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Algal laboratory evolution at a cold temperature leads to overproduction of lipid under both cold and warm conditions

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Research Article

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Abstract

Background

Microalgae that both grow fast and overproduce lipids are desirable materials for biofuel production. Evolutionary adaptation under certain stressful conditions is associated with greater levels of lipid accumulation within cells; and the lipid overproduction trait may be maintained even when algal cells are grown under benign conditions where growth rate is high. Therefore, cold-evolved algae and warm cultivation conditions could be a perfect combination for population-level lipid production. Here we test this hypothesis by working with *Chlorella sorokiniana* isolates from an earlier laboratory evolution experiment, in which algal populations have evolved under either constantly benign (25°C) or constantly cold conditions (15°C). The growth performance and lipid content per cell of each algal isolate were measured at both temperatures.

Results

The combination of 15°C-evolution algae and 25°C culture conditions gave the highest levels of lipid content per cell as well as a fairly fast growth rate, and not surprisingly, extremely high population-level lipid production. Analysis of evolutionary changes relative to the ancestral strain failed to identify evolutionary trade-offs: adaptation to a certain environment did not cause decreased growth performance in the alternative environment, and an increase in lipid content per cell was not associated with reduced growth rate.

Conclusions

These findings indicate that laboratory evolution under stressful conditions may enhance environmental adaptability and lipid accumulation of microalgae, offering a potential basis for further improving lipid productivity in biotechnology applications.

1. Introduction

Microalgae are promising materials for biofuel production [1]. Research efforts have been devoted to collecting algal materials and optimizing cultivation conditions that give high lipid production [2–7]. Fast algal growth and high lipid production per cell are both intended [7–10]. However, a trade-off may exist between these two traits [11]. Conditions that stimulate lipid accumulation rarely allow for faster growth [12, 13]. Specifically, elevated lipid accumulation is often involved in physiological responses of algal cells to unfavorable conditions [14], such as nitrogen deprivation [15, 16], high salinity [17–19], high light intensity [20, 21], and cold exposure [14, 22–26]. Under such stressful conditions, microalgae often alter their lipid biosynthesis pathways to produce and accumulate neutral lipids, particularly in the form of triglycerides (TAG). The adaptive functions of greater lipid content within cells include serving as an

energy reserve for survival and repair, and acting as a sink for excess photosynthetic energy and carbon to mitigate oxidative stress [9, 27, 28]. Algal growth rate, however, is typically suppressed under those stressful conditions.

A number of strategies have been proposed to circumvent the conflict between rapid growth and lipid production. For example, two-phase cultivation methods have been developed, where algae are first cultured under conditions optimal for growth, and then exposed to a stress factor that triggers lipid accumulation. The costs associated with this strategy, particularly the time cost, might be very high [3, 29]. Alternatively, certain cultivation conditions may balance the cell growth rate and lipid accumulation targets [10]. Here we suggest a new specific strategy in which an enhanced lipid phenotype could subsequently be expressed under cultivation conditions that favor rapid growth. To achieve such an aim, one may choose an algal strain with an intrinsic capacity of rapid growth and use the laboratory evolution approach to generate algal strains with greater lipid accumulation capacity.

While phenotypical plasticity allows for immediate physiological responses to environmental stresses, genetic changes would cause evolutionary adaptation during long-term exposure [30, 31]; and a rich body of research has demonstrated the potential of evolutionary adaptation to environmental change in microalgae [31–36]. We expect that high lipid accumulation becomes a stable, potentially heritable trait in algal populations that evolve under environmental stresses such as cold temperatures.

The present study investigated the possibility that previous adaptation to cold conditions can improve lipid production in benign environments, with an industrially important alga, *Chlorella sorokiniana*. We worked with evolved isolates from an earlier laboratory evolution experiment [37], where algal populations had evolved under constantly benign (25°C) or constantly cold conditions (15°C). Here our reciprocal transplant assays suggested that cold-evolution increases lipid content per cell in benign conditions. Surprisingly, the cold-evolved algal isolates also showed slightly greater growth rate in the benign conditions compared with those that had evolved in the latter environment.

2. Materials and Methods

2.1 Algal isolates and culture conditions

Algal isolates studied