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Location probing by males complicates sexual dynamics and successful mate-guarding in squid groups

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Fitness is defined by the successful passing of an individual's genes to the next generation. Cephalopods that mate in groups, in particular coastal squid and cuttlefish species, have evolved complex sexual tactics to maximize reproductive success (Morse and Huffard 2019). Through dynamic skin patterning accompanied by typical body postures, individuals send visual signals in the form of displays to potential mates and rival competitors, often engaging in escalating conflicts (Moynihan and Rodaniche 1982, Mather 2016). Moreover, males can simultaneously exhibit receptive color patterns to females and agonistic patterns or even deceptive female patterns to rival males (Brown et al. 2012, Hanlon and Messenger 2018). Therefore, given the diverse number of tactics used to both attract a mate and deter competitors, effective female mate-guarding by consorting males is critical to reproductive success in group-mating cephalopod species. Indeed, literature on coastal squids has described mate-guarding as the pre- and post-copulatory role of males, who are generally observed positioned around the female while she lays eggs in crevices, exhibiting agonistic displays to ward-off rivals to prevent insemination by other males' sperm (Moynihan and Rodaniche 1982, Mather 2016, Hanlon and Messenger 2018, Morse and Huffard 2019).

The bigfin reef squid *Sepioteuthis lessoniana*, like the closely related *S. sepioidea*, is a group-living species that forms shoals and/or schools, and exhibits complex social and sexual behaviors (Moynihan and Rodaniche 1982, Boal and Gonzalez 1998, Sugimoto and Ikeda 2012), including the presence of sneaker males or 'female mimics' (Wada et al. 2005). However, to date it has not been the focus of detailed behavioral research in the wild. In this study, we observed *S. lessoniana* behavior during mating seasons in two distant geographical locations, and found new behavioral elements in the sexual courtship of *S. lessoniana*. These elements add complexity to existing social interactions and impact sexual strategic choices faced by individuals, with potential implications for the reproductive fitness of both males and females.

Using SCUBA, and after allowing an initial habituation period to the divers, we recorded interactions of individuals and groups in Indonesia (Lembeh, 1.4249° N, 125.2258° E; total 1 h of recording) and Egypt (El Quseir, 26.1014° N, 34.2803° E; total 2 h of recording), between July–September 2013 and May–July 2019, respectively. The focal squids (consort male, female, and main rival male) were distinguished by size, strategy, prominent markings potentially left by attacks, or typical displays (Mather et al. 2010). During filming, we observed that, in addition to females entering crevices to lay eggs as previously described for loliginid squid species (Morse and Huffard 2019), the male also displayed the same behavior (Video S1). Within this context, we annotated when males entered the same crevices where females laid eggs, as well as other notable occurrences, such as visual displays, male-male attacks, and copulations.

We observed that visual displays, in terms of skin and body patterns, were mostly similar to the ones described for *S. sepioidea* (Moynihan and Rodaniche 1982, Mather 2016, Hanlon and Messenger 2018), with some degree of variation (see Lin et al. 2017). For example, the male stripe display (Fig. 1a, also featured in Fig. 1c), and females exhibiting an almost uniform purple display when sexually receptive (Fig. 1b). Dominant males also

FIG. 1. Some typical patterns and displays by *S. lessoniana* from the Red Sea. (a) The stripe display, with a noticeable white stripe across the center of the mantle. (b) Female displaying sexual receptivity with an almost uniform purple color. (c) Double signaling by the consort males, exhibiting sexual courting pattern to the female (bright purple) and an agonistic pattern to the competing male (dark brown and white flashes, see Video S1).

used double signaling, exhibiting an agonistic pattern (brown with white flashes) on one side to competing male(s), and a sexual courting pattern to the female on the other (bright purple; Fig. 1c). Notably, during agonistic bouts, we also identified an undescribed display, performed particularly by the dominant male (Fig. 2). During this display, white flashing is often visible (Fig. 2a), complemented sometimes by a zebra color pattern (Fig. 2b, Video S2). Interestingly, the tentacles were markedly exposed and set on a downward direction, with the arms splayed around the mouth or also facing downwards (tentacle exhibition display; Fig. 2). This conspicuous behavior makes the squid highly visible, deterring potential rivals (e.g. Video S1).

