

Bidirectional sex change in mushroom stony corals

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Sex change occurs when an individual changes from one functional sex to another. The direction of sex change occurs mainly from male to female (protandry) or vice versa (protogyny), but sometimes may be bidirectional (repetitive). Here, for the first time in stony corals, we report on a protandrous sex change exhibited by two mushroom corals, *Fungia repanda* and *Ctenactis echinata*, with the latter also exhibiting bidirectional sex change. Compared with *C. echinata*, *F. repanda* exhibited relatively earlier sex change, significantly slower growth and higher mortality rates, in accordance with sex-allocation theory. Sex ratio in both the species was biased towards the first sex. The bidirectional sex change displayed by *C. echinata* greatly resembles that of dioecious plants that display labile sexuality in response to energetic and/or environmental constraints. We posit that, similar to these plants, in the studied corals, sex change increases their overall fitness, reinforcing the important role of reproductive plasticity in scleractinian corals in determining their evolutionary success.

Keywords: coral reproduction; *Fungia*; sex change; sequential hermaphroditism; reproductive strategies; reproductive success

1. INTRODUCTION

Stony corals (order Scleractinia), the frame builders of coral reefs, are sessile organisms that exhibit an extraordinary diversity of life-history traits (at the individual, species and population levels) that determine their reproductive patterns. Through symbiosis with unicellular algae (zooxanthellae), which largely supply their energetic requirements (photosynthates), hermatypic (reef-building) corals are major sources of the high primary productivity of coral reefs (e.g. Falkowsky *et al.* 1984; Sorokin 1993). Despite intensive studies and increasing knowledge over the last three decades of the various reproductive modes in scleractinian corals (Fadlallah 1983; Schlesinger & Loya 1985; Babcock *et al.* 1986; Heyward *et al.* 1987; Harrison & Wallace 1990; Richmond & Hunter 1990; Hayashibara *et al.* 1993; Hall & Hughes 1996; Kramarsky-Winter *et al.* 1997; Schlesinger *et al.* 1998), we are still far from recognizing the large variety of this group's reproductive strategies.

Although the majority of animals reproduce as gonochores (having separate sexes), many reproduce as hermaphrodites that can be either simultaneous (functioning as both sexes simultaneously) or sequential (exhibiting sex change as a normal part of their life history). Sequential hermaphroditism in animals (e.g. Warner 1975; Charnov 1979, 1982; Policansky 1981) and plants (e.g. Freeman *et al.* 1976, 1980; Charnov & Bull 1977; Policansky 1981, 1982; Zimmerman 1991; Korpelainen 1998; Yamashita & Abe 2002) has been the subject of a variety of theoretical and empirical evolutionary studies. Complex and sometimes conflicting evolutionary forces determine whether animals and plants are male, female or hermaphrodite throughout their lives, or whether they switch from one functional sex to another. Although in most animals the change in sex will occur only once in an individual's lifetime, either from

male to female (protandry) or vice versa (protogyny), it may sometimes be bidirectional (i.e. repetitive; Munday *et al.* 2006).

Part of the sex-allocation theory (i.e. the way in which organisms allocate resources to male and female function; Charnov 1982), the size-advantage hypothesis (SAH), predicts that sex change is favoured when reproductive success (fitness) increases more quickly with size or age in one sex than in the other (Ghiselin 1969; Warner 1975; Charnov 1979, 1982). The direction of sex change (protandrous or protogynous) is determined by the relative reproductive success over the course of a lifetime for the two sexes. The optimal size at sex change occurs when the potential subsequent lifetime reproductive output as the second sex exceeds that of remaining as the first sex. A protandrous sex change is favoured if female fitness increases at a slower rate than male fitness when young or small, but accelerates above that of the male when larger sizes or older ages are reached. The reverse holds true for a protogynous sex change. The evolution of such labile sex-allocation mechanisms enables the organisms to maximize their genetic contribution to the next generation under the prevailing circumstances. If there is no difference in the reproductive value between the sexes across the course of a lifetime, selection favours separate sexes with no sex change (i.e. gonochorism; Ghiselin 1969; Warner 1975, 1988; Charnov 1982; Iwasa 1991).

Most studies on animal sex change in either direction (i.e. from male to female or female to male) have been conducted on fishes, the largest and only vertebrate group in which this phenomenon occurs (Munday *et al.* 2006). In invertebrates, it is known to occur in some echinoderms, crustaceans, molluscs and polychaete worms (Policansky 1982; Munday *et al.* 2006). Sex change in both directions (bidirectional sex change) has been reported from several species of animals whose local structure is socially controlled and occasionally changes,

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Table 1. Notation and computations used.

N_m	number of breeding males
N_f	number of breeding females
SR	sex ratio in the population = $N_m/(N_m + N_f)$
W	weight in grams (g)
W_{\max}	weight (g) of the largest observed individual
$A_v W_{sc}$	average weight (g) at sex change
W_r	relative size (W) at sex change = $A_v W_{sc}/W_{\max}$
n	number of reproductive corals = $N_m + N_f$
NR	number of non-reproductive corals
N_t	total number of living individuals in the monitored population = $n + N_{NR}$
$A_v \pm$ s.d.	average \pm 1 s.d.
M-F; F-M	direction of sex change: male to female and female to male
F-M-F; M-F-M	bidirectional sex change from female to male to female and from male to female to male

i.e. in some coral-reef fishes (e.g. Munday *et al.* 1998; Munday 2002; Muñoz & Warner 2003; Manabe *et al.* 2007) and in some mobile invertebrates (e.g. Warner *et al.* 1996; Collin 2006; Munday *et al.* 2006).

