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A life-history trade-off gene with antagonistic pleiotropic effects on reproduction and survival in limiting environments

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Although life-history trade-offs are central to life-history evolution, their mechanistic basis is often unclear. Traditionally, trade-offs are understood in terms of competition for limited resources among traits within an organism, which could be mediated by signal transduction pathways at the level of cellular metabolism. Nevertheless, trade-offs are also thought to be produced as a consequence of the performance of one activity generating negative consequences for other traits, or the result of genes or pathways that simultaneously regulate two life-history traits in opposite directions (antagonistic pleiotropy), independent of resource allocation. Yet examples of genes with antagonistic effects on life-history traits are limited. This study provides direct evidence for a gene—*RLS1*, that is involved in increasing survival in nutrient-limiting environments at a cost to immediate reproduction in the single-celled photosynthetic alga, *Chlamydomonas reinhardtii*. Specifically, we show that RLS1 mutants are unable to properly suppress their reproduction in phosphate-deprived conditions. Although these mutants have an immediate reproductive advantage relative to the parental strain, their long-term survival is negatively affected. Our data suggest that *RLS1* is a *bona fide* life-history trade-off gene that suppresses immediate reproduction and ensures survival by downregulating photosynthesis in limiting environments, as part of the general acclimation response to nutrient deprivation in photosynthetic organisms.

1. Introduction

Although life-history trade-offs are central to life-history evolution [1–3], their mechanistic basis is often unclear (e.g. [3–6]). Traditionally, trade-offs have been understood in terms of competition for limited resources among traits (e.g. reproduction, somatic growth, maintenance) within an organism (aka adaptive resource allocation [7]). Reduced nutrient availability is known to substantially magnify an apparent trade-off, while increased nutrient availability can diminish (or remove) negative interactions between traits (e.g. [8]). Such plastic responses are thought to be determined by priority rules that determine how resources are allocated in response to different nutrient inputs [7]. In some animal lineages, laboratory and field experiments showed that under nutrient-limiting or stressful conditions, energy and resources were directed to maintenance or storage functions, taking precedence over reproduction [7].

Trade-offs can occur between physiological traits that are expressed at the same or at different times of the life cycle, and can result from genetic factors (pleiotropy), environmental factors, or combinations of these two types of factors that result in negative interactions between traits [7,9]. Although artificial selection and experimental evolution have shown that trade-offs can have a genetic basis (see [10]), few studies have been able to pinpoint the underlying

molecular mechanisms [6]. For instance, while trade-offs could be the result of adaptive resource allocation at the organismal level [2,11], this differential allocation can be caused by a trade-off between protein biosynthesis (growth) and energy metabolism (survival)—probably mediated by signal transduction pathways at the level of cellular metabolism [2]. Trade-offs can also be produced as a consequence of the performance of one activity generating negative consequences for other traits. For example, aerobic metabolism generates reactive oxygen species (ROS) that, if not fully neutralized, can be damaging to biological molecules and negatively affect other activities or life traits, such as lifespan [5,12,13]. Furthermore, trade-offs could be the result of signalling genes or pathways that simultaneously regulate two life-history traits in opposite directions, independent of resource allocation [6,14]. For instance, yeast mutants in the Ras2/cAMP pathway cannot arrest their reproduction in limiting environments and are more sensitive to stress due to their inability to induce the transcription of stress-related genes (e.g. [15]). Nevertheless, examples of genes with antagonistic effects on two life-history traits are limited [16].

Because the loci involved in life-history trade-offs are expected to affect traits in opposite ways (i.e. show antagonistic pleiotropy [1]), life-history trade-offs are generally thought to limit the set of possible trait combinations, and thus restrict the range of possible evolutionary outcomes [10]. However, during transitions in individuality—such as the transition to multicellularity and eusociality, trade-offs constraining the evolutionary trajectories of solitary individuals can be uncoupled through the evolution of specialized cells in multicellular individuals [17,18] and castes in eusocial insects [19]. Specifically, in unicellular individuals, the same cell contributes to both survival and reproduction, but these activities are not performed at the same time; the cell switches from investing in survival or in reproduction (i.e. a trade-off). In multicellular individuals, however, cells can specialize in either activity (the trade-off is broken), and this leads to the differentiation of survival-enhancing cells (soma) and reproductive cells (germ). At a mechanistic level, we argued that the evolution of soma and germ involved the co-option of mechanisms underlying survival-reproduction trade-offs in unicellular lineages, by changing their expression from a temporal into a spatial context [20,21].

