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Evolutionary biology

Environmentally induced responses co-opted for reproductive altruism

Aurora M. Nedelcu[1,2,*]

[1]*Department of Biology, University of New Brunswick, PO Box 4400, Fredericton, New Brunswick, Canada, E3B 5A3*
[2]*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA*
**anedelcu@unb.ca*

Reproductive altruism is an extreme form of altruism best typified by sterile castes in social insects and somatic cells in multicellular organisms. Although reproductive altruism is central to the evolution of multicellularity and eusociality, the mechanistic basis for the evolution of this behaviour is yet to be deciphered. Here, we report that the gene responsible for the permanent suppression of reproduction in the somatic cells of the multicellular green alga, *Volvox carteri*, evolved from a gene that in its unicellular relative, *Chlamydomonas reinhardtii*, is part of the general acclimation response to various environmental stress factors, which includes the temporary suppression of reproduction. Furthermore, we propose a model for the evolution of soma, in which by simulating the acclimation signal (i.e. a change in cellular redox status) in a developmental rather than environmental context, responses beneficial to a unicellular individual can be co-opted into an altruistic behaviour at the group level. The co-option of environmentally induced responses for reproductive altruism can contribute to the stability of this behaviour, as the loss of such responses would be costly for the individual. This hypothesis also predicts that temporally varying environments, which will select for more efficient acclimation responses, are likely to be more conducive to the evolution of reproductive altruism.

Keywords: reproductive altruism; evolution; acclimation; *Volvox*; *Chlamydomonas*; soma

1. INTRODUCTION

Reproductive altruism is an extreme form of altruism best exemplified by sterile castes in social insects and somatic cells in multicellular organisms (e.g. Buss 1987; Maynard Smith & Szathmáry 1997; Queller 2000). Although reproductive altruism is central to the emergence of multicellularity and eusociality, the mechanistic basis for the evolution of this behaviour is yet to be deciphered (e.g. Queller 2000; Toth & Robinson 2007). In eusocial insects, caste evolution is thought to have involved the remodelling of conserved regulatory circuits present in their solitary ancestors, including pathways associated with basic life-history traits such as nutrition, metabolism and reproduction (e.g. Toth & Robinson 2007). Similarly, we have suggested that the evolution of soma in multicellular lineages involved the co-option of life-history genes whose expression in their unicellular ancestors was conditioned on environmental cues (as an adaptive strategy to enhance survival at an immediate cost to reproduction), through shifting their expression from a temporal (environmentally induced) into a spatial (developmental) context (Nedelcu & Michod 2004, 2006).

To address this proposal, I am using a group of closely related green algae (Volvocales) comprising both unicellular species (e.g. *Chlamydomonas reinhardtii*) and multicellular forms with a complete division of labour between reproductive and somatic cells (e.g. *Volvox carteri*) (Kirk 1998). *Volvox carteri* consists of approximately 2000 permanently biflagellated sterile somatic cells and up to 16 non-flagellated reproductive cells. The terminal differentiation of somatic cells in *V. carteri* is dependent on the expression of *regA*—a master regulatory gene that encodes a transcription factor thought to repress several nuclear genes coding for chloroplast proteins (Kirk *et al.* 1999; Meissner *et al.* 1999). As a result, the growth (dependent on photosynthesis) and reproduction (dependent on growth) of somatic cells are suppressed. Repression of their reproductive potential is costly for somatic cells, but this behaviour is beneficial for the group as a whole, since it ensures its motility. In other words, somatic cells are altruistic, and *regA* is directly responsible for this behaviour (see discussion in Nedelcu & Michod 2006).

