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# A Pre-Zygotic Barrier to Hybridization in Two Con-Generic Species of Scleractinian Corals

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OBSERVATION ARTICLE

# A pre-zygotic barrier to hybridization in two con-generic species of scleractinian corals [v1; ref status: indexed, http://f1000r.es/1uz]

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## Abstract

Hybridization is often cited as a potential source of evolutionary novelty in the order *Scleractinia*. While hybrid embryos can be produced *in vitro*, it has been difficult to identify adult hybrids in the wild. Here, we tested the potential for hybridization between two closely related species in the family Fungiidae. We mixed approximately 5000 eggs of *Ctenactis echinata* with sperm from *Ctenactis crass*. No hybrid embryos were produced. This observation adds to a growing body of evidence for pre-zygotic barriers to hybridization in corals and challenges the claim that hybridization is a major source of evolutionary novelty in the order.

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## Observation

Hybridization is a controversial topic in coral reef ecology[1,2]. While small numbers of hybrid embryos can be produced in a few species *in vitro*[3], the evidence for hybrids in the field is often equivocal because the genetic techniques used for corals cannot distinguish between hybridization and incomplete lineage sorting[4]. In fact, only one of the over 1300 species in the order is generally accepted to be unequivocally of hybrid origin: *Acropora prolifera*[1,5]. Nonetheless, hybridization is often invoked as a source of evolutionary novelty in the order *Scleractinia*[6,7].

Here, we report an incidental observation on the potential for hybridization between two closely related scleractinian corals species in the family Fungiidae, *Ctenactis echinata* and *Ctenactis crassa*. These species are sympatric, often dominating large multi-specific assemblages of fungiid corals throughout the central Indo-Pacific[8]. Colonies of these species are superficially very similar (Figure 1A and B) but can readily be distinguished by the shape of the costal dentitions[9]. Both species are gonochoric, that is each colony is either male or female, and reproduce by broadcast spawning, releasing gametes into the water column for fertilization[8] (Figure 1C and D). At our study site on Sesoko Island (26°38'13.00"N; 127°51'56.24"E), Okinawa, Japan, spawning occurs following the full moons from July to August[8]. Furthermore, both species release gametes at the same time[8] and consequently there is the potential for hybridization. In the days before the predicted date of spawning in July 2013, we collected four colonies of *C. echinata* and six colonies of *C. crassa*, to produce larvae for other experiments.

While the species are relatively easy to identify, determining the sex of each individual prior to spawning is impossible without destructive sampling to expose the gametes. Consequently, we placed each individual in a separate 20 L bucket containing sea water in the open air at approximately 20:00 h in order to sex each individual once gametes had been released. On the night of 27 July between 22:30 and 23:30 h three *C. echinata* and five *C. crassa* spawned revealing that the three spawning *C. echinata* were female, while four *C. crassa* were females and one was a male. The size of the eggs of each species at the time of release was distinct with a range in maximum diameter of 244–266 µm in *C. echinata* and 133–155 µm in *C. crassa*. In contrast to earlier work on *C. echinata*[10], we saw no symbiotic algae in the eggs of either species. We collected approximately 5000 eggs from the three *C. echinata* females and mixed them with sperm from the *C. crassa* male. As a positive control we mixed eggs from the four *C. crassa* females with the *C. crassa* sperm. Approximately 100 eggs were observed under a stereo-dissecting microscope for cleavage, indicating fertilization, every 2 to 6 h over the next 24 h. At no point did we observe cleavage in the cross between species indicating that no hybrid embryos were produced. In contrast, over 90% of *C. crassa* eggs in the positive control were fertilized within 2 h. We conclude that despite synchrony in the time of gamete release between these two closely related sympatric species there appears to be strong pre-zygotic mechanism to avoid hybridization. While our observations are preliminary and in only one direction (i.e. we did not cross *C. echinata* males with *C. crassa* females) we predict that hybridization between these species is unlikely. This observation adds to a growing body of evidence indicating strong pre-zygotic barriers to hybridization in many scleractinian corals[11–13].

