by the survival function in R 4.1.1 (R Development Core Team, 2021). 231 Additionally, to examine whether sex ratio deviated from 0.5, we performed binomial tests 232 for each collection day. 233 If *T. angustipennis* has a polyandrous mating system, it is expected that female 234 mating frequency increases with increasing time throughout the reproductive season. To 235

236 number of fathers as the objective variable and collection day, sample type (females in 2017,

examine this, we constructed a GLM (log link and Poisson distribution) with the estimated

237 oothecae in 2017, or females in 2018), and the number of analyzed offspring as explanatory variables. Field-collected oothecae in 2018 did not hatch (see above). All GLM analyses were
performed using JMP ver. 14 (SAS Institute, 2018), in which overdispersion was corrected by
the function implemented in the software.

241

242 Results

243 *Reproductive phenology*

244 We collected 498 individuals over the 2 years (2017 = 47 males, 32 females, 118)245 nymphs; 2018 = 108 males, 61 females, 132 nymphs). In both years, numerous individuals 246 were collected from late August to mid-September, and then the number decreased (Fig. 2a, 247 b). Very few individuals were recorded in August 24, 2017, probably due to low abilities of 248 the collectors on this day. This motivated us to correct variation in collecting ability in 2018. 249 We found a female with a spermatophore attached to the abdominal terminalia (i.e., just after 250 mating), a female mounted by two males (Fig. 1), and a pair engaged in cannibalistic mating 251 on September 27, 2017. We also found a pair engaged in cannibalistic mating on September 252 26, 2018.

Almost all individuals were still nymphs in mid-August. Eclosion started from late August, and almost all individuals had become adults by mid-September (Fig. 2c, d). The estimated median dates of eclosion were August 28.4 (\pm 2.03 s.e.) and 30.7 (\pm 2.01) for males and females in 2017, and August 29.1 (\pm 2.01) and 31.4 (\pm 1.20) for males and females in 2018 (Fig. 3). Although males tended to eclose earlier, there were no significant differences in the timing of eclosion between sexes and between years (Table 1).

The adult sex ratio tended to be biased to males during September, which differed significantly from the null hypothesis (i.e., 0.5) at the end of the month (Fig. 2e, f). The adult sex ratio (i.e., the frequency of males) then decreased in October, but there was no significant departure from the null hypothesis. Fluctuations in sex ratio in early seasons were due to thesmall number of adults in the sample.

264 Mating status was examined in 82 (N = 26 in 2017; N = 56 in 2018) out of 93 (N =265 32 in 2017; N = 61 in 2018) females. The remaining 11 females were failed at detecting the 266 spermatheca due to technical problems. One female in 2018 with no observable sperm 267 oviposited fertile eggs, and two females in 2018 were failed at examination but oviposited 268 fertile eggs. These three females were recorded as mated. As a result, 46% (12 of 26) and 269 29% (16 of 56) of females were considered mated in 2017 and 2018, respectively. This result 270 revealed that mated females were rare until mid-September, and the frequency started to 271 increase thereafter (Fig. 2g, h). The estimated median dates of female first mating were 272 September 25.6 (\pm 4.18) and 25.0 (\pm 3.02) in 2017 and 2018, respectively. There was no 273 significant difference in the timing of female first mating between years (Table 1).

274 Oothecae were first found from the end of September, and increased in October (Fig. 275 2i, j). The estimated median dates of oviposition were October 8.4 (\pm 3.21) and 8.1 (\pm 1.81) in 276 2017 and 2018, respectively. There was no significant difference in the timing of oviposition 277 between years (Table 1).

278

279 *Female mating frequency*

As expected, *T. angustipennis* had a polyandrous mating system. Based on the simple allele count estimates, 62.1% (18/29) of analyzed clutches included multiple paternity, with 1.93 \pm 0.88 fathers (mean \pm s.d.). Based on maximum likelihood (ML) estimates, 96.6% (28/29) of analyzed clutches included multiple paternity, with 6.34 \pm 3.29 fathers (Fig. 4). Thus, as expected, this simple method based on allele count may underestimate, but the results strongly indicate that females of this species frequently mate with multiple males.

