ARTICLE

Cryptic bachelor sex change in haremic colonial groups of the coral-dwelling damselfish *Dascyllus reticulatus*

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Abstract

Protogynous sex change has been widely reported as a life-history strategy in polygynous reef fish. *Dascyllus reticulatus* is known to form haremic colonial groups on branching corals and is thought to undergo protogynous sex change based on gonad histology. We conducted a 2-year underwater survey on 13 haremic groups to examine the sex-change patterns of *D. reticulatus* on reefs of southern Japan. We observed 11 instances of protogynous sex change. One involved a take-over sex change by the largest female following the disappearance of the dominant male. In other contexts, females undergo sex changes in the presence of males or larger females. These sex-change individuals commonly had limited mating opportunities as females. Of the ten sex changers, five subordinate females became bachelor males showing no sexual behavior despite their ability to release sperm. Owing to behavioral crypticity, these bachelor males remained within their groups without suffering attacks from territorial males. Furthermore, sex changers showed higher growth rates than females. Two bachelor sex changers subsequently obtained mating status, of which one became a female again after inter-group migration. It is suggested that female *D. reticulatus* often overcomes reproductively isolated situations via the growth advantages of protogynous sex change and sexual plasticity.

Keywords Pomacentridae · Haremic colonial group · Protogyny · Bachelor sex change · Reversed sex change

Introduction

Sex change, also referred to as sequential hermaphroditism, has been observed in a diverse range of plants and aquatic animals (Policansky 1982; The Tree of Sex Consortium 2014), including teleost fishes (Sadovy de Mitcheson and Liu 2008; Kuwamura et al. 2020). The adaptive significance of sex change can be explained in terms of the size-advantage model (Ghiselin 1969; Warner 1975; Warner et al. 1975), which predicts that protogyny (female-to-male sex change) is selectively advantageous in fishes with a polygynous mating system, in which the increase in male reproductive success with size is more pronounced than that in females, as large males generally monopolize mating. Protogyny is predicted to occur when an individual reproduces more successfully as a female when small or young, but as a male with increasing age and size. The strong correspondence between protogyny and polygyny has been empirically confirmed in several species of reef fish (Warner 1984; Munday et al. 2006; Kuwamura et al. 2020). Thus, protogynous sex change can be interpreted as a life-history strategy that contributes to higher reproductive success than gonochoristic sexuality in polygynous mating systems.

In fish exhibiting harem polygyny, in which a single territorial male monopolizes mating opportunities with multiple cohabiting females, large individuals often maintain social dominance over smaller cohabiting individuals (e.g., Robertson 1972; Kuwamura 1984; Sakai and Kohda 1997). As a consequence of the social dominance relationships within local groups, subordinate females generally do not undergo sex changes in the presence of a dominant male, whereas the largest female often changes sex to take over a harem in the event of the disappearance of the dominant male. Such sex-change patterns have been reported in a range of fish families (Labridae: Robertson 1972; Warner and Robertson 1978;

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Hoffman 1985; Warner and Swearer 1991; Scaridae: Choat and Robertson 1975; Pomacanthidae: Moyer and Nakazono 1978; Sakai 1997; Pomacentridae: Fricke and Holzberg 1974; Fricke 1977; Coates 1982; Cirrhitidae: Kadota et al. 2012), and are commonly referred to as take-over sex change (Sakai 1997). This type of transition enables dominant individuals to enhance their reproductive success through sex change, as predicted by the size-advantage model.

A tactical variation in sequential hermaphroditism involves sex change in females that occurs in the presence of a dominant male. Some females have been observed to undergo sex change in the presence of males and immediately form a group as new dominant males, acquiring a portion of the harem females (Labridae: Robertson 1974, Moyer 1991; Pomacanthidae: Moyer and Zaiser 1984, Sakai 1997; Cirrhitidae: Kadota et al. 2012), which is referred to as harem-fission sex change. This type is assumed to be associated with particular social conditions, in which dominant males fail to maintain adequate social interaction with females (Lutnesky 1994; Sakai 1997). A further pattern of sex change occurring in the presence of males is referred to as a bachelor sex change (Sakai 1997), meaning that sex-change females subsequently leave the harem to become bachelor males (Pomacanthidae: Aldenhoven 1984; Moyer and Zaiser 1984; Labridae: Hoffman et al. 1985; Pinguipedidae: Ohnishi 1998; Scaridae: Muñoz and Warner 2003). This pattern has been suggested to confer tactical advantages with respect to growth and/or survival and also increase the opportunity of rapidly assuming dominance in male-loss groups (Aldenhoven 1984, 1986; Moyer and Zaiser 1984; Hoffman et al. 1985; Iwasa 1991). As a consequence of these transitions, it is suggested that sex-changing individuals increase the likelihood of attaining a dominant male status even when sex change is performed during male existence.

The teleost genus *Dascyllus* (Pomacentridae) comprises of 10 species of demersal egg spawners characterized by variations in adult body size and sexual patterns (Yogo 1987; Allen 1991; Godwin 1995). Relatively small species of *Dascyllus* utilize branching-type corals as shelter and breeding sites, in which they form polygynous social groups (Fricke 1977; Schwarz 1980; Coates 1982; Schwarz and Smith 1990; Allen 1991; Sakanoue and Sakai 2019). The fish of this genus have long been suggested to have the ability to change sex (Fricke and Holzberg 1974), and the hermaphroditic sexuality of the polygynous *Dascyllus* species has been demonstrated, mainly on the basis of gonad histology (e.g., *D. aruanus*: Asoh 2003; *D. carneus*: Asoh and Yoshikawa 2003; *D. flavicaudus*: Asoh 2004) and experimental manipulations (e.g., *D. aruanus*: Coates 1982; Tanaka 1999; Kuwamura et al. 2016). To date, however, such sex changes in female *Dascyllus* damselfish are yet to be verified under natural conditions. In an attempt to demonstrate female sex change in the field, Coates (1982) sought to induce sex changes by experimentally removing males from natural groups of *Dascyllus aruanus*, although they failed in this regard, owing to the frequent immigration of other males. Accordingly, it was concluded that the *Dascyllus* species rarely undergo sex changes in nature. Thus, despite the presence of hermaphroditic gonads in *Dascyllus* species, it is yet to be sufficiently ascertained why protogynous sex change has been rarely observed in *Dascyllus* under natural conditions.