Here, for the first time in stony corals, we report on two fungiid species, *Fungia repanda* and *Ctenactis echinata*, exhibiting protandrous sex change, with the latter also exhibiting bidirectional sex change. These corals reveal reproductive tactics analogous to those of plants, which exhibit labile sexuality in response to energetic and/or environmental constraints. Our major aim during the initial stages of this research was to study the reproductive modes of several fungiid species co-occurring in the same habitat. However, our discovery of the two fungiid species changing sex, first observed during their breeding period of August 2006, mediated a change in the focus of our study to that of the timing and relative sizes at sex change, growth rates, mortality rates and sex ratios of the two populations.

Our knowledge of the processes that contribute to fitness measures (i.e. fitness gain curves, *sensu* Ghiselin 1969; Warner 1975; Charnov 1982) allows us to formulate hypotheses to test predictions of the sex-allocation theory. Some of the most notable successes of the sex-allocation theory (Charnov 1982) have been in explaining cases in which individuals adjust their offspring sex ratio in response to local conditions (Charnov 1982; West *et al.* 2002; Allsop & West 2004; Collin 2006). However, sex-allocation theory has been much less successful in predicting and explaining variation in the overall population sex ratio (West *et al.* 2002). The reason for this is that there are conceptual and practical difficulties in measuring reproductive success (fitness) in plants and animals, since this often depends upon life-history details that are rarely known (e.g. West *et al.* 2002; Allsop & West 2004). A notable exception to this is in sex-changing organisms, where the theory predicts that organisms should have a sex ratio biased towards the 'first sex' (i.e. the sex at which individuals begin their life; Charnov 1982; Warner 1988; Munday 2002; West *et al.* 2002; Allsop & West 2004; Collin 2006). Given that the relative number of males and females in a population should be inversely related to the relative reproductive success of each sex (Charnov 1993; Allsop & West 2004), it follows that when the second-sex (i.e. male) fertility exceeds the first-sex (i.e. female) fertility, sex ratios should be female biased (Allsop & West 2004). The latter authors analysed data on adult sex ratios in 121 sex-changing animal species and found support for the theoretical prediction that the

sex ratio is biased towards the first sex. However, Collin (2006) presented data on the size, sex and grouping of individuals of 19 species of calyptraeids, a family of protandrous marine gastropods, and found that, across all species, the sex ratio was not significantly more often biased towards the first sex than it was towards the second sex. Hence, it was interesting to test in the two sex-changing fungiid populations, whether the sex ratios observed match theoretical predictions.

The SAH has been largely applied to mobile animals that exhibit social control of sex change, e.g. some fishes (e.g. Munday *et al.* 1998; Munday 2002; Muñoz & Warner 2003; Manabe *et al.* 2007) and some mobile invertebrates (e.g. Warner *et al.* 1996; Collin 2006; Munday *et al.* 2006). However, its applicability has not been addressed in sessile or very low mobility sex-changing animals that lack any obvious social interactions. The SAH predicts that sex change will occur earlier in populations exhibiting slower growth rates and higher mortality rates (Ghiselin 1969; Warner 1975; Charnov 1979, 1982; Iwasa 1991; Munday 2002; Munday *et al.* 2006). The detailed data obtained on these parameters for the fungiid populations during 2004–2007 enabled us to test these hypotheses on these sessile corals, which in all probability lack social control of sex allocation (table 1).

2. MATERIAL AND METHODS

(a) Study site and sampling

Fungiids are solitary, single-polyped, 'free-living' species, which differentiates them from the majority of scleractinians that are sessile, clonal species. However, the term free-living may be misleading, since they are capable of minimal movement (a few centimetres per year, mainly by small-sized individuals; Chadwick & Loya 1992), enabling them to somewhat improve their microhabitat environmental conditions (e.g. shading, overturning, competition for space). The reproductive modes of corals in the family Fungiidae are relatively poorly known (Kramarsky-Winter & Loya 1998). During the initial stages of the research, and as part of a wider research scope aiming to study reproductive strategies in the Fungiidae, in June 2004, we haphazardly collected 10–20 individuals of 10 fungiid species from a patch reef near Sesoko, Okinawa, Japan (26°39' 50" N, 127°52' 24" E). The site is inhabited by tens of thousands of specimens (probably greater than 12 fungiid species) densely distributed between 3 and 16 m in depth. Samples of both the species (figure 1a–d) comprised individuals ranging from small to

very large (*F. repanda*, 85–380 mm in diameter and *C. echinata*, 125–385 mm in length; figures 2 and 3). These were transferred to the laboratory and placed in five 2 m³ running seawater containers (figure 1e,f).

