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Heritable variation in growth and biomass productivity in the clonal freshwater macroalga *Oedogonium*

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ABSTRACT

In an analogous manner to which selective breeding and propagation of heritable traits have improved terrestrial crops, the directional selection of target strains of commercial algae produced through clonal propagation is essential to realising their full potential for biomass applications and develop cost effective production. As a first step towards establishing a trait selection programme in clonal freshwater macroalgae of the genus *Oedogonium*, we determined whether there is heritable variation in growth and biomass productivity in 6 genetically distinct strains. Intraclonal variability in growth was quantified within clonal lineages of single filaments in laboratory cultures, and intraclonal variability in biomass productivity was quantified within clonal lineages of intensive cultures containing multiple filaments maintained in outdoor cultures. We found significant estimates of genetic variance (Broad-sense heritability, H^2: 0.16–0.51) in growth among clonal replicates of 4 strains in laboratory cultures, and significant estimates of genetic variance ($H^2 > 0.69$) in biomass productivity in two strains in outdoor intensive cultures. These results demonstrate that there is an important heritable component to variation in growth and biomass productivities among theoretically identical clonal lineages for these strains. Such data is rare for freshwater macroalgae and suggest that growth and biomass productivity for these strains could be improved by maintaining selected clonal lines.

1. Introduction

A key step in the development of a cost effective production industry for any novel target species is the establishment of a successful breeding programme. Such programmes utilise directional selection (selection for one end of the phenotypic distribution of a trait) to gradually improve the phenotypic expression of a trait of interest, such as growth or productivity, over successive generations [2,12]. To enable directional selection two conditions need to be satisfied: first there must be variation in the trait of interest, and second, some of this variation must be heritable [2]. This first condition may be difficult to satisfy for organisms which reproduce through clonal propagation. Vegetative fragments generated through clonal propagation are often considered to be identical copies of the tissue they originated from [23]. However, genetic variation can occur in clonally reproduced cell lineages through a number of mechanisms including somatic line mutations, intragenomic recombination, mobile genetic elements, gene duplication, and ploidy changes [25]. Somatic line mutations are one of the most common causes of genetic variation in clonally reproduced cell lineages. These mutations arise during mitotic cell divisions and are passed onto all daughter cells when they occur in undifferentiated tissues with the capacity for future cellular division [20]. Because clonal reproduction does not involve genetic recombination, any genetic variation can be passed onto subsequent clonal generations meaning that, for all intents and purposes, it is heritable [25,35].

The evolutionary consequences of intraclonal genetic variation have received little attention and have focused on only a few groups of multicellular organisms [25]. However, a number of studies have demonstrated phenotypic variation within clonal lineages of macroalgae [13,14,30]. Moreover, recent studies have demonstrated that natural selection on phenotypic variation in clonal lineages can lead to evolutionary change in those lineages [15,17] and artificial selection of clonal cell lineages can lead to significant differences in trait expression relative to randomly selected control lines [16]. These findings indicate that there is considerable potential for directional cell-lineage selection to act upon phenotypic variation in clonal lineages and change the expression of selected traits over multiple clonal generations. Furthermore, they suggest that breeding programme protocols and procedures developed for sexually reproducing organisms can be successfully applied to clonally propagated lineages.

Modern breeding programmes for plants and livestock have been in existence for more than a century, revolutionising production through the development of high-yielding varieties [4,5]. To determine whether it would be possible to establish an analogous breeding programme in a clonally propagated alga, we tested whether heritable variation exists in clonal lineages of freshwater macroalgae (large, multicellular algae). Freshwater macroalgae are an emerging novel target for a diverse

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range of applications, including biofuels [8,19], the bioremediation of waste waters [9,18,26,31], animal feed [3], fertiliser and soil conditioners [1] and as a tool for carbon sequestration [1]. When cultivated intensively, freshwater macroalgae such as *Oedogonium* – the genera identified as a target for biomass applications [10] – grow predominantly via vegetative (clonal) propagation and sexual reproduction is rare. Target strains of *Oedogonium* for intensive cultivation have only recently been identified based on the performance of wild type strains isolated from natural environments [10,11]. In order to realise the full potential of freshwater macroalgae and deliver on biomass applications, directional selection in target strains is essential.

Most industrial applications and end-use products of macroalgae require large amounts of biomass to be produced in a given area due to the high costs of construction of algal facilities [28,34]. While fast growth rates are desirable in target species, the critical measure for the majority of end-product applications is "areal" biomass productivity — the amount of dried ash-free biomass per unit area (m^2) per time (day) as a product of the rate of growth and density of the biomass [6,10,22]. Therefore, the aim of this study was to determine whether there is heritable variation in growth and biomass productivity between clonal lineages of 6 genetically distinct strains of the freshwater macroalgal genus *Oedogonium*. Previous studies of heritability in macroalgae have quantified variation in clonal lineages of single thalli (e.g. [15]). However, demonstration of significant heritability of growth rate of individual thalli/filaments may not translate to significant heritability of biomass productivity when multiple thalli/filaments are cultured together if, for example, positive traits in some filaments within a culture are diluted by negative traits in others. There is also potential for growth to co-vary with traits such as tolerance to variable temperature and light irradiance that are important at the mass culture scale but are not evident under controlled environment laboratory trials. Consequently, we tested for heritable variation in both single filaments in laboratory cultures and at a larger scale of intensive outdoor cultures. Our specific aims were to first determine whether there is heritable variation in growth between clonal lineages of single filaments of *Oedogonium*, and second, to determine whether there is heritable variation in biomass productivity between clonal lineages of these same strains containing multiple filaments (~100,000 s) under intensive cultivation. Testing for heritability at a culture scale will enable us to determine whether any individual filament effects can be scaled up to a size relevant for biomass production. A finding of heritable variation would demonstrate that there is potential for growth and/or biomass productivity of *Oedogonium* to be improved through a trait selection programme and would be the first demonstration of this for freshwater macroalgae.