Dascyllus reticulatus is a small coral-dwelling damselfish that commonly occurs in subtropical and tropical waters in the eastern Indian Ocean and the western Pacific (Allen 1991; Godwin 1995). In captivity, it has been observed that an individual that was previously breeding as a female changed sex to a male after the death of a larger dominant male (Tanaka 1999). On the basis of histological analyses, Schwarz and Smith (1990) and Asoh (2005) established that *D. reticulatus* individuals of various sizes from natural populations possess transitional gonads (development of testicular tissues in the ovarian gonad structure), indicating the potential occurrence of functional sex change in nature. However, there is no direct evidence of a protogynous sex change in *D. reticulatus* under natural conditions.

Herein, we report the conditions and processes of functional sex changes in *D. reticulatus* confirmed on the reefs off Kuchierabu-jima Island, southern Japan. Using a combination of continuous field observational surveys and periodic sexing of identified individuals within a population of *D. reticulatus*, we confirmed 11 cases of protogynous sex change. These transitions involved not only the take-over type of sex change but also a type giving rise to bachelor males, which appears to differ from previously reported observations of bachelor sex changers, in that the concerned individuals remained within the original colonial group. Based on our observations, we provide a detailed description of the process of sex change, individual characteristics, and the social contexts promoting sex changes in *D. reticulatus*, and discuss the tactical advantages of sex change with a particular emphasis on the novel type of bachelor sex change.

Materials and methods

Study area

This study was conducted on reefs in Nishiura Bay, Kuchierabu-jima Island (30° 28′ N, 130° 10′ E Fig. 1), Kagoshima, southern Japan. The island faces the Kuroshio current in a subtropical biogeographical region, and more than 600 fish species have been recorded in the surrounding reefs (Gushima and Murakami 1976; Kimura et al. 2017). We established a study area of 50 m × 100 m within the bay,
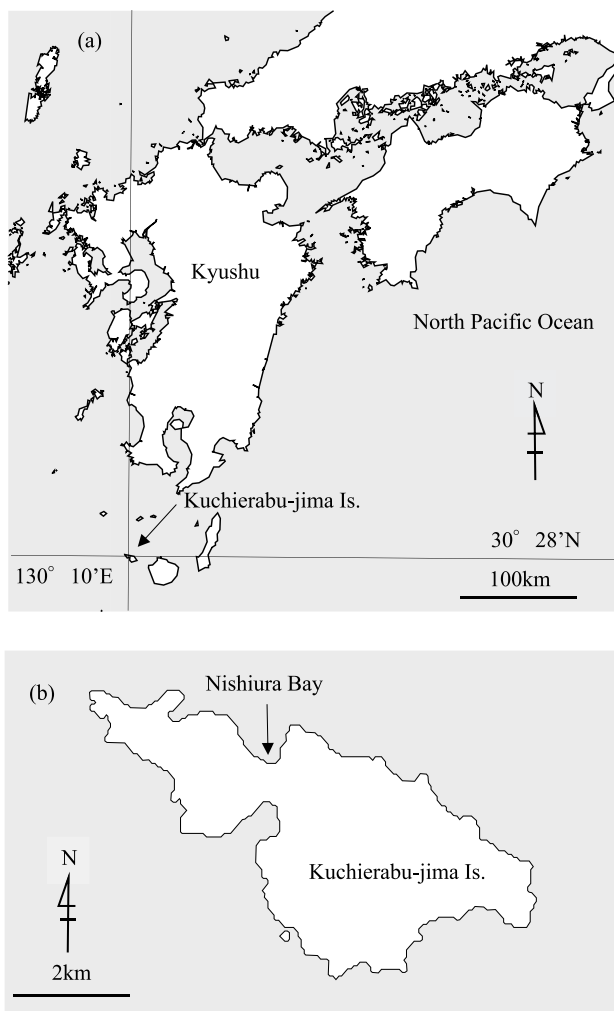
Fig. 1 Location of Kuchierabu-jima Island, southern Japan (**a**) and Nishiura Bay, the northern part of Kuchierabu-jima Island (**b**)

encompassing a population of *D. reticulatus* in a flat reef zone at depths of 4–8 m. The study area was in the same locality, where the colonial group structure of *D. reticulatus* was previously surveyed (Sakanoue and Sakai 2019). The population was observed daily by SCUBA diving during the *D. reticulatus* breeding season, from June to October 2016 and June to November 2017. During the study period, the water temperature ranged from 22.4 to 32.3 °C. Owing to the seasonal occurrence of stormy conditions with high waves and strong northerly winds, we were unable to conduct underwater surveys during the winter and early spring seasons (December to April).

In previous studies on the reproductive ecology of *D. reticulatus,* it has been considered that the damselfish has a sexuality of the monandric protogyny, i.e., all males are derived from sex change of females (Schwarz and Smith 1990). Recently, some small individuals which developed testes directly from juvenile gonads were also confirmed

(Asoh 2005). Although small-sized males were confirmed in the survey population in the present study, their abundance was considerably low and their body sizes were larger than females (Sakanoue and Sakai 2019). We referred that all male individuals including the small ones observed during the survey period were derived from females in this study, i.e., a monandric population.