Each sampled coral was tagged individually with a numeric plastic tag attached to it by a nylon fishing cord (figure 2a,b) inserted through a thin hole drilled at the edge of the coral skeleton with a portable dental drill. No adverse effects on the corals were observed due to the tagging procedures. In the laboratory, using Vernier calipers, we measured the length (*L*, mm) and width (*W*, mm) of each coral to the nearest 0.1 mm. The wet weight (*W*, g) of each coral was weighed to the nearest 0.1 g, after removing excess moisture. After two weeks of acclimatization in the large running seawater containers, each coral was placed in an individual 40 × 20 × 20 cm aquarium (figure 2a,b), all of which were then placed in the large containers (figure 1e,f). On 3 July 2004 (full moon), we began nightly observations of the corals for possible spawning (figure 1c,d) between 17.00 and a few hours after dawn, for eight consecutive nights (after closing the seawater supply and lowering the water level in the large containers, thus avoiding any water connection between individual aquaria). We recorded the sex of each individual coral by noting the unique shape of the shed gametes (figure 1e,f). We verified our observations using microscope preparations of the gametes. In the morning, we renewed the running seawater supply. Similar procedures and observations were repeated during August 2006 and July to September 2007. As a result of the sex change exhibited by *F. repanda* and *C. echinata*, first observed during their breeding period of August 2006, we subsequently increased the sample sizes of both the species, now becoming the major species to be studied (table 2). After each annual set of measurements and observations, the tagged corals were transferred back to their natural habitat and retrieved in June of the following year (i.e. one to two weeks before the breeding season). To ascertain the reproductive state of the corals, we collected five large specimens of each species, on a monthly basis, from January 2007 to October 2007, for histological studies.

(b) Data analysis

Fisher's exact probability test of independence was used to test differences in the mortality rate between the two fungiid populations during 2004–2007. The χ^2 -test was used to test the level of deviation from a sex ratio of 0.5 in each fungiid population. A *t*-test was used to test the possible differences in the average weight (in grams) at sex change between the two populations, and to compare (after arcsine transformation of the data) the relative weight (W_r) at sex change between the two populations.

3. RESULTS

Both *F. repanda* and *C. echinata* have a similar depth distribution (3–6 m in depth), residing side by side on the reef in a patchy spatial distribution and microhabitats that vary in quality. Some individuals were observed in narrow crevices with low illumination, while others resided in well-illuminated areas of the reef; some were partially buried in sand, some were situated upside down and a few were partially injured, but the majority of individuals in both populations appeared healthy. While many other coral species had died at this reef site during the 1998

catastrophic bleaching event in Okinawa (Loya *et al.* 2001), most fungiids had survived.

In 2004, most individuals of *F. repanda* and *C. echinata* spawned five nights after the full moon of July and August, for three consecutive nights (table 2). In both the species, spawning started at approximately 22.00 and continued throughout the night, with different specimens spawning at different hours until the next morning (figure 1c,d). Some specimens spawned six and seven nights after the full moon, a few on all the three nights, and a few specimens did not spawn at all. In August 2006, spawning again occurred five to seven nights after the full moon (no observations were made in July), at precisely the same time as in 2004. Of the *F. repanda* breeding population, 28% ($n=14$) had changed sex (M–F), and among the *C. echinata* breeding corals, 55% ($n=9$) had changed sex (3M to 2F and 3F to 3M). Ten new individuals of *C. echinata* were added to the population in July 2006 (6M, 3F and 1NR; table 2).

In 2007, we monitored the populations starting on the full moon of 31 May. No spawning took place on any of the following seven nights. In July, five to seven nights after the full moon of 30 June, most individuals of *C. echinata* spawned extensively, 80% of those from the original 2006 population had changed sex, out of which approximately 27% changed to the sex recorded in 2004 (i.e. exhibited bidirectional sex change). The sex distribution among the 57 new individuals added to the *C. echinata* population in June was 30M, 11F and 16NR (table 2). Unlike previous years, *F. repanda* did not spawn in July 2007. In August, five to seven nights after the full moon of 30 July, both the species spawned, at the same time as in the previous years. Nevertheless, due to the relatively high mortality in this species (21% of the original 2004 population ($n=14$) and 28% of the remaining 2006 population ($n=14$)), only two corals spawned, out of the 10 which had survived from the 2006 population, and neither of them changed sex. However, out of the 40 new individuals added to the *F. repanda* population in June 2007, 60% were males, 23% were females and 17% were NR.

All the histological preparations of corals, which were collected at the end of May 2007 (i.e. one month prior to their spawning time), exhibited either male or female gonads.

The size (weight) at first reproduction and weight range of individuals in which sex change occurred differed between the species (table 3; figure 3). The smallest reproducing individuals in both the species were all males (the first sex). The weight of first-sex individuals in *F. repanda* ranged from 100 to 300 g ($n=6$), while in *C. echinata* it was from 300 to 700 g ($n=16$; figure 3). While the weight at sex change in *F. repanda* occurred at a relatively small average weight (515 ± 91 g) and within a narrow range (385–685 g), in *C. echinata* sex change occurred at a significantly larger ($p < 0.0001$) average weight (1337 ± 393 g) and within a much wider range (700–2200 g), which is an intermediate weight range for this species (table 3; figure 3). At sizes above 1800 g, the vast majority of individuals in *C. echinata* were females (9 out of the 10 individuals were females, i.e. sexual size dimorphism). In *F. repanda*, none of the very large specimens (above 1800 g; $n=8$) reproduced during the 3-year study (figure 3).

