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学位論文題目	Elucidation of reproductive behaviors of inshore and oceanic squids using molecular and anatomical techniques (分子生物学及び形態学手法を用いた沿岸性及び外洋性イカ類の繁殖行動の解明)
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論文内容の要旨

Squids are divided into two major groups as myopsid or inshore squids and oegopsid or oceanic squids based on their differential habitat and morphologies. In some squids, such as those in the family Loliginidae inhabiting inshore waters, upon copulation, females receive male-delivered sperm capsules, spermatangia, from dimorphic sized males and store at two different body locations: the buccal membrane and the distal end of the oviduct. This insemination site dimorphism is associated with alternative reproductive strategies. However, in inshore kabi squid *Loliolus sumatrensis*, a species of Loliginidae, the females possess three insemination sites: buccal membrane (BM), basal left IV arm (ARM) and lateral head behind the left eye (EYE), therefore we studied such the unusual phenomena. First, we attempted to identify rules and patterns of usage of these sites in relation to size differences in male individuals, and the mating history, maturity, fecundity, as well as growth indices of female individuals. Unlike most other loliginid squids, no dimorphism was found in male size. Besides, no significant differences in sperm size (flagellum and head) were observed among spermatangia at the three insemination sites. The seasonal dynamics of a population in the Seto Inland Sea revealed a set priority for the initial use of insemination sites as BM, followed by ARM and then EYE, whereas the maximum number of stored spermatangia was greater in EYE>ARM>BM. At the individual level, however, once all insemination sites of a female have started to be used, subsequent inseminations by other males could occur anywhere depending on the most suitable site given the current situation. Thus, the choice of site to be inseminated was not dependent on full occupancy of preferred insemination sites, but the mating history of the female. Female maturity status was correlated with the usage pattern of insemination sites but not with the number of stored spermatangia at any insemination site. These imply that males inseminate at different sites in a female according to the mating history and maturity status of the female. However, because the inseminations at three different locations in a female kabi squid occurred by

monomorphic males, the females were further investigated to explore their promiscuity level. Hence, we developed microsatellite markers and genotyped the paternity of each spermatangium on three sites. The microsatellite-based genotyping showed that each female mated with 7 to 9 males, suggesting that *L. sumatrensis* are polyandrous. Even multiple paternity was also found at every single site and the actual number of sires per site was relatively higher at ARM than at the other two sites. Surprisingly, simultaneous usage of all three sites in a female occurred by a few males, those shared a major part of the paternity in the respective female, indicating that sperm allocation occurs within a female. These results suggest that a male kobi squid inseminates at different locations within a female depending on the current sperm storage as well as developmental status of female, and the suitability of insemination place for maximizing fertilization success.

In contrast to inshore kobi squid, females of oceanic diamond squid *Thysanoteuthis major*, upon copulation, receive and store male-delivered sperm not in the form of spermatangia, but in multiple sperm storage organs, known as seminal receptacles (SRs), located ventrally on their buccal membrane. However, apart from the mating season, they are often observed in pairs of one male and one female of similar sizes, indicating behavioral monogamy, and this pairing lifestyle is unique among the squids. Therefore, further study was carried out to determine whether diamond squids are truly monogamous, by genotyping the stored sperm in SRs with newly developed microsatellite markers. The microsatellite-based fragment length analysis (FLA) showed that one female mated with at least 2 to 5 males, indicating that they are not monogamous. Even each SR within the females also exhibited multiple paternity. Interestingly, the FLA electrograms showed multi-allelic peaks with significant similarity in peak patterns among SRs within female. Despite mixed paternity being evident, we found that paternity share in all the SRs within a female was biased to presumably a single male, which supports their pairing behavior and is somewhat related to extra pair copulations. These infer that regardless of pairing, the female diamond squids practice extra pair copulations outside the pair-bonded partner to have genetically diversified offspring. In conclusion, both inshore and oceanic squids perform varying degrees of behavioral strategies for copulation, sperm insemination, storage, and fertilization eventually to produce offspring of high-quality.

論文審査結果の要旨

申請者は沿岸性イカ類の1種であるヒメジンドウイカと外洋性種であるソデイカを研究対象に基本的な繁殖生態学の知見を得るため、形態学的手法と分子生物学的手法を駆使して総合的に研究を行った。

沿岸性種ヒメジンドウイカにおいては、まず形態学的に区別ができない近縁種との違いをミトコンドリアゲノムの遺伝子配列を比較することで明らかにし、その上で形態観察により、本種は同属のヤリイカ科では珍しい繁殖様式をとることを明らかにした。すなわち、通常ヤリイカ科はメスが体内と体外の各1箇所、計2箇所に精子を保存するのに対し、ヒメジンドウイカでは、体外に3箇所貯蔵することを見つけた。何故3箇所もあり、またどのように使い分けているか明らかにするのに、まず次世代シーケンス法により大規模な塩基配列を取得し、その中から個体識別を可能とする遺伝子領域を抽出した。これにより3箇所につけられた精子の父親が簡単なPCR法で明らかにできるようになった。この方法を用いて明らかになったことは、1) 多くの雄が1匹の雌に精子を受け渡し、いわゆる乱婚であること、2) さらに交尾に優位な雄が存在し、その

雄が3箇所全てに自分の精子を受け渡すことが示された。この現象は「精子配分」と言われる現象に類似する。しかし通常よく知られた「精子配分」では、雄がメスの質や状態を見極め、受け渡す精子量を雄自身が調整する。ところが本種においては、雄がメスの3箇所に受け渡す精子量を配分、調整するという動物界で大変珍しい行動が起こっていることを明らかにした。

次に申請者は外洋性種であるソデイカを対象とし研究を行った。ソデイカは体サイズが1メートルにもなる大型イカで、重要な水産資源でもある。漁師、研究者のこれまでの観察で、ソデイカはオスメスが成熟するよりずっと以前に「つがい」となり、行動を共にすると言われていた。そこで申請者はソデイカの「つがい」が一夫一婦制につながるほど強固であるかを確かめるため、先の手法と同様に遺伝子検査でメスの乱婚度を調査した。その結果、調査したメス5個体全てにおいて2匹以上のオスの精子が発見された。これより、ソデイカも他のイカ類と同様、乱婚であることが初めて示された。しかし予想される父親の数は少なく、また精子の多くは1個体のオス由来であったことから、「つがい」行動の意義として、ペアになったオスがメスの産む卵をできるだけ多く獲得するために進化したものと考察した。

口頭試験の質疑応答において会場の複数の大学院生から英語で質問を受け、それに対し全て回答することができた。審査員より、予備審査で指摘した助言をもとに博士論文の修正がなされているという発言があった。申請者は博士論文研究の間に査読付論文を1報発表し、現在2報目の論文執筆およびデータのとりまとめに取り掛かっている。

以上のことから、申請者の論文は博士の学位授与に値するものと審査委員が全員一致し判定した。