286 Unexpectedly, the female mating frequency did not increase with increasing time throughout the reproductive season, but included cases of high mating frequencies just after 287 288 the emergence of mated females (Fig. 4). The GLM explaining the ML estimates for the 289 number of fathers was marginally non-significant ($\chi^2_4 = 8.94$, p = 0.063). The effects of collection day and sample type were not significant (collection day, $\beta = 0.008 \pm 0.007$, $\chi^2_1 =$ 290 1.18, p = 0.28; sample type, $\chi^2_2 = 0.67$, p = 0.72). The effect of the number of analyzed 291 offspring tended to be positive ($\beta = 0.023 \pm 0.013$, $\chi^2_1 = 3.31$, p = 0.069), suggesting the 292 293 dependency of estimates on sample size. The GLM explaining allele count estimates of the number of fathers was not significant ($\chi^2_4 = 1.65, p = 0.80$). 294

295

296 Discussion

297 We determined the reproductive schedule in a wild population of the praying mantid 298 T. angustipennis in western Japan. As expected from the hypothesis that this species has a 299 polyandrous mating system, we provided genetic evidence for female multiple mating with 300 estimates of female mating frequency in the wild. Our ML estimates of female mating 301 frequency were high relative to a previous study on this species (Inoue & Matsura, 1983) and 302 to a congeneric species (Watanabe et al., 2011). Mutations and genotyping errors could bias 303 estimation of parental assignment, but our estimation using Colony took these effects into 304 account (Wang, 2004; Wang & Santure, 2009). To our knowledge, this is the first 305 comprehensive record of female reproductive phenology and multiple mating in wild mantid 306 populations.

The estimated median times of eclosion and first date of female mating indicate that adult females reach reproductive maturation at ~26 days after adult moult (Fig. 3). We know from previous studies on mating behavior that new adults of this species take about a month to mate (e.g., Kadoi *et al.*, 2017), and our current results demonstrate that this is also the case 311 in the wild. A relatively long period to sexual maturation suggests that adult females require a 312 large amount of nutrients for their reproduction. Since variation in prey nutrients largely 313 influence female reproductive output (Matura & Morooka, 1983; Maxwell et al., 2010; Barry 314 & Wilder, 2013), foraging success in this period may be an important factor for female 315 reproductive fitness in this predatory insect. The requirement of a plentiful supply of nutrients 316 in this period may also be relevant to the evolution of sexual cannibalism because female 317 nutritional condition may influence the propensity of mate attraction (Lelito & Brown, 2008; 318 Barry, 2010) and subsequent occurrence of sexual cannibalism (Maxwell, 2000; Maxwell et 319 al., 2010; Barry, 2015). Since cannibalized males can constitute a significant amount of 320 female nutritional intake in the congeneric species T. aridifolia (Hurd et al., 1994; Brown & 321 Barry, 2016), it is warranted to investigate how sexual cannibalism contribute to the 322 nutritional intake and reproductive output of T. angustipennis females that require long time 323 for reproductive maturation.

324 The estimated median times for female first mating and oviposition indicate that 325 females require ~13 days from the first mating to oviposition (Fig. 3), suggesting that a 2 326 week window allows for females to mate with other males. We observed a possible case of 327 female multiple mating (Fig. 1), and our genetic analysis of paternity revealed that females 328 mated with multiple males in the field. Female mating frequency did not increase constantly 329 through reproductive seasons, as indicated by the non-significant effect of collection day in 330 the GLM explaining the number of fathers in a clutch (Fig. 4). This suggests that female 331 multiple mating occurs shortly after reproductive maturation, and then females may mate 332 infrequently. This is congruent with the fact that mated female mantids cease to emit 333 pheromones to attract males (Lelito & Brown, 2008; Barry et al., 2011). Thus, the time 334 window for female remating may be shorter than the above estimate. If this is the case, female 335 mating frequency likely depends on the local density of males that can reach the female