Individual corals of the two fungiid populations changed sex at different relative sizes, W_r : *F. repanda*

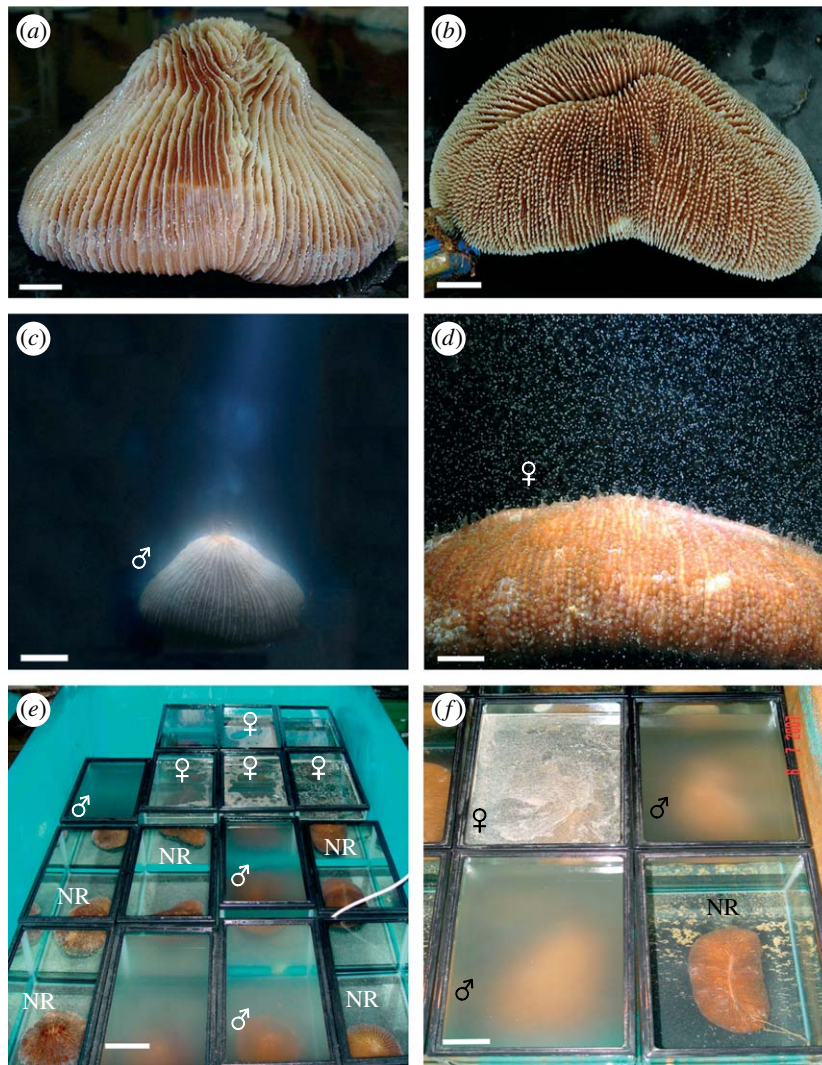


Figure 1. Photographs of the experimental fungiid species and gamete release by the corals in aquaria. (a) *Fungia repanda*; scale bar (SB), 2.5 cm and (b) *C. echinata*; SB, 1.5 cm. (c) Male *F. repanda* shedding spermatozoa through the mouth that remained suspended in the water; SB, 3.5 cm. (d) Female *C. echinata* expelling eggs through the mouth. The eggs were negatively buoyant and settled on the aquarium floor; SB, 3.5 cm. (e) Example of adjacent aquaria demonstrating the difference in their appearance during sperm and egg release by the fungiids: aquaria with opaque, cloudy water contain males (δ) and those with foamy-like surface contain females (♀). Aquaria with transparent water contain corals that did not release gametes (non-reproductive; NR); SB, 10 cm. (f) Typical appearance of aquarium containing a female during reproduction (upper left), a male during reproduction (upper right and lower left aquaria) and a NR coral (lower right); SB, 5.0 cm.

individuals changed sex at a relatively small size, while *C. echinata* changed sex at intermediate sizes (table 3; figure 3). The average annual percentage increase in growth (weight) in *F. repanda* was significantly lower ($p < 0.005$) than in *C. echinata* during 2004–2007 (4.5 versus 8.5%, respectively; table 3). Furthermore, during 2004–2007, significantly higher mortality rates ($p < 0.05$) were recorded in *F. repanda* than in *C. echinata* (41 versus 4.7%, respectively; table 3). In 2007, both *F. repanda* and *C. echinata* were significantly ($p < 0.05$) biased towards males (table 3).

