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Sexually Dichromatic Protogynous Angelfish *Centropyge ferrugata* (Pomacanthidae) Males Can Change Back to Females

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ABSTRACT—Protogynous hermaphroditism, female-to-male sex change, is well known among reef fishes where large males monopolize harems of females. When the dominant male disappears from a harem, the largest female may change sex within a few weeks. Recently, from experiments with some protogynous haremic fishes in which two males' cohabitated, it was confirmed that sexual behavior and gonads were completely reversible according to individual social status. However, the ability to reverse secondary-developed sexual body coloration has never been examined in any protogynous fish. We conducted two male cohabitation experiments with the protogynous haremic angelfish, *Centropyge ferrugata*, which has conspicuous sexual dichromatism on the dorsal fin. Smaller males of *C. ferrugata* soon performed female-specific mating behaviors when they became subordinated after losing a contest. They then completed gonadal sex change to females 47 or 89 d (n=2) after beginning cohabitation. In the course of the reversed gonadal sex change, male-specific coloration on the dorsal fin changed to that of a female. Thus, the sex of *C. ferrugata*, including secondary developed sexually dichromatic characteristics, can be completely reversible in accord with their social status.

Key words: reversed sex change, sexual dichromatism, mating behavior, social status, marine angelfish

INTRODUCTION

Sequential hermaphroditism (sex change) is a widely documented phenomenon in plants and animals (Policansky, 1982). The size-advantage model explains the evolution of sex change as a life-history strategy alternative to gonochorism (Ghiselin, 1969; Warner, 1975, 1988). This model predicts that direction of sex change will be determined by mating systems. According to the model, protogynous (female to male) sex change will be selectively favored in fishes with a polygynous mating system where large males monopolize mating to the detriment of smaller ones.

* Corresponding author: Tel. +81-824-24-7941; FAX. +81-824-22-7059. E-mail: sakai41@hiroshima-u.ac.jp This prediction has been confirmed in many reef fishes (see Warner, 1984, 1988; Nakazono and Kuwamura, 1987; Kuwamura and Nakashima, 1998). In many cases, sex change is socially controlled; the dominant, largest, fish in a polygynous group usually becomes male as a result of sex determination by social status (Robertson, 1972; Warner, 1984; Ross, 1990; Kuwamura and Nakashima, 1998).

Recently, both-ways sex change (or bi-directional sex change) has been reported from several polygynous species of some fish families such as Gobiidae and Pomacanthidae (see Kuwamura and Nakashima, 1998). Even in fishes often demonstrating protogynous sex change in nature, it has been confirmed that the largest dominant male will change sexual behaviors and gonads back to a female's when it becomes subordinated again after cohabitation with an even larger male (reversed sex change; e.g., Sunobe and Nakazono, 1993; Hioki and Suzuki, 1996; Kuwamura *et al.*, 2002). However, species previously studied lacked apparent sexual differences in body colorations.

Sexual dichromatism or dimorphism is widely developed in protogynous fishes maintaining lek-like polygynous mating systems where females often change their mates, e.g., wrasses and parrotfishes (Robertson and Warner, 1978; Warner and Robertson, 1978; Thresher, 1984; Warner, 1984). In these fishes, larger and brighter males apparently have advantages in male-male territorial competition and/or in female mate choice (Warner and Schultz, 1992; Kuwamura et al., 2000). Whereas, haremic fishes, where females stably inhabit within a male territory and seldom change mates, usually lack apparent sexual dichromatism or dimorphism (Robertson and Hoffman, 1977). Among polygynous fishes, reversed (or both-ways) sex change has been previously demonstrated only in monochromatic species mostly maintaining haremic mating systems (e.g., Hioki and Suzuki, 1996; Kuwamura et al., 2002). It has been unclear whether males of sexual dichromatic protogynous fishes can completely change back to females.

Exceptional among haremic fishes, some Centropyge angelfishes (Pomacanthidae) are known for maintaining conspicuous sexual dichromatism, e.g., C. interrupta and C. ferrugata (Moyer and Nakazono, 1978; Moyer, 1990). As the size advantage model predicted, protogynous sex change has been observed widely in the genus (Moyer, 1990). The sex change is socially controlled by a dominance relationship among harem members (Moyer and Nakazono, 1978; Sakai, 1997; Sakai et al., 2003). The purpose of the present study was to examine whether change of social status would induce males of the sexually dichromatic angelfish C. ferrugata to perform reversed sex change. We report whether the sex-changing male can transit not only sexual behaviors and gonads but also body colorations into the female type. In comparisons with the results of related studies, we briefly discuss the process and social conditions of the both-ways sex change in reef fishes.

