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Evolutionary biology

A monogamous pipefish has the same type of ovary as observed in monogamous seahorses

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Syngnathid fish (pipefish and seahorses) are unique among teleost fish in that their ovary consists of a rolled sheet with germinal ridge(s) on the dorsal side running along the entire length of the sheet. A distinct difference is seen in the ovarian structure between polygamous *Syngnathus* pipefish and monogamous seahorses (*Hippocampus* spp.), the former having one germinal ridge and the latter with two ridges. This study examined the ovarian structure and the mode of egg production in a monogamous pipefish *Corythoichthys haematopterus*. The ovary of *C. haematopterus* had two germinal ridges like that observed in monogamous seahorses. There were two distinct groups of follicles in the ovary, one being a cohort of extremely small follicles and the other a cohort of follicles developing and increasing in size with the passage of time. We suggest that the ovarian structure and the mode of egg production in this pipefish are adaptations to monogamy.

Keywords: ovarian structure; Syngnathidae; pipefish; mating pattern; monogamy

1. INTRODUCTION

Mating patterns of animals are directly or indirectly constrained by their morphologies, especially those related to reproduction. Fish in the family Syngnathidae (pipefish and seahorses), famous for remarkable adaptations for paternal care in the brood pouch (Wilson *et al.* 2001), are unique among teleost fish in their ovarian structure (Wallace & Selman 1981). Their ovary consists of a rolled sheet, which has a stem cell compartment on the dorsal side, called a germinal ridge, running along the entire length of the sheet (Begovac & Wallace 1987; Selman *et al.* 1991). Development of the follicles starts at the germinal ridge and developing follicles are arranged in a sequence according to their development (Begovac & Wallace 1987, 1988; Selman *et al.* 1991). There is a distinct difference in the ovarian structure between pipefish of the genus *Syngnathus* and seahorses (*Hippocampus* spp.), the only two genera in which ovarian structure has thus far been examined in this family. The *Syngnathus* ovary, examined in *Syngnathus scovelli* (Begovac & Wallace 1987), has a single germinal ridge at one edge along the length of the sheet, with the most advanced follicles at the other, so-called mature edge. By contrast, the *Hippocampus* ovary has two germinal ridges, one at each edge, with the most advanced follicles situated on the ventral side midway between the edges (Selman *et al.* 1991).

The mode of egg production in *Syngnathus* is classified as the asynchronous type (Wallace & Selman 1981; Kornienko & Drozdov 1999), in which oocytes of all stages are present at any time in the ovary. Fish of this type are expected to deposit eggs without a long interval. Actually, females of *Syngnathus typhle* allocate their eggs among several males within a short period of time (less than 48 hours; Berglund *et al.* 1988). By contrast, *Hippocampus* females are unable to replenish mature eggs over a long period of time (9–45 days) after each spawning (Foster & Vincent 2004), suggesting that egg production is more synchronous.

Syngnathus and *Hippocampus* also have different mating patterns; the former is polygamous (polyandrous or polygynandrous) but the latter is monogamous (Jones & Avise 2001; Foster & Vincent 2004). Females of *Syngnathus* can prepare a clutch at a higher rate than males can complete brooding, allowing for females to obtain greater reproductive success by mating with multiple males (Berglund *et al.* 1989). On the other hand, *Hippocampus* females have a low potential for multiple matings within a period of time during which males complete brooding (Vincent 1994). These contrasting features of the two groups invite an investigation of the relationship of mating patterns with the ovarian types in this family.

Corythoichthys haematopterus is a monogamous pipefish that inhabits shallow waters of the Indo-Pacific (Matsumoto & Yanagisawa 2001). This pipefish exhibits long-term pair bonding: the same partners mate repeatedly up to approximately 10 times in a breeding season with non-brooding intervals of 1 or 2 days, after which they mate again in the next breeding season as long as they live (Matsumoto & Yanagisawa 2001). After each spawning, females of this pipefish cannot replenish mature eggs for 10–19 days (Sogabe *et al.* 2007). If mating patterns are linked with the ovarian type, females of *C. haematopterus* are expected to have the seahorse-type ovary rather than the *Syngnathus* pipefish-type, to produce mature eggs synchronously. To test this prediction, we conducted a histological study of *C. haematopterus* ovaries at successive developmental stages.

2. MATERIAL AND METHODS

(a) *Sample collection*

Sampling was made during scuba diving on the boulder slope (2–8 m depth) at Morode Beach on the west coast of Shikoku Island, Japan, in August 2007. Prior to sampling, we observed greeting behaviour and copulations of *C. haematopterus* within a fixed area (140×10 m) of the slope to identify pair members and the day of their spawning. We collected two females each day, from day 2 to day 11 of spawning (the day before the expected day of next spawning). On the spawning day, we collected two females on three occasions, at the time of greetings (soon after sunrise) and just before and soon after spawning (approx. 1.5 hours after sunrise).