We identified *regA*'s closest homologue in *C. reinhardtii* and showed that this gene (presently known as *rls1*; Duncan *et al.* 2007) is induced in the dark, when the expression of chloroplast proteins is downregulated (Nedelcu & Michod 2006). Based on those findings, we hypothesized that *rls1* is generally induced under conditions when the temporary downregulation of photosynthesis is beneficial in terms of survival, though costly in terms of immediate reproduction (Nedelcu & Michod 2006). Such conditions include acclimation—a suite of physiological responses that restore the balance between produced and used energy, in response to various environmental changes (Grossman 2000). To test this hypothesis, the current study investigated the expression of *rls1* under several environmental stress factors (phosphorus, sulphur and light deprivation) and compared the timing of *rls1* induction with changes in *C. reinhardtii*'s reproductive potential.

2. MATERIAL AND METHODS

(a) *Algal strain and growth conditions*

A wall-less *C. reinhardtii* strain (CC2454) was obtained from the *Chlamydomonas* Center (http://www.chlamy.org/). Synchronized cultures were grown in Tris-acetate-phosphorus (TAP) medium (Gorman & Levine 1965), on a shaker (approx. 150 r.p.m.) at 25°C, on a 12 h L : 12 h D cycle. Phosphorus- and sulphur-deprived cultures were grown in TAP lacking phosphorus and sulphur, respectively (Chang *et al.* 2005); light-deprived cultures were grown in regular TAP, but flasks were covered in aluminium foil. To inhibit the photosynthetic electron flow, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; 50 mM stock in 95% ethanol) was added to a final concentration of 10 μM (Wykoff *et al.* 1998).

(b) *Gene expression analyses*

RNA was extracted from *ca* 1×10^7 cells, using the Qiagen RNeasy Plant Mini Kit, and the DNA was removed using an Invitrogen DNase I. cDNA synthesis and PCR were carried out with the

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Invitrogen Thermoscript RT–PCR system, the Invitrogen Platinum PCR SuperMix High Fidelity and the PCRx Enhancer System. Primers were designed across introns to distinguish between cDNA and genomic amplification products (Nedelcu & Michod 2006).

3. RESULTS

Chlamydomonas reinhardtii cultures were started at low density and grown in parallel under four distinct sets of conditions: (i) control (replete medium); (ii) without phosphorus; (iii) without sulphur; and (iv) dark. Under our conditions, in replete medium, cells grow four times in volume (during the light phase) and divide twice (during the dark phase) to produce four daughter cells; thus, the control culture quadrupled every 24 h during the logarithmic growth phase and entered the stationary phase by day 3 (figure 1*a*). On the other hand, as expected (e.g. Sager & Granick 1953; Wykoff *et al.* 1998), the reproductive potential of both nutrient- and light-deprived cultures was negatively affected (relative to the control culture), with the sulphur-deprived culture showing the most dramatic decline (figure 1*a*).

Interestingly, *rls1* was expressed not only in the absence of light (Nedelcu & Michod 2006), but also under phosphorus and sulphur deprivation, as well as in the control culture as it entered the stationary phase (figure 1*b*). Furthermore, in all four treatments, the induction of *rls1* corresponded with a decline in the reproduction potential of the population, independent of the culture's age and density. For instance, in the sulphur-deprived culture, *rls1* was expressed as soon as day 2, at only 1.3×10^{6} cells ml^{-1}, whereas in the control culture, *rls1* was induced starting with day 3, after the culture reached 1.1×10^{7} cells ml^{-1}; note that in both cases, cultures are in the stationary phase (figure 1*c*).

Acclimation involves both specific responses (e.g. scavenging for a specific nutrient) and general responses. The latter entail various photosynthetic and metabolic changes that ultimately result in the temporary cessation of cell growth and division (Grossman 2000). The fact that (i) *rls1* is expressed under multiple stress conditions (figure 1*b*); (ii) its induction coincides with a decline in reproduction potential (figure 1*c*); and (iii) it codes for a putative transcriptional repressor (Nedelcu & Michod 2006), suggests that *rls1* is part of the general acclimation response and might function as a regulator of acclimation in *C. reinhardtii*. In support of this suggestion is the finding that an inhibitor of the photosynthetic electron flow that triggers acclimation-like responses (i.e. DCMU; Wykoff *et al.* 1998) also induces the expression of *rls1* (figure 1*d*).

