



OBSERVATION ARTICLE

REVISED Fused embryos and pre-metamorphic conjoined larvae in a broadcast spawning reef coral [v2; ref status: indexed, <http://f1000r.es/54p>]

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Abstract

Fusion of embryos or larvae prior to metamorphosis is rarely known to date in colonial marine organisms. Here, we document for the first time that the embryos of the broadcast spawning coral *Platygyra daedalea* could fuse during blastulation and further develop into conjoined larvae, and the settlement of conjoined larvae immediately resulted in inborn juvenile colonies. Fusion of embryos might be an adaptive strategy to form pre-metamorphic chimeric larvae and larger recruits, thereby promoting early survival. However, future studies are needed to explore whether and to what extent fusion of coral embryos occurs in the field, and fully evaluate its implications.

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report



report

1 **Andrew Baird**, James Cook University
Australia

2 **Baruch Rinkevich**, National Institute of
Oceanography Israel

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REVISED Amendments from Version 1

According to the comments from the reviewers, we mainly made the following changes:

- 1) We improved the text in many instances as suggested by Prof. Rinkevich. For instance, in Materials and Methods, we used "combined" instead of "mixed"; we replaced "each type of fusion" with "chimeric larvae". Moreover, to demonstrate the sectorial fusion, we described these conjoined larvae as multi-headed in the Discussion.
- 2) We have corrected the wrong idea of the sexually produced embryos not being genetically distinct and rewritten this part in the Discussion.
- 3) Prof. Rinkevich suggested that the results of Mizrahi *et al.*, 2014 would change our statement of "for the first time". Our observation presented the embryonic chimeras as a result of fusion of embryos in a broadcast spawning coral, whereas Mizrahi *et al.*, 2014 revealed that the larvae of brooding coral *Tubastraea coccinea* could metamorphose and aggregate in swimming groups. Thus, we did document for the first time the fusion between individuals at the embryonic stage in reef corals and the inborn colonies of multiple polyps upon settlement. Moreover, we followed the settlement and growth of these chimeric larvae. Overall, it is appropriate to state that we documented the embryonic chimeras and inborn colonies "for the first time" in reef corals.
- 4) We point out the absence of water turbulence in this study as suggested by Prof. Baird and state that it may be a factor triggering the fusion of embryos. We added the references of Mizrahi *et al.*, 2014 and Gauthier *et al.*, 2008 to further discuss the potential implications of this phenomenon.

We are really grateful for the comments from the two reviewers and the efforts of the editorial office.

See referee reports

Introduction

In sessile colonial marine invertebrates (e.g., sponges, cnidarians, bryozoans and ascidians), fusion among conspecifics during early ontogeny could immediately lead to a marked increase in juvenile size, thereby enhancing the performance in growth, survival and competition^{1,2}. In addition, the allogenic fusion is expected to form chimeras which possess greater genetic variability and wider ranges of physiological resistance¹. Larvae of colonial marine organisms tend to settle in a gregarious manner^{3–7} and their juveniles often come into physical contact through growth and then fuse^{8–10}. These life history traits increase the opportunities for fusion, and important rates of chimerism due to allogenic fusion have been detected in wild natural populations of corals and ascidians^{11,12}. Nevertheless, fusion of embryos or larvae during planktonic and dispersive phase (i.e. prior to settlement and metamorphosis) is rarely known to date.

Modular marine invertebrates like sponges and cnidarians usually spawn their gametes in a high synchrony^{13–15}, thus also providing the chance of contact and fusion among embryos or larvae. For instance, sticky eggs released by the oviparous sponge *Cliona celata* were found to adhere to each other and form flattened egg mass, within which larvae fused in twos or threes. The compound larvae metamorphosed into sponges with single oscula, indicating the cytotoxic fusion among embryos or larvae¹³. More recently, larvae of two sponges and sun coral *Tubastraea coccinea* have been demonstrated

to fuse and generate swimming chimeras^{16–18}. Furthermore, sexually produced embryos of a non-colonial sea anemone *Urticina felina* were observed to fuse naturally during internal brooding, generating pre-metamorphic cytotoxic and sectorial chimeras^{19,20}. These findings suggested that the direct contact between embryos and larvae would facilitate fusion either during internal brooding or pelagic phase.

