Sexual cannibalism increases male material investment in offspring: quantifying terminal reproductive effort in a praying mantis

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Models of the evolution of sexual cannibalism argue that males may offset the cost of cannibalism if components of the male body are directly allocated to the eggs that they fertilize. We tested this idea in the praying mantid Tenodera sinensis. Males and females were fed differently radiolabelled crickets and allowed to mate. Half of the pairs progressed to sexual cannibalism and we prevented cannibalism in the other half. We assess the relative allocation of both male-derived somatic materials and ejaculate materials into the eggs and soma of the female. Our results show that male somatic investment contributes to production of offspring. The eggs and reproductive tissues of cannibalistic females contained significantly more male-derived amino acids than those of non-cannibalistic females, and there was an increase in the number of eggs produced subsequent to sexual cannibalism. Sexual cannibalism thus increases male material investment in offspring. We also show that males provide substantial investment via the ejaculate, with males passing about 25% of their radiolabelled amino acids to females via the ejaculate even in the absence of cannibalism.

1. Introduction

Sexual cannibalism occurs when a female consumes a male before, during or after mating. Rates of sexual cannibalism are highly variable among species [1–3]. In praying mantids that exhibit sexual cannibalism, it occurs in 13–28% of natural encounters in the field [4–5], thus imparting significant mortality on males during the breeding season. Unlike some sexually cannibalistic spiders [6–10], male mantids can mate repeatedly and potentially fertilize multiple females during their lifetime [4,11–13]. Thus, all else being equal, male mantids should be under significant selection to reduce the cost of falling prey to a mate.

Sexual cannibalism necessarily eliminates the possibility of future mating for the male, but this cost might be at least partially ameliorated if he mates successfully with the predatory female and his value as a food item allows this female to produce more offspring [14–20]. Male sacrifice may evolve if the chance of encountering more than one female in a lifetime is small and the consumption of the male by the female operates as parental investment [21] that increases the female’s reproductive output [14]. In spiders where sexual cannibalism occurs, the paired pedipalps, which are the organs of sperm transfer, regularly become damaged as a result of copulation, such that each can be used only once, and thus strictly limiting mating frequency [6–10]. Buskirk et al. [14] developed a model which predicts that male complicity in sexual cannibalism will evolve if the number of additional offspring produced as a result of sexual cannibalism exceeds the number of additional offspring the male could sire if he avoided cannibalism and searched for additional mates [22]. Male sacrifice may also evolve as a means of increasing paternity when the sex ratio is male biased and thus males risk sperm competition despite low individual male mating frequencies [23,24].
Elgar & Schneider [22] reviewed the evidence of fecundity benefits of sexual cannibalism, with an emphasis on spiders, and found little support for fecundity benefits overall. However, in most mantids males represent large prey for females, and prior studies have shown that when females consume larger prey, they lay larger ootheca (egg cases) that contain more eggs [18,25–27]. Ootheca may weigh 30–50% of a female’s biomass and thus represent a tremendous investment [28]. Hurd et al. [4] estimated that in one population of the Chinese mantis, Tenodera sinensis, males made up 63% of the diet of adult females. Thus male bodily nutrients, when consumed by their mates, may represent an extreme form of male reproductive investment [29]. If these resources increase the number or fitness of offspring subsequently fathered by the male, it can also be considered an extreme case of male parental investment [21].

We tested the extent to which male-derived materials were allocated to the eggs that were potentially available for a male to fertilize. We used radioisotopes to follow the flow of nutrients from males to females subsequent to mating, in the presence and absence of sexual cannibalism. We also determined whether nutrients derived from the soma and the ejaculate were differentially allocated to the eggs. Different patterns of incorporation would occur if the ejaculate nutrients functioned specifically to incorporate nutrients into eggs. No difference would indicate generalized use of male-derived nutrients [30].

2. Material and methods

(a) Collection and rearing
Mantids were captured as final-instar juveniles from wild populations in Chautauqua County, NY and TN, USA. Mantids were fed crickets (Acheta domestica) ad libitum until maturity, after which they were maintained on a diet of one cricket per day. Mantids were housed as described previously [13,31,32]. We placed individual mantids into 500 ml plastic terrariums and maintained them at 22°C with an ambient light cycle.