MATERIALS AND METHODS

Males of C. ferrugata are all derived from sex changing females (i.e., no primary males), and stably maintain territories including harems of smaller females (Sakai, 1997; Sakai and Kohda, 1997). Sexual dichromatism of Centropyge angelfishes apparently appears in the posterior parts of their soft dorsal fins (reviewed by Moyer, 1990). Those of males of C. ferrugata are streaked with broad, bright blue bands on a dark blue background, while those of females are colored dark blue in whole (cf. Moyer and Nakazono, 1978). It is known that the bright blue colorations contain wavelength in both the UV (300-400nm) and blue (400-500nm) regions of the spectrum in congeneric species, C. argi and C. bispinosus (Marshall, 2000). Additionally, the posterior edge of a dorsal fin of a male C. ferrugata has a considerably acute shape, while the female's is rounded. Dominant males of C. ferrugata stably maintain the coloration and shape of the dorsal fin (Moyer, 1990). Sex is determined from the dominance relationship among harem members; the largest becoming a male (Sakai, 1997). Sex changing females gradually turn their soft dorsal fins into the male type.

We carried out aquarium experiments at the Sesoko Station of the Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. Just before the experiment, we collected male *C. ferrugata* using hand nets and screen nets with SCUBA on the fringing reefs around Sesoko Island (26° 39'N; 127° 57'E). We confirmed sperm release by pushing the abdomen before every experiment. All the males used in the present experiment (8 individuals) were mature and functional.

We kept two males in a mesh cage of 1.0 m \times 0.5 m with a depth of 0.5 m. The cage was set in a square tank of 3.8 m \times 2.9 m at a depth of 0.5 m with running seawater. A number of plastic pipes and blocks were set on the bottom as shelters. We fed lettuce and Tetra Doro Marin (Pfizer Inc.) every morning and afternoon.

We conducted two sets of two males' cohabitation experiments. The first experimental pair was set on June 7, 2000 (male 94 mm and 79 mm in total length (TL)). The larger dominant male suddenly died on June 15 after severe chasing between the males, though there were no apparent injuries on its body. We added a new larger male (91 mm TL) into the cage on July 7. On the day of the new male release, we separated the two males using a 10 mm mesh barrier set in the middle of the cage. From July 8, we removed the barrier and kept the pair cohabitating until July 24 (the end of the experiment).

The other experimental pair was set on September 5, 2000 (male TLs 105 mm and 93 mm). The smaller male of this pair had been used as the larger one in the previous experiment. Just prior to the start of the experiment, we had kept the male in single condition for 11 days. We confirmed that the male still maintained the male-type fin coloration and released sperm on the day of two males' cohabitation. To try to decrease the risk of a fierce chase, we again separated the two males using the mesh barrier during the first 10 days. Even in the presence of the barrier, the two swam closely and often showed aggressive displays towards each other through the mesh barrier. We temporarily opened the barrier for 30 min on the evenings of September 15 and 16. We removed the barrier from 15:00 on September 17. The larger male suddenly died on September 26 (21 days after the start). This pattern of the larger male dying continued, as new larger males were set within a day of the previous male's death. In all three other large males (101-106 mm in TL, n=3) subsequently died on September 30, October 10 and 27 due to anorexia or dejection troubles. A new larger male (103.4 mm in TL) was set on October 29, and it survived until the end of the experiment. The smaller individual remained subordinate to any of the five introduced larger males throughout the experiment. The breeding season of C. ferrugata on the coral reefs of Sesoko Island ends late November or early December when the water temperature usually drops below 23°C (Sakai, 1997). We finished the cohabitation experiment on December 3, and fixed the gonad of the smaller individual in Bouin's solution for histological examination. We embedded the gonad of the smaller individual of the second experimental set in paraffin. Serial 7-µm transverse sections were stained with hemotoxylin and eosin. To compare the gonadal structure with typical examples of sexually functional individuals, we made gonadal transverse sections additionally for a breeding pair (male 96 mm, female 79 mm in TL) whose mating behaviors had been preliminarily observed for 6 months on reefs of Sesoko Island.