To explore this possibility, we are using the volvocine lineage—a group of haploid green algae that comprises both unicellular species (e.g. *Chlamydomonas reinhardtii*) and multicellular species with fully differentiated somatic and germ lines, such as *Volvox carteri*. In *V. carteri*, a single gene—known as *regA*, is both necessary and sufficient to determine somatic cell fate [22]. *regA* is only expressed in somatic cells and codes for a putative transcription factor that is thought to repress the expression of nuclear-encoded chloroplast proteins [23,24], which in turn will negatively affect photosynthesis and thus the cell growth and division of somatic cells. We have previously suggested that *regA* evolved from a life-history gene that was involved in trading off reproduction for survival in its unicellular ancestors [20]. In this context, we showed that *regA*'s closest homolog in the unicellular *C. reinhardtii* (known as *RLS1*; <u>R</u>eg<u>A</u>-<u>L</u>ike-<u>S</u>equence 1) is expressed under conditions that require the suppression of immediate reproduction to ensure survival [20,21]. However, the direct role of this putative life-history trade-off gene in the survival and reproduction of *C. reinhardtii* and its ability to

trade off these two life-history traits has not been examined. Here, we directly tested the hypothesis that *RLS1* is a life-history trade-off gene by investigating the reproduction and long-term survival of a *C. reinhardtii* RLS1 mutant and its wild-type parental strain under limiting environmental conditions.

2. Material and methods

(a) Strains and growth conditions

A *C. reinhardtii* genomic *RLS1* mutant (LMJ.RY0402.057072) and its wild-type parental strain (CC-4533 *cw15 mt-*) were obtained from the *Chlamydomonas* Resource Center (https://www.chlamy-collection.org/). The RLS1 mutant was generated by random insertion of a paromomycin resistance cassette [25]. Stock cultures of both strains were grown in Tris-Acetate-Phosphate (TAP) medium (https://www.chlamycollection.org/methods/) on a rotary shaker (100 r.p.m.) at 25°C, under a 12 h light (150 µmol quanta $m^{-2} s^{-1}$): 12 h dark regime.

Experimental cultures were grown in either the normal light : dark (LD) cycle or in continuous dark (CD). The LD regime ensures the population is synchronized, as cells grow during the light phase (when they perform photosynthesis) and divide in the dark. Cultures grown in CD are strictly heterotrophic, allowing us to assess the impact of light and photosynthesis on cell growth and division in optimal and nutrient-limiting conditions. Nutrient-deprived cultures were grown in phosphate-depleted medium (Tris-Acetate; TA) prepared by substituting potassium phosphate with potassium chloride [26]. Experimental cultures were initiated at 5×10^5 cells ml^{-1}, with three biological replicates (see electronic supplementary material for additional detail). Population growth was assessed daily (at 2.5 h into the light cycle) by counting the cells using a haemocytometer; for each biological replicate, four technical replicates (i.e. four aliquots per culture) were averaged.

(b) Cell viability

Dead and live cells were assessed using a fluorescent exclusion dye (SYTOX Green; excitation/emission = 504/523 nm; Invitrogen). To evaluate long-term viability (i.e. cells that are both alive and able to exit quiescence and reproduce when optimal conditions return) we used a simplified serial dilution agar plating method [27] (see electronic supplementary material for additional detail). Briefly, equivalent culture aliquots containing 250 cells were plated on TAP agar plates and the visible growing colonies (corresponding to the cells that were both alive and viable) were counted for each plate. The number of non-viable cells (alive but not able to produce colonies) was calculated as the difference between the number of live cells (as assessed using the SYTOX green) and the number of colonies (viable cells) [28]. Viable, non-viable and dead cells were then expressed as percentage of the total number of cells in the culture.

(c) Statistical analyses

Statistical analyses were performed using GraphPad Prism 9 (www.graphpad.com/). To assess the significance of the potential differences in number of cells (i) between the wild-type and RLS1 mutant strain at each time point and (ii) over time (for each strain), we used two-way ANOVA analyses (95% confidence interval; Tukey's multiple comparison tests). For the viability analyses, we performed unpaired two-sample *t*-tests (95% confidence interval).

(d) Identification of the *RLS1* mutation

To confirm the disruption of *RLS1* and characterize the specific *RLS1* mutation, clonal cultures were established and DNA was extracted using the Qiagen DNA Plant Extraction Kit. The genomic area assumed to be affected was amplified using primers corresponding to the *RLS1* regions flanking the insertion cassette as well as cassette-specific primers (following the protocol provided at https://www.chlamylibrary.org) (electronic supplementary material, figures S1 and S2).

3. Results and discussion

(a) Genetic and phenotypic characterization of the *RLS1* mutant

PCR amplification and sequencing confirmed that the *RLS1* coding region is disrupted in the mutant strain. Specifically, the cassette is inserted three nucleotides upstream of the 3′-end of the first coding exon, resulting in a truncated mutant protein containing part of the cassette (figure 1a). Notably, the insertion interrupts the SAND domain—a conserved DNA binding domain responsible for the function of SAND-containing transcription factors [29].

While growing the mutant cultures, we noticed that during exponential phase, in contrast to the wild-type parental strain, the RLS1 mutant grows mainly as cell clusters (figure 1b). The phenotype appears to be due to the inability of the daughter cells to hatch out from under the mother cell wall, which results in non-flagellated clusters of cells. However, as the cultures enter the stationary phase, the populations express a mix of single cells and small clusters (see inset in figure 1b), and revert to single-celled individuals in late stationary phase.

