

## **Exploring traits of engineered coral entities to be employed in reef restoration**

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### **Background**

A chimeric organism is an entity that possess cells derived from two or more conspecifics<sup>1</sup>. Natural chimerism is widely distributed in nature in a variety of phyla such as fungi, plants, vertebrates (including mammals) and marine solitary and colonial invertebrates<sup>2</sup>. In many sessile marine colonial invertebrates, including corals, direct allogeneic tissue-to-tissue contacts of newly settled conspecific juveniles (aggregated settlement) results in either fusion and thus, creating a chimeric entity, or rejection, the initiation of tissue necrosis. Although aggregated settlement and fusion was first described over a century ago<sup>3</sup> and thereafter in numerous coral species, the literature is deficient regarding the ecological and evolutionary costs vs. benefits and the molecular machinery associated with coral chimerism. Previous studies suggested an increased size and enhanced survival for coral chimeras under lab setting<sup>4</sup>. It was also claimed that chimeric entities, driven by genotypic diversity within the colony, may present a wider genetic expression<sup>5,6</sup>. Such improved ecological traits may maximize the resilience of the chimeric coral colonies, resulting in an augmented capacity to withstand environmental changes. The above suggest the consideration of coral chimerism as an important, yet still neglected, evolutionary rescue instrument, that can be used for applied needs<sup>2</sup>. It is well known that climate change and anthropogenic disturbances are rapidly leading coral reefs to degradation and biodiversity loss and that passive management acts (such as MPAs) have failed to reverse this trend<sup>7</sup>, emphasizing the need for novel active restoration tactics. It is thus claimed that coral chimerism may be used as a novel potential implement in the active reef restoration tool-box.

In my Ph.D. studies, I am studying various ecological and evolutionary aspects of coral chimerism as a new tool in the active reef restoration tenet. The present work is the first to test, under prolonged *in situ* (field) conditions, the hypothesis that coral chimerism is an evolutionary rescue instrument. For that purpose, I conducted experiments that evaluated, under real field conditions, the outcomes of chimerism on two levels of biological organizations: a) the outcomes of chimerism on the whole entity traits (growth, survivorship, pattern formation), and b) the outcomes of chimerism on gene expression traits (specific transcriptomic landscapes, behaviors of stress-responsive genes).

### **Methods**

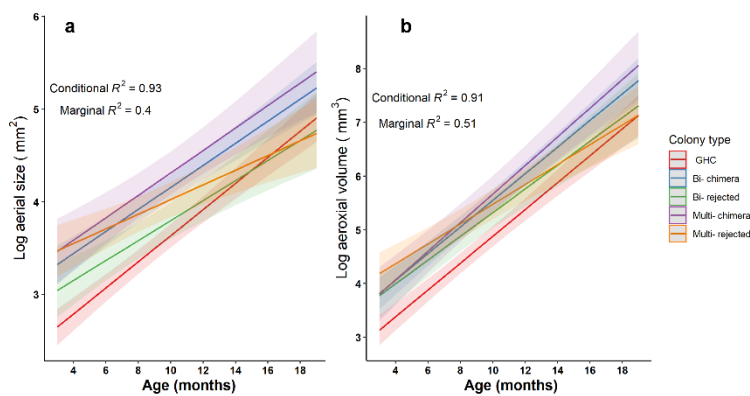
This study focuses on the branching brooding coral *Stylophora pistillata*, a model coral species (e.g., coral reproduction, chimerism in corals). Planulae were collected and reared to settle in aggregation in Petri dishes and created: i) chimeras (bichimeras – for 2 partners/entity; multichimeras- for > 2 partners/entity), ii) rejected entities (bi- and multi-, respectively), and iii) genetically homogenous colonies (GHC; regular colonies). After six months in the I.U.I. water tables, all coral entity types were

translocated to a mid-water floating nursery (10 meters depth) in the north part of the Gulf, designated for active restoration<sup>8</sup>, and maintained side by side for 12-16 months under natural conditions. To assess ecological traits of the whole holobiome following aggregated settlement, the coral entities were photographed periodically during their stay in the nursery to assess differences in growth and survivorship. Aerial size, height and aeroxial ecological volume (the colony's volume, including spaces between the branches) were measured for each photo. To assess differences in gene expression between chimeras and GHCs and their symbiotic dinoflagellates, selected chimeras and GHCs were assigned to two *in-situ* groups: (1) 'relaxed', control colonies (chimeras and regular colonies)– that were maintained under ambient conditions (10 meters on the nursery; deep platform) and (2) 'stressed' colonies (chimeras and regular colonies)- move to a shallow platform at 2 meters depth for two days.