Mating behavior of *C. ferrugata* is reported as follows (Moyer and Nakazono, 1978; Sakai and Kohda, 1995). From ca. 1 hr before sunset, males start courtship displays to females. A male performs the lateral display (soaring) to each of the females within its territory in turn. Around sunset, a female ascends into the water column with a male's snout attached to the female cloaca (nuzzling). After repeating the nuzzling ascent several times, females release hundreds of pelagic eggs into the water column at the top of the last ascent (Sakai and Kohda, 1995; Sakai, 1996). Females soon hide in coral crevices to sleep after spawning. Males will sleep after all the females of their harem have hidden. We called the 1hr before sunset the mating period in the present study. Females usually spawn everyday though social conditions may affect spawning frequencies (see Sakai, 1997). For each experimental pair, we recorded the time of the occurrences of courtships and aggressive interactions throughout the mating period almost every day. When any experimental individuals performed the nuzzling ascent in the female position, we judged it as an individual that had completed behavioral sex change into a female.

Eggs of *C. ferrugata* are ca. 0.7 mm in diameter and pelagic (Hioki *et al*, 1990). When experimental pairs conducted the nuzzling ascent, we soon after gently scooped the surface of the water within and around the cage using a hand-held plankton-net (0.4 mm mesh) to check whether eggs were released or not. When eggs were found, we confirmed whether they were fertilized and developing using a microscope. Both at the start and at the end of each experiment, we took close-up photographs of experimental individuals to compare color patterns and shapes of dorsal fins.

RESULTS

In the first experimental pair, for the first three days the two males swam fast without any direct contact and often hid in the shelters. On June 10, both males started to swim calmly. The subordinate male subsequently experienced single condition for 22 days due to the sudden death of the dominant male. On July 9 the new larger male had performed soaring toward the smaller male for the first time. Just before sunset of that day, the latter accepted nuzzling while in the female position, though its dorsal fin still had male-specific blue bands. This was 32 days after the start of cohabitation for the smaller individual. The smaller individual showed the nuzzling ascent in a female position on 60% of the 15 subsequent evenings. The smaller individual released eggs at 19:05 (15 min before sunset) on July 24. These eggs were fertilized. We then confirmed sperm release from the larger dominant male by pushing its abdomen. Thus, the smaller male completed its gonadal sex change to a functional female 47 days after the start of the experiment. By that time, blue lines on the posterior part of the dorsal fin of the smaller individual had already disappeared, like a female (Fig. 1A). The posterior edge of the dorsal fin of the smaller individual was ragged, but the shape had not completely become round until the end of the experiment (Fig. 1A).

In the second experimental pair, both males showed aggressive displays towards each other through the mesh barrier during the first 10 days. When we temporarily opened the barrier on September 15, the largest male severely attacked the smaller male. We opened the barrier again for 20 min on the next day. Then, the smaller male frequently showed a lateral display against attacking larger male as it gave up the contest competition. The dominance relationship between the two males was considerably settled afterwards. The smaller subordinate individual first received the larger male's nuzzling on September 17. This was 12 days after the start of cohabitation. The smaller individual even maintained apparent male-specific coloration on the dorsal fin at that time. It showed the nuzzling ascent in female position on 59.6% of the subsequent 52 evenings observed. Though all five larger males led the smaller individual to perform the female mating behaviour, we could not



Fig. 1. Change of the sexually dimorphic coloration on soft dorsal fins of *C. ferrugata* along with the reversed sex change. (A) Contour and posterior parts of the dorsal fin of the smaller individual in the first experimental pair had turned dark (arrow) by the first spawning day, July 24, 2000. (B) The subordinate individual in the second experimental pair changed its male-specific coloration on the dorsal fin into the female type (arrow) at the end of the experiment, December 3, 2000. This individual had formerly been the larger male in the first experimental pair (upper individual in A; see the same spot-pattern on the head area). (C) A typical example of a female's dorsal fin is shown. Each bar indicates 1 cm.

