



OBSERVATION ARTICLE

REVISED A pre-zygotic barrier to hybridization in two con-generic species of scleractinian corals [v2; ref status: indexed, <http://f1000r.es/27i>]

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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 *C. crassa*. 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.

Article Status Summary

Referee Responses

Referees	1	2
v1 published 20 Sep 2013	 report 1	 report 1
v2 published 01 Nov 2013 REVISED		

- 1 **Yossi Loya**, Tel Aviv University Israel
- 2 **Bernie Degnan**, University of Queensland Australia

Latest Comments

No Comments Yet

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Competing Interests: No competing interests were disclosed.

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UPDATED Changes from Version 1

In response to feedback from the reviewers, we have updated our Observation Article as follows. In response to comments by Yossi Loya, we now note in the text that we did not quantify the viability of the *Ctenactis echinata* eggs by crossing them with *C. echinata* sperm because no sperm was available at the time. We have also corrected one citation. In response to a comment by Bernie Degnan, suggesting we had only looked at 100s of eggs, we now state that none of the approximately 5000 *C. echinata* eggs exposed to *C. crassa* sperm were fertilized. In response to email correspondence with Bert Hoeksema, we have changed the name of the species in Figure 1 C & D from *Fungia repanda* to *F. fungites*. We have also provided more detail on how the *Ctenactis* species were identified and have corrected the relevant citation.

See referee reports

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 sorting⁴. 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 *C. crassa*. These species are sympatric, often dominating large multi-specific assemblages of fungiid corals throughout the central Indo-Pacific⁸. These species can generally be distinguished on the basis of the density of septa and the shape of septal dentitions, however, in Okinawa, these features are very similar and the most useful diagnostic character is a strong arch in the corallum of *C. crassa* (Figure 1A and B)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 fertilization⁸ (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 August⁸. Furthermore, both species release gametes at the same time⁸ 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

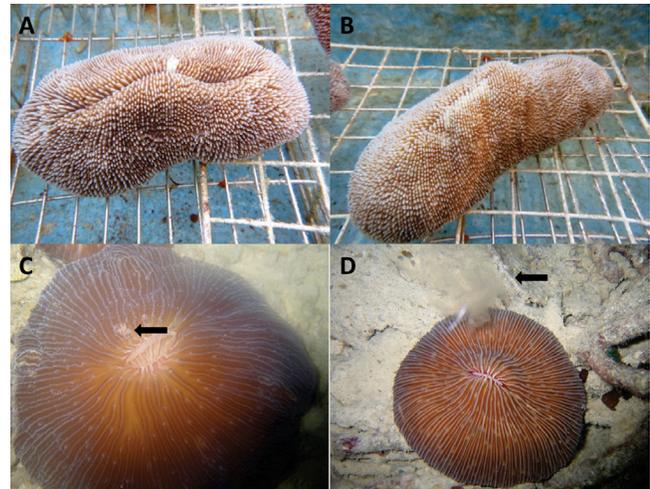


Figure 1. Study species and broadcast spawning in fungiid corals. Live *Ctenactis echinata* (A) and *C. crassa* (B) in aquaria prior to being isolated for spawning. Each colony is approximately 20 cm in length. Coral species in the family Fungiidae, such as these colonies of *Fungia fungites*, are gonochoric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

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. The viability of the *C. crassa* sperm was tested by mixing it with *C. crassa* eggs, however, we could not quantify the viability of the *C. echinata* eggs because no *C. echinata* sperm was available on the evening of the experiment. Nonetheless, eggs from these colonies of *C. echinata* did produce viable larvae for use in later experiments. 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 and none of the approximately 5000 eggs remained intact after 24 h. 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}.

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.

Grant information

Funding was provided by the Australian Research Council Centre of Excellence for Coral Reef Studies COE561432 (AHB), a Queensland Smart Futures Fellowship (JF) and a Sesoko Tropical Biosphere Research Station Travel Award 2013 (VRC).

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

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[PubMed Abstract](#) | [Publisher Full Text](#)

Current Referee Status:

Referee Responses for Version 1



Bernie Degnan

School of Biological Sciences, University of Queensland, Brisbane, Australia

Approved: 24 October 2013

Referee Report: 24 October 2013

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.

1 Comment

Author Response

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

Posted: 26 Oct 2013

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



Yossi Loya

Department of Zoology, Tel Aviv University, Tel Aviv, Israel

Approved: 22 October 2013

Referee Report: 22 October 2013

- 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.

1 Comment**Author Response**

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

Posted: 26 Oct 2013

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