336 within this short time window. The large variation in female mating frequency (Fig. 4) might 337 be explained by the possible variation in local male density. From the standpoint of the male, 338 the opportunity to mate with a female may be restricted to a short time period after female 339 maturation. The weak tendency of protandry (Figs. 2, 3), although non-significant in the 340 survival model analysis (Table 1), suggest that males attain mating success by early eclosion, 341 but the benefit is minimal due to large variation in the timing of female eclosion (Fig. 3). 342 When females mate with multiple males, sperm from different males compete for 343 fertilization of the limited number of eggs through sperm competition (Simmons, 2001). The 344 observed high rate of clutches including multiple paternity (62.1–96.6%) and the high 345 frequency of female remating (1.93-6.34 males per clutch) indicate that males of T. 346 angustipennis engage in moderate to strong sperm competition. Sexual selection via sperm 347 competition is expected to favor offensive and defensive male traits that increase fertilization 348 success (Simmons, 2001). In Ciulfina klassi, sexual selection operates on male genital 349 morphology, which enables rapid sperm transfer to the female (Holwell et al., 2010). Specific 350 male genital morphology may be beneficial for flushing out rival sperm from within the 351 spermatheca, as an offensive adaptation to sperm competition. Sperm competition also 352 provides insight into male adaptation to sexual cannibalism because a cannibalized male 353 could increase his fitness in the context of competitive fertilization by expending more effort 354 in his final mating (i.e., terminal investment) (Clutton-Brock, 1984; Andrade & Kasumovic, 355 2005). Sexually cannibalized males of *Pseudomantis albofimbriata* extend the duration of 356 copulation, although this did not increase fertilization success in sperm competition in the 357 double mating experiment (Barry et al., 2011). These males might expend more effort on 358 mate guarding as a defensive adaptation to sperm competition. Nevertheless, little is known 359 about sperm competition in mantids, including *T. angustipennis*, warranting further study.

361	Acknowledgments
-----	-----------------

362 We thank K. Miura and E. Watanabe for their advice on microsatellite development and

363 analysis, and G. Holwell, an anonymous reviewer and an associate editor for their

364 constructive comments. The authors declare that there are no conflict of interests. The data

that support the findings of this study are available from the corresponding author upon

366 reasonable request.

367

368 References

369 Andersson, M. (1994) Sexual Selection. Princeton University Press, Princeton.

370 Andrade, M.C.B. (1996) Sexual selection for male sacrifice in the Australian redback spider.

371 *Science*, **271**, 70–72.

Andrade, M.C.B. & Kasumovic, M.M. (2005) Terminal investment strategies and male mate
choice: extreme tests of Bateman. *Integrative and Comparative Biology*, 45, 838–847.

374 Attard, C.M., Holwell, G.I., Schwartz, T.S., Umbers, K.D.L., Stow, A., Herberstein, M.E. &

- Beheregayay, L.B. (2009) Microsatellite markers for the praying mantid *Ciulfina rentzi*(Liturgusidae). *Molecular Ecology Resources*, 9, 1480–1482.
- Barry, K.L. (2010) Influence of female nutritional status on mating dynamics in a sexually
 cannibalistic praying mantid. *Animal Behaviour*, **80**, 405-411.

Barry, K.L. (2015) Sexual deception in a cannibalistic mating system? Testing the Femme
Fatale hypothesis. *Proceedings of the Royal Society B*, 282, 20141428

- 381 Barry, K.L., Holwell, G.I. & Herberstein, M.E. (2008) Female praying mantids use sexual
- 382 cannibalism as a foraging strategy to increase fecundity. *Behavioral Ecology*, **19**, 710–
- **383** 715.

- Barry, K.L., Holwell, G.I. & Herberstein, M.E. (2009) Male mating behaviour reduces the
 risk of sexual cannibalism in an Australian praying mantid. *Journal of Ethology*, 27,
 386 377–383.
- Barry, K.L. & Kokko, H. (2010) Male mate choice: why sequential choice can make its
 evolution difficult. *Animal Behaviour*, **80**, 163–169.
- Barry, K.L., Holwell, G.I. & Herberstein, M.E. (2011) A paternity advantage for speedy
 males? Sperm precedence patterns and female re-mating frequencies in a sexually
 cannibalistic praying mantid. *Evolutionary Ecology*, 25, 107–119.
- **392** Barry, K.L. & Wilder, S.M. (2013) Macronutrient intake affects reproduction of a predatory
- **393** insect. *Oikos*, **122**, 1058–1064.
- Birkhead, T.R., Lee, K.E. & Young, P. (1988) Sexual cannibalism in the praying mantis *Hierodula membranacea. Behaviour*, 106, 112–118.
- 396 Brown, W.D. & Barry, K.L. (2016) Sexual cannibalism increases male material investment in
- 397 offspring: quantifying terminal reproductive effort in a praying mantis. *Proceedings of*398 *the Royal Society B*, 283, 20160656.
- Buskirk, R.E., Frohlich, C. & Ross, K.G. (1984) The natural selection of sexual cannibalism. *American Naturalist*, 123, 612-625.
- 401 Christensen, T. & Brown, W.D. (2018) Population structure, movement patterns, and
- 402 frequency of multiple matings in *Tenodera sinensis* (Mantodea: Mantidae).
- 403 *Environmental Entomology*, 47, 676–683.
- 404 Clutton-Brock, T.H. (1984) Reproductive effort and terminal investment in iteroparous
 405 animals. *American Naturalist*, 123, 212–229.
- 406 Du, M., Liu, Y.H. & Niu, B.Z. (2015) Isolation and characterization of polymorphic
- 407 microsatellite markers in *Bagarius yarrelli* using RNA-Seq. *Genetics and Molecular*
- 408 *Research*, 14, 16308-16311.