(b) Radiolabelling
We applied radiolabels to mantids by feeding them radiolabelled crickets. Our procedure for radiolabelling crickets followed Gwynne & Brown [33] and Brown [34]. Crickets were starved for 2 days before applying the radiolabel. Ten millilitres of radiolabelled L-amino acid mixture (MP Biomedicals, Santa Ana, CA, USA; approx. 0.5 μCi of 14C or 5 μCi 3H) were placed by pipette onto a 1 cm2 of lettuce contained within a glass Petri dish. The drop of radiolabelled amino acid was left to dry under a fume hood and then placed in the cage of an individual cricket. Crickets were held for 2 days to allow them to consume the label.

Male and female mantids were randomly assigned to one of the two radiolabelling treatments and were fed one appropriately labelled cricket every 2 days for a total of three radiolabelling crickets. On alternate days, females that had consumed the labelled cricket were fed an unlabelled cricket to maintain them on a moderate diet of about one cricket per day. Females on an ad libitum diet generally eat about two cricket per day; thus we provided a diet that neither starved nor satiated females. Total radioisotope incorporated by the mantids was measured as the total titre of radioisotope quantified by liquid scintillation counting (see below) summed across all somatic and reproductive tissues. Male T. sinensis incorporated on average 8.51 ± 3.20 μCi of 14C or 160.08 ± 52.55 μCi of 3H, according to isotope treatment, which was equivalent to about 1.7% and 1.1%, respectively, of the radioisotope initially provided to the crickets that the males consumed. Although mean dosages were very different, there was also large variation in amounts incorporated by males, and thus a non-significant difference between isotope treatments ($F_{1,20} = 4.24, p = 0.06$), in amount of isotope within males.

(c) Experimental design
Six days after consuming their first radiolabelled cricket, females were paired with a male that had received the opposite radiolabel. We measured male pronotum length as an estimate of body size. Both males and females were weighed and assigned to either cannibalism or no-cannibalism treatments. Males were presented to females in 3-l plastic cages and were allowed to mate. In all cases, we eliminated precopulatory cannibalism by orienting the male to the back of the female, away from her predatory strike. For pairs assigned to the cannibalism treatment, we allowed cannibalism to occur any time after the onset of copulation, and we maintained pairs together until the female consumed the male. For pairs assigned to the no-cannibalism treatment, we observed them until copulation ended and then immediately separated males and females. We recorded the duration of copulation for all pairs.

After mating trials, males of the no-cannibalism treatment were immediately frozen, as were any unused parts of males from the cannibalism treatment. We later dissected non-cannibalized males into reproductive organs (testes and accessory glands) and soma (rest of body). Females were maintained on a diet of one cricket per day for up to 60 days, to allow them to digest male-derived nutrients and allocate them to somatic and reproductive tissues. During oviposition, female mantids produce a spongy matrix into which they deposit their eggs, which is collectively called the ootheca. We recorded the date of oviposition and we collected and froze oothecae as females produced them. As females died, they were frozen and subsequently dissected into reproductive (eggs and ovarian material) and somatic (rest of body) tissues. We also measured female pronotum length. Eggs from oothecae were counted to yield a count of total eggs laid (oviposited within ootheca) and total eggs produced (from oothecae and ovaries).

Eggs (i.e. total ovarial eggs or total oothecal eggs), oothecal matrix and body parts were placed into scintillation vials with 300–500 μl of tissue solubilizer (Solvable by Packard, Meriden, CT, USA). All vials were then placed in a 50–60°C water bath. These materials were left overnight to dissolve all other than the exocuticle. We then added 5 ml of scintillation fluid (Ultima Gold by Packard, Meriden, CT, USA) and all samples were counted in a liquid scintillation counter (Beckman LS 6800, Beckman Coulter, Inc., Fullerton, CA, USA). Quenching was measured by external standardization. Samples were counted for 10 min in triplicate.

(d) Analysis
We calculated three measures of the allocation of radioactive amino acids from males to females. First, we calculated the proportion of male radiolabel transferred to the female. This addresses differences in overall material investment between cannibalized and non-cannibalized males. We calculated the total radiolabel incorporated by each male as the sum of radiation across all male tissues and the tissues of the female with which he mated, including only counts for the isotope (14C or 3H) fed to the male. For females, we generated separate totals of (i) total radiation derived directly from crickets and (ii) total radiation derived from males as the sum of radiation...
from separate isotopes across all female tissues, eggs and ootheca matrix. To measure the material transferred between mates, we calculated the proportion of total radiolabel that was initially incorporated by males but recovered within females, eggs and oothecae. This proportional measurement controls for differences in the absolute amount of radiolabel initially incorporated by males.