For broadcast spawning corals, synchronous spawning events usually result in billions of naked embryos floating at the sea surface in the form of spawn slicks^{21,22}. The direct contact between naked embryos highlights the possibility of fusion of coral embryos while sticking together in slicks. Moreover, previous studies have demonstrated there is a window in ontogeny, before allorecognition system matures, when newly settled polyps can fuse²³. Time for allorecognition maturation in reef corals varied from 4 months following settlement in brooding species²⁴, to 1–3 years in spawning species^{9,10}. This further supports the possibility of fusion at embryonic stage when allorecognition may be weak in corals. As yet, the possible occurrence of fused embryos and conjoined larvae in broadcast spawning corals has not been investigated.

Here, we happened to test this unexplored probability of fusion of embryos in broadcast spawning reef corals. We experimentally mimicked spawn slicks using gametes collected from 4 mature colonies of *Platygyra daedalea*, and followed the fate and development of embryos within lab-generated slicks.

Materials and methods

Ten gravid colonies of *P. daedalea* (20–30 cm in diameter) were collected at depth between 2–4 m from Luhuitou fringing reef in Sanya, China (18°12'N, 109°28'E). Corals were maintained in an outdoor tank with flowing sand-filtered seawater in Tropical Marine Biological Research Station in Hainan, Sanya. Four colonies spawned around 22:00 on May 18, 2014 (5 nights after full moon). Egg-sperm bundles were collected using pipettes, then combined and gently agitated to facilitate bundle disintegration and cross-fertilization. Fertilization was allowed to take place for about 2 hours, after which eggs (ca. 300, 000) were washed two times with fresh seawater and suspended in a 15 cm-diameter jar. Because of the logistical constraints, eggs were left undisturbed and they formed dense slicks on the seawater surface. The next morning around 08:30, embryos were inspected under a dissecting microscope and we accidentally discovered that some embryos fused. Embryos were washed and seawater was changed twice daily thereafter. Two days after fertilization, 500 larvae were randomly sampled to count the proportion of chimeric larvae. Seven days after fertilization, chips of crustose coralline algae *Hydrolithon onkodes* were used to induce the settlement of larvae and the recruits were reared in the lab at 28°C until June 26.

Results

Embryos became bowl shaped (cushion stage) 8 h after fertilization. Notably, some embryos fused (Figure 1A) and a substantial proportion even stuck together into dense aggregates (Figure 1B). It could be deduced that fusion of embryos took place some time during blastulation. Mortality of embryos within the first 2 days was extremely high (>50%) and the dense aggregates all died and

decomposed. Unitary larvae became pear-shaped and began to rotate actively 20 h after fertilization, while conjoined larvae were highly variable in shape. Bi-fused larvae were dominantly peanut-shaped, and multi-fused larvae were arranged in chains or triangles, or in the form of the letter “T” or “L” (Figure 1C, D).

Of the 500 randomly sampled larvae, 174 (34.8%) were conjoined with 2–4 partners. Conjoined larvae clearly showed their spatial arrangement after elongation and fusion was apparently without polarity. Larvae either joined at the aboral end (Figure 1E, F), or united side by side (Figure 1G), or even fused perpendicularly (Figure 1K). Furthermore, 56 out of the 174 conjoined larvae (32.2%) united at the aboral extremity and only these larvae were potentially competent to metamorphose normally into inborn colonies (Figure 1H, I), which were prominently larger in size than the single settlers (Figure 1J). In contrast, perpendicularly bi-fused larvae settled incompletely, with one partner metamorphosing and firmly attaching while the other still being parallel to the substrate and not able to settle (Figure 1K), ultimately leading to the death of the whole entity 3 days later. Since the coralline algae provided here was not suitable for the settlement of *P. daedalea* larvae, only 12 inborn colonies were obtained in total and they persisted for 26 days post-settlement when the study ended (Figure 1L).

Discussion

The present study documented for the first time the fusion of embryos and inborn colonies in a broadcast spawning coral. Fusion of *P. daedalea* embryos was spontaneous, resulting simply from the aggregation and contact of embryos in mimicked slicks, which was analogous with that in sponge *C. celata*¹³. While unlike the cytotoxic compound larvae in sponge *C. celata*, the chimeric

P. daedalea larvae were multi-headed, suggesting sectorial fusion of coral embryos and supporting the assumption that corals typically exhibit sectorial fusion¹.