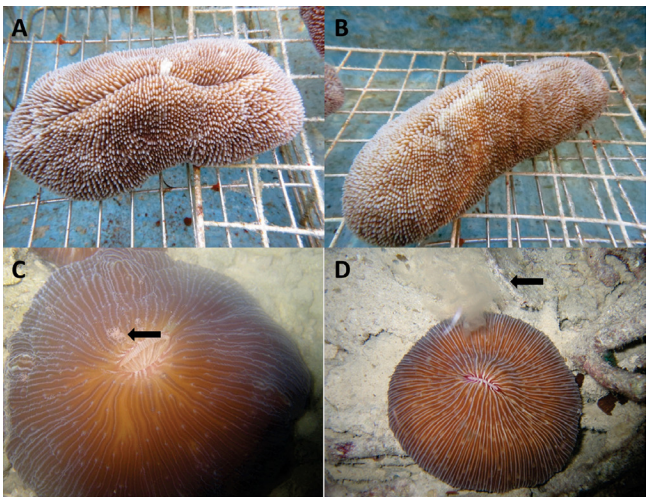


**Figure 1. Study species and broadcast spawning in fungiid corals.** Live *Ctenactis echinata* (**A**) and *C.crassa* (**B**) in aquaria prior to being isolated for spawning. Each colony is approximately 20 cm in length. Coral species in the family Fungiidae, such as these colonies of *Fungia repanda*, are gonochoric broadcast spawners: each individual releases either eggs (**C**) or sperm (**D**) into the water column where fertilization takes place (arrows indicate gametes).

### Author contributions

AHB, VRC & JF conceived the study and performed the experiment. All authors contributed to writing the manuscript.

### Competing interests

No competing interests were disclosed.

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*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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# Open Peer Review

## Current Referee Status: ✓ ✓

**Version 1**

Referee Report 24 October 2013

**doi:**10.5256/f1000research.2411.r1878

**Bernie Degnan**
School of Biological Sciences, University of Queensland, Brisbane, Australia

This Observation Article reports the lack of cross-fertilization between *Ctenactis echinata* and *Ctenactis crassa*, closely related fungiid corals that naturally release gametes at the same time. The authors recognise the limitations of this observation - only small numbers of eggs (100's) were observed and only eggs from *C. echinata* were available to be fertilized (i.e. they did not cross *C. echinata* males with *C. crassa* females) - but rightly point out that this study provides further evidence that hybridization is not as widespread amongst scleractinian corals as often portrayed in the literature. However, the analysis of reciprocal crosses and a larger numbers of eggs is necessary before it can be said with some confidence that these congeners can not hybridize.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

***Competing Interests:*** No competing interests were disclosed.

Author Response ( *F1000Research Advisory Board Member* ) 26 Oct 2013

**Andrew Baird**, School of Marine Biology and Aquaculture, James Cook University, Australia

Dear Bernie,

Thank you for your comments. We would just like to point out that while we only examined 100 eggs every few hours under the microscope, we used approximately 5000 eggs in the fertilization experiment, all of which had broken down after 24 h suggesting none had fertilized. We have added a sentence to the revised text to draw attention to this.

***Competing Interests:*** no competing interests

Referee Report 22 October 2013

**doi:**10.5256/f1000research.2411.r2160

**Yossi Loya**
Department of Zoology, Tel Aviv University, Tel Aviv, Israel

- The title is appropriate for the content of the article. The abstract represents a suitable summary of the work. Please correct: *crass* to *crassa*, in the 3rd line of the Abstract.

- Article content: The design, methods and analysis of the results been clearly explained and are appropriate for the topic being studied. Figures 1C and 1D appear to be irrelevant to the article since they show different species. I suggest deleting them. A proper reference to the statement *'spawning occurs following the full moons from July to August'* (reference 8 in the manuscript) is: Loya Y. & K. Sakai (2008). Bidirectional sex change in mushroom corals. *Proc. Roy. Soc. Biol.* B 275: 2335-2343.

- Data and Conclusions: The authors note that they did not cross *C. echinata* males with *C. crassa* females; however they also did not test the positive control of crossing *C. echinata* males with *C. echinata* females (all their experimental *C. echinata* specimen were females). Nevertheless, this does not diminish their prediction that hybridization between these species is unlikely. The paper contributes further information to the controversial topic of potential hybridization and breeding incompatibilities within the mating systems of broadcast spawning reef corals.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

***Competing Interests:*** No competing interests were disclosed.

Author Response ( *F1000Research Advisory Board Member* ) 26 Oct 2013

**Andrew Baird**, School of Marine Biology and Aquaculture, James Cook University, Australia

Dear Yossi,

Thank you for your comments. We have corrected the typos you identified, changed the reference as requested and added a sentence to clarify the controls that were used to test for gamete viability. Images 1 C & D are presented as an example of the spawning behavior of fungiids.

***Competing Interests:*** none