(b) *RLS1* suppresses reproduction in phosphate-deprived environments

In *C. reinhardtii*—as in other photosynthetic organisms, the lack of nutrients (e.g. phosphate, sulfur, nitrogen) limits the consumption of NADPH and ATP generated via photosynthesis, due to the slow-down of anabolic processes and the decreased demand for reductant [30]. Consequently, the photosynthetic electron transport chain becomes reduced and the redox potential of the cell increases [30,31]. Excessive reduction of the electron transport chain can lead to over-excitation of chlorophyll molecules and the accumulation of high potential electrons that can interact with oxygen and create ROS [32]. Although ROS are by-products of normal metabolism and act as secondary messengers in various signal transduction pathways (e.g. [33–35]), increased intracellular levels of ROS (oxidative stress) can alter cellular functions and damage many biological structures, most importantly, DNA (e.g. [33]). Thus, it has been suggested that in order to decrease the potential damaging effect of excess light energy under nutrient limitation—and increase survival, photosynthesis needs to be downregulated [30,32,36]. This acclimation process is a well-known general stress response that coordinates nutrient availability with the metabolism of the photosynthetic cell and its growth and division potential, resulting in a temporary inhibition of cell division (cell cycle arrest), and thus a decrease in reproduction [30,31]. We predicted that if *RLS1* acted as a life-history gene that increases survival at a cost to immediate
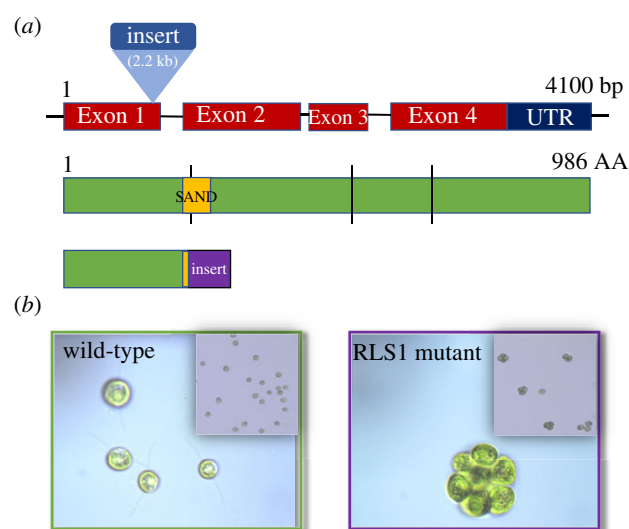

Figure 1. Genetic (a) and phenotypic (b) characterization of the *Chlamydomonas reinhardtii* RLS1 mutant. (a). *RLS1* gene structure (top row) showing the location of the paromomycin resistance cassette [25]; the insertion results in a truncated protein that lacks a functional SAND domain and includes a region of the translated cassette. (b) Micrographs of the wild-type parental and the RLS1 mutant strains. (Online version in colour.)

reproduction (as we previously hypothesized; [20]), loss of *RLS1* should reflect in differences between the RLS1 mutant and its parental wild-type strain in the ability to both suppress reproduction and survive in nutrient-deprived environments.

First, to address the role of *RLS1* in suppressing the reproduction of *C. reinhardtii* in nutrient-limited conditions, we compared the population growth of the two strains grown in phosphate-depleted media (figure 2 and electronic supplementary material, figure S3). As predicted, in a phosphate-deprived medium under an LD regime, the RLS1 mutant reproduced more than the wild-type strain and the final population exceeded the size of its wild-type counterpart by 50% (i.e. 6.2×10^6 versus 4.1×10^6 cells ml^{-1}; $p < 0.0001$; figure 2a). However, the reproductive advantage of RLS1 mutant was lost when cultures were maintained in constant dark (figure 2b); in fact, its population size only reached 57% of that of the wild-type strain (1.6×10^6 versus 2.8×10^6 cells ml^{-1}; $p < 0.0001$). These differences in the RLS1 mutant's response to phosphate deprivation between LD and CD regimes are consistent with *RLS1*'s involvement in the response to nutrient deprivation being linked to photosynthetic activities. Other mutants in genes involved in acclimation to nutrient stress in *C. reinhardtii* are also known to respond differently in light and dark. For instance, mutants that are unable to downregulate the photosynthetic electron transport during nutrient deprivation die sooner than the wild-type when grown in the light due to accumulation of photo-oxidative damage; but when maintained in the dark, they can survive nutrient deprivation as well as the wild-type strains do [32,36,37].