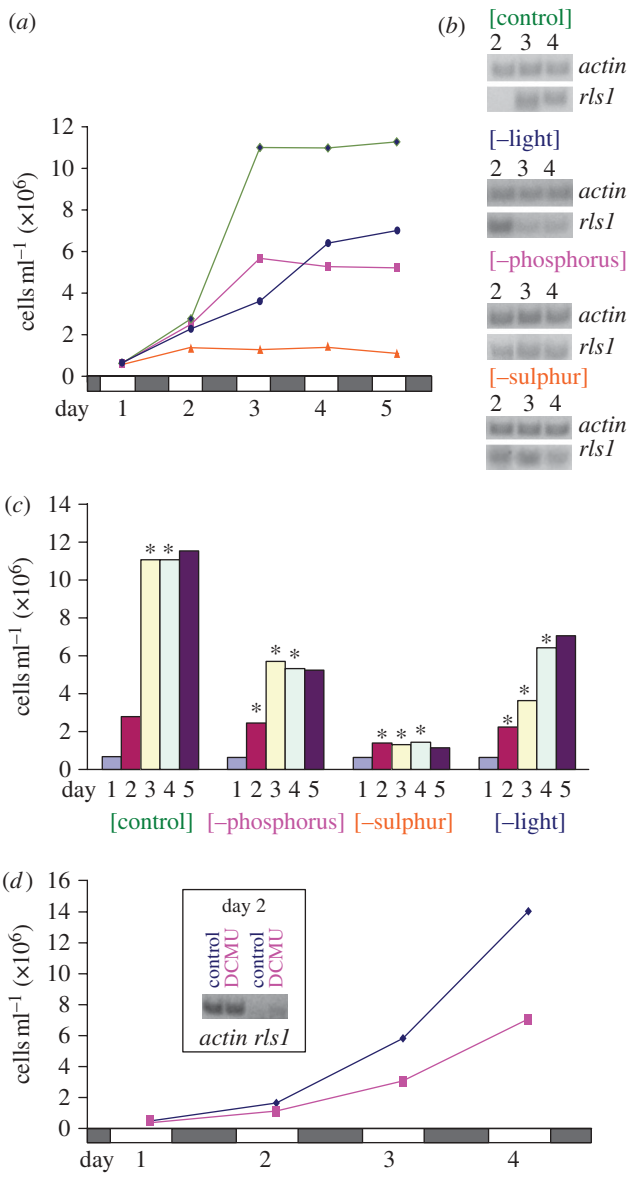

Figure 1. *Chlamydomonas reinhardtii* growth and *rls1* expression during phosphorus, sulphur and light deprivation. (*a*) Growth curves of control and deprived *C. reinhardtii* cultures; open and solid boxes indicate the succession of light and dark phases. Green line, control; blue line, minus light; purple line, minus phosphorus; orange line, minus sulphur. (*b*) Expression of *rls1* (RNA was extracted 4 h before the onset of dark; semi-quantitative RT–PCR; Nedelcu & Michod 2006) in the control and deprived cultures from (*a*); several independent experiments showed similar expression patterns (data not shown). (*c*) Correspondence between time of *rls1* induction (indicated by asterisks) and decline in reproduction potential of control and deprived *C. reinhardtii* cultures from (*a*) (note that as cells divide during the dark phase, the induction of *rls1* in a given day will affect the division in the next dark phase and will reflect in the cell counts of the following day). (*d*) Induction of *rls1* in the presence of the photosynthetic electron transport inhibitor, DCMU (RNA was extracted 24 h after the addition of the inhibitor; i.e. 5.5 h into the light phase of day 2). Blue line, control; purple line, DCMU.