Fig. 2. Gonadal structure of a subordinate individual of *C. ferrugata* after the two males' cohabitation experiment. The individual examined spent 89 days as a subordinate individual in the second experiment (Fig. 1B, see text). (A) The gonad maintained a large ovarian cavity with ovigerous lamella structure. (B) Perinucleolus stage oocytes (pn) were often seen in the gonad. Cysts including spermatogenic cells (sc) were also partly present. Gonad structures of a breeding male (C) and female (D) are also shown: various stages of spermatogenesis cells occupy the seminal lobule of the testis, and various stages of oogenesis cells are present in the ovigerous lamellae of the ovary.

confirm egg release by the end of the experiment. Blue bands on the soft dorsal fin of the smaller individual had almost disappeared by the end of the experiment (Fig. 1B) at which time the posterior part of the dorsal fin was ragged and had not adopted a completely round shape (Fig. 1B). We fixed the gonad of the smaller individual on day 89 after the start of cohabitation (77 days after the first nuzzling ascent). The gonad of the smaller individual still contained spermatogenic tissues as a vestige of a functional testis (Fig. 2). It also included considerably developed ovarian parts though it lacked vitellogenic oocytes or preovulated eggs in the gonad (Fig. 2). Thus, the smaller subordinate individual was in the process of the gonadal sex change to become a functional female.

DISCUSSION

The present study demonstrates that male-to-female sex change occurs not only in behaviors and gonads, but also in fin coloration when males became subordinated again in the haremic protogynous species *Centropyge ferru*- *gata.* This is the first report showing that the change of social status induces the reversed sex change even in sex-ually dichromatic characters.

A subordinate male in the first experimental set experienced single condition in the course of the reversed sex change. Therefore, experience of single state seems to relate with the reversed sex change of males. However, the male that had experienced single condition for 11 days until the start of the second experiment still maintained the maletype fin coloration and released sperm. Moreover, the presence of bachelor males directly derived from sex change of females or from losing all females from harems has often been reported in nature populations of *Centropyge* angelfishes as stable individual status (Moyer and Zaiser, 1984; Aldenhoven, 1986; Sakai, 1997). Thus, it is implausible that the single state itself induced a reversed sex change to females in *C. ferrugata*.

The period needed for the male-to-female sex change seems much longer than that required for the female-tomale sex change after male disappearance in many fishes (Table 1). It took around 10 days to settle aggressive rela-

Table II Baladon for and boar mayo box on ango in root none	Table 1.	Duration for the	both-ways s	sex change in	reef fishes
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			Female to male sex change			Male to female sex change				
		Mating system	Sex changer	Spawning	Ref.	Sex changer	Spawning	1st female mating	End of attacks	Ref.
Gobiidae	Gobiodon histrio	Monogamy	L	(< 28)	1	S	(< 28)	-	No data	1
	G. micropus	Monogamy	L	(< 49)	2	S	(< 49)	-	No data	2
	G. oculolineatus	Monogamy	L	(< 49)	2	L	(< 49)	-	No data	2
	G. okinawae	Unknown	L	(< 21*)	3	S	(< 21*)	-	No data	3
	G. quinquestrigatus	Monogamy	L	(30–49)	2	S	(23–49)	_	No data	2
	G. rivulatus rivulatus	Monogamy	L	(< 49)	2	S	(< 90)	_	No data	2
	Paragobiodon echinocephalus	Monogamy	L	27	4	S	24	-	No data	4
	Lythrypnus dalli	Unknown	L	< 14	5	No data	14–53	-	No data	5
	Trimma okinawae	Polygyny	L	6–14	6	S	4–11	_	No data	6
Labridae	Labroides dimidiatus	Harem	L	14–18	7	S	53–77	1–56	1–20	8
Pomacanthidae	Centropyge acanthops	Harem	L	8	9	S	91*	18	2	9
	C. ferrugata	Harem	L	15–22	10	S	47, 89*	32, 12	3, 11	11
	C. fisheri	Harem	L	6	9	S	35*	7	1	9
	C. flavissimus	Harem	L	No data	9	S	81	56	4	9
	Apolemichthys trimaculatus	Unknown	L	25	12	S	123*	No data	40	12
Cirrhitidae	Cirrhitichthys aureus	Unknown	L	230	13	L	207	54	No data	13