Interestingly, the reproductive advantage of RLS1 mutants was also lost in phosphate-replete medium; in fact, the loss of *RLS1* had a negative impact on population growth in cultures grown under either an LD regime or CD (figure 2c,d). Specifically, under an LD regime, the mutant population achieved only around 75% of the final wild-type population size (8.3×10^6 cells ml^{-1} versus 1.1×10^7; $p <$
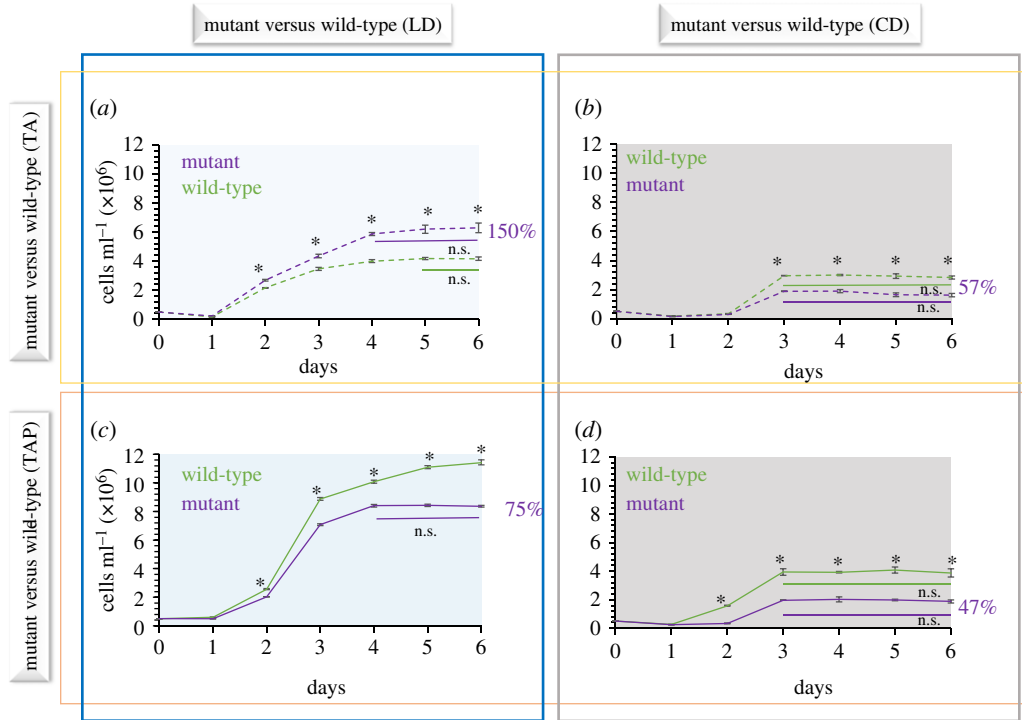
Figure 2. Comparisons between the population growth of wild-type and RLS1 mutant strains under either LD (*a,c*) or CD (*b,d*) regimes, in phosphate-depleted (TA; *a,b*) or phosphate-replete (TAP; *c,d*) media. Solid and dashed lines indicate phosphate-replete and phosphate-depleted media, respectively. Error bars represent 2xSE (three biological replicates); asterisks denote significant differences ($p < 0.0001$) between the two strains at each corresponding time point. Straight lines below growth curves indicate time periods characterized by non-significant (n.s.) growth (i.e. stationary phase) for each strain. Percentages indicate the final mutant population size relative to that of the parental strain. (Online version in colour.)

0.0001; figure 2*c*). Also, the mutant population entered the stationary phase on day 4 (i.e. no difference in population size between days 4 and 5, and days 5 and 6; $p = 0.7414$ and $p = 0.4813$, respectively) while the wild-type population continued to grow from day 4 to 5 and 6 ($p < 0.0001$ and $p = 0.0015$, respectively). In CD, the difference in population growth between the two strains was even more pronounced, as the mutant only achieved 47% of the wild-type population size (1.8×10^6 versus 3.8×10^6 cells ml^{-1}; $p < 0.0001$; figure 2*d*). The more significant negative effect associated with the loss of *RLS1* in cultures grown in CD suggests that *RLS1* is also involved—directly or indirectly, in regulating reproduction in the dark. Such a possibility is supported by the fact that *RLS1* was found to be induced in cultures maintained in the dark [20,21]. Since the expression of *RLS1* in the dark coincided with the downregulation of a chloroplast light-harvesting protein, it has been suggested that *RLS1* might be involved in the suppression of chloroplast biosynthesis in the dark to avoid investment of resources and energy in a structure whose function is limited in the absence of light [20]. Consequently, those resources and energy can be re-allocated to growth and reproduction. In this scenario, the loss of *RLS1* will prevent the reallocation of resources from chloroplast biosynthesis to cell growth and reproduction, which can explain the lower population levels of the RLS1 mutant grown in either CD or a 12 h light:12 h dark regime (figure 2*c,d*).

To further address the exact role of *RLS1* in suppressing the reproduction of *C. reinhardtii* in nutrient-limiting environments, we compared the growth curves of the wild-type and RLS1 mutant strains in phosphate-depleted media in either LD or CD regimes (figure 3; electronic supplementary material, figure S4). Phosphate is an important macronutrient