Second, we calculated the fraction of total radiolabel in eggs and female reproductive tissues that originated from the male. This allows us to examine whether male contribution to offspring production increases with cannibalism. Third, we measured distribution of male radiolabel to female reproduction versus female soma. This enables testing of whether male-derived isotopes were differently distributed between female reproduction and soma depending upon sexual cannibalism. This would be the case if females used ejaculate materials differently from those of male soma. For this analysis, we first calculated the total radiolabel passed from male to female. We then determined the proportion of this total that was allocated to female reproduction (including eggs and ootheca matrix). If ejaculate nutrients are designed to be preferentially used for reproduction, we predict greater proportional allocation in the no-cannibalism treatment where the ejaculate is the only potential source of male-derived nutrients. No difference between treatments would suggest generalized use of male somatic and ejaculate nutrients by the female [30].

We analysed our proportional data using the general linear model (GLM). All proportions were arcsine transformed for analysis to meet assumptions of normality. This approach is justified particularly when proportions generally fall within the middle of the distribution (between 0.2 and 0.8) [35], which is the case with our data. In each case, we analysed the effect of cannibalism on reproductive allocation using a GLM with backward elimination of non-significant covariates. Cannibalism treatment (y/n) and the form of radioisotope incorporated by males were included as independent variables. Covariates included male and female pronotum length (size), duration of mating, and a male and female body mass index (BMI) that was calculated using the residuals of a nonlinear regression of body mass and pronotum length [36]. Body mass increased significantly with pronotum length in males ($r = 0.68, n = 21, p < 0.001$) but not females ($r = 0.06, n = 21, p = 0.68$). We also analysed the data using body mass (g) per se, rather than BMI. The results are qualitatively similar and are given in electronic supplementary material, table S1.

### 3. Results

Mating lasted on average 3.51 ± 0.24 h. Females that cannibalized males gained significantly more mass during mating (cannibalism: mass gain = $0.62 ± 0.06$ g; no cannibalism: mass gain = $0.05 ± 0.06$ g). Males that were cannibalized by females transferred considerably more of their dose of radioisotope to females (figure 1a and table 1; $F_{1,20} = 63.75, p < 0.0001$); on average cannibalized males passed 88.9% (95% CI: 73.6–98.7%) of their radioisotope whereas non-cannibalized males passed 25.1% (95% CI: 17.9–36.8%) of their label. The proportion transferred to females decreased with male BMI and increased with female BMI (table 1). No other covariates were significant (table 1).

#### (a) Effect of sexual cannibalism on male investment in offspring

We next asked how sexual cannibalism affected female allocation of male-derived materials into eggs and offspring. Sexual cannibalism resulted in a significant increase in the proportion of radioisotopes within female reproductive tissues (i.e. the combination of ovaries, egg and ootheca matrix) that were of male origin (figure 1b and table 1). On average, 38.8% (95% CI: 32.2–45.5%) of radioisotope allocated to female reproduction was of male origin when cannibalism occurred, compared to 21.1% (95% CI: 15.2–27.5%) without cannibalism. Allocation also increased with male BMI; no other covariates were significant. Thus, eggs that were produced subsequent to sexual cannibalism possessed significantly more amino acids of male somatic origin compared with eggs produced without cannibalism, and larger males made a larger investment in offspring.

#### (b) Relative allocation of radioisotope within males and females

We then assessed if relative allocation of male-derived isotopes to female reproduction versus female soma differed with and without sexual cannibalism. Different patterns of incorporation would occur if the ejaculate nutrients functioned specifically to incorporate nutrients into eggs. No
difference would indicate generalized use of male-derived nutrients [30]. Figure 1c shows that 67.8% (95% CI: 52.2–84.3%) of isotope received from the male ejaculate in the absence of cannibalism was allocated to reproduction. Similarly, when females cannibalized males, and thus received isotope both from male soma and ejaculate, the proportion allocated to reproduction was 77.2% (95% CI: 61.9–93.8%). These patterns of allocation did not differ significantly (table 1) and thus there is no evidence that females allocate male ejaculate investment differently from male somatic investment.