Among polygynous mating systems, we use the term 'harem' for fishes where females stably live within male territories. For 'sex changer', the relative body size of the sex change individual in the condition of the cohabitation of two or more individuals (L: large individual, S: small one) is shown. Duration (days) needed for the sperm release (F to M sex change) or egg release (M to F) in the context of mating behaviors from the start of cohabitation experiments is shown in 'spawning' (start = 0). Data within parentheses are taken from experimental studies that were not designed as accurately estimating duration for sex change. Due to lack of 'spawning' data, days needed for changes in structures of genital papillae (for Gobiidae) or gonads (for Pomacanthidae) are alternatively shown with asterisks. '1st female mating' means days needed for the first performance of the female-role spawning ascent without egg release, which are exclusively performed in pelagic egg spawners (see Thresher, 1984). 'End of attacks' shows days needed for settlement of territorial competition between two males. Scientific name of fish is based on respective reference papers. For sources, 1: Munday *et al.* (1998), 2: Nakashima *et al.* (1996), 3: Cole and Hoese (2001), 4: Nakashima *et al.* (1995), 5: Reavis and Grober (1999), 6: Sunobe and Nakazono (1993), 7: Robertson (1972) and Nakashima *et al.* (2000), 8: Kuwamura *et al.* (2002), 9: Hioki and Suzuki (1996), 10: Sakai (1997) and Y. Sakai (unpublished data), 11: present study, 12: Hioki and Suzuki (1995), 13: Kobayashi and Suzuki (1992)

tionships between males of *C. ferrugata* in the present study. Similarly, several weeks were often needed for settlement of male-male competition in other experimental fishes (Table 1). In contrast, female-female competitions would seldom occur in the process of protogynous sex change due to presence of clear dominance relationships among females in haremic fishes (Hioki and Suzuki, 1996; Nakashima *et al.*, 2000; Sakai *et al.*, 2003). As suggested by Kuwamura *et al.* (2002), differences in the sex change duration between the two directions may have resulted from the presence of the time for settlement of male-male territorial competitions in the process of the sex change.

In the present study, after the settlement of male-male competition, subordinate males of *C. ferrugata* soon performed female mating behaviors (nuzzling ascent) even before they could release eggs. After the completion of behavioral sex change, subordinate males may gradually proceed with gonadal sex change. Similar observations on the process of sex changing have also been reported in other fishes producing pelagic eggs, i.e., Labridae, Pomacanthidae and Cirrhitidae (Table 1), and it has been

suggested that the precedence of the behavioral sex change may have functions to decrease the risks of losing future mates (Nakashima *et al.*, 2000; Sakai *et al.*, 2002 and 2003). Even in the present study, the rapid transition in female sexual behaviors by the subordinate male of *C. ferrugata* may have had a function to retain the larger male as a mate.

The fin coloration almost turned to female type at the completion of gonadal sex change (see Fig. 1). Similar coincident change of body coloration with gonadal sex change has been often reported in the context of protogynous sex change in polygynous fishes (e.g., Robertson and Warner, 1978; Warner and Robertson, 1978). Gonadal androgen (e.g., 11-ketotestosterone) is suggested to have a function to control development of sexually attractive ornaments or colorations in males even in fishes as well as other vertebrates (Andersson, 1994; Karino, 1996; Carlisle *et al.*, 2000). Thus, change of gonadal hormone from androgen to estrogen during the process of gonadal sex change may mediate reversed change in sexual dichromatism in *C. ferrugata*.

When subordinate individuals of C. ferrugata started to perform female mating behaviors, they still maintained the male-specific coloration on their dorsal fins. Dominant males of *C. ferrugata* accepted such subordinate males as mates. Similarly in the process of protogynous sex change by sexually dichromatic wrasses, it has also been known that smaller females spawn with a sex-changing female even before its completion of color change (Godwin et al., 1996; Sakai et al., 2002). Thus, sexually dichromatic characters could not primarily control mating relationship between individuals starting sexual behaviors. Male-specific fin coloration of C. ferrugata may have a function in facilitating sex recognition and/or female mate choice, as has been demonstrated in many dichromatic fishes (Warner and Schultz, 1992; Karino, 1996; Kuwamura et al., 2000). Besides that, complete change of fin coloration into the female type may lead to subdominant individuals having a decreased risk of suffering accidental wounds as a result of male-male territorial competition, especially during a non-mating period. Further studies on the evolutionary meaning of the sexual dichromatism in Centropyge angelfishes are expected to reveal the significance of the completion of reversed sex change including body colorations.

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