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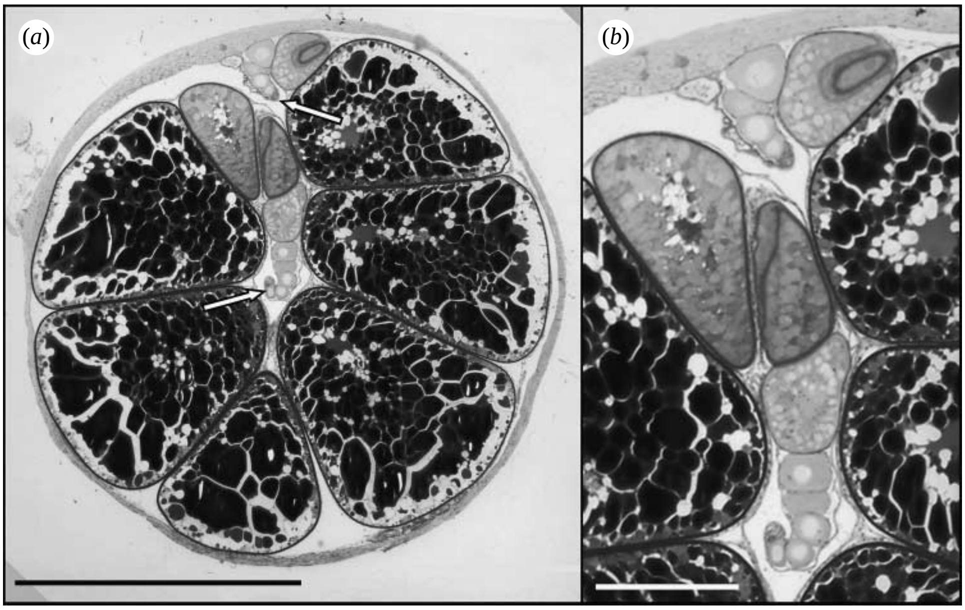

Figure 1. (*a*) Transverse section of the ovary of *C. haematopterus* 7 days after spawning, showing two germinal ridges (arrows) and serially arranged developing follicles arising from each. Scale bar, 1 mm. (*b*) Enlarged image of the region of two germinal ridges. Scale bar, 250 µm.

A total of 26 females captured were individually placed into a sealable plastic bag and brought to the laboratory (UWA Marine Institute beside Morode Beach). Within 4 hours of collection, the fish were brought to the laboratory, killed with an overdose of clove oil and then dissected to remove the ovary. The paired ovaries were separated and each was preserved in 10% formalin; one was used for microscopic observation and the other for measurement of the size and number of follicles.

(b) *Histological procedures*

A portion of the preserved ovary was cut out and preserved in 1% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 24 hours at room temperature. It was then dehydrated with a series of ethanol and acetone treatments, and embedded in Spurr's resin. Tissues were sectioned at a thickness of 2–3 µm and stained with 1% toluidine blue in 0.1 M sodium phosphate buffer (pH 7.4). We followed Begovac & Wallace (1988) to stage oocyte development (stage I, oogonia; stage II, oocyte in the primary growth phase; stage III, cortical alveoli formation phase; stage IV, vitellogenesis phase; stage V, maturation phase; and stage VI, mature egg).

(c) *Measurement of follicle size distribution*

All follicles removed from each of the 13 ovaries were photographed with a digital camera. The number and size of developing follicles were calculated from digital images using the IMAGEJ program (National Institute of Health, USA). We used a projected area of each follicle as an index of size.

3. RESULTS

Transverse sections of *C. haematopterus* ovary exhibited a rolled structure, and developing follicles were serially arranged between the ovarian wall and the luminal epithelium (figure 1*a*). Two germinal ridges were visually recognized on the dorsal side ($n=13$, figure 1*b*).

Two dominant groups of follicles in the ovary were observed (figure 2; see figure S1 in the electronic supplementary material). One was a cohort consisting of extremely small follicles (stages I–III) whose size distribution did not change with time after spawning. The other was a cohort of developing follicles that linearly increased in size with time (stages IV and V). The growth rate, measured from follicles soon after spawning and on the day of next spawning was $5.7 \times 10^{-2}\ \mathrm{mm}^2\ \mathrm{d}^{-1}$ ($n=1$).

On the day of spawning, all mature oocytes were ovulated into the ovary lumen (stage VI; see figure S1 in the electronic supplementary material). In the ovary sampled at the greeting time of the spawning day, mature eggs were scattered within the ovary lumen. In the ovary sampled just before spawning, mature eggs adhered to each other and lined up in a single layer. Soon after spawning, only oogonia and oocytes in early developmental phases (stages I–III) remained in the ovary ($n=1$).