for all organisms, and *C. reinhardtii* grown in phosphate-depleted media was shown to have a significantly lower population growth [38,39]. As expected, under an LD regime, the wild-type responded to phosphate deprivation by suppressing its reproduction such that the maximal population size after 6 days was only ca. 35% of the level achieved in phosphate-replete medium (1.14×10^7 versus 4.16×10^6 cells ml^{-1}; $p < 0.0001$; figure 3*a*). However, under the same conditions, the RLS1 mutant population continued to grow and reached up to 75% of its growth capacity in optimal conditions (8.36×10^6 versus 6.28×10^6 cells ml^{-1}; $p < 0.0001$; figure 3*b*), confirming that the mutant is less able to suppress its reproduction under phosphate deprivation. Also, as expected if the suppression of reproduction under phosphate deprivation was related to photosynthetic activities, the response of the wild-type to the lack of phosphate was less pronounced in CD (figure 3*c*). Specifically, in phosphate-depleted media, compared to only 35% in the LD regime (figure 3*a*), the wild-type strain maintained approximately 73% of its growth capacity in the dark (3.87×10^6 versus 2.82×10^6 cells ml^{-1}; $p < 0.0001$; figure 3*c*; electronic supplementary material, figure S5*c*). On the other hand, compared to the LD regime (figure 3*b*), the mutant showed almost no response to phosphate deprivation when grown in CD (95%; $p = 0.0002$; figure 3*d*; electronic supplementary material, figure S5*d*). Overall, the differences in the way the wild-type and RLS1 mutant strains responded to phosphate deprivation in both LD and D regimes indicate that the involvement of *RLS1* in suppressing reproduction in a diurnal cycle is linked to changes in photosynthetic activities that are known to be associated with the acclimation to nutrient deprivation [30]. This is consistent with the fact that *RLS1* can also be induced by a photosynthetic electron transport inhibitor that
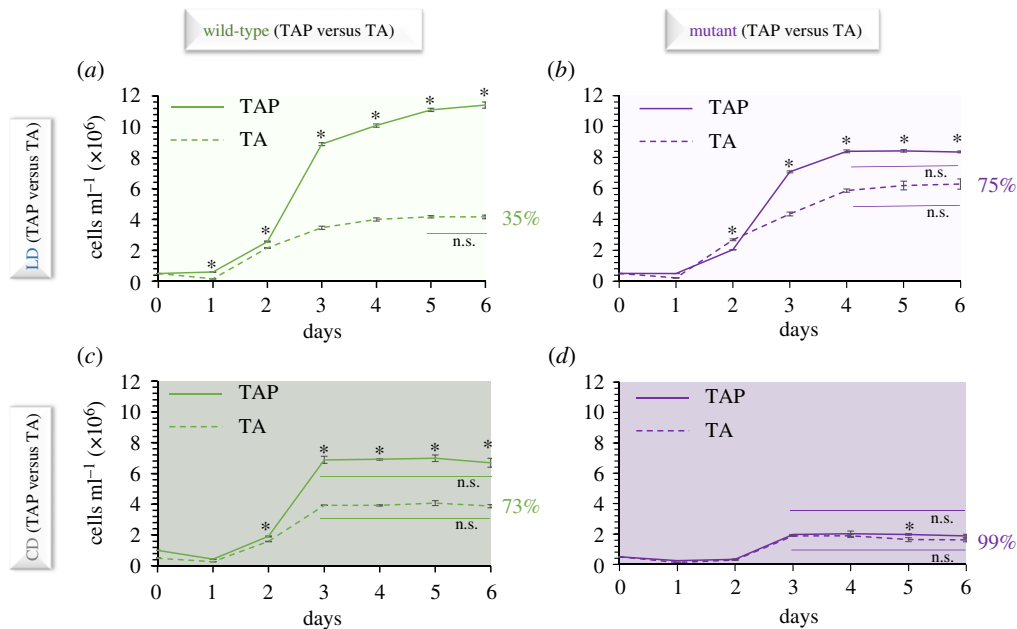
Figure 3. Comparisons between the responses to phosphate-deprivation in a LD regime (*a,b*) and in CD (*c,d*) of the *C. reinhardtii* wild-type (*a,c*) and RLS1 mutant (*b,d*). Solid and dashed lines indicate phosphate-replete (TAP) and phosphate-depleted (TA) media, respectively. Error bars represent 2xSE (three biological replicates); asterisks denote significant differences $p < 0.0001$ between the two strains at each corresponding time point. Straight lines below growth curves indicate time periods characterized by non-significant (ns) growth (i.e. stationary phase) for each strain. Percentages indicate the final population size grown in phosphate-deplete relative to phosphate-replete media. (Online version in colour.)

elicits acclimation-like responses [21]. Furthermore, the differences between the RLS1 mutant and the wild-type populations when grown in the dark (in either phosphate-replete or depleted media) are consistent with an additional role of *RLS1* in optimizing reproduction in the dark. In fact, when grown in the dark, the RLS1 mutant is not able to maintain the same relative population size as the wild-type strain (see electronic supplementary material, figure S5).

(c) *RLS1* ensures long-term survival

Under nutrient deprivation, *C. reinhardtii* individuals enter a resting/quiescent state that is excited when optimal conditions are restored. This non-reproductive and lowered metabolic state is thought to prevent oxidative damage and ensure long-term survival [40,41]. To address if *RLS1* is a *bona fide* life-history gene that trades off reproduction for survival in limiting conditions, we compared the long-term viability (i.e. the ability of cells to successfully exit dormancy and reproduce) of the wild-type and RLS1 mutant strains in stationary cultures grown in phosphate-depleted media for up to 40 days (figure 4; electronic supplementary material, figure S6). First, we found that although the RLS1 mutant continued to maintain a higher population size relative to the wild-type over a long period of time (up to 40 days; figure 4*a*; $p < 0.0001$), a lower number of cells formed colonies (e.g. 36 versus 82 at Day 24, and 16 versus 42 at Day 40; $p < 0.01$ and $p < 0.0001$, respectively) when the same number of cells (i.e. 250) were plated on TAP agar plates (figure 4*b*; electronic supplementary material, figure S7*a*). The number of growing colonies reflects the proportion of viable cells in each population.