Field observations and recapture examinations

At the beginning of the study period in each survey year, we captured all *D. reticulatus* individuals within the study area using screen and hand nets. They were anesthetized using diluted clove oil (0.05%), and thereafter, we measured standard length (SL) to the nearest 0.1 mm using calipers, and sexed individuals based on microscopic observations of the shape of its urogenital papilla and gamete release. This was confirmed by gently pressing the abdomen (Sakanoue and Sakai 2019; Fricke 1980). Males possess a long conical papilla, whereas females have a short, thick papilla. A small undeveloped papilla is indicative of juvenile status (Thresher 1984; Mizushima et al. 2000). Small individuals (SL < 35 mm) with a small undeveloped papilla and an inability to discharge gametes when pressed were defined as juveniles. To enable individual identification, a visible implant elastomer tag (Northwest Marine Technology Inc., Shaw Island, WA, USA) was injected subcutaneously into the lateral body. Individuals with an SL of less than 20 mm were not tagged to avoid any potential detrimental effects. After allowing the captured fish to recover from the anesthesia, all individuals were released to the branching corals at the site of capture. A total of 56 and 88 individuals were subsequently identified in 2016 (density: 0.011 individuals/m^2) and 2017 (density: 0.018 individuals/m^2), respectively. To accurately determine the composition of each group, and the growth rate and functional sex of individuals, we recaptured fish to undertake SL measurements and sexing of all individuals after 2 months and repeated this at the end of the survey period. Data on individual growth and gonadal sex changes were obtained using the recapture procedure.

Colonial groups of *D. reticulatus* are formed on branching corals, including those in the genera: *Acropora*, *Pocillopora*, and *Stylophora* (Sakanoue and Sakai 2019). We defined damselfish individuals using the same sheltering coral as comprising a single group, and accordingly recorded seven (A, B, C, D, E, F, and G) and six (A2, C2, D2, E2, H, and I) colonial groups in 2016 and 2017, respectively (A2, C2, D2, and E2 were formed on the same corals as A, C, D and E in 2016, respectively). In this regard, we previously identified two different types of polygynous colonial groups in *D. reticulatus*, depending on the depth of the coral branches (Sakanoue and Sakai 2019). Relatively large individuals often inhabit

corals with long branches (*Pocillopora eydouxi*) and form haremic colonial groups with a female-biased sex ratio (groups A, A2, G, and I), which we designated "long-branch groups" (Sakanoue and Sakai 2019). In contrast, relatively small adult individuals and juveniles coinhabit short-branched corals (*Pocillopora elegans* and *Acropora* aff. *divaricata*) under high-density conditions to form multi-male colonial groups without biased sex ratio (groups B, C, C2, D, D2, E, E2, F, and H), which we designated "short-branch groups" (Sakanoue and Sakai 2019). There were no other colony groups within a 100 m radius of the study area.

In our preliminary survey, we confirmed that *D. reticulatus* in each group started spawning during the morning (07:30 to 09:30), thus, behavioral observations were conducted between 07:00 a.m. and 12:00 a.m. to monitor the mating activities of *D. reticulatus*. To observe reproductive activity, including sexual behavior, we used a total of 99 (30 males, 47 females, and 22 juveniles) individuals within eight groups that we were able to continuously monitor throughout the survey (*Dascyllus* individuals of groups B, C, and D were disappeared in September 2016 due to heavy disturbance by typhoons): 11 individuals in two groups (A and E) and 88 individuals in six groups (A2, C2, D2, E2, H, and I) in 2016 and 2017, respectively. Of 11 observed individuals in 2016, six individuals survived until the start of the 2017 survey (see Table 2). For each of the monitored groups, the duration of the observation period ranged from 10 to 120 min, depending on the extent of mating activity within the group. Morning behavioral observations were conducted for 40–69 successive days in each group, during which we recorded the time and location of male courtship display that guide females to the nest while performing signal jumps (Sale 1971), spawning, and parental egg care behavior. Prior to spawning, females often visited male mating nests (Tanaka 1999). Spawning events were confirmed visually by the presence of eggs in the nest, swollen abdomen, and extended ovipositor of females.

If an individual disappeared from the original colony group, whether or not it moved to another group within the study area was confirmed from the population compositions. If the individual disappeared from the study area, we searched the surrounding area outside the study area, and if it was not found, we assumed that it died due to predation.

Based on field observational surveys of mating behavior and colonial group composition, we obtained data related to social changes, including: individual disappearance, recruitment, and the occurrence of sex-change individuals. To clarify the social differences among individuals who underwent protogynous sex change, we conducted comparative analyses among them, focusing on size rank in the original group, spawning success, growth, and survival.

Statistical analyses

All statistical analyses were performed using R 3.2.2 (R Development Core Team, 2020), with statistical significance set at *P* value < 0.05, and the results are expressed as mean ± standard deviation (SD).

In terms of behavioral crypticity, we identified two types of sex changers (see "Results"): individuals who exhibited male sexual behavior during and after the sex change (non-cryptic sex changers) and those who did not (cryptic sex changers). To analyze the differences in social conditions associated with these two behavioral types, we statistically compared the body size (mm SL) of sex-changing individuals, size ranks of sex changers as females in the original groups, and relative body size ratio (%) of sex changers compared with that of the largest female in the original groups (SL [mm] of a sex-changing individual/SL [mm] of the largest female in the group × 100). Given that the data did not meet the assumptions of homoscedasticity, for the purposes of comparison, we used Welch's two-sample *t* test (Ruxton 2006).

For comparative analyses of reproductive success, growth, and survival among individuals that underwent/did not undergo protogynous sex change, we applied general linear mixed (GLMM) and linear mixed (LMM) models using the "glmer" and "lmer" functions of the "lme4 package", respectively. In performing these analyses, we detected no evidence of overdispersion in any of the models used in this study.

To compare the reproductive success of females, we used spawning data obtained in 2017 for females in the short-branch groups. We observed the highest frequency of sex-change events (see "Results"). For analyses, we applied a GLMM with a Poisson distribution, in which group ID was specified as a random effect and the number of observed female spawning events was defined as the response term. Sex-change and non-sex-change females were used as potential explanatory terms. To compare the number of spawning events, we used the number of morning behavioral observation days in each group as the offset term.