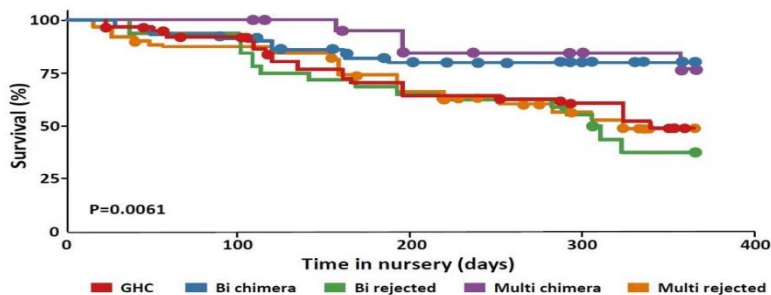
## Results and Discussions

### Whole entity traits

Results revealed an 'early astogenic stage' increased aerial size and aeroxial ecological volume (but not height) in chimeric and rejected entities, as compared to GHCs. However, as of the age of 18 months, no significant difference in all of these parameters was revealed (Fig. 1). These results are of importance since colonial size augmentation at early life stage may gain benefits for fitness by occupying space<sup>9,10</sup>, by decreased size-dependent mortality<sup>11,12</sup> and by sustaining earlier sexual maturation<sup>13,14</sup>. Indeed, despite the size equality, the survival of the chimeras (both bi- and multi-) was significantly higher than the rejected entities and GHCs (Fig. 2). Chimerism further influenced pattern formation as chimeras initiated more than the typical single up-growing branch (the first branch that is developed in a colony). Higher survival rates and different pattern formation for chimeras indicate that fusion positively affects some ecological traits for the whole chimeric entity.



**Figure 1:** The best-fitted mix linear models for (a) areal size (mm<sup>2</sup>), and (c) aeroxial volume. Covariate (X-axis) is the age in months, random factors are the samples. Each line represents a linear model for each colony type as described in the caption. Conditional R<sup>2</sup> (the proportion of variance explained by the whole model) and marginal R<sup>2</sup> (proportion of variance explained by the fixed factor alone) are attached to each sub-figure.



**Figure 2.** Kaplan–Meier survival curves (with the p-value) for all colony types along a period of one year in the **midwater coral nursery under ambient field conditions**. The Y-axis depicts the cumulative survival probabilities and the X-axis is the time in the nursery in days. Circles on lines indicate census dates.

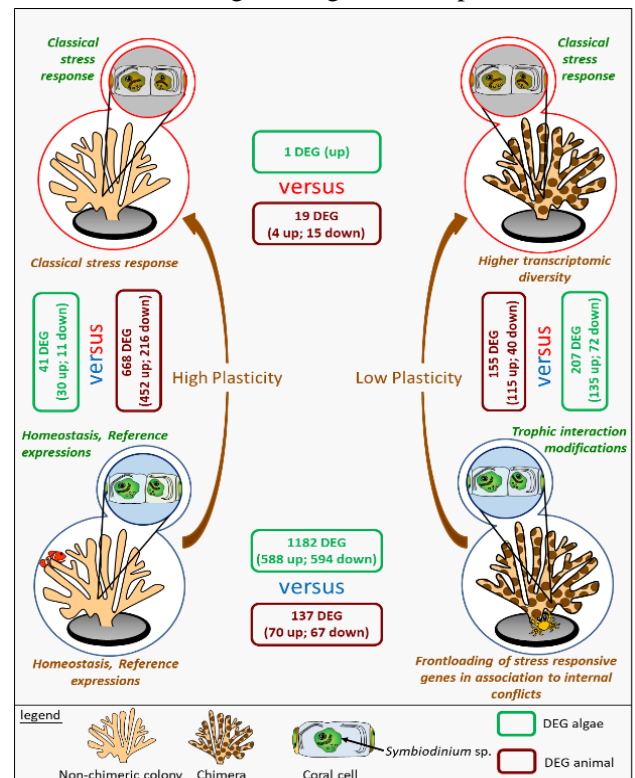
### Gene expression traits

For the gene expression studies, while ITS2 amplicon sequencing revealed that there was not a difference in the microbiome and *Symbiodinium* sp. hosted by the colony type in chimeras and GHCs residing in both platforms, the transcriptome analyses revealed that even under the ‘relaxed’ conditions regimen (the environmental settings at the lower platform), chimeras and GHCs hosts expressed differently numerous genes. The zooxanthellae, as well, while maintained under the same environmental conditions, were affected by the chimeric status, revealing hundreds of genes differentially expressed between chimeras and GHCs. Yet, under the stress conditions inflicted in the shallower platform, gene expressions landscapes between the groups equalized, and only negligible differences in gene expression were recorded (just 1 and 19 expressed genes in the algae and coral host, respectively; Fig. 3). I further noted that most of the differentially expressed genes in GHCs between the shallow and the deep platforms were stress-related genes already expressed at higher levels in the deep platform ‘relaxed’ chimeras. Our findings suggest a beforehand climate-attuned genomic signature in the chimeric corals’ transcriptomic landscape characterized by frontloading of stress-responsive genes, making chimeras already prepared to encounter environmental stresses.