To address if the cells that did not resume reproduction were dead or permanently arrested (non-viable; senescent), we calculated the difference between the number of live cells (assessed using a viability dye) and the number of viable cells (inferred from the number of colonies) [28]. Interestingly, we found that the lower proportion of viable cells in the RLS1 mutant populations is paralleled by a higher proportion of non-viable/senescent cells (figure 4*c* and electronic supplementary material, figure S7*b*). During quiescence, photosynthetic cells need to downregulate photosynthetic activities to avoid potential photo-oxidative damage [28,41]. The possibility that the decreased ability to resume reproduction (and thus cell viability) in the RLS1 mutant population *RLS1* is due to the accumulation of photo-oxidative damage is consistent with a role for *RLS1* in increasing long-term survival by downregulating photosynthesis.

(d) *RLS1* is a life-history gene with antagonistic effects on reproduction and survival in limiting environments

Overall, our data provide strong evidence that *RLS1* is directly involved in the ability to trade off reproduction for survival in nutrient-limiting conditions. Specifically, we found that, compared to the parental strain, RLS1 mutants are less able to suppress their reproduction in phosphate-depleted media (figure 3). In fact, in a normal LD regime, the RLS1 mutants maintain higher population densities relative to the wild-type strain (figure 2*a*), which could provide them with a reproductive competitive advantage in phosphate-deprived environments. However, this reproductive advantage is not only lost in optimal conditions (figure 2*c*), but is also counteracted by a reduced long-term viability (figure 4). The opposite effects that the loss of *RLS1* has on the reproduction and survival of *C. reinhardtii* grown in phosphate-depleted media provide direct evidence that *RLS1* is a life-history trade-off gene with antagonistic effects on the two
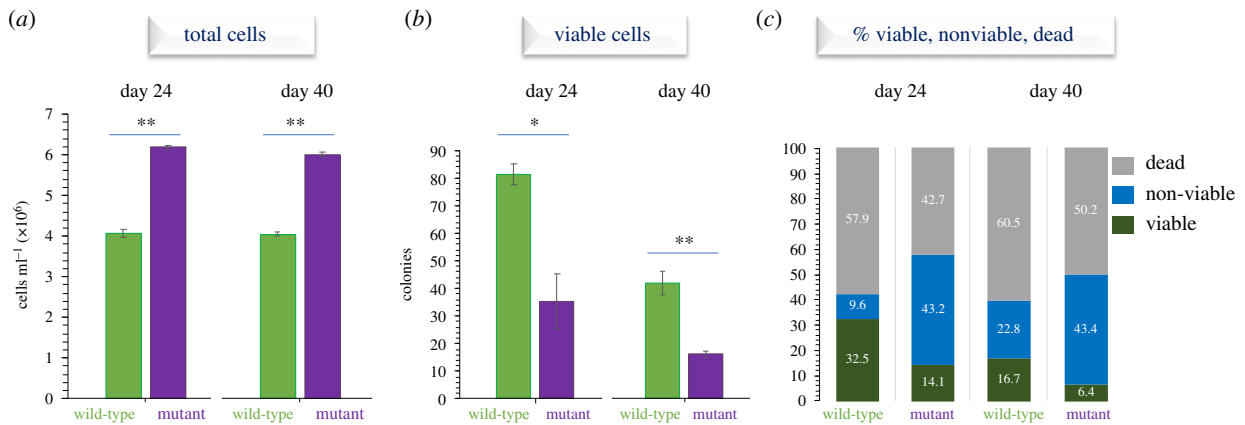
Figure 4. Comparisons between the *C. reinhardtii* wild-type and RLS1 mutant populations grown for 24 and 40 days in phosphate-depleted media, in terms of (*a*) population size (i.e. total number of cells), (*b*) long-term viability (i.e. viable cells estimated as the number of growing colonies from a total of 250 plated cells), and (*c*) the relative proportion of viable (alive and reproducing), non-viable (alive but non-reproducing/senescent) and dead cells. Asterisks (*) and (**) denote $p < 0.01$ and $p < 0.0001$, respectively. (Online version in colour.)

fitness components—reproduction and survival, under nutrient-limiting conditions.

We have previously shown that *RLS1* was induced in both phosphate- and sulfur-deprived media [21]. Here we focused on phosphate deprivation as, compared to sulfur, it has a less immediate effect on reproduction [21]. However, many aspects of the general responses to nutrient deprivation—including the downregulation of photosynthesis and temporary cessation of reproduction, are similar during both phosphate- and sulfur-deprivation [30,31]. Nevertheless, the specific role of *RLS1* in other nutrient-deprived environments (including nitrogen—which can also induce sexual differentiation in *C. reinhardtii*) needs to be fully investigated.