4. DISCUSSION

How can general acclimation responses be co-opted for reproductive altruism? In photosynthetic organisms, the flux of electrons through the electron-transport system (ETS) has to be balanced with the rate of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) consumption; imbalances between these processes can result in the generation of damaging reactive oxygen species (e.g. Wykoff *et al.* 1998). When a nutrient (such as sulphur or phosphorus) becomes limiting in the environment, ATP and NADPH consumption declines (figure 2). This results in an excess of excitation energy and a subsequent change in the redox state of the

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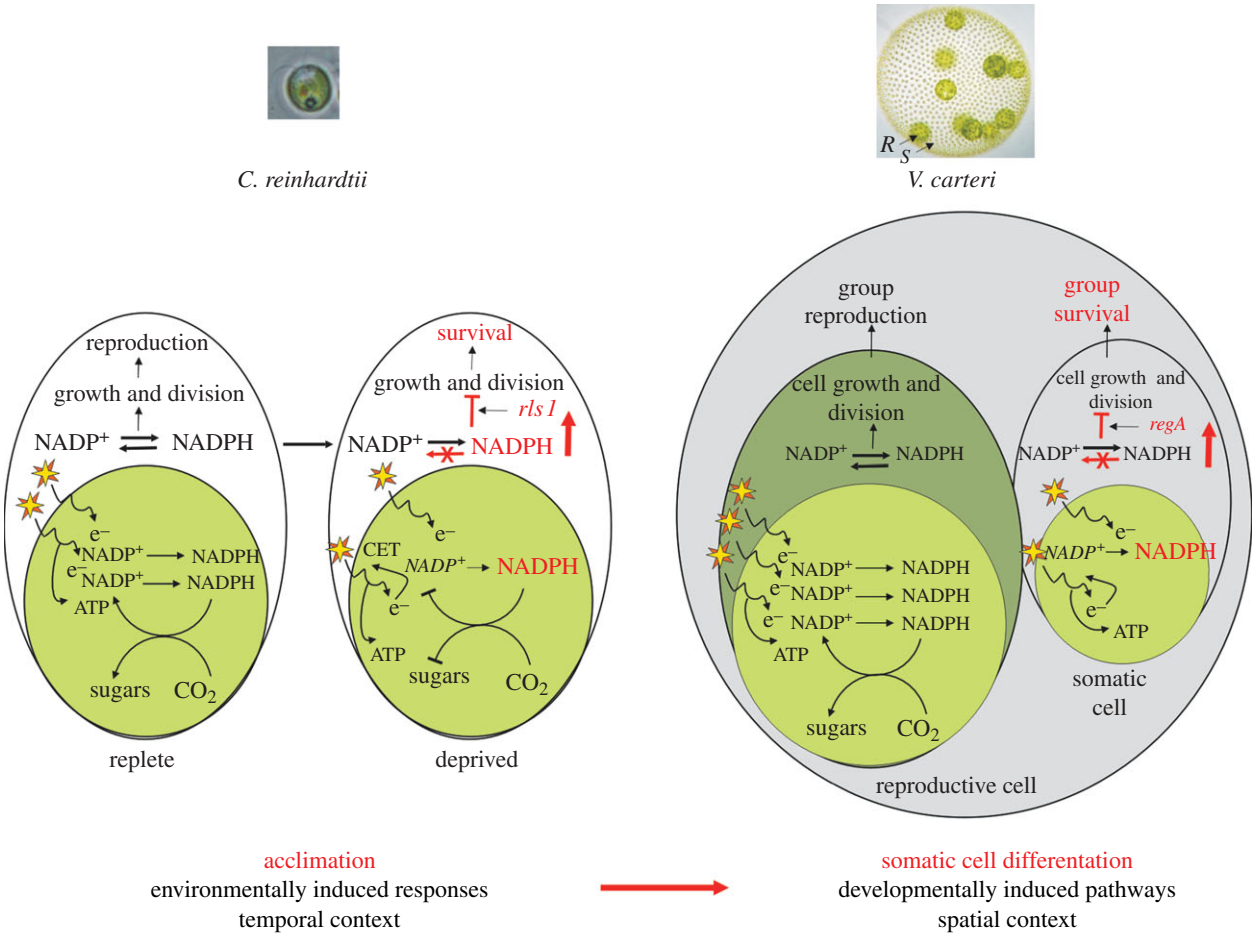