- 409 Elgar, M.A. (1992) Sexual Cannibalism in Spiders and Other Invertebrates. In: Elgar, M.A. &
- 410 Crespi, B.J. (eds) *Cannibalism: ecology and evolution among diverse taxa*. Oxford
- 411 University Press, Oxford, pp 128–155.
- 412 Elgar, M.A. & Schneider, J.M. (2004) Evolutionary significance of sexual cannibalism.
- 413 *Advances in the Study of Behavior*, **34**, 135–163.
- 414 Faircloth, B.C. (2018) MSATCOMMANDER: detection of microsatellite repeat arrays and
 415 automated, locus-specific primer design. *Molecular Ecology Resources*, 8, 92–94.
- 416 Fromhage, L. & Schneider JM (2005) Safer sex with feeding females: sexual conflict in a
 417 cannibalistic spider. *Behavioral Ecology*, 16, 377–382.
- 418 Gemeno, C. & Claramunt, J. (2006) Sexual approach in the praying mantid *Mantis religiosa*419 (L.). *Journal of Insect Behavior*, 19, 731–740.
- 420 Grabherr, M.G., Haas, B.J., Yassour, M. et al. (2011) Full-length transcriptome assembly
- 421 from RNA-Seq data without a reference genome. *Nature Biotechnology*, **29**, 644–652.
- 422 Holwell, G.I., Winnick, C., Tregenza, T. & Herberstein, M.E. (2010) Genital shape correlates
- 423 with sperm transfer success in the praying mantis *Ciulfina klassi* (Insecta: Mantodea).
- 424 *Behavioral Ecology and Sociobiology*, 64, 617–625.
- 425 Hurd, L.E., Eisenberg, R.M., Fagan, W.F., Tilmon, K.J., Snyder, W.E., Vandersall, K.S., Datz
- 426 S.G. & Welch J. D. (1994) Cannibalism reverses male-biased sex ratio in adult mantids:
 427 female strategy against food limitation? *Oikos*, 69, 193-198.
- 428 Inoue, T. & Matsura, T. (1983) Foraging strategy of a mantid, *Paratenodera angustipennis* S.:
- 429 mechanisms of switching tactics between ambush and active search. *Oecologia*, 56,
 430 264–271.
- 431 Jones, O.R. & Wang, J. (2010) COLONY: a program for parentage and sibship inference
- 432 from multilocus genotype data. *Molecular Ecology Resources*, **10**, 551–555.

- 433 Kadoi, M., Morimoto, K. & Takami, Y. (2017) Male mate choice in a sexually cannibalistic
- 434 species: male escapes from hungry females in the praying mantid *Tenodera*435 *angustipennis*. *Journal of Ethology*, **35**, 177-185.
- 436 Koshikawa, S., Miyazaki, S., Cornette, R., Matsumoto, T., Miura, T. (2008) Genome size of
- 437 termites (Insecta, Dictyoptera, Isoptera) and wood roaches (Insecta, Dictyoptera,