Interestingly, *RLS1*'s antagonistic pleiotropic effects have been maintained in its closest homologue in *V. carteri* (i.e. *regA*). A functional RegA protein not only suppresses the reproduction of somatic cells but also protects them against stress, as RegA mutant cells have been found to be more sensitive to stress relative to their wild-type counterparts [42]. Furthermore, RLS1 mutants are not only more affected by long-term nutrient deprivation, but they are more sensitive to heat stress as well (electronic supplementary material, figure S8). This difference in sensitivity to heat stress between the wild-type and *RLS1* mutant strains is also analogous to that observed between somatic cells (expressing *regA*) and gonidia (not expressing *regA*) in *V. carteri* [43]. But how can *RLS1* regulate these two life-history traits? Below we suggest that *RLS1*'s role as a life-history trade-off gene involves the downregulation of photosynthesis (and thus growth and reproduction) as part of the general acclimation response to increase survival in limiting conditions.

(e) *RLS1*'s life-history trade-off activity is linked to photosynthesis

In unicellular organisms, reproduction is linked to cell growth, which is dependent on nutrient availability. Under nutrient deprivation, cell growth and reproduction are repressed, culminating with the induction of quiescence/dormancy that will ensure long-term survival [40,44–46]. In unicellular photosynthetic organisms, cell growth is dependent not only on nutrients (inorganic substrates) but also on light. In *C. reinhardtii*, when nutrients (e.g. phosphorus,

sulfur, nitrogen) are limited (including during stationary phase and conditional senescence), imbalances between excitation energy and cell's reducing power result in the downregulation of photosynthesis, as an adaptive response to avoid potential light-induced, oxidative damage [31,36,47,48]. For instance, a 75% decrease in maximal *in vivo* photosynthetic O_2 evolution was observed within 4 days of phosphate deprivation or 1 day of sulfur deprivation in *C. reinhardtii* [30]. In addition to downregulating photosynthesis, the general acclimation response in *C. reinhardtii* also involves the temporary cessation of reproduction [31]. However, the signalling pathways coordinating these two processes that underlie a basic life-history trade-off are not well understood.

Theoretically, the suppression of reproduction in nutrient-limiting conditions can involve three hypothetical distinct scenarios. First (figure 5, scenario (*i*)), the suppression of reproduction and increased survival can be a direct response to the re-allocation of nutrients, possibly involving a trade-off between protein biosynthesis (growth) and energy metabolism (survival). Alternatively (figure 5, scenario (*ii*)), reproduction could be suppressed in response to nutrient stress-production of ROS; such oxidative stress could induce a temporary cell cycle arrest (suppression of reproduction) to repair the ROS-induced DNA damage, which will result in an increase in survival. Lastly (figure 5, scenario (*iii*)), redox signals associated with imbalances between excitation energy and reducing power under nutrient deprivation will induce the downregulation of photosynthesis—a response that can avoid potential photo-oxidative damage (and increase survival), but will also limit cell growth and thus reproduction.

These three hypothetical scenarios reflect the different proposed mechanisms underlying life-history trade-offs, and make distinct predictions as far as the role of *RLS1* in the survival–reproduction trade-off under nutrient limitation. The first scenario is consistent with resource allocation and is independent of light; consequently, if RLS1 is involved in this resource-based trade-off, the loss of *RLS1* should have the same effect on reproduction in both LD and CD regimes. The second scenario envisions that *RLS1* is activated by ROS and acts as a general cell cycle regulator (similar to the animal *p53*, for instance) that induces a temporary cell cycle
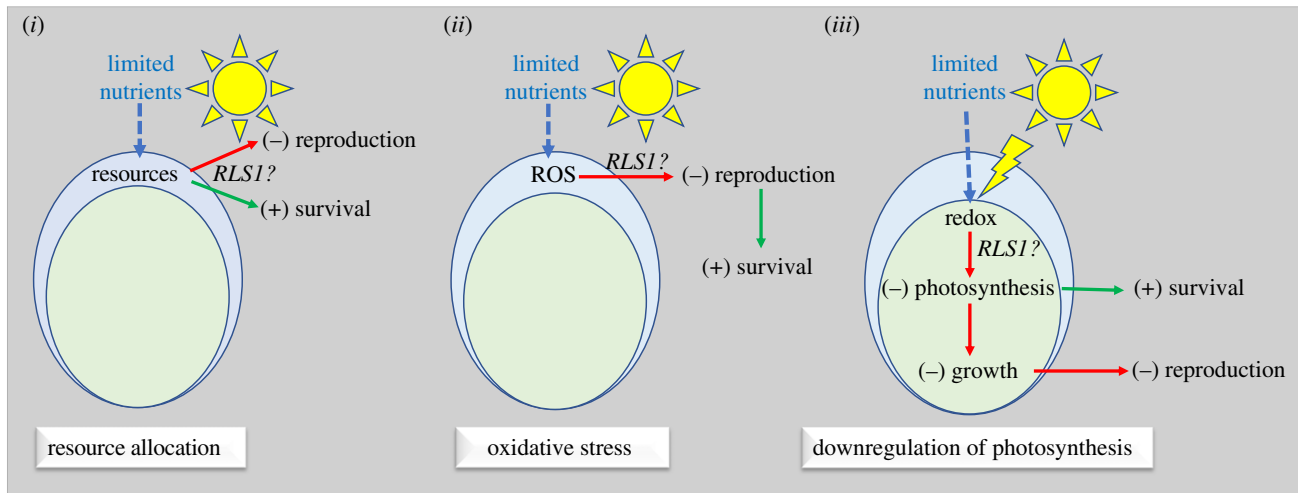
Figure 5. Three hypothetical mechanistic scenarios that could account for the involvement of *RLS1* in the reproduction-survival trade-off in *C. reinhardtii* in response to nutrient-deprivation. Scenario (*i*) is independent of light and involves allocation of resources to survival (at a cost to reproduction). Scenario (*ii*) assumes ROS produced in response to nutrient stress having a negative effect on reproduction by inducing cell cycle arrest, which will have a positive effect on survival by repairing the DNA damage. Scenario (*iii*) envisions downregulation of photosynthesis having antagonistic effects on survival and reproduction. See text for discussion. (Online version in colour.)