To compare the growth of individuals belonging to the short-branch groups in 2017 (C2, D2, E2, and H), we applied a LMM in which group ID was specified as a random effect. In this model, we defined the growth value (mm/day) of individuals as a response term. The growth value was calculated as the difference in the measured SL (mm) between the start and the end of the study period in 2017 divided by the number of days of the period (124–134 days). To verify the factors that affected individual growth, we included the body size of individuals (measured at the beginning of the survey), three sexual types (sex-change females, non-sex-change females, and males), and the two-way interaction term (between body

size and sexual type) as potential explanatory terms in the full model. The final model was obtained by selecting the model based on the variable reduction method using a likelihood ratio test. If a significant difference was detected among the three types, we performed a post-hoc test using the LMM. *P* values were adjusted using the Bonferroni procedure ($P = 0.0167$).

We compared the individual survival data of six groups (A2, C2, D2, E2, H, and I) which we continually observed in 2017. We applied a GLMM with a binomial distribution, in which group ID was specified as a random effect, and individual survival (death = 0, survival = 1) was defined as a response term. To verify the factors that affected individual survival, we included the body size of individuals (measured at the beginning of the survey), two different colonial groups (long-branch groups = 0, short-branch groups = 1), and sexual types (males = 0, females including sex changers = 1) as potential explanatory terms in the full model. The final model was obtained by selection using variable reduction and likelihood ratio tests.

Results

Sex-change patterns

During the course of the 2-year survey, we identified a total of 11 *D. reticulatus* females (23.4% of 47 females) whose shape of the urogenital papilla had changed to a male morphological type, along with confirmation of sperm ejection based on a laboratory sexing procedure performed at recapture (Table 1). Among the 11 sex-change individuals, nine were previously confirmed to be functional females by microscopic observation of egg production in the laboratory and six had been observed to spawn eggs in nature (Table 1). Thus, we detected protogynous sex changes among females in the *D. reticulatus* study population.

In the long-branch group, where a haremic *D. reticulatus* male monopolized mating activities with three females (group I), the largest *D. reticulatus* female underwent a sex change subsequent to the disappearance of the dominant male (I–f1; Table 1) and thereby assumed the role of the new dominant male that monopolized mating with the remaining

Table 1 Individual conditions and processes of protogynous sex changers in *Dascyllus reticulatus* on reefs of Kuchierabu-jima Island

Individual code	Before the sex change					After the sex change		
	SL (mm)	Behavioral observation period	The last spawning as a female	Number of spawning events	Disappearance of large territorial males	Male sexual behavior	The first spawning as a male	SL (mm)
Long-branch group								
I–f1	55.1	40 days (July 24–Sep. 1, 2017)	Aug. 28	5	Uncertain date during Sep. 2–Nov. 5, 2017	Nov. 5, 2017	(No data)	56.0[b]
Short-branch groups								
C2–f1	39.0	60 days (July 5–Sep. 2, 2017)	No spawning	0	No	Aug. 9, 2017	Aug. 26, 2017	44.7
C2–f2	38.2		July 25, 2017	1	No	Aug. 30, 2017	No	44.7
C2–f4	31.3		Aug. 25, 2017	2	No	(No data)	(No data)	40.0[b]
D2–f7	36.1	61 days (July 6–Sep. 4, 2017)	July 15, 2017	1	No	(No data)	(No data)	46.6[b]
E–f2	40.5	52 days (Sep. 10–Oct. 31, 2016)	Oct. 2, 2016	1	Oct. 12, 2016	No	No	42.8
E–f4	36.6		Oct. 3, 2016	1		No	No	38.6
E2–f3	39.1	66 days (June 28–Sep. 1, 2017)	No spawning	0	July 18, 2017	No	No	47.8
E2–f4	36.1		No spawning	0		No	No	46.6
H–f2	39.2[a]	67 days (June 29–Sep. 3, 2017)	No spawning	0	No	Aug. 25, 2017	No	44.4
H–f8	35.7[a]		No spawning	0	No	No	No	42.7

All 11 sex-change individuals, nine females were initially confirmed as functional females based on microscopic observations of egg production except for two individuals (a) that were confirmed to possess female-type urogenital papillae

Gonadal sex changes (sperm release) were confirmed during recapture at the end of the behavioral observation period, with the exception of three individuals (b) at the end of the study period (November 5–13, 2017)

The individual codes are a combination of the group name, sex, and size rank among females (e.g., E–f2 indicates the second-largest female in group E)

two females. Prior to undergoing sex change, the female had frequently spawned with the former dominant male of the group (Table 1). Following the disappearance of this male, the sex-change female began performing male sexual behavior (signal jumps and nest building) toward cohabitant females (i.e., exhibited behavioral sex change). The completion of gonadal sex change was also confirmed by the occurrence of sperm discharge at recapture immediately after behavioral observations. This was the only instance of a take-over-type sex change observed in this study.

The remaining ten cases of protogynous sex change were detected among individuals in the short-branch group (Table 1). Of these, two (C2–f2 and D2–f7; Table 1) underwent gonadal sex changes in the presence of large males, although no associated behavioral observations were made. An additional three females, two in group C2 (C2–f1 and C2–f2) and one in group H (H–f2), underwent gonadal sex change in the presence of males and exhibited male-type sexual behaviors (Table 1). Of these, only one individual (C2–f1) obtained mating opportunities with a female in the same colonial group within the survey period (Table 1), and thus appeared to be an example of a harem-fission sex change. The other two sex changers became nest-holding males, although they were yet to obtain any mating opportunities by the time monitoring was discontinued.

In contrast, the other five sex-change females (E–f2, E–f4, E2–f3, E2–f4, and H–f8) showed no evidence of male-type sexual behavior during the process of gonadal sex change or even after completing the gonadal transition, despite the development of functional testes (Table 1). Owing to a lack of sexual behavior, the occurrence of gonadal sex change was initially confirmed based on laboratory sexing at the time of recapture. Having undergone gonadal sex change, these individuals remained within their original groups in corals as non-breeding bachelor males and were accordingly designated "cryptic bachelor sex changers".