4. DISCUSSION

In a recent review on diversity and flexibility of sex-changing strategies in animals, Munday *et al.* (2006) indicated that understanding the selective mechanisms that influence the variation in individual animal and plant mating strategies, under different ecological conditions, is the key to understanding the adaptive significance and benefits gained by sex change in these organisms. Theories

related to sex change in plants and animals have often progressed along disparate lines. Our discovery of plant-like sex allocation in an animal–plant association (zooxanthellate corals), in which both are largely dependent on similar energetic resources, has the potential to bring our understanding of the evolutionary significance of sex change in the plant and the animal kingdoms closer together. Thus, the daily energy requirements of corals are largely supplied by their endosymbiotic zooxanthellae (Muscatine *et al.* 1983; Falkowsky *et al.* 1984). Furthermore, Rinkevich (1989) showed that in the coral *Stylophora pistillata*, energetic resources for gonadal development are largely supplied by the translocation of photosynthates from the zooxanthellae. Here, we discuss some intriguing analogies between the corals and sexually labile plants with respect to some of their life-history traits and energy allocation.

(a) Reproductive strategies

Although both *F. repanda* and *C. echinata* share many life-history traits, they differ in some aspects of their

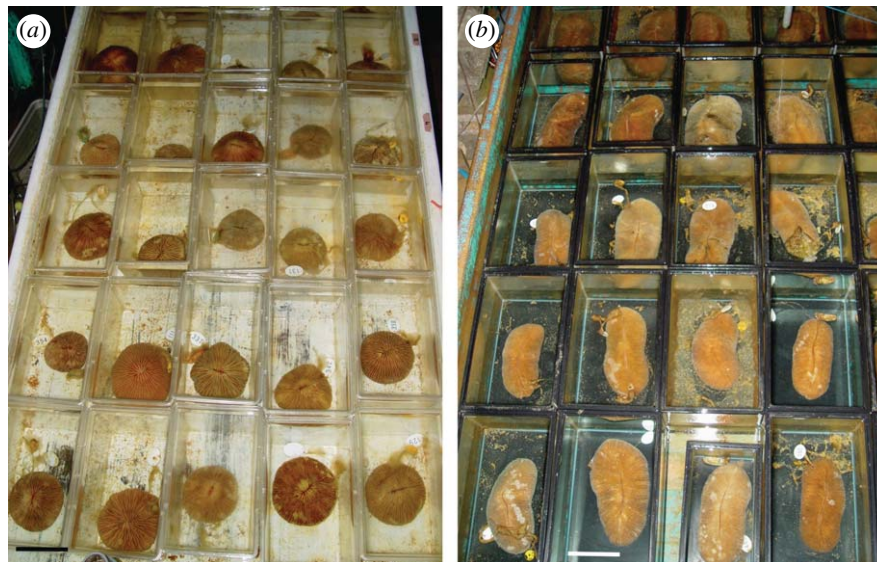


Figure 2. Experimental set-up: each tagged coral was placed in an individual $40 \times 20 \times 20$ cm aquarium, all of which were then placed in the large running seawater containers. (a) *Fungia repanda*; SB, 15 cm. (b) *Ctenactis echinata*; SB, 15 cm.

reproductive strategies. While the vast majority of scleractinian corals are simultaneous hermaphrodites (Harrison & Wallace 1990; Hayashibara *et al.* 1993), all the corals whose reproduction has been studied within the family Fungiidae have been reported to be gonochoristic (Fadlallah 1983; Krupp 1983; Harrison & Wallace 1990; Kramarsky-Winter & Loya 1998). We found both *F. repanda* and *C. echinata* to be protandrous species with the capacity for bidirectional sex change. In some simultaneous hermaphrodites, small individuals during their first year of reproduction are often initially male only (or predominantly male), with eggs being produced at a later age (Rinkevich & Loya 1979; Harrison & Wallace 1990; Hall & Hughes 1996). Our histological study revealed that in neither fungiid were male and female gonads found together prior to spawning. This rules out the possibility of cyclical hermaphroditism, as reported for three species of the deep-sea ahermatypic coral *Caryophyllia* (Waller *et al.* 2005).

Both the fungiids exhibit broadcast spawning (i.e. releasing either male or female gametes into the water column, where fertilization takes place; hence, their mating system is random; figure 1c,d). The SAH postulates that protandry is strongly favoured where pair spawning is random in relation to size (Warner 1975, 1988; Charnov 1982), although protandry is less favoured in broadcast spawners where mating is effectively random, because sperm competition tends to favour large size in males (Muñoz & Warner 2003; Munday *et al.* 2006).