arrest. Loss of *RLS1* would result in increased reproduction (as in cancer cells) and potentially lower long-term survival due to accumulation of unrepaired damage/mutations. However, its effect on reproduction should be similar in both LD and CD regimes. In the third scenario, redox signals associated with imbalances between excitation energy and availability of reducing power under nutrient limitation [21] would trigger the expression of *RLS1*. As a transcription factor, *RLS1* could act to induce the downregulation of photosynthetic activities, which would have an antagonistic pleiotropic effect on the two life-history traits; that is, it will promote survival at a cost to immediate reproduction. As the postulated redox signal requires light, the loss of *RLS1* should not increase reproduction in a CD regime. Our data are consistent with this scenario.

Overall, our findings and the models discussed above suggest that *RLS1* acts as a master acclimation regulator that downregulates photosynthesis in response to nutrient-limiting conditions to ensure survival, though at a cost to immediate reproduction. Consistent with this possibility, *RLS1* was shown to be expressed not only in phosphate-depleted media but also under sulfur-deprivation as well as in cultures in the stationary phase, at the time when reproduction declined [21]. Furthermore, expression of *RLS1* can be induced by inhibitors of the photosynthetic electron transport chain that trigger acclimation-like responses, and coincides with the downregulation of a light-harvesting chloroplast protein [21]. Notably, the homologue of *RLS1* in *V. carteri* (*regA*) can also be induced by environmental stress associated with light exposure after prolonged dark periods and its loss results in decreased viability [42]. By contrast, the loss of genes shown to be involved in specific responses to nutrient deprivation (i.e. nutrient acquisition/scavenging) in *C. reinhardtii* are known to negatively affect population growth in limiting environments [32,39,49].

Nevertheless, in addition to contributing to survival in nutrient-deprived environments by avoiding photo-oxidative damage in the light (as part of the general photosynthetic acclimation response), *RLS1* might also optimize resource allocation in the dark (if alternative sources of carbon are available) by reducing the unnecessary investment in the photosynthetic apparatus, which will optimize reproduction in the dark. Interestingly, in *V. carteri*, *regA* is also thought to downregulate the expression of chloroplast proteins in somatic cells [23,24]. However, since *V. carteri* is unable to use organic substrates, the inability to express chloroplast proteins results in a permanent repression of cell growth and reproduction—that is, somatic cell differentiation [50]. Altogether, our data suggest that *RLS1* plays an important role in balancing reproduction and survival to increase fitness in limiting conditions, which argues for this gene acting as a *bona fide* life-history trade-off gene.

4. Conclusion

This study suggests that single-celled species are good model-systems to investigate the fundamental genetic and molecular mechanisms underlying life-history trade-offs. In addition to their suitability for experimental work, in these lineages the organismal and cellular level coincide, and thus cellular-level responses (e.g. cell cycle arrest) are directly reflected in organismal-level processes (e.g. cessation of reproduction). Our data show that in the unicellular species, *C. reinhardtii*, a single gene—*RLS1*, can adaptively adjust both survival and reproduction as part of the general acclimation response that ensures survival at a cost to immediate reproduction by regulating photosynthetic activities in response to nutrient and light availability.

Genes with potential antagonistic pleiotropic effects (independent of resource allocation) might also be responsible for some of the observed life-history trade-offs in multicellular organisms. For instance, in the nematode *Caenorhabditis elegans*, a natural variant (associated with a small deletion in the *cis*-region of *eak 3*—a gene involved in the synthesis and activity of a hormone) that has increased sensitivity to dauer induction (a dormant stage induced in unfavourable conditions) is also negatively affected in reproduction [16]. Furthermore, similar to our RLS1 mutant, *eak-3*

variants have been shown to have a fitness advantage in stressful environments (through increased dauer production), but be outcompeted in favourable environments (because of their decreased reproduction). Deciphering the specific pathways regulated by genes with antagonistic pleiotropic effects on fitness will provide a deeper understanding of the general mechanisms underlying life-history trade-offs.

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