### Conclusions

My study is the first to explore the ecological and molecular traits affecting the fitness of chimeric coral colonies *in natura*. As emerged here, chimeric coral colonies display improved ecological and molecular proficiencies that may augment robustness, withstanding climate change impacts, making chimerism as an evolutionary rescue mechanism. Therefore, harnessing this chimeric mechanism as a novel applied tool in active reef restoration may serve as one of the groundbreaking approaches in reef restoration. Employing this approach should be further considered for augmenting coral adaptation in the changing world<sup>2,15</sup>. Studying coral chimerism under natural conditions (the PhD topic) is not a trivial task and more has to be investigated before a better understanding can be achieved regarding this unique natural phenomenon.

**Figure 3:** Schematic representation of key results and conclusions supporting that chimeras are robust entities characterized by frontloading of stress-responsive genes, lower transcriptomic plasticity, and higher transcriptomic diversity. When acclimated at 10m depth (blue circle), non-chimeric colonies (unicolor corals) and chimeras (bicolor corals) differentially express numerous cnidarian (brown box) and algal (green box) genes (blue ‘versus’). In response to translocation to 2m depth for 48h (red circle), the transcriptomic responses of non-chimeric colonies drastically differed from their chimeric counterparts (blue and red ‘versus’), while at 2m depth, these differences were negligible (red ‘versus’). Brown boxes: cnidarian DEGs; green boxes: *Symbiodinium* sp. DEGs.



## References

1. Rinkevich, B. & Weissman, I. L. Chimeras in colonial invertebrates: a synergistic symbiosis or somatic-cell and germ-cell parasitism? *Symbiosis* **4**, 117–134 (1987).
2. Rinkevich, B. Coral chimerism as an evolutionary rescue mechanism to mitigate global climate change impacts. *Glob. Chang. Biol.* **25**, 1198–1206 (2019).
3. Duerden, J. E. Aggregated Colonies in Madreporarian Corals. *Am. Nat.* **36**, 461–471 (1902).
4. Amar, K. O., Chadwick, N. E. & Rinkevich, B. Coral kin aggregations exhibit mixed allogeneic reactions and enhanced fitness during early ontogeny. *BMC Evol. Biol.* **8**, 126 (2008).
5. Rinkevich, B., Shaish, L., Douek, J. & Ben-Shlomo, R. Venturing in coral larval chimerism: A compact functional domain with fostered genotypic diversity. *Sci. Rep.* **6**, 1–7 (2016).
6. Maier, E., Buckenmaier, A., Tollrian, R. & Nürnberger, B. Intracolony genetic variation in the scleractinian coral *Seriatopora hystrix*. *Coral Reefs* **31**, 505–517 (2012).
7. Bates, A. E. *et al.* Climate resilience in marine protected areas and the ‘Protection Paradox’. *Biol. Conserv.* **236**, 305–314 (2019).
8. Shafir, S., Van Rijn, J. & Rinkevich, B. A mid-water coral nursery. in *Proceeding of 10th international coral reef symposium* 1674–1679 (2006).
9. Raymundo, L. J. & Maypa, A. P. Getting bigger faster : mediation of size-specific mortality via fusion in juvenile coral transplants. *Ecol. Appl.* **14**, 281–295 (2004).
10. Connell, J. H. Population ecology of reef-building corals. *Biol. Geol. coral reefs* **2**, 205–245 (1973).
11. Christiansen, N. A., S., W., S., H. & Tibbetts, I. . R. Grazing by a small fish affects the early stages of a post-settlement stony coral. *Coral Reefs* **28**, 47–51 (2009).
12. Penin, L. *et al.* Early post-settlement mortality and the structure of coral assemblages. **408**, 55–64 (2010).
13. Wallace, C. C. & Harrison, P. L. Reproduction , dispersal and recruitment of scleractinian corals. in *Ecosystems of the World, 25. Coral Reefs* (ed. Dubinsky, Z.) **25**, 133–207 (Coral Reefs. Elsevier Science Publishing Company, Inc., 1990).
14. Rinkevich, B. & Loya, Y. The reproduction of the Red Sea coral *Stylophora pistillata*. I. Gonads and planulae. *Mar. Ecol. Prog. Ser.* **1**, 145–152 (1979).
15. Rinkevich, B. The active reef restoration toolbox is a vehicle for coral resilience and adaptation in a changing world. *J. Mar. Sci. Eng.* **7**, 201 (2019).
16. Puill-Stephan, E., van Oppen, M. J. H., Pichavant-Rafini, K. & Willis, B. L. High potential for formation and persistence of chimeras following aggregated larval settlement in the broadcast spawning coral, *Acropora millepora*. *Proc. R. Soc. London. Ser. B Biol. Sci.* **279**, 699–708 (2012).

17. Mizrahi, D., Navarrete, S. A. & Flores, A. A. V. Groups travel further: pelagic metamorphosis and polyp clustering allow higher dispersal potential in sun coral propagules. *Coral Reefs* **33**, 443–448 (2014).