Following their respective transitions, we observed marked changes in the social status of the two bachelor sex changers. The first of the two, the 4th ranking female (E–f4) in terms of size, had become a bachelor male in group E, which included two larger individuals (E–f1 and E–f2; Table 2A) at the end of the monitoring period in October 2016. At the beginning of the following breeding season, this bachelor male had already acquired dominant male status (E2–m1) and established a mating nest, and actively performed courtship displays to a female (E2–f1). However, the male disappeared prior to spawning with the female, presumably as a consequence of predation (Table 2B). This was the only example (in this study) of a cryptic bachelor sex changer, acquiring dominant male status in its original group.

The second of these two cryptic bachelor sex changers (E–f2) was observed to undertake inter-group migration to a long-branch group, in which a large dominant male (A2–m1) monopolized mating of haremic group females (Table 2C). At the end of October 2016, this bachelor male was the second-largest "male" in its group of origin (group E) (Table 2A). Following inter-group transfer during the non-breeding season, it became the smallest member of long-branch group A2, and was observed to spawn as a female (A2–f5) with the dominant male at the time of the first recapture (June 26, 2017). Thus, these observations indicate that this individual underwent a subsequent reversed sex change to a functional female (Table 2C). The A2–f5 thereafter stably maintained spawning opportunities as a female, with four spawning events subsequently observed over the course of the 69 days during which the group was monitored.

We also recorded a second instance of sex-change-related inter-group migration. A small cryptic bachelor sex changer, originally the 8th size-ranked female in group H (H-f8; Table 1), moved to the long-branch group A2 (August 8, 2017). Sperm release was confirmed at the end of the study period, and this individual retained a bachelor male status and suffered from persistent attacks of the large dominant male in group A2 (Table 2C). However, we were unable to further detect this bachelor male. Thus, in this study, we identified two cryptic bachelor males that undertook inter-group migration to a long-branch group.

Social and habitat conditions associated with the occurrence of sex change

Among the five recorded occurrences of cryptic bachelor sex change, four emerged in colonial groups in which large nest-holding males had disappeared (Table 1). In addition, the presence of larger cohabiting females in these groups tended to indicate the original subordinate social status of the cryptic sex changers (Table 1). This contrasts with the case of those sex changers observed to actively exhibit male sexual behavior (I–f1, C2–f1, C2–f2, and H–f2; Table 1). The size ranks (1.50 ± 0.58, $n = 4$) were found to be significantly higher than those of the cryptic sex changers (4.20 ± 2.28, $n = 5$; $t = 2.55$, $P = 0.05$). With respect to the size difference between the largest female in the group, the mean size (SL) of the cryptic bachelor sex changers was 80.5% that of the largest female (0.81 ± 0.06, $n = 5$). However, the four sex changers exhibiting male sexual behavior had a significantly higher percentage body size compared with that of the largest females (0.96 ± 0.07, $n = 4$; $t = 3.70$, $P = 0.01$). There was, however, no significant difference in the body sizes of the sex changers exhibiting sexual behavior (42.87 ± 8.16 mm, $n = 4$) and the cryptic sex changers (37.60 ± 2.09 mm, $n = 5$; $t = 1.26$, $P = 0.28$; Table 1). Thus, whereas the sex

Table 2 Compositions of *Dascyllus reticulatus* colonial groups promoting cryptic bachelor sex changes (bold) and those related to post-sex change events

Individual code	Sep. 10, 2016		Number of spawning events	Social changes	Oct. 31, 2016	
	SL (mm)	Gonadal sex			SL (mm)	Gonadal sex
(A) Short-branch group E in 2016						
E–m1	55.5	Male	5	Disappeared on Oct. 12		
E–f1	50.2	Female	3		50.2	Female
E–m2	44.7	Male	0	Disappeared on Sep. 21		
E–f2	**40.5**	**Female**	**1**	**Cryptic bachelor sex change**	**42.8**	**Male**
E–f3	39.4	Female	0	Disappeared on Sep. 21		
E–f4	**36.6**	**Female**	**1**	**Cryptic bachelor sex change**	**38.6**	**Male**
E–f5	35.0	Female	0	Disappeared on Sep. 21		
E–f6				Recruited on Sep. 21	41.2	Female
Individual code	June 28, 2017		Number of spawning events	Social changes	Sep. 1, 2017	
	SL (mm)	Gonadal sex			SL (mm)	Gonadal sex
(B) Short-branch group E2 in 2017						
E2–m1 (E–f4)	45.6	Male	0	Disappeared on July 13		
E2–f1 (E–f6)	45.4	Female	0	Disappeared on July 18		
E2–m2	**44.1**	**Male**	**2**	**Disappeared on July 18**		
E2–f2	44.1	Female	2		48.0	Female
E2–f3	**39.1**	**Female**	**0**	**Cryptic bachelor sex change**	**47.8**	**Male**
E2–f4	**36.1**	**Female**	**0**	**Cryptic bachelor sex change**	**46.6**	**Male**
Individual code	Jun. 26, 2017		Number of spawning events	Social changes	Sep. 2, 2017	
	SL (mm)	Gonadal sex			SL (mm)	Gonadal sex
(C) Long-branch group A2 in 2017						
A2–m1 (A–m1)	63.5	Male	15		63.5	Male
A2–f1 (A–f1)	59.2	Female	4		60.5	Female
A2–f2 (A–f2)	52.7	Female	4		53.6	Female
A2–f3	54.6	Female	3		55.5	Female
A2–f4	51.6	Female	0		54.5	Female
A2–f5 (E–f2)	**48.0**	**Female**	**4**	**Moved from group E and reversed sex change**	**55.5**	**Female**
H–f8				**Moved from group H on Aug. 8**	**42.7**	**Male**

The pre-change identities of individuals are shown in parentheses

changers exhibiting male sexual behavior were derived from dominant or near-dominant females, the cryptic sex changers were invariably from among the smaller females in the groups.

We found that sex-change individuals in the short-branch groups typically experienced poor spawning opportunities as females (Table 1). However, it was not only sex-change individuals who were unable to obtain mating opportunities. Among the 24 non-sex-change females in the short-branch groups, 11 also showed no spawning activity during 60–67 days of monitoring in 2017. As a consequence of inactive reproductive condition in the short-branch group, the number of spawning events of females in the short-branch groups that underwent sex changes in 2017 (0.50 ± 0.75 times, $n=8$) was not significantly different from that of other non-sex-change females in the groups (1.12 ± 1.62 times, $n=24$; Poisson GLMM: $x^2=0.62$, $P=0.43$).