Corals often spawn during seasonally calm periods and low-amplitude tides^{21,22,25} and spawn slicks extending up to few km in length were often observed in the field^{21,26}. Given that slicks remained aggregated 1–2 d after spawning^{21,22} and embryos can fuse during embryogenesis within 8 h post-fertilization, fusion of coral embryos is highly favored *in situ*. On the other hand, although mass coral spawning events usually involved several species, significant temporal differences in spawning to ensure fertilization and reproductive isolation have been demonstrated for many sympatric species^{27–29}, which considerably increase the encounters between embryos of the same species in slicks. Taken together, fusion of coral embryos might be a naturally occurring phenomenon. However, the density of embryos here was 1700 cm² and likely to be much higher than that in the field. Moreover, water turbulence that the embryos would experience was absent in this study. Therefore, it is possible that our experimental conditions eventually led to the formation of embryonic chimeras. Likewise, larvae brooded by *T. coccinea*, when kept at high density in still water, could metamorphose and aggregate in clusters with extended lifespan¹⁸. Thus, whether fusion of coral embryos occurs in natural spawn slicks and the dispersal potential of these chimeric larvae remain to be determined.

At last, an important observation was that the chimeric larvae were able to settle firmly and form inborn colonies. The inborn colonies here originated from fusion of embryos and settlement of chimeric larvae, contrasting the traditional concept that the asexual budding of the primary polyp leads to the formation of

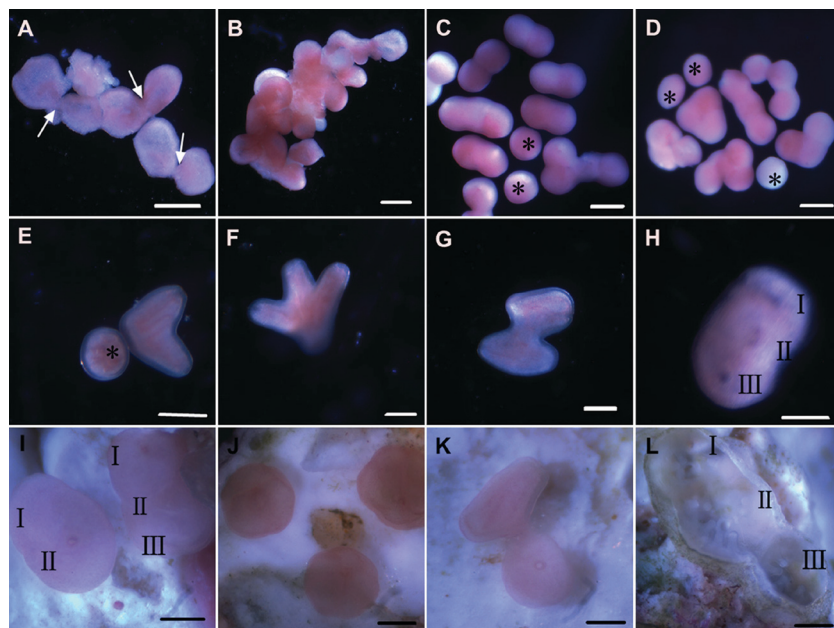


Figure 1. *Platygyra daedalea*. (A) Fused embryos (arrows point to the fusing areas). (B) A dense aggregate comprising more than 20 embryos. (C–G) Unitary (asterisks) and conjoined larvae. (H, I) Inborn colonies. (J) Single settlers. (K) Incomplete settlement of perpendicularly bi-fused larvae, with the left partner being parallel to the substrate. (L) An inborn colony 26 days post-settlement. Roman numerals indicate visible individuals within inborn colonies. Scale bars 250 μ m.

a young coral colony³⁰, and thus fusion of embryos could be an unexpected shortcut to colony formation in reef corals. Furthermore, the inborn colonies persisted for about one month and exhibited no sign of rejection, suggesting the possibility that the embryonic chimeras might contribute to recruitment in the natural environment¹⁷. These facts raise questions as to the ecological implications of inborn colonies formed as a consequence of fusion of embryos in corals. Firstly, larger coral colonies composed of multiple fused partners are known to yield remarkable gains, such as enhanced survival and growth^{5,8}. Hence, the larger initial size and the status of multi-polyp at settlement may confer these inborn colonies better capacities to compete for space and survive partial mortality.