438 Cryptocercidae). *Naturwissenschaften*, **95**, 859.

- Lelito, J.P. & Brown, W.D. (2006) Complicity or conflict over sexual cannibalism? Male risk
 taking in the praying mantis *Tenodera aridifolia sinensis*. *American Naturalist*, 168,
 263–269.
- Lelito, J.P. & Brown, W.D. (2008) Mate attraction by females in a sexually cannibalistic
 praying mantis. *Behavioral Ecology and Sociobiology*, 63, 313–320.
- 444 Matura, T. & Morooka, K. (1983) Influences of prey density on fecundity in a mantis,
 445 *Paratenodera angustipennis* (S.). *Oecologia*, 56, 306-312.
- 446 Maxwell, M.R. (1998) Lifetime mating opportunities and male mating behaviour in sexually
 447 cannibalistic praying mantids. *Animal Behaviour*, 55, 1011-1028.
- 448 Maxwell, M.R. (2000) Does a single meal affect female reproductive output in the sexually
 449 cannibalistic praying mantid *Iris oratoria? Ecological Entomology*, 25, 54–62.
- 450 Maxwell, M.R., Gallego, K.M. & Barry, K.L. (2010) Effects of female feeding regime in a

451 sexually cannibalistic mantid: fecundity, cannibalism, and male response in

- 452 *Stagmomantis limbata* (Mantodea). *Ecological Entomology*, **35**, 775–787.
- 453 Moya-Laraño, J., Pascual, J. & Wise, D.H. (2004) Approach strategy by which male
- 454 Mediterranean tarantulas adjust to the cannibalistic behaviour of females. *Ethology*,
 455 110, 717–724.
- 456 Peakall, R. & Smouse P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic
- 457 software for teaching and research-an update. *Bioinformatics*, **28**, 2537-2539.

- 458 R Development Core Team (2021) R: A Language and Environment for Statistical
- 459 *Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- 460 SAS Institute (2018) *JMP version 14*. SAS Institute Inc., Cary.
- 461 Scardamaglia, R.C., Fosacheca, S. & Pompilio, L. (2015) Sexual conflict in a sexually
- 462 cannibalistic praying mantid: males prefer low-risk over high-risk females. *Animal*463 *Behaviour*, 99, 9–14.
- 464 Schneider, J.M. (2014) Sexual cannibalism as a manifestation of sexual conflict. *Cold Spring*465 *Harbor Perspectives in Biology*, 6, a017731.
- 466 Shuster, S.M. & Wade, M.J. (2003) Mating Systems and Strategies. Princeton University
- 467 Press, Princeton.
- 468 Simmons, L.W. (2001) Sperm Competition and Its Evolutionary Consequences in the Insects.
 469 Princeton University Press, Princeton.
- 470 Umbers, K.D.L., Holwell, G.I., Stow, A.J. & Herberstein, M.E. (2011) Molecular evidence
- 471 for variation in polyandry among praying mantids (Mantodea: *Ciulfina*). *Journal of*
- **472** *Zoology*, **284**, 40-45.
- Wang, J. (2004) Sibship reconstruction from genetic data with typing errors. *Genetics*, 166,
 1963–1979.
- Wang, J. & Santure, A.W. (2009) Parentage and sibship inference from multilocus genotype
 data under polygamy. *Genetics*, 181, 1579–1594.
- 477 Wang, R., Li, C., Stoeckel, J., Moyer, G., Liu, Z., & Peatman, E. (2012) Rapid development
- 478 of molecular resources for a freshwater mussel, *Villosa lienosa* (Bivalvia: Unionidae),
 479 using an RNA-seq- based approach. *Freshwater Science*, **31**, 695–708.
- 480 Wang, L., Wang, Z., Chen, J., Liu, C., Zhu, W., Wang, L. & Meng, L. (2015) De Novo
- 481 transcriptome assembly and development of novel microsatellite markers for the

- 482 traditional Chinese medicinal herb, *Veratrilla baillonii* Franch (Gentianaceae).
- 483 *Evolutionary Bioinformatics*, **2015**, 11.
- 484 Watanabe, E., Adachi-Hagimori, T., Miura, K., Maxwell, M.R., Ando, Y. & Takematsu, Y.
- 485 (2011) Multiple paternity within field-collected egg cases of the praying mantid
- 486 *Tenodera aridifolia. Annals of the Entomological Society of America*, **104**, 348-352.
- Welke, K.W. & Schneider, J.M. (2012) Sexual cannibalism benefits offspring survival. *Animal Behaviour*, 83, 201–207.
- 489 Zhang, H., Wei, L., Miao, H., Zhang, T. & Wang, C. (2012) Development and validation of
- 490 genic-SSR markers in sesame by RNA-seq. *BMC Genomics*, **13**, 316.