(c) Egg production

The interval between mating and oviposition averaged $12.1 \pm 1.6$ days. This period significantly decreased with female BMI ($F_{1,18} = 4.99, p = 0.045$) and increased with ootheca mass ($F_{1,18} = 6.76, p = 0.023$), but was not significantly affected by cannibalism ($F_{1,18} = 2.55, p = 0.14$). Average fecundity in the experiment was $258 \pm 23$ eggs. As expected, female BMI prior to oviposition was positively associated with total egg production (table 1), demonstrating that BMI is a good indicator of female fecundity. Neither treatment nor any other covariate significantly affected total egg production. Initial oothecae may have included eggs developed prior to mating and which could not have been affected by the treatment. When we exclude the first ootheca, females that cannibalized males produced significantly more eggs (figure 2 and table 1). Subsequent to the first ootheca, females that cannibalized males produced an average of $88.4$ eggs (95% CI: 55.4–133.9 eggs), whereas females in the non-cannibalism treatment produced $37.5$ eggs (95% CI: 10.8–79.4 eggs). Again, female BMI was also positively related to egg production.

4. Discussion

The eggs and the reproductive tissues of females contained significantly more male-derived amino acids subsequent to sexual cannibalism. Thus when males are subject to sexual cannibalism, their soma provides direct material investment in offspring. To the extent that this investment increases...
total offspring production, male somatic investment may at least partially offset the cost of loss of future mating opportunity in *T. sinensis*, as outlined in the model of Buskirk *et al.* [14]. In the current study, we found that, subsequent to the first ootheca, egg production increases with a mean difference of 50.9 eggs as the soma of the cannibalized male is used for production of additional oosacs. Previous studies have shown that larger prey result in greater egg production in both *T. sinensis* [25,26] and *Tenedolena angustipennis* [27]. Given that male mantids are among the largest prey available and that females in the field are typically food limited [37,38], and in one study males made up 63% of female diet during the breeding season [4], it seems likely that sexual cannibalism increases egg production under field conditions. Our results clearly show that male somatic investment contributes to oosac production. This is further exemplified by our discovery that male material allocation to eggs and ovaries increases with male body size. Studies of two other mantis species, *Hierodula membranacea* and *Pseudomantis albofimbriata*, have shown that fecundity increases with sexual cannibalism [18,20]. Whether the additional eggs produced after sexual cannibalism are sufficient to create conditions under which sexual cannibalism is an adaptive male strategy will depend upon (i) the extent to which a cannibalized male fertilize these additional eggs and (ii) the cost of additional reproductive opportunities that are necessarily lost as an outcome of cannibalism [14]. Based on the numbers from our study, cannibalized males potentially gain the opportunity to fertilize an additional 50.9 eggs with their current mate but lose the opportunity to fertilize the 258 eggs within the average ootheca of an additional female. Thus, the availability of additional mates is crucial, and males should be risk averse when there is about a 20% (50.9/258) chance or greater of remating. If mating opportunities are sufficiently low, as occurs in some spiders [6–10], the opportunity to fertilize even a portion of the additional eggs may select for greater risk taking behaviour by males [13] and even self-sacrifice [14,15].

Although sexual cannibalism increases male material investment in oosacs, male material investment is not entirely lacking in the absence of cannibalism. Our results also provide evidence of the transfer of amino acids via ejaculatory expenditure. Indeed, male *T. sinensis* contain massive accessory glands [39,40] and mating lasts an average of 3.5 h ([26]; this study), suggesting that males may make a significant material contribution to mates via the ejaculate, even when they are not cannibalized. Subsequent to mating, males (those not cannibalized) possessed 27% of their remaining radiolabel within their reproductive tissues and 73% within their soma. With or without sexual cannibalism, we found no significant difference in the distribution of male-derived materials between female reproduction and female soma. Thus although females that cannibalize males receive a greater amount of total material, there is no evidence that amino acids consumed during cannibalism are used proportionately to a greater or lesser degree than materials passed via the ejaculate.

**References**

10. Herberstein ME, Gasket AC, Schneider JM, Vella NGF, Elgar MA. 2005 Limits to male copulation frequency: sexual cannibalism and sterility in St Andrew’s cross spiders (*Araneae*: *Araneidae*).

**Data accessibility.** Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.50566.

**Authors’ contributions.** W.D.B. and K.L.B. conceived the study and collected the data. W.D.B. analysed the data and drafted the manuscript. Both authors contributed to the final version of the manuscript.

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