Ctenactis echinata exhibited bidirectional sex change at intermediate sizes. Interestingly, a similar bidirectional sex change, occurring at intermediate sizes, was reported for a dioecious (i.e. separate sexes) invasive tree in Japan: trees that were consistent males were the smallest; inconsistent trees, switching sex between years (bidirectional), were intermediate in size; and consistent females were the largest. The maintenance of female reproduction was dependent upon the resource status of individual trees, mediating flowering frequency or reversal to male (Yamashita & Abe 2002). Bidirectional sex change has also been reported in a variety of other plants (Freeman *et al.* 1976, 1980; Charnov & Bull 1977; Policansky 1981;

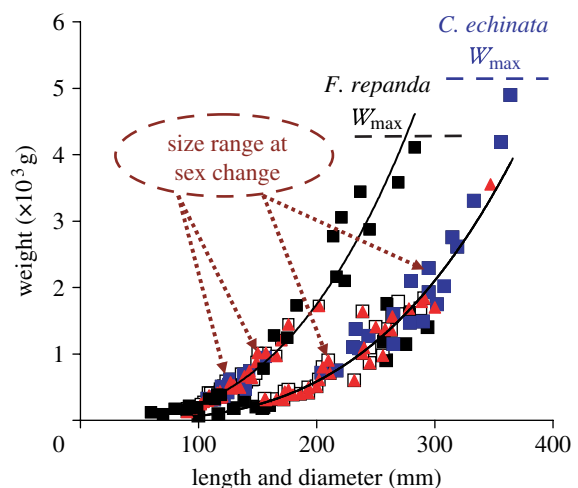


Figure 3. Allometric relationship and size range at sex change of the two fungiid corals: *C. echinata* and *F. repanda*. Length values (in mm) are for *C. echinata* and diameter values (in mm) are for *F. repanda*. N_t (for *C. echinata*) = 84, including 29M, 19F, 14NR and 22EI, and N_t (for *F. repanda*) = 98, including 32M, 17F, 22NR and 27EI. Red triangles, males (M); blue squares, females (F); black squares, non-reproductive (NR); white squares, extra individuals (EI) added to the sample that were only measured (i.e. their sex is unknown). W_{max} = weight (g) of the largest individual collected from the study site (table 3).

Zimmerman 1991; Korpelainen 1998) that become male during periods of poor environmental conditions (e.g. low soil fertility, dry soils, shading, extreme temperatures), and change to female following an improvement in environmental conditions. For example, Freeman *et al.* (1976) demonstrated in five dioecious plant species that males were proportionately more abundant at xeric sites than at mesic sites, while females were over-represented at mesic sites. Zimmerman (1991) showed that some orchids change sex to male if grown in the shade and to female if grown in the sun. Similarly, we suggest that resource partitioning in sex allocation constitutes a flexible response of individual fungiid corals to local environmental conditions, i.e. becoming male in *C. echinata* may be a response to micro-environmental stresses (e.g. overturning, shading, injury, and diseases), while

Table 2. Record of key population statistics and life-history traits of the studied sex-changing mushroom corals *F. repanda* and *C. echinata* during 2004–2007. (n.a., not applicable.)

	<i>F. repanda</i>			<i>C. echinata</i>		
	2004	2006	2007	2004	2006	2007
total corals collected (N_t)	17	17	14	11	11	21
no. of corals retrieved alive	n.a.	14	10	n.a.	11	20
no. of corals retrieved dead	n.a.	3NR	3M, 1F	n.a.	0	1F
observed breeding (n)	8M, 5F	5M ^a , 9F	1M, 1F	5M, 6F	5M, 4F	7M, 8F
non-reproductive (NR) ^b	4	0	1M, 7F	0	2	3M ^c , 2F
same sex in successive years	n.a.	5M, 5F	0	n.a.	2M, 2F	3M
no. of individuals changing sex and direction of sex change	n.a.	4M to 4F	0	n.a.	2M to 2F and 3F to 3M	8M to 8F ^d and 4F to 4M ^e
bidirectional sex change	n.a.	n.a.	0	n.a.	n.a.	2(M–F–M); 2(F–M–F)
new individuals added	0	0	24M, 9F, 7NR	0	6M, 3F, 1NR	30M, 11F, 16NR
no. of individuals returned to the reef	8M, 5F, 4NR	5M, 9F	25M, 10F, 15NR	5M, 6F	11M, 7F, 3NR	37M, 19F, 21NR

^a One NR from the 2004 population reproduced as M.

^b Same individuals were observed to breed in the previous year.

^c Same individuals from the 2006 population.

^d A total of M–F plus F–M–F.

^e A total of F–M plus M–F–M.

Table 3. Life-history statistics of the scleractinian corals *F. repanda* and *C. echinata*: summary of the main results obtained during 2004–2007. The annual growth rate of each individual was calculated for the 2004–2007 population as $(W_{2007} - W_{2004})/W_{2004} \times (100/3)$. Numbers denoted by superscripts indicate the level of statistical significance between comparable attributes of the two species tested for the given time period. See table 1 for notation and computations and figure 3 for regression curves.

variable/statistic	<i>F. repanda</i>	<i>C. echinata</i>
direction of sex change	protandry	protandry; bidirectional
regression of D and L versus W	$W = 6 \times 10^{-4} D^{2.795}$	$W = 3 \times 10^{-5} L^{3.156}$
R^2 -value and sample size	($R^2 = 0.9517$; $N_t = 98$)	($R^2 = 0.9489$; $N_t = 84$)
percentage mortality (2004–2007)	41 ^a ($N_t = 17$)	4.7 ^a ($N_t = 21$)
sex ratio in 2007	0.71 ^b ($n = 35$)	0.66 ^b ($n = 56$)
W (g) of the largest coral (W_{\max})	4107	4898
A_v W (g) at sex change	515 ± 91 ^c ($n = 4$)	1337 ± 393 ^c ($n = 11$)
relative weight (W_r) at sex change	0.12 ± 0.02 ^d ($n = 4$)	0.27 ± 0.08 ^d ($n = 11$)
range of W (g) at sex change	385–685	650–2090
percentage bidirectional sex change in 2007	0	27 ($n = 15$)
A_v annual percentage increase in W (2004–2007)	4.5 ± 2.5 ^e ($N_t = 9$)	8.5 ± 3.8 ^e ($N_t = 15$)

^a A significant difference (Fisher's exact probability test of independence, $p = 0.032$).

