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Behavioral Dynamics That Would Lead to Multiple Paternity within Egg Capsules of the Squid *Loligo pealei*

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Studies of sexual selection are important for advances in evolutionary theory. They also provide clues to help manage populations of commercial species. The long-finned squid *Loligo pealei* is a valuable resource (1) that is fished mainly on spawning grounds. A central question is how such targeted fishing might affect the species' reproductive behavior. One possible consequence would be that a particular fishing method removes, for example, the largest, fittest males, so that genetic recruitment would be affected directly. Because *L. pealei* has a brief one-year life cycle (2), the deleterious effects of such a scenario would manifest themselves quickly.

Our hypothesis is that the mating system of *L. pealei* is robust and flexible, and that there is a high degree of genetic mixing, not only at communal egg beds, but within the individual egg capsules. We are currently testing this hypothesis through behavioral studies. Previously, Hanlon (3) identified some of the principal behaviors on the spawning grounds, including those of the ''sneaker'' males, and noted the high potential for multiple paternity within egg capsules. We now report new findings from field observations during May 1997 (6 h of videotape from 22 SCUBA dives in Vineyard Sound), supplemented with 130 h of laboratory trials conducted from May through August. Squids used in the laboratory trials were jig-caught and were studied over several weeks.

Field observations suggest at least four behaviors through which males of various sizes gain copulations with females. First, a large male may pair temporarily with a female, mate her, and guard her from rival males before, during, and after mating and oviposition events. On spawning grounds, females are rarely unpaired for any length of time, so lone large males must first supplant a paired male before pairing with the female. Second, a lone large male sometimes foregoes the fighting, guarding, and pairing phases and quickly grabs a paired female and copulates with her; this behavior has been recorded when the female is very close to the egg beds (Video 1). Third, small males commonly act as ''surreptitious'' sneakers by remaining at a distance from the egg beds, then rapidly jetting towards females when her large consort male is not vigilant, mating her, and escaping rapidly. Fourth, small males may act as ''bold'' sneakers by swimming near the egg beds and intercepting pairs that approach the beds. Of these male behaviors, the second and fourth were observed for the first time in *L. pealei* during 1997.

Pairing with a female confers at least two benefits for males at the spawning grounds: copulation success and an ''owner advantage'' in fights. Males paired with a female were successful in 30 of 34 observed copulation attempts (88%). In contrast,

http://www.mbl.edu/html/BB/home.BB.html

Video 1. *Video sequence of an extra-pair copulation by a large lone male. At the start of the segment, the female is in the center of the screen, just to the right of the egg bed. Her paired male is just above her. As she begins to place her arms into the bed, a lone, large male darts in from the left, grabs her mantle, and proceeds to copulate in the parallel position. After 14 s of copulation, the female's paired male starts to wedge himself between her and the copulating male. Nevertheless, the copulation lasts for a total of 28 seconds, which is a typical duration for parallel copulations. This video can be viewed at the URL listed above.*

unpaired males that were longer than the female were successful in only 10 of 38 attempts (26%), and unpaired males that were of the same mantle length as the female were successful in 13 of 64 attempts (20%). This lower copulatory success for unpaired males might reflect female choice; indeed, we have observed females jet from males that attempt to copulate in the field and laboratory. With regard to agonistic bouts at the spawning grounds, paired males remained with the female when challenged by another male in 23 of 24 contests. This high fighting success of paired males might explain the alternative large male behavior; *i.e.,* repeatedly defeated lone large males might resort to a form of ''sneaky'' mating.

At the spawning grounds, only large males behaved as paired consorts to females, which were always smaller (mean ± SE mantle length of females jigged in May: 17.2 ± 0.4 cm, n = 26). In the laboratory, a similar trend was observed in six trials that involved four males (two large males, *i.e.,* mantle length greater than 17 cm, and two small males, *i.e.,* mantle length less than or equal to 17 cm) and two females. We conducted scan surveys every 10 min. At each scan, a male was considered to have been guarding a female if he was swimming within two body lengths of her and was actively positioning himself between her and the other males. In these trials, only one female was being guarded during a given scan. Of 72 scans, one of the largest two males was guarding a female in 62 scans, whereas one of the shortest two males was guarding in 10 scans (χ^2 with Yates correction = 36.13, $P < 0.0001$).