Nevertheless, we detected significant differences among the three sexual types in short-branch groups (males: 0.06 ± 0.01 mm/day, $n=9$; females: 0.06 ± 0.01 mm/day, $n=10$; sex changers: 0.07 ± 0.01 mm/day, $n=6$) with respect to growth during the survey period (LMM: $F=4.27$, $P<0.05$). Growth was found to be significantly negatively correlated with body size (LMM: $F=4.54$, $P<0.05$; Table 3). The two-way interaction term (between body size and sexual type) was removed from the final model during the stepwise process ($P>0.05$), which indicated that the regression coefficients (slopes) were all parallel among

Table 3 Factors affecting the growth rate of *Dascyllus reticulatus* in the short-branch groups on reefs of Kuchierabu-jima Island

Explanatory variable	Estimate	SE	df	F	P
Intercept	0.12	0.03	–	–	–
Body size (mm SL)	−0.002	<0.01	1	4.54	<0.05
Males vs. females	0.01	0.01	1	2.44	0.13
Sex changers vs. females	0.02	0.01	1	9.20	<0.01
Sex changers vs. males	0.01	0.01	1	1.36	0.25

Data were obtained during the survey period from June to November in 2017

The final model was selected based on the likelihood ratio test using linear mixed models

Table 4 Factors affecting the survival rate of *Dascyllus reticulatus* on reefs of Kuchierabu-jima Island

Explanatory variable	Estimate	SE	df	x^2	P
Intercept	9.38	0.59	–	–	–
Body size (mm SL)	−0.14	0.48	1	5.51	<0.05
Group type (long-branch or short-branch)	−3.65	0.01	1	4.89	<0.05

Data were obtained during the survey period from June to November in 2017

The final model was selected based on a likelihood ratio test using a binomial general linear mixed model

the three sexual types and the growth value was higher for smaller individuals (Fig. 2). Furthermore, a post hoc (LMM) test revealed no significant differences between the growth of females and that of males (LMM: $F = 2.44$, $P = 0.13$), or between males and sex changers (LMM: $F = 1.36$, $P = 0.25$). In contrast, the growth of sex-change individuals in the short-branch group was significantly higher than that of non-sex-changing females in these groups (LMM: $F = 9.20$, $P < 0.01$).

During the course of the survey, we occasionally observed instances of predation on *D. reticulatus* individuals, notably by carnivorous fish, such as the groupers *Cephalopholis urodeta* and *Variola albimarginata,* and the sandperch *Parapercis millepunctata*. In applying analyses of GLMM with a binomial distribution, the sexual type (male or female) was removed from the final model during the stepwise process ($P > 0.05$). We established that the disappearance of *D. reticulatus* individuals in the short-branch group (66.1%; 37 of 56 individuals) was significantly higher than that in the long-branch group (27.3%; 3 of 11 individuals) (binomial

GLMM: $x^2 = 4.89$, $P < 0.05$; Table 4). Furthermore, we found that the survival of individuals was significantly negatively correlated with body size (binomial GLMM: $x^2 = 5.51$, $P < 0.05$; Table 4), indicating that regardless of belonging coral types, larger individuals have a higher risk of mortality.

Discussion

Among fish within family Pomacentridae, protogynous sex change has previously been confirmed in *D. aruanus* based on in situ male-removal experiments conducted on reefs (Coates 1982; Kuwamura et al. 2016). To the best of our knowledge, however, this study is the first in which protogynous sex change has been observed in pomacentrids under natural conditions, and the first to characterize the different patterns of protogynous sex change in the family. In addition, consistent with the findings of Kuwamura et al. (2016) who reported the phenomenon of reversed sex change in *D. aruanus* based on removal experiments, we recorded

Fig. 2 Relationship between the body size (mm SL) of males (circles; $n = 9$), females (triangles; $n = 10$), and sex changers (rhombus; $n = 6$) of *Dascyllus reticulatus* at the beginning of the survey period and their subsequent growth rates (mm/day) in the short-branch groups. Solid, dotted, and broken linear regression lines show the relationships for males, females, and sex changers, respectively. Data were obtained from June to November 2017
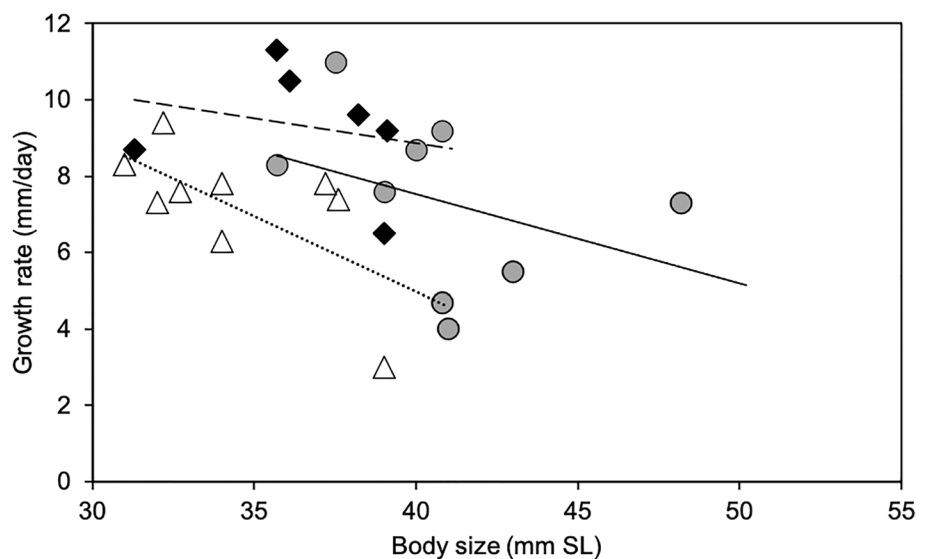
an instance, where a female *D. reticulatus* that had initially undergone sex change to a bachelor male subsequently exhibited a reversed sex change back to a female (Table 2). This is the first time this has been observed under natural conditions.