Fusion of coral embryos also shed new light on the chimerism in scleractinian corals, which was often attributed to fusion of gregariously settling larvae^{5,7}, or of juveniles that come into contact through growth^{7,9,10}. However, our study documented fusion between individuals in *P. daedalea* occurred at the embryonic stage, earlier than any other corals studied to date. Since the embryos here were produced sexually from 4 parent colonies and they were genetically distinct, fusion of embryos could be a novel mechanism for chimerism in scleractinian corals. In that case, the increased genetic diversity within these inborn colonies may translate into versatile physiological qualities, thus enabling them to better cope with environmental changes unless negative interactions occur^{1,31,32}.

Overall, this is the first report of embryonic chimeras in reef corals. Fusion of coral embryos could be an adaptive strategy to form larger and chimeric recruits, thereby promoting growth and survival during the vulnerable early stages^{5,8}. Clearly, future studies are required to explore whether fusion of embryos occurs in the field and fully evaluate its biological and ecological implications.

Ethics statement

Coral sampling was permitted by the Administration of Sanya Coral Reef National Nature Reserve, the Department of Ocean and Fisheries of Hainan Province.

Author contributions

LJ conceived and performed the study. All authors wrote the manuscript and gave final consent for publication.

Competing interests

No competing interests were disclosed.

Grant information

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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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Version 2

Referee Report 02 April 2015

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Baruch Rinkevich

Israel Oceanography & Limnological Research, National Institute of Oceanography, Haifa, Israel

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.

Version 1

Referee Report 17 February 2015

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Baruch Rinkevich

Israel Oceanography & Limnological Research, National Institute of Oceanography, Haifa, Israel

This short research article by Jiang *et al.* is timely, adds to our understanding on the magnitude of natural chimerism, and meets research standards with **methodological details that made available** to allow others to replicate this study. I recommend indexation pending responding on the below minor editorial/clarification points:

- Abstract (line 2)- An unclear sentence. Suggest revising from: 'Here, we observed for the first time the embryos....' to 'Here, we document for the first time that embryos...'. See also below the note appended to the citation of Mizrahi et al., 2014 and define your statement, 'for the first time'.
- 'Conjoined larvae' (as from Abstract line 4 and along the manuscript). This term and the following terms 'chimera' and 'fusion' are mutually used, irrespective to the somehow different biological statuses they present (for example, 'conjoined' is a very sterile term indicating being, coming, or brought together so as to meet, touch, overlap, or unit). I would suggest to use the term 'Conjoined larvae' in the Results section when first describing the process of larval joining and then use the terms 'fusion' and 'chimerism' in all other parts of the manuscript to state the biological outcome.

- Related to the aforementioned- it will be most valuable to show evidence for the ‘fusion’ outcomes, such as histological sections, or that no disjointing between the conjoined larvae occurred following the employment of physical force.
- Introduction and Discussion- There are four papers that I would suggest the authors to add and to cite. The first and the most important is the study by Mizrahi *et al.* (2014). In this paper the authors showed that *Tubastraea coccinea* offspring can metamorphose and aggregate/fuse in groups of up to eight polyps in the water column, before settlement. This may also change the way Jiang *et al.* refer to their finding (statements such as ‘the first time’ should be revised). The second paper is the study on sponge chimeras by Gauthier and Degnan (2008). This paper reveals the potential intermixing of cells between fused partners. The third paper is the old report by Duerden, the first observation documenting ‘aggregated colonies’ (Duerden, 1902). It will also be valuable to cite Rinkevich (2011).
- Introduction 2nd paragraph, 1st sentence: ‘Modular marine invertebrates tend to aggregate after release’ is redundant to the former introduction text. Delete or rephrase.
- M & M: ‘... then mixed and gently agitated...’ Specify if the mixed eggs/sperm were from the same genotype or from all/several genotypes.
- M & M: ‘...to count the proportion of each type of fusion’. Which ‘types’ of fusion the authors imply to? Detail/explain.
- Discussion, below Fig 1: ‘...*P. daedalea* larvae still retained a degree of individuality...’. What is the meaning of ‘individuality’ here? What is the meaning of ‘a degree’? Morphologically? Physiologically? Define and rephrase.
- Discussion, the sentence: ‘It should be pointed out that though embryos here were produced sexually from 4 parent colonies, it did not denote they were genetically distinct’. Something is wrong with the sentence. If larvae are sexually produced they should be genetically distinct from each other. Rewrite.