491

493	
494	Figure legends
495	
496	Fig. 1. A Tenodera angustipennis female mounted by two males in the rice field. Observed on
497	September 27, 2017.
498	
499	Fig. 2. Phenology of the number of individuals collected (a, b), eclosion (c, d), sex ratio (e, f),
500	female mating status (g, h), and the number of oothecae collected (i, j). * $p < 0.05$, ** $p < 0.01$
501	from binomial tests of sex ratio ($H_0 = 0.5$).
502	
503	Fig. 3. Timing of eclosion, female first mating, and oviposition estimated by parametric survival
504	models. Median and 2.5%, 25%, 75%, and 97.5% quantiles are shown.
505	
506	Fig. 4. Phenology of female mating frequency. Filled diamonds and circles refer to field-caught
507	females and field-collected oothecae in 2017, respectively. Open diamonds refer to field-
508	caught females in 2018.
509	

511 Table 1. Parametric survival models for estimating the phenology of eclosion, female first

	β	s.e.	Z	р		
Eclosion (distribution = logistic)						
Intercept	13.699	2.007	6.83	< 0.0001		
Sex (male/female)	-2.311	1.510	-1.53	0.13		
Year (2018/2017)	0.666	2.029	0.33	0.74		
Female first mating (distributi	on = log-log					
Intercept	3.680	0.102	36.14	< 0.0001		
Year (2018/2017)	-0.016	0.127	-0.13	0.9		
Oviposition (distribution = Weibull)						
Intercept	3.991	0.059	68.22	< 0.0001		
Year (2018/2017)	-0.007	0.068	-0.10	0.92		

512 mating, and oviposition in *Tenodera angustipennis*

513

514

516	Legends of supp	lemental tables
-----	-----------------	-----------------

518 Table S1. Characteristics of eight newly developed microsatellite markers in *Tenodera*

angustipennis

521 Table S2. Generalized linear models explaining variation in collector ability





Calender date





Locus	Forward primer	Reverse primer	Size range (bp)	Na	Ho	He	<i>P</i> of HWE	Accession No.
Tang25872	CCGGCAAAGAGAAGTCGTTC	TCAATGCGCAGATCATCGC	138-168	9	0.528	0.767	< 0.001	LC655310
Tang26190	CACAGCTGACACAATGTTGC	ACAGCTCTACTCTCATGCTCC	373-410	4	0.629	0.511	< 0.001	LC655311
Tang26442	AGGGCGATCTTGACAAACAC	GATTACCCTAGAGCGGCTGG	151-220	14	0.667	0.720	< 0.001	LC655312
Tang29392	ATCACACATTCAGTCAGCGC	GTGTCCATGTCTTCCATTCC	346-350	3	0.389	0.377	ns	LC655313
Tang30982	ACCAAGGACTAGATGCGGAC	GAGGAGGTTTATCGTTGGTG	223-244	7	0.722	0.731	ns	LC655314
Tang33394	ACAGCACCATGAGTTCTGTG	CCGTCGCAATCTACAAGACG	417-425	5	0.676	0.750	ns	LC655315
Tang33405	GATGCCGAACTTCATGCTG	GTTCTTGTCTTGCCTCACG	235-357	22	0.914	0.918	< 0.05	LC655316
Tang33507	ATAATTCATTGCGACCGGGC	GAAGGCAGAAATAGCGGCAC	389-405	9	0.722	0.839	< 0.05	LC655317

1 Table S1. Characteristics of eight newly developed microsatellite markers in *Tenodera angustipennis*

 $N_{\rm a}$, number of alleles; $H_{\rm o}$, observed heterozygosity; $H_{\rm e}$, expected heterozygosity; HWE, Hardy-Weinberg equilibrium

	β	s.e.	Likelihood χ^2	р
Intercept	0.482	1.164	0.16	0.69
Collector A	-0.448	0.377	1.64	0.20
Collector B	0.185	0.171	1.14	0.29
Collector C	-0.147	0.221	0.46	0.50
Collector D	0.794	0.231	9.80	0.0017
Collector E	-0.836	0.295	10.40	0.0013
Collector F	0.039	0.179	0.05	0.83
Collection time	-0.008	0.011	0.53	0.47
Number of collectors	-0.233	0.156	2.02	0.16

3 Table S2. Generalized linear models explaining variation in collector ability

4 Estimated coefficients were used to correct collection effort in 2018 results (Fig. 1).

5