The *D. reticulatus* females surveyed in the study underwent sex change not only to assume dominance of a haremic group subsequent to the loss of the dominant male (in the long-branch group) but also in an attempt to obtain females as new nest-holding males in the presence of a dominant male (in three short-branch groups). The processes of these two sex-change patterns closely resemble take-over sex change and harem-fission sex change, respectively, which have been confirmed in several protogynous species of fish (Robertson 1972, 1974; Moyer and Nakazono 1978; Aldenhoven 1984, 1986; Kuwamura 1984; Lutnesky 1994; Sakai 1997; Kadota et al. 2012). Sex change in these species has been suggested to be socially controlled by behavioral and visual interactions with local group members, particularly dominant individuals (Robertson 1972; Ross et al. 1983; Warner 1988; Munday et al. 2006). In this regard, during the 2-year survey, we recorded only a single take-over sex change event in long-branch groups of *D. reticulatus*, in which the large dominant male monopolized mating in a harem of females with low individual density (Sakanoue and Sakai 2019). Therefore, it is plausible that dominant males exercise social control over the sex change of females in low-density harem groups.

Short-branch groups of *D. reticulatus* tend to be characterized by high individual density and low mating activity, with the individual density of the short-branch groups being approximately four times higher than that of long-branch groups (Sakanoue and Sakai 2019). Furthermore, we have established that, despite being reproductively active, most short-branch group males only mate with a single female (Sakanoue and Sakai 2019). It has also been suggested that in some haremic protogynous fishes, females with limited mating opportunities tend to initiate sex change even in the presence of males (Moyer and Zaiser 1984; Sakai 1997; Hamaguchi et al. 2002), which appears to be a consequence of the social conditions in short-branch groups of *D. reticulatus*. Moreover, it has been proposed that an increase in group size or the density of cohabiting individuals within a group often results in the weakening of social control, thereby promoting protogynous sex change in females (Yogo 1987; Lutnesky 1994; Sakai 1997). Consequently, the high population density and lack of mating interactions in short-branch groups may be important factors that promote sex change in individuals, even in the presence of males.

Bachelor males derived from protogynous sex change have been recognized in a range of different reef fishes. Moyer and Zaiser (1984) observed bachelor sex changers in some haremic *Centropyge* angelfishes wandering as floaters inspecting surrounding harems, suggesting that this status may be conducive to assessing the conditions of local groups, thus enabling them to rapidly attempt to take over the control of harems that are currently without males. Owing to intense male–male competition with respect to establishing mating territories, in the case of the bluehead wrasse *Thalassoma bifasciatum,* the bucktooth parrotfish *Sparisoma radians,* and the sandperch *Parapercis snyderi*, bachelor males can endure prolonged periods before attaining mating male status (Hoffman et al. 1985; Warner 1988; Ohnishi 1998; Muñoz and Warner 2003). Thus, as males, bachelor sex changers typically disperse from their groups of origin, contrasting with the behavior of the *D. reticulatus* cryptic bachelor sex changers observed in this study, which would appear to represent a novel form of bachelor sex change in terms of cohabitation within the group.

Genus *Dascyllus* individuals are generally considered to experience high predation pressure (Sale 1971; Fishelson et al. 1974; Sakanoue and Sakai 2019), particularly in the short-branch groups typically cohabitated by cryptic bachelor sex changers, in which individuals were found to be notably vulnerable to predation and suffer higher mortality rates (Table 4). Accordingly, it is assumed that such high predation pressure would markedly restrict the active movement of *D. reticulatus* beyond the confines of the host coral, notably in the case of bachelor sex changers. Male sexual behavior typically increases the likelihood of being attacked, not only by predatory fish but also by conspecific breeding males, owing to the necessity to emerge from safe sheltering habitats and general conspicuousness (Thresher 1984). This would be particularly applicable in the case of males of the coral-dwelling *D. reticulatus*, among which large individuals are at a greater risk of mortality, as indicated by the results in this study. It is speculated that large individuals would be more conspicuous to their predators because of their actively behavior including courtships and maintenance. Thus, the observed behavioral concealment of *D. reticulatus* bachelor sex changers is believed to be an adaptive form of survival.

Nevertheless, we observed a cryptic sex changer (E–f4) of *D. reticulatus* that performed male sexual behavior after acquiring dominant status (Tables 1 and 2), thereby indicating the potential of the cryptic bachelor sex changers of *D. reticulatus* to exhibit male sexual behavior. Similar periodic control of sexual behavior has previously been reported among the small males of diandric labrid fishes (Warner and Robertson 1978; Warner 1984; Sakai et al. 2002a). For example, on the basis of the findings of a male-removal study on the diandric labrid, *Halichoeres melanurus*, Sakai et al. (2002a) confirmed that the social status of females affected sexual behavior during the transitional phase of sex change, with large females tending to engage in male sexual behavior during the process of protogynous sex change. This would conceivably also apply in the case of *D. reticulatus*,

given that all sex changers exhibiting male sexual behaviors were comparatively larger in size as compared to females. However, none of the four cryptic bachelor sex changers observed in this study were characterized by large body size. They were commonly found cohabiting with larger females. Accordingly, we speculate that a subordinate size rank within the local groups may be associated with the concealment of sexual behaviors in cryptic bachelor sex changers.

In various protogynous fishes, it has previously been confirmed that sex-changing females often exhibit male sexual behavior toward smaller females even before completing the gonadal transformation to functional males (Godwin et al. 1996; Nakashima et al. 2000; Sakai et al. 2002a, b). In contrast, even after completing the gonadal transformation, the cryptic bachelor sex changers observed in this study did not exhibit sexual behavior (i.e., sperm release). In this context, it has been suggested that the display of sexual behavior by sex changers functions in retaining smaller females as future mates (Nakashima et al. 2000). In addition, all sex changers including the cryptic bachelor males never been observed an alternative male mating tactics as sneaker which is confirmed in other harem polygynous fishes (Warner et al. 1975). Conversely, it is inferred that females who undergo sex change to cryptic bachelor males do not do so to attain immediate male mating status.