We also consistently observed a unique behavior across both sites during egg laying. Of the 15 individual events in which females laid eggs (once or multiple times), 13 were preceded by males entering the same crevice (˜87% probability of laying eggs after male probing; two-tailed exact binomial test: $P = 0.005$; Fig. 3a). The remaining two events occurred less than 90 s after filming started, which makes it possible that the male could have previously entered the location. Moreover, as only once did a competitor instead of the consort male enter after the female, events are therefore probabilistically dependent as reversing their order would markedly change the probability of occurrence (to ˜6%). When performing this behavior, the male not only hovered close to the crevice, but effectively entered more than half-body length inside and remained for a period of seconds, withdrawing afterwards to give place to the female (Video S1). Each sex exhibited specific skin patterns during probing and laying

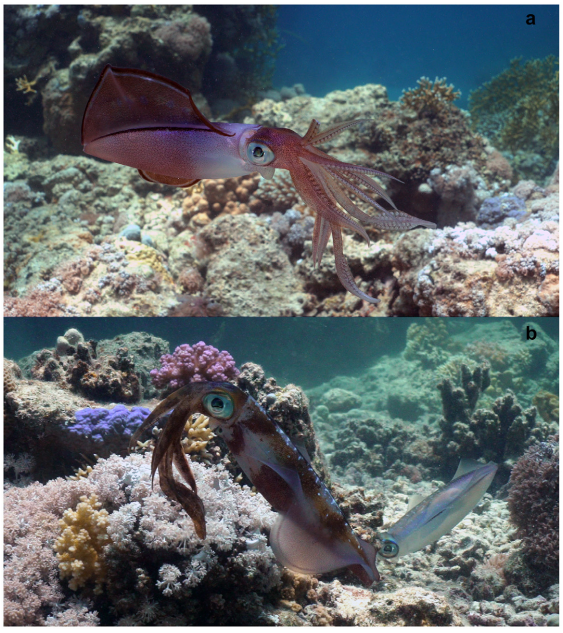

FIG. 2. Males performing the tentacle exhibition display, with variations. (a) White flashing and arms spread around the mouth. (b) With zebra color pattern, and arms down with tentacles (and the female laying eggs in the background, see Video S2).

eggs, both assuming a generally white appearance, but the female with an iridescent green/yellow across the center of the mantle (Fig. 3b), and the male with brown spots around the mantle and a clear blue line (Fig. 3c). In some

