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Partitioning of genetically distinct cell populations in chimeric juveniles of the sponge *Amphimedon queenslandica*

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Summary
Natural chimerism, the fusion between genetically distinct conspecifics, is a process known to occur in various marine benthic invertebrates. Sponges (phylum Porifera) have proven to be a useful model to study the origin and evolution of allorecognition. Like some other invertebrates, they display an ontogenetic shift in their allorecognition response: genetically different individuals can fuse during early development, but, in most instances, not as adults. However, there is a limited understanding of the cellular organisation of sponge chimeras and the onset of this allorecognition response, which prevents integration of incompatible genotypes. Here we follow the behaviours and fates of cells derived from genetically distinct larvae of the demosponge *Amphimedon queenslandica* that have fused together at metamorphosis. By labelling individual larvae with different fluorescent dyes, we can follow cell movement in the postlarval chimeras. We observed that cells from the two individuals readily mixed for 2 weeks after the initial fusion. After that time, differently labelled cells began to sort into different postlarval cellular territories, with one lineage giving rise to choanocytes and the other to pinacocytes and cells of the mesohyl. These results suggest that a rapid ontogenetic shift in the allogeneic response of *A. queenslandica* occurs about 2 weeks after the initiation of metamorphosis and that the molecular basis of this response is also involved in creating differential cell affinities that underlie the construction of the sponge body plan. Compatible with this proposition is the observation that cells from postlarvae that are allowed to develop for 2 weeks before contact do not fuse and form a distinct boundary between genotypes. The successful chimeras remained stable for the duration of the experiment (3 weeks) raising the possibility that reproductive chimeras might persist in the natural environment, with a single genotype giving rise to germ cells.

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Introduction

Suitable space for settlement and growth is often limited for sessile marine invertebrates that live on the benthos and in close proximity to one another. Competition for habitat can be intense and the risk of overgrowth and episettlement is high. This can lead to chimerism, which is defined as the fusion between genetically distinct conspecifics. This phenomenon has been documented in different phyla of protists, fungi, plants and animals (reviewed in Buss [1] and Grosberg [2]). It can be potentially detrimental for organisms that do not sequester their germline or only do so late in their ontogeny [1,3], as it might lead to germline competition and parasitism. To evaluate the evolutionary significance of chimerism, several studies have measured its fitness costs and benefits. While fusion between different genotypes has proven to be beneficial in some organisms, such as slime moulds and algae, no definite benefits have been demonstrated in marine invertebrates, even when the fusions tested were between close kin (reviewed in Rinkevich [4]; [5]). To our knowledge, only one study has evaluated the beneficial effect of genetic flexibility in marine invertebrates—chimeras of the ascidian *Botryllus schlosseri* were shown to exploit the joint genomic fitness of the fused partners, depending on the environmental conditions. However, fitness tests were not performed and chimeras still displayed germ cell parasitism [6].

Various marine invertebrates possess recognition mechanisms sensitive enough to discriminate not only closely related species but also genetically different individuals from the same species (conspecifics or allogenenics) (reviewed in Grosberg [2]). These genetic allorecognition systems appear to have arisen independently in different invertebrate groups [7]. It has been proposed that these mechanisms emerged as an adaptation to counter the risks associated with chimerism. Interestingly, there is an apparent absence of allorecognition in early stages of ontogeny, in both vertebrates and invertebrates [8–15], which could have contributed to the retention of chimerism through evolution. It has been proposed that this absence could be due to changes associated with the costs and benefits of fusion across different life cycle stages [12]. However, it is possible that larvae and early juveniles simply lack a functional immune system and thus are unable to reject fusion, at least initially [5,15,16].

This ontogenetic shift in allorecognition raises questions regarding the long-term stability of chimeras that are established early in development. Are these associations transitory and cease to persist once maturation of the allorecognition system is reached, or alternatively, can the fused partners develop tolerance to one another and maintain their chimeric association through time? Genotyping experiments in the hydrozoan *Hydractinia symbiolongicarpus* suggest that histoincompatible embryonic chimeras are unstable after the onset of alloimmunity at metamorphosis, with only one genotype being detectable in 1-month-old polyps. The surviving polyps do not display allotolerance either, as they only fuse with polyps sharing the same fusibility/rejection characteristics [15]. However, there are studies that demonstrate that chimerism is not just a transitory state under ontogenetic control. For instance, chimeras of *B. schlosseri* show resorption of one of the adult partners, but the blood, the soma and the germ cells of the remaining partner are in many cases chimeric, pointing to cell lineage parasitism. Moreover, there are cases where the whole mass of gonads, as well as the soma, are derived from the resorbed individual [17–20].

These observations also imply that if distinct cell lineages are disproportionately maintained in a chimera, it might be difficult to detect both genotypes. One study followed the fate of cells in a chimera during development, in which halves of two different embryos of *H. symbiolongicarpus* were grafted together, with one of the partners stained with neutral red [21]. Interestingly, half-embryos that rejected the fusion retained a few red-stained cells in the unstained half after separation post-metamorphosis. However, in this study, the genetic relatedness of the grafted embryos was not ascertained, since they were obtained from a multi-parental pool, and observations were curtailed shortly after metamorphosis. Therefore, it would be extremely valuable to establish how the cell populations of two genetically distinct chimeric partners interact throughout development in other invertebrates, to further document the transitory/permanent nature of chimerism.

Sponges are part of the marine benthic community and display allorecognition responses. Although the genetic nature behind this recognition process is unknown, highly polymorphic genes have been proposed as potential factors [22,23]. In adult sponges, most histocompatibility studies have found that tissue fusion occurs exclusively between isogeneic individuals (reviewed in [24]). However, there are a few reports of allograft (genetically different individuals of the same species) [25–27] and even xenograft (individuals of different species) acceptance [28]. A study on chimeric gemmules of the sponge *Ephydatia muelleri* also suggests interstrain histocompatibility [29]. While the formation of adult chimeras in the field is equivocal, there is evidence from various laboratory studies that sponge larvae may settle together to form chimeric individuals. These results have been obtained with assays performed between larvae of isogeneic or allogeneic partners [5,12,13,29–36]. However, it has not been determined at which developmental stage the poriferans reach allomaturity and whether chimeras remain stable after this.

Unlike colonial invertebrates such as ascidians and hydrozoans, sponge larval chimeras completely merge upon fusion and it is difficult to follow the fate of each individual. Therefore, it has not been established whether fusion entails conflict or cooperation and if both individuals are maintained in the chimera over time. If cooperation occurs, it is also unknown how the different genotypes distribute themselves within a chimera, whether there is cellular intermixing, or compartmentalisation with cells of each individual taking specialised roles in the chimera.

Fluorescent labelling has previously allowed to track the fate of the outer cell layer of larvae in the demosponge *Amphimedon queenslandica*, during metamorphosis and postlarval development. These cells migrate inwards and transdifferentiate into three cell types in the juvenile sponge: the flagellated choanocytes, the pinacocytes and cells of the mesohyl [37]. A similar pattern has been observed in the sponge *Halisarca dujardini*, in which a cell marker, specific to the larval flagellated cells, can be detected in the choanocyte and the upper pinacocyte of