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, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 26 Feb 2015

Lei Jiang, Chinese Academy of Sciences, China

- We revised the sentence in abstract as suggested. Our observation presented the embryonic chimeras as a result of fusion of embryos in a broadcast spawning coral, whereas the results of Mizrahi *et al.*, (2014) revealed that the larvae of brooding coral *Tubastraea coccinea* could metamorphose and aggregate in swimming groups. Thus we did document for the first time the fusion between individuals at the embryonic stage in reef corals. Moreover, we followed the settlement and growth of these chimeric larvae. Overall, it is proper and discreet to state “for the first time”.

- We have followed the reviewer's advice to use "conjoined larvae" in the Results and Abstract sections where described the larval joining.
- This was only an accidental observation and it was a pity that we did not preserve samples for histological sections, nor did we employ physical force to see whether the conjoined larvae would disjoint.
- As aforementioned, we did observe the embryonic chimeras and follow the settlement and post-settlement growth of chimeric larvae in a broadcast spawning coral for the first time. Mizrahi *et al.* (2014) revealed that the swimming polyp clusters survived longer and did not confirm whether these clusters can settle. Therefore, the findings of Mizrahi *et al.* (2014) were not contradictory to our statement of "the first time". We have added the suggested references. But as for Rinkevich (2011), we think it is more suitable to cite the classical reference of Rinkevich *et al.* (1987) which mainly focused on the chimerism in colonial marine invertebrates.
- We deleted the latter part of this sentence and made it more specific as to the synchrony in gametes release.
- We used the word "combined" to show that the bundles from 4 colonies were brought together for cross fertilization.
- We replaced "each type of fusion" with "chimeric larvae".
- We made it more specifically that these conjoined larvae were multi-headed to demonstrate the sectorial fusion.
- We were sorry for this mistake because we were a little confused at first when referring to the results of Puill-Stephan *et al.* (2012). They found that fusion between sexually produced larvae resulted in non-chimeric colonies of one genotype. Thus we got a misunderstanding that the sexually produced larvae were not genetically distinct absolutely. However, they only use nine microsatellite loci to genotype. We have corrected and rewritten this part.

Competing Interests: No competing interests were disclosed.

Referee Report 16 February 2015

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Andrew Baird

School of Marine Biology and Aquaculture, James Cook University, Townsville, QLD, Australia

This is well written article and an interesting observation although some of the literature cited is a little dated (e.g. Harrison & Wallace 1990). To my knowledge this is first time that anyone has followed these embryonic chimeras through to settlement and that fact that they can settle and grow is an important observation. However, embryonic chimera formation is an artifact of the experimental conditions i.e. the very high densities of embryos in still water in the bowls. Indeed, embryonic chimera formation is quite

common, particularly when working with larvae of merulinds at higher temperatures. It is very rare to see chimeras in spawn slicks or on settlement tiles, at least on soaks of short duration, although this has yet to be quantified. Therefore, embryonic chimera formation is highly unlikely to be of much ecological or evolutionary significance. Indeed, a phenomenon of much more ecological significance is the fact that many embryos break up during development under conditions likely to prevail in the wild (see Heyward and Negri, 2012).

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 26 Feb 2015

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Spawn slicks are commonly observed in the field and more importantly, they remained aggregated 1-2 days after spawning (Oliver *et al.*, 1987; Gilmour *et al.*, 2009). It is true that these conditions are favorable for the formation of embryonic chimeras. Moreover, the high density and absence of water turbulence in this study might trigger the fusion of embryos. Under similar experimental conditions, larvae of *A. millepora* tended to settle in aggregation (Puill-Stephan *et al.*, 2012), and larvae of *T. coccinea* formed swimming polyp clusters (Mizrahi *et al.* (2014)). Therefore, though fusion of coral embryos has never been reported in the wild, we speculate it might occur in the field and remains to be determined especially corals often broadcast spawn their gametes during calm periods and low-amplitude tides.

Competing Interests: No competing interests were disclosed.