2. Methods

2.1. Sample collection and isolation

Oedogonium is a cosmopolitan genus of filamentous freshwater green macroalgae that has a worldwide distribution and is a common component of natural ecosystems. It is an unbranched, uniseriate green algae made up of small cylindrical cells. *Oedogonium* is a robust and competitively dominant genera that has been identified as a key target for the bioremediation of freshwater waste streams [3,26] and as a feedstock biomass for bioenergy applications [10,19]. Strains were originally isolated from samples of macroalgae collected from naturally occurring water bodies, irrigation channels and wetland areas in three distinct geographic regions of Australia — Riverina (35°S, 145°E), SE Queensland (26°S, 151°E) and North Queensland (19°S, 146°E). Two strains originating from each of the 3 regions were used in the experiment — Riv4 and Riv6 from Riverina, Tar1 and Tar4 from Tarong, and Tsv1 and Tsv2 from Townsville. Detailed collection information and methods for species identification are provided in Lawton et al. [11] for strains Riv4, Tar1, Tar3, Tsv1, and Tsv2 and as supporting information for Riv6 (Supporting information, Appendix A). Following isolation, strains were maintained in nutrient enriched autoclaved freshwater in a temperature and light controlled laboratory (12:12 light:dark cycle, 50 μmol photons m^{-2} s^{-1}, 23 °C) for at least 1 year prior to experiments. All strains are genetically distinct [11].

2.2. Clonal lineages

When cultured, *Oedogonium* grows through diffuse growth of cells in filaments with vegetative (clonal) propagation of these filaments through fragmentation. Sexual reproduction is rare and while we occasionally observe female reproductive cells (oogonia), we have never observed male reproductive structures in the cultures. Consequently, any new biomass that grows within a culture is a clone of the original biomass contained within that culture. Once the biomass within a culture has increased to the point where the original biomass now constitutes only a small percentage (<10%) of the total culture, the biomass contained within that culture could be considered to be a new "generation". In this way, we created multiple generations to produce clonal lineages of *Oedogonium* strains. Stock cultures were established by isolating a single filament from each strain and allowing this to grow until sufficient biomass was reached to begin the experiments.

2.3. Heritability in single filaments

Intraclonal variability in growth was quantified within clonal lineages of single *Oedogonium* filaments of each of the 6 strains maintained in laboratory cultures. At the start of the experiment, a parental (F_0) generation was established by isolating a single filament of each strain from stock cultures and cutting these filaments to a standardised length of 6 mm. Each filament was maintained in a sterile 60 mm petri dish in nutrient enriched (MAF growth medium, Manutech Pty Ltd, 13.4% N, 1.4% P; 0.05 g L^{-1}) dechlorinated freshwater in culture cabinets at 24.5 °C with 12 hour light:12 hour dark cycles and a light level of 50 μmol m^{-2} s^{-1} photosynthetically active radiation (Supporting information, Appendix B, Table S1). These conditions correspond to the ambient summer conditions in Australia used in a previous growth experiment with these strains [11]. Growth was measured by photographing each filament under a dissecting microscope (Olympus model SZ61) at the start and end of a 7 day period and then determining the 2-dimensional surface area of filaments using ImageJ [32]. Specific growth rates were calculated for each individual filament of each strain using the equation SGR (% day^{-1}) = $\mathrm{Ln}(B_f / B_i) / T * 100$, where B_f and B_i are the final and initial surface areas (mm^2) and T is the number of days in culture. At the end of the 7 day period, 5 new filaments were cut from the biomass grown in each replicate during the previous week of the experiment. These new filaments were distributed into new, independent petri dishes and were considered to be the next generation (F_1). This process was repeated a further 2 times to create a total of 3 clonal generations (F_{1-3}) of known ancestry for each of the 6 strains. As no replicates of strains Riv4 or Tsv2 survived past the first generation, these strains were excluded from analysis in this experiment.

2.4. Heritability in intensive cultures

Intraclonal variation in biomass productivity was quantified within clonal lineages of 6 strains of *Oedogonium* maintained in intensive, outdoor cultures. Stock cultures of each strain were grown in 5 L plastic buckets in a greenhouse with ambient natural light at the Marine and Aquaculture Research Facility Unit, James Cook University. Cultures were provided with aeration by a continuous stream of air entering the cultures through multiple inlets around the base of the buckets. Culture water was enriched (0.05 g L^{-1}) with MAF growth medium (Manutech Pty Ltd). All experimental replicates were maintained under the same conditions. Stock cultures were maintained for a period of at least 3 weeks prior to the start of each experiment to allow