This result indicates behavioral flexibility among males in the laboratory. In particular, a few small males guarded females in the presence of larger males. This might indicate motivational differences between males that become more evident when squid are confined. That is, some large males might have opted not to compete for females in the trials, thereby creating the opportunity for more highly motivated small males to pair with females. In contrast, large squids at the communal egg beds are probably highly motivated to reproduce and thus displace any small males that might attempt to guard females. As in the laboratory, motivational differences might exist between males in the field. Some reproductively inactive squids can usually be seen several meters from the egg beds, suggesting that some of them might have temporarily dropped out of the competition for females.

Behavioral flexibility in mating position has also been documented. Two copulatory positions are known: parallel and head-to-head (4,5). In a field video subsample, large males attempted 14 parallel copulations and one head-to-head. In contrast, small males attempted 9 head-to-head and 9 parallel copulations. Long-term focal observations of 10 males in the laboratory (each male attempting 4–40 copulations) demonstrate that small as well as large males (mantle length range: 14–27 cm) will attempt to copulate in both positions within the same day.

Sperm are placed in different locations in the two mating positions. In parallel copulations, the male places one or more spermatophores near the female's oviduct. In head-to-head copulations, one or more spermatophores are attached to, and eventually transferred into, the female's seminal receptacle (6). During oviposition, the eggs pass out of the oviduct and into the mantle cavity where, presumably, there are swarms of swimming sperm (from a male parallel mating). The eggs are then drawn out through the funnel and into the female's arms. How sperm in the seminal receptacle (just below the mouth) are released or controlled by the female is unclear, but she probably has the option of releasing stored sperm while holding the egg capsule in her arms before depositing it on the substrate. This suggests the possibility of female choice over potential sires at this late stage.

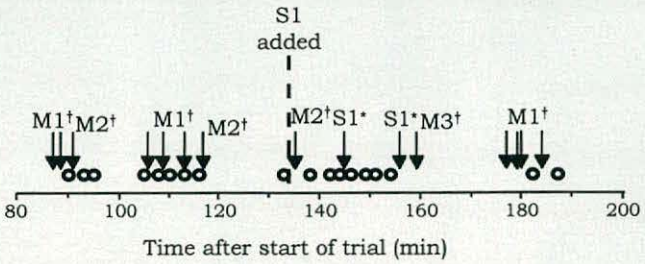

Figure 2. *Multiple copulations and oviposition in a laboratory trial. This trial started when two males and a female were added to a tank already containing a male and female (the ''resident'' squid). M1: resident large male, M2: introduced large male, M3: introduced large male, S1: introduced small male. Each open circle represents the oviposition of one egg capsule by the resident female (she laid 18 capsules in this trial). Arrows represent copulations by males, where *signifies head-to-head copulation, †parallel copulation.*

Females mate with multiple partners, and many different sequences can occur. In the field, for example, we observed females mating with two or more consecutive males. In the laboratory, we saw four sequences of mating and oviposition in small, mixed-sex groups of squid. Each sequence involved one female who mated with two or more males during several hours of oviposition. In some cases, as many as three different males copulated immediately before the laying of a single egg capsule (Fig. 1). Given such opportunities for competition between the sperm of potential sires, the question of relative fertilization success among males becomes crucial. We are currently developing DNA markers (microsatellite loci and RAPD) to assess the paternity of these offspring (7).

In summary, these new observations document several male mating behaviors. Males may temporarily pair with females; males in such pairs experience high copulatory and fighting success. Alternatively, males may employ one of several ''sneaker'' tactics. Copulation involves one of two positions, each of which involves a distinct location of sperm placement. In addition to these behavioral differences between males, individual males can copulate in both positions, even within the same day. The social dynamics of *L. pealei* suggest a high level of genetic mixing in communal egg beds, as well as within individual egg capsules.

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