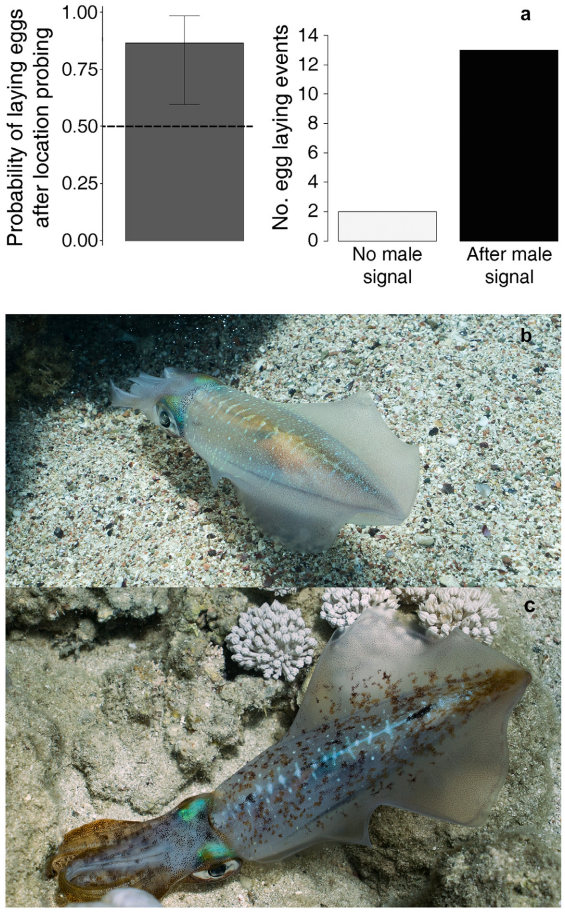

FIG. 3. Location probing data and displays (see also Video S1). (a) Data and exact binomial estimated probability of egg laying occurring with or without male location probing. (b) Typical female skin pattern while laying eggs, bright iridescent tones in a mostly white body concentrated around the eyes and the dorsal mantle. (c) Typical male skin pattern while probing the location, also characterized by a mostly white body but with a blue line across the mantle intersected by smaller blue lines, and darker tones of brown in the arms as well as in the tip and across the mantle.

occasions, sand and debris were seen being ejected out of the crevice when males entered (second event in Video S1). However, it is not clear if this ejection was performed intentionally with the arms/funnel, or if it was an unintentional by-product stemming from fin movement. It is therefore uncertain what is the purpose of this location probing behavior, for example if the male is inspecting the crevice for potential predators that could feed on the eggs, marking a specific site within the location/crevice for the female to lay the eggs, or cleaning the location for the female to deposit the eggs on a stable surface.

Pre-probing locations carries a clear risk for the male, as he abdicates from mate-guarding momentarily and leaves the female vulnerable to the approaches of competing males. When in groups, the main competing male would attempt to seize these opportunities to copulate with the female (with the consort male rushing to position himself between the two, often attacking the competitor, e.g. Video S1), successfully doing so in at least one occasion (Video S3). Evidence suggests that female *S. lessoniana* can store sperm and may have cryptic female choice of sperm (Morse and Huffard 2019), which could lead to the complete ejection of the consort male's sperm and, therefore, greatly reduce his reproductive fitness. Conversely, the opportunity of copulating with another male while the male consort is absent may increase the reproductive success of the female (and the competing male), by providing a more diverse gene pool with which to fertilize her eggs.

S. lessoniana is considered to be a species complex composed of several different cryptic species across the Indian and Pacific Oceans, many with overlapping ranges (Cheng et al. 2014). However, distinguishing between cryptic species in the wild is difficult because, while these species are genetically distinct, they are hard to distinguish morphologically. Notably, individuals from considerably distant geographic regions (˜10,000 km between the Red Sea and Indonesia) both show the tentacle exhibition display and the probing behavior. This suggests that either: (1) the individuals observed are the same species, or (2) these behaviors are shared across cryptic species, either as a conserved trait or as a product of convergent evolution. The occurrence of location probing behaviors may also be dependent on habitat features. *S. lessoniana* have been observed to lay eggs on multiple different types of substrate, from within branching corals to seagrass beds, on mooring lines, and on open sandy substrate (S. H. Cheng, personal observation). In "open environments" where the substrate is visible, such as sandy substrates, location probing may not be present; however, further observations of egg laying are required to understand the potential role that habitat structure may have in shaping male and female behaviors. While its purpose is still unknown, location probing by males seems to be a frequent and important aspect of mating and egg laying in crevices for these populations. From an evolutionary perspective—as this behavior carries potentially high costs to the consort male due to abandoning mate-guarding—there may be significant selective advantages, such as both partners avoiding egg predation. Moreover, location probing by consort males could also be selected for by females, as it additionally provides the female with the opportunity to attain spermatophores from other males. Further observations and studies will help us better understand the ecological (geographical distribution, and habitat specificity) and evolutionary (phylogeny, development, and purpose) drivers behind these behaviors, as well as how they shape individual decision-making in complex social and ecological contexts.

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Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3529/suppinfo

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Data (Sampaio et al. 2021) are available in Figshare at: https://doi.org/10.6084/m9.figshare.14763504.