Figure 2. A model for the co-option of acclimation responses into somatic cell differentiation in *V. carteri* (*R* and *S* denote reproductive and somatic cells); see text for discussion. Although many components are involved, for simplicity, changes in the redox status are symbolized by the over-reduction of the NADP pool (vertical red arrows) owing to either decreased NADPH consumption in nutrient-deprived *Chlamydomonas* or excess of excitation energy (owing to a higher surface/volume ratio) in *Volvox* somatic cells. The switch to cyclic electron transport (CET), which can maintain ATP synthesis (and thus vital processes) in acclimated *Chlamydomonas* cells (Eberhard *et al.* 2008) and possibly in *Volvox* somatic cells, is also indicated.

photosynthetic apparatus, which will trigger a suite of short- and long-term acclimation responses, including the downregulation of photosynthesis (to avoid the production of toxic oxygen radicals) and the subsequent cessation of cell growth and division (e.g. Wykoff *et al.* 1998; Pfannschmidt *et al.* 2009). Other environmental factors (e.g. cold, water stress) are also known to result in changes to cellular redox status and trigger acclimation responses (e.g. Eberhard *et al.* 2008). Thus, in principle, any factor that can elicit a similar redox change could prompt acclimation-like responses and ultimately induce cessation of cell division. In a group context, if the induction of such a redox change is restricted to a subset of cells, and if the suppression of reproduction in this subset of cells is beneficial to the group, reproductive altruism can evolve and be fixed.

In *V. carteri*, the expression of *regA* is restricted (by an unknown mechanism) to cells whose size at the end of embryonic divisions falls below 8 μm (Kirk *et al.* 1993). As cell surface area and volume change at different rates, we propose that in these small cells, the ratio between membrane-bound proteins (including ETS and ETS-associated components) and soluble factors (including $NADP^+$ and ADP) becomes skewed—relative to the ratio in larger cells, towards the former. Consequently, these small cells could experience an imbalance between the flux of electrons and the availability of final acceptors (i.e. $NADPH^+$), which would result in a change in the intra-cellular redox status and the induction of acclimation-like responses, culminating with the suppression of their division (figure 2).

Hence, by simulating the general acclimation signal (i.e. a change in cellular redox status) in a developmental rather than environmental context, responses beneficial to a unicellular individual could be co-opted into an altruistic behaviour at the group level. As pleiotropy to other essential functions has been proposed to limit selfishness and allow stable cooperation to evolve (Foster *et al.* 2004), the co-option of environmentally induced responses for reproductive altruism can also contribute to the stability of this behaviour, as the loss of such responses would be costly for the individual. Furthermore, this hypothesis predicts that reproductive altruism is more likely to evolve in lineages with enhanced acclimation mechanisms, which can effectively and adaptively adjust their survival and reproduction potential in response to environmental changes. Because environments that vary in time (such as those in which volvocalean algae live; Kirk 1998) will select for enhanced and efficient acclimation responses (note that temporally varying environments have been shown to select for

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phenotypic plasticity—i.e. generalists, in *C. reinhardtii*; Reboud & Bell 1997), such environments are likely to be more conducive to the evolution of reproductive altruism. In this context, it is noteworthy that cast differentiation in social wasps is also thought to have evolved in variable environments, and specific adaptations to seasonal environments that control sequential shifts between life-cycle phases have been proposed as prerequisites to the evolution of sociality in this group (Hunt & Amdam 2005). As all organisms need to adjust their metabolism and reproduction potential to environmental changes, it is likely that analogous mechanisms apply to the evolution of reproductive altruism in other systems.

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