^b A significant deviation from a sex ratio of 0.5 (χ^2 -test: *F. repanda*, $p = 0.011$; *C. echinata*, $p = 0.016$).

^c A significant difference (t -test, $p = 0.0004$).

^d A significant difference (t -test, arcsine-transformed data, $p = 0.0002$).

^e A significant difference (t -test, arcsine-transformed data, $p = 0.004$).

becoming female follows improved environmental conditions and energy resources.

(b) Differential costs of female and male reproduction

In a similar pattern to most scleractinian corals (Schlesinger & Loya 1985; Harrison & Wallace 1990; Schlesinger *et al.* 1998), the onset of oogenesis in the females of both *F. repanda* and *C. echinata* precedes by one to three months that of spermatogenesis in the males. Nevertheless, maturation and release of gametes is synchronous in both the sexes, suggesting that the female energy investment in the lipid-rich eggs is more costly than the male investment in sperm. A similar reproductive pattern was reported for other fungiid species (Kramarsky-Winter & Loya 1998). The size/weight of first reproduction was much smaller in

F. repanda than in *C. echinata* and the smallest reproducing individuals in both the species were all males (i.e. an energetically less costly resource investment). This suggests that energy is directed initially towards coral growth, in order to reduce the high risk of mortality experienced by small individuals (Loya 1976; Harrison & Wallace 1990).

Differential costs associated with female and male reproduction in plants have been similarly observed by various researchers when applying the SAH to flowering plants (e.g. Charnov & Bull 1977; Freeman *et al.* 1980; Charnov 1982; Schlessman 1988; De Jong & Klinkhamer 1994). The greater energetic costs of female relative to male reproduction are usually due to the plant's investment in both flowering and fruiting (Bierzychudek 1984; Schlessman 1988; Zimmerman 1991; Klinkhamer & de Jong 1997; Korpelainen 1998).

(c) Effects of the environment and individual size on resource allocation to reproduction, fecundity and sex expression

For many organisms, body size can have a major influence on energy allocation and trade-off for growth versus reproduction. Before the onset of sexual reproduction, individuals must attain a threshold size. Thereafter, reproductive output often increases as a function of body size (e.g. in herbaceous plants and trees, invertebrates, fishes, amphibians and some mammals; Caswell 1989). Similarly, in sex-changing organisms, switches in sex are often size or age related (Ghiselin 1969; Warner 1975, 1988; Charnov 1982; Policansky 1982).

Similar to the findings for many other animals, our histological study reveals that in both of the studied fungiid species, gonad size increases with individual body size. Hence, in both the fungiids, female fecundity increases with an individual's size, as has been shown for other scleractinian corals (Hall & Hughes 1996), including some fungiid species (Kramarsky-Winter & Loya 1998). Furthermore, in larger corals, relatively more resources are allocated to reproduction than in smaller ones (Hall & Hughes 1996). An individual's size also influences the photosynthesis–irradiance relationship in corals and plants, indicating that net primary production of an entire colony or individual plant increases with increasing body size (or canopy in plants; Jokiel & Morrissey 1986).

Kramarsky-Winter & Loya (1998) reported on sexual dimorphism with respect to size in *Fungia scutaria* from the Red Sea, where small individuals were predominantly males, whereas very large individuals were all females. They hypothesized that these findings indicate either protandry or that males reach sexual maturity at a smaller size than females. In view of the similarities in the reproductive traits of *F. scutaria* and *C. echinata* (e.g. sexual size dimorphism, population sex ratio, timing and length of gametogenesis, season and timing of spawning and being gonochoric broadcasters), we further predict *F. scutaria* to be not only a protandrous sequential hermaphrodite but also, and, most probably, a bidirectional sex changer.

It has been postulated that plants with environmental sex determination (Charnov & Bull 1977) tend to be more female at good quality sites versus poor quality sites (Freeman *et al.* 1976, 1980; Lovett Doust & Cavers 1982; Zimmerman 1991). In many species of plants, sex expression is also correlated with size, i.e. large plants are usually 'more female' than small plants (Policansky 1981; Lovett Doust & Cavers 1982; Bierzychudek 1984; Schlessman 1988; Korpelainen 1998; Yamashita & Abe 2002). Models that predict an evolutionarily stable sex ratio have been developed to explain this phenomenon (Charnov & Bull 1977; Freeman *et al.* 1980; Charnov 1982; Schlessman 1988; Iwasa 1991; De Jong & Klinkhamer 1994), and the majority of these are derived from the SAH for sequential hermaphroditism in animals. Larger plants are believed to have greater resources or resource-gathering potential and, consequently, the increase in reproductive success is the greatest for females. The resource-limitation model of the sex-allocation theory (Charnov 1982) predicts that an increase in plant size and associated resource availability will maximize plant reproductive success through an increase in female

expression. Male reproductive success is thought to require fewer resources and to reach an asymptote at an upper limit in large plants.