4. DISCUSSION

Our results showed that the ovary of *C. haematopterus* with two germinal ridges is highly similar to that of *Hippocampus* but not to that of *Syngnathus*. All three genera of *Corythoichthys*, *Hippocampus* and *Syngnathus* are members of the major subfamily Urophori in which male brooding occurs using the tail (Wilson *et al.* 2003). Recently, molecular phylogeny revealed that *Hippocampus* and *Syngnathus* are closely related, constituting a monophyletic lineage within the subfamily (Wilson *et al.* 2003), despite being given different common names, seahorses and pipefish. This lineage is distantly connected with the lineage of *Corythoichthys* in the subfamily (Wilson *et al.* 2003). This means that the similarity of ovarian structure between *C. haematopterus* and *Hippocampus* is not directly due to phylogenic relatedness. Selman *et al.* (1991) inferred that a duplication of the germinal ridge in the ovary of *Hippocampus* occurred during seahorse evolution from a pipefish lineage. However, our finding indicates either that the character state of two germinal ridges is more ancestral than that inferred by Selman *et al.* (1991), or has evolved independently at least twice within the subfamily.

This study revealed that the mode of egg production in *C. haematopterus* differs from that reported in *Syngnathus* (Wallace & Selman 1981; Kornienko & Drozdov 1999). In *Syngnathus*, whose ovary is

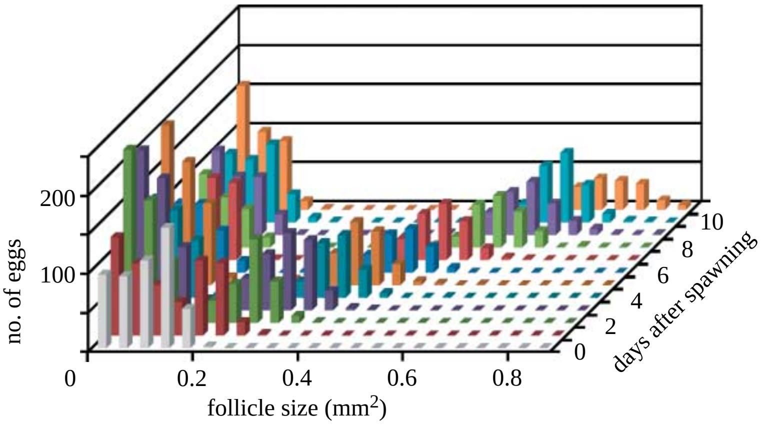

Figure 2. Temporal changes in the size distribution of developing follicles in the ovary of *C. haematopterus* in relation to the time from previous spawning.

classified as the asynchronous type (Wallace & Selman 1981), several stages of developing follicles can be distinguished at a time (Begovac & Wallace 1988; Kornienko & Drozdov 1999). By contrast, there are two groups of follicles in the ovary of *C. haematopterus*: one being a cohort of extremely small follicles and the other a cohort of follicles developing and increasing the size with the passage of time. This type of ovary is categorized as the group-synchronous type (Wallace & Selman 1981).

Naturally, the ovary type has important implications for how eggs are spawned. Females of *Syngnathus* can repeat spawning within a short period of time, enabling the allocation of eggs among several males (Berglund *et al.* 1988). In *C. haematopterus*, females extrude all mature eggs as a single sheet to transfer them to the male's brood pouch (Matsumoto & Yanagisawa 2001). After spawning, female *C. haematopterus* are unable to spawn a new clutch for 10–19 days (Sogabe *et al.* 2007). Females of *Hippocampus* spp. are also unable to replenish mature eggs for a long period of time (9–45 days) after spawning (Foster & Vincent 2004). This suggests that mating patterns of syngnathids are constrained more or less by the ovarian type.

All species of *Corythoichthys* and *Hippocampus* observed thus far are monogamous (Gronell 1984; Matsumoto & Yanagisawa 2001; Foster & Vincent 2004), contrasting with polygamous mating patterns in *Syngnathus* spp. (Jones & Avise 2001). Monogamy, in particular genetic monogamy, is a rare mating pattern in animals and is generally considered to be evolutionarily less stable than other mating patterns (Emlen & Oring 1977). However, if monogamy involves morphological and/or physiological adaptations, the monogamous mating pattern can be more resistant to ecological fluctuations, as suggested in *C. haematopterus* by Sogabe & Yanagisawa (2007) from the fact that the potential reproductive rate did not differ between the sexes at any temperature. The structural similarity of the ovary between *Corythoichthys* and *Hippocampus* may indicate that both groups have an ovarian adaptation to monogamy. Further studies are desirable on the relationship between mating patterns and ovarian structure in syngnathids.

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