A take-over sex changer in the long-branch harem and three high size-rank sex changers in the short-branch groups showed sexual behavior as males, which implies that a change of sex is undertaken to achieve reproductive success in males. However, this would not apply to subordinate females in the short-branch groups, which were characterized by behavioral crypticity as males. Accordingly, we suggest that a bachelor sex change of *D. reticulatus* is not invariably undertaken to gain reproductive status as a male. Under conditions deemed unfavorable with regard to survival or reproductive success, such as a lack of reproductive opportunities (i.e., short-branch group in this study), females may, for example, undergo sex change to attain bachelor male status as a tactic to evade potentially detrimental outcomes and enhance future prospects. Sex change might thus be seen as a tactic that facilitates tolerance to current unfavorable conditions in short-branch groups.

We suspect that moving from a short-branch group to a long-branch harem could represent a particularly important life-history strategy in coral-dwelling damselfish. In the population studied, it has previously been established that females in long-branch groups are characterized by an approximately threefold higher spawning frequency than those cohabiting in short-branch groups (Sakanoue and Sakai 2019). In this context, it is anticipated that gaining access to a long-branch group would enhance subsequent mating opportunities. Moreover, assimilation into a long-branch group would be advantageous in terms of individual

survival, given the lower rates of predation in these groups. Thus, for females in short-branch groups, migration to a long-branch harem could be an essential strategy for attaining higher reproductive success. This supposition is partially supported by our observation of the migration of two cryptic bachelor males to a long-branch group (Table 2). In this context, cryptic bachelor sex changes promoting rapid growth may serve as a backup mechanism facilitating inter-group migration.

The growth advantage is often emphasized as a life-history factor promoting sex change (e.g., the growth-advantage model; Iwasa 1991; Nakashima et al. 1995). It has been suggested that bachelor sex changers maximize their growth potential at the expense of current reproductive opportunities, and achieving rapid growth is beneficial in terms of the acquisition of social status that facilitates the dominance of mating opportunities (Moyer and Zaiser 1984; Warner 1988). As observed in this study, the cryptic bachelor sex changers of *D. reticulatus* did not appear to immediately acquire mating status as males, which is consistent with the pattern typically observed in bachelor sex changers (Aldenhoven 1984, 1986; Moyer and Zaiser 1984; Warner 1988; Ohnishi 1998). However, as found in this study, cryptic bachelor sex change can contribute to the subsequent acquisition of mating status by the bachelor males of *D. reticulatus* (Table 2), thereby indicating a tactic facilitates rapid growth. It is suggested that such rapid growth would be advantageous in terms of inter-group migration, as observed in the study, which is consistent with the precepts of the growth-advantage theory of bachelor sex change (Moyer and Zaiser 1984; Warner 1988). Further research is warranted to examine the extent of the inter-group movement of individuals subsequent to a cryptic bachelor sex change and to establish the means which they use to acquire reproductive opportunities.

Perhaps the most surprising aspect of this study is our finding that a sex-change bachelor male of *D. reticulatus* acquired mating status as a female again, via the sequential processes of group migration and reversed sex change (Table 2), which represents a novel facet of our current understanding of the characteristics of bachelor sex changers. A second bachelor male was also observed to undertake inter-group movement to long-branch group A2 as a small member (H-f8). Although we were unable to observe this bachelor male, it is plausible that this individual also acquired mating opportunities as a female, as in the case of E–f2. A similar reversion of small males to females triggered by group intrusion, as a consequence of cohabitation with larger males, has been observed in a range of protogynous fishes in recent years, including *Dascyllus* (Munday et al. 2010; Kadota et al. 2012; Kuwamura et al. 2016), and the reversed sex changes recorded in these studies appear to be consistent with the patterns observed in the current study.

Collectively, these findings tend to indicate that the sex of *D. reticulatus* is determined by dominance relationships based on the size of individuals within the local group, as is the case with the take-over type of sex change. However, the fact that a bachelor sex changer opted to join a group containing larger males is something that has not been considered in previous theories.

The cryptic bachelor sex change was the most frequently observed sex change pattern in the study population, which may account for the failure of previous observational studies to confirm sex changes in *D. reticulatus* in nature. Based on the findings of this study, we can hypothesize that cryptic bachelor sex changes occur in subordinate females that previously had limited opportunities to lay eggs. However, some females that did not undergo sex change were unable to gain mating opportunities, thereby indicating that there is a certain possibility of adopting a future role as a cryptic bachelor male. Furthermore, the fact that bisexual gonads have been identified in histological studies of field-sampled individuals from natural colonies of *Dascyllus* species [*D. aruanus* (Asoh 2003), *D. carneus* (Asoh and Yoshikawa 2003), *D. flavicaudus* (Asoh 2004)], and *D. reticulatus* (Schwarz and Smith 1990; Asoh 2005) could indicate that cryptic sex change is a common phenomenon in various coral-dwelling *Dascyllus* damselfish. We anticipate that future studies focusing on the functions and mechanisms of cryptic bachelor sex changes in haremic colonies of *Dascyllus* damselfishes will advance our current understanding of the functional aspects of sexual plasticity in the life history of coral-dwelling fishes.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical notes All procedures performed in this study followed the Guidelines for the Proper Conduct of Animal Experiments and related activities laid down by the Hiroshima University Animal Research Committee (No. 020A170410 certified on April 10th, 2017), the ASAB/ABS Guidelines for the Use of Animals in Research (Guidelines for the Treatment of Animals in Behavioral Research and Teaching; https://doi.org/10.1016/j.anbehav.2019.11.002), the Guidelines for the Use of Fishes in Research by the Ichthyological Society of Japan (http://www.fish-isj.jp/english/guidelines.html), and the Guideline for Ethological Studies by the Japan Ethological Society (http://www.ethology.jp/guideline.pdf).

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