(d) Correlates of sex allocation in sequential hermaphrodite fungiids and sexually labile plants

The studied fungiids and some dioecious plants that exhibit labile sexuality in response to energetic and/or environmental constraints display intriguing analogies in several of their ecological and physiological characteristics, as well as life-history traits: for example, being attached to the substrate and hence lacking social interactions; having similar energetic pathways (photosynthates); a photosynthesis–irradiance relationship that is influenced by an individual's size; exhibiting size-related sexual dimorphism; having greater energetic costs of female relative to male reproduction; maintenance of female reproduction being largely dependent on the physiological/resource state of the individual; displaying bidirectional sex change at intermediate sizes; and more. Freeman *et al.* (1980) presented evidence that in patchy environments that vary in quality, individuals of a large number of dioecious plant species are able to alter their sex in response to changes in the ambient environment and/or changes in size or age. They suggested that such lability of sexual expression probably enhances an individual's genetic contribution to the next generation (see also Iwasa 1991). Hence, we posit that sex change in fungiid individuals enhances their survival value and reproductive success in a similar way to that which occurs in dioecious plants that display labile sexuality in response to energetic and/or environmental constraints.

The great similarity in the life-history traits of *C. echinata* to some plants that display labile sexuality might explain the size range and timing of sex change in this species in response to energetic and/or environmental constraints. We suggest that *C. echinata* individuals of intermediate sizes have insufficient energy to reproduce as females every year. Thus, if female fecundity scales with size at a faster rate than energy gain scales with size, then there might be a stage at which the corals would benefit from sex change to female (i.e. in accordance with the SAH and the resource-limitation model of sex allocation in plants), but may not have quite enough energy to do so. By alternating energy investment in the relatively costly female reproduction between years, they can 'recover' on alternate years by channelling energy to the less costly male reproduction, until they reach the critical size at which they have the capability to acquire enough energy to remain female, thus probably increasing their overall reproductive success. Indeed, in *C. echinata*, 90% of the largest individuals (above 1800 g; $n=10$) were observed to be females, while the smallest reproducing individuals (300–700 g; $n=16$) were all males (sexual size dimorphism, figure 3), supporting the SAH predictions. By contrast, and in variance with the SAH, the largest individuals of *F. repanda* (above 2000 g) did not reproduce. Additionally, no asexual reproduction (i.e. budding) was observed in these corals. It is possible that this species exhibits senescence after attaining a certain size/age. Such a phenomenon was demonstrated for the colonial coral *S. pistillata* (Rinkevich & Loya 1986); hence, it is even more probable for a solitary polyp such as *Fungia*. If this is true, it is also possible that, due to energetic

constraints, very large/old individuals of this species may not breed every year. Clearly, further studies over longer periods of time are required before drawing more definite conclusions.

(e) Corroboration of hypotheses derived from the sex-allocation theory and SAH

Our data on the sex ratios in the two fungiid populations enabled us to test and corroborate the hypothesis derived from the sex-allocation theory that the sex ratio of the two fungiid populations should be biased towards the first sex (Charnov 1982; Warner 1988; West *et al.* 2002; Allsop & West 2004). In 2007, both the species indeed showed a significant bias towards the first sex: in *F. repanda*, sex ratio = 0.71 ($n=35$) and in *C. echinata*, sex ratio = 0.66 ($n=56$; $\chi^2 < 0.05$; table 3). However, in both 2004 and 2006, the sex ratio in both the species did not significantly differ from a sex ratio of 0.5 ($\chi^2 > 0.05$; table 2). This may have been due to the relatively small sample sizes in the populations monitored in these years, or it may be that the sex ratio in these populations is a flexible response by individuals to local environmental conditions (see Charnov 1982). At this stage of our knowledge, we support Allsop & West's (2004) conclusion that future progress in this area requires additional and more detailed species-specific studies, in particular of protandrous invertebrates.

The timing, growth rates and mortality rates of the two fungiid populations at sex change (table 3) enabled the corroboration of some predictions derived from the SAH, i.e. sex change will occur earlier in populations with a slower growth rate and a higher mortality rate (Warner 1988). This pattern has been documented in several protogynous and protandrous species and recent analyses are revealing the mechanisms responsible for this variation (see Munday *et al.* 2006). The observed patterns in the studied fungiids match theoretical predictions: i.e. compared with *C. echinata*, *F. repanda* exhibited relatively earlier sex change, slower growth and higher mortality rates (table 3). A similar relationship was shown in plant individuals that change sex (Iwasa 1991).

Our study exemplifies the view that models which can be applied on the scale of individuals are likely to provide important insights into the factors underlying the evolution of sex change in animals and plants. We believe that longer term studies on individuals of sex-changing animals will reveal that they employ a greater variety of sex-allocation strategies (see Munday *et al.* 2006) in order to increase their fitness.

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