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Contact with Squid Egg Capsules Increases Agonistic Behavior in Male Squid (*Loligo pealei*)

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Agonistic (aggressive) behavior is costly (1). For this reason, males should fight only when it will increase their reproductive fitness. In some species, the last male to mate with a female before she oviposits sires most of the offspring (2). If *Loligo pealei* has a similar system of sperm precedence, a male should drive away nearby males when females are about to lay their eggs. In *L. pealei*, conspecific egg capsules induce other females to lay their eggs (3). Therefore, the presence of egg capsules could provide male squid with the information that females are about to begin ovipositing. To determine whether male squid might use this information, we tested the effect of squid egg capsules on male agonistic behavior. An earlier report suggested that squid egg capsules may increase male agonistic behavior (4). We also explored which stimuli connected with the egg capsules might influence male behavior.

Agonistic behavior in *L. pealei* is similar to that observed in *Loligo plei* (5). First there is a variety of signaling through skin patterns; then the behavior escalates to Fin Beating, Chasing, (or Fleeing) and finally to Open Arm Charges. In the highly aggressive Open Arm Charge, one squid rushes toward the other squid with his arms in a tight cone. Once he is close to his opponent, he throws open his arms. Sometimes the charging squid contacts and wrestles with the conspecific, but more often he stops short of the other squid.

The effect of egg capsules on male agonistic behavior was tested as follows. Male squid were isolated for 14 to 21 h prior to a trial. Squid were randomly assigned as either the "resident" or "intruder." Resident squid were isolated in circular tanks (3 m in diameter, 0.5 m deep). Intruder squid were isolated in rectangular tanks (132 × 76 × 43 cm). The mantle lengths of the resident and intruder (16–21 cm) were size-matched to within 1 cm. Water temperature in the tanks was maintained between 14.5°–22.5°C, and the tanks were exposed to an ambient light cycle (June–Aug.). Squid were fed *Fundulus* spp. on the evening before the trials, which were run early the next day. Each squid was used only once. During a trial, an intruder male was introduced into the circular tank containing the resident. Incidences of Fin Beating, Chases, Open Arm Charges, and other behaviors were recorded. After 20 min (n = 23) or 30 min (n = 10), squid egg mops (16–20 egg capsules attached to an airstone), were placed in the center of the tank for 20 min (n = 16) or 30 min (n = 5) and then removed. One min (median: 1 min, 1st and 3rd quartiles: 1 min, n = 21) after the addition of an egg mop, squid swam towards it, blew water on it, and finally touched it with their arms. In one set of control trials, airstones were added without egg capsules (Table I).

As Table I shows, the presence of egg mops induced a large increase in Open Arm Charges, while the airstone alone had no effect. The increase in Open Arm Charges never occurred prior to physical contact with the egg capsules (49/49 trials).

In a further set of experiments, we placed an egg mop under a perforated cover. The cover blocked squid from seeing the eggs, but allowed chemical substances to flow from under the cover into the tank. Experiments in which a bolus of dye was introduced under the cover indicated that substances could pass into the tank from this location. While the egg mop was under the cover, the number of Open Arm Charges did not increase (Table I: Covered egg mop). Once the cover was removed, and the squid had seen and touched the egg capsules, the number of Open Arm Charges increased (7/7 trials).

Table I

The effect of different treatments on the number of Open Arm Charges

Treatment	Number of Open Arm Charges/10 min Median (1st quartile, 3rd quartile)	n
Nothing added to tank	0 (0, 0)	33
Airstones only	0 (0, 0)	5
Egg mop	9.7 (6.5, 17.3)*	21
Covered egg mop	0 (0, 0)	7
Egg mop in glass box	0 (0, 1.5)	8

* Significantly different from "Airstones only" treatment ($Z = 3.41$, $P < 0.001$).

We also placed an egg mop in an uncovered glass box, which was lowered into the tank containing the squid. The glass box allowed visual, and possibly chemical, stimuli to enter into the squids' tank, but prevented the squid from contacting the egg mop. Squid visually inspected the egg mop through the glass box, but there was no increase in the number of Open Arm Charges (Table I: Egg mop in glass box). When the egg capsules were placed outside of the glass box, agonistic behavior increased after the squid contacted the egg mop (7/7 trials). Contact with the egg capsules appears to be critical for inducing the effect.

These results suggest that contact with squid egg capsules increases agonistic behavior in male squid. Although visual cues may be important for locating the egg mop, the stimulus that 'turns on' agonistic behavior may be a chemical embedded in the egg capsule that the male must touch to sense. By using a cue from the egg capsule to regulate their agonistic behavior, male squid may be able to target the expression of this costly behavior to times when it will produce the maximum reproductive benefit.

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