Spontaneous male death and monogyny in the dark fishing spider

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Methods

(a) Animal collections

Immature male and female spiders were collected during the spring (April-May) of 2006-2010 in Lancaster County near Lincoln, Nebraska (U.S.A.). Male and female Dolomedes tenebrosus were collected at night using a light-emitting diode (LED) headlamp. Night collection takes advantage of the eyeshine seen in D. tenebrosus (and other fishing spiders), which assists us to locate and capture the spiders in the field. Field caught individuals were transported back to the laboratory and housed individually in 87.3 mm x 87.3 mm x 112.7 mm plastic containers (763C, AMAC Plastics, Petaluma, CA). Containers were housed in a climate-controlled room (24–27°C) and placed on a light:dark cycle that matched the outside summer environment (April-August, 13:11-15:9 light:dark). Female spiders were fed two 2-week-old (1/4”) crickets, Acheta domesticus, three times a week and male spiders were fed two 1-week-old (1/8”) crickets, A. domesticus, once a week (Bassett's Cricket Ranch, Visalia, CA). In addition, all spiders were provided water ad libitum. All spiders were weighed (Ohaus Explorer balance 0.0001 g) twice, first when transported to the laboratory and once again prior to their assigned experimental trial.

(b) Mating trials

Staged mating trials were videotaped (Sony DCR-HC96 MiniDV Handycam) during the summer of 2006 (n = 4) and 2007 (n = 20). All individuals used were field caught virgins, which moulted to maturity in the laboratory (spiders are not sexually mature prior to their final moult). Mature male and female spiders were used only once during the mating trials. Males were assigned to females at random and females were
placed individually in a 252.4 mm x 90.5 mm (diameter x depth) plastic arena (250C, Pioneer Plastics, North Dixon, KY) for a minimum of 24 hours prior to the introduction of the male. The arena floor was covered with a disc of filter paper (Double Rings, 102 Qualitative, 24 cm) and at the center of the arena was a 47.6 mm x 84.1 mm plastic vial (40 DRAM, Thornton Plastics, Salt Lake City, UT) covered in fiberglass mesh on which the female could climb and position herself. On average, mating trials lasted 1.5 hours from the introduction of the male to the insertion of his first pedipalp. Video S1 (see the electronic supplementary material) shows one of the first mating trials from 2006.

(c) Heartbeat measurements

To determine when males died following mating, we measured the time from palpal insertion to the termination of the heartbeat postcopulation during the summer of 2007 (n = 8), 2009 (n = 4), and 2010 (n = 3). Only male spiders that were fully intact (i.e. males that had not been punctured by the female and/or damaged when removed from the mating arena) were used for heartbeat measurements. In order to measure the heartbeat, males were removed from the arena following the insertion of their first pedipalp and their heartbeat was monitored by counting the pulse rate of the abdomen (opisthosoma) every 15 minutes via stereo microscope (Leica M216, Buffalo Grove, IL) postcopulation until it terminated.

(d) Palpal dissections

In the summer of 2009 we removed and dissected the pedipalps of mature virgin male spiders (n = 5) to determine if *D. tenebrosus* males charge both of their pedipalps with sperm. Following a simplified method similar to Bukowski and Christenson [1], we removed the pedipalps of each male under a stereo microscope (Leica M216, Buffalo Grove, IL). The pedipalps were then crushed with metal forceps and placed on a microscope slide with a drop of water. Finally each pedipalp was viewed using a light microscope (Leica DM4000 B, Buffalo Grove, IL) and the presence or absence of sperm cells was recorded.
(e) Field monitoring
In the summer of 2010 we collected males from the field and held them in the laboratory until their maturity moult, at which time they were released in the field and their mate search behaviour was monitored. Following their maturity moult, male spiders were marked with a paint pen (DecoColor, Uchida of America, Torrance, CA) and returned to the field (to their original collection location) for observations. Over a series of nights, June-July 2010, male spiders (n = 18) were released at their initial collection points and monitored. By following individual male spiders throughout the night, we were able to quantify the probability of a *D. tenebrosus* male locating and contacting a *D. tenebrosus* female in the field.

(f) Silk trials
In the summer of 2009 we videotaped (Sony DCR-HC96 MiniDV Handycam) the behaviour of male spiders (n = 15) when presented with the silk from a virgin and a non-virgin female spider. Mature virgin and non-virgin female spiders were used only once during the silk trials and mature male spiders were used twice (repeated measures design). Female spiders were placed in an individual 252.4 mm x 90.5 mm (diameter x depth) plastic arena (250C, Pioneer Plastics, North Dixon, KY) which had the floor covered with a disc of filter paper (Double Rings, 102 Qualitative, 24 cm). Female spiders (virgin and non-virgin) then spent a minimum of 24 hours in the arena laying down silk. With the female spiders removed, male spiders were individually introduced into a silken arena. The order of the presentation (virgin vs. non-virgin silk) was randomized and males were presented with virgin or non-virgin silk a minimum of 24 hours apart. During the silk trials, we recorded male activity (i.e. time spent moving) and male courtship signals (visual and seismic) for a period of 15 minutes.
Figures and Table

<table>
<thead>
<tr>
<th></th>
<th>weight (mg)</th>
<th>CW (mm)</th>
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<tr>
<td>females</td>
<td>761.3 ± 29.2</td>
<td>8.0 ± 0.2</td>
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<tr>
<td>males</td>
<td>53.5 ± 3.4</td>
<td>3.1 ± 0.1</td>
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<td>ratio</td>
<td>14:1</td>
<td>2.5:1</td>
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**Figure S1.** Female-biased sexual size dimorphism in the dark fishing spider, *Dolomedes tenebrosus*. Females (*n* = 25) weigh more than males and have wider cephalothoraxes (prosoma), measured in carapace width (CW), than males (*n* = 25). Data are mean ± s.e. Image by Karina I. Helm.
Figure S2. Changes in the heartbeat of males \((n = 15)\) postcopulation. Following palpal insertion and expansion of the haematodochal bulb (i.e. copulation), the mean time to heartbeat cessation was 164 ± 9 minutes. Copulation in *Dolomedes tenebrosus* results in 100% male mortality.
Figure S3. The haematodochal bulb of the male postcopulation. In *Dolomedes tenebrosus* males the haematodochal bulb (*circled*) remains in the expanded state following copulation. Photograph by Steven K. Schwartz.
**Table S1.** The three published araneoid examples of self-sacrifice behaviour in the form of spontaneous death associated with copulation.

<table>
<thead>
<tr>
<th>species</th>
<th>spontaneous death characteristics</th>
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<tbody>
<tr>
<td><em>Argiope aemula</em></td>
<td>Male death is associated with the insertion of their second pedipalp.</td>
<td>Sasaki &amp; Iwahashi [2]</td>
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<tr>
<td><em>Argiope aurantia</em></td>
<td>Male death is triggered by the insertion and inflation of the distal bulb of their second pedipalp.</td>
<td>Foellmer &amp; Fairbairn [3]</td>
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<tr>
<td><em>Tidarren sisypoides</em></td>
<td>Male death is associated with the insertion of their first and only pedipalp (due to palp-amputation).</td>
<td>Knoflach &amp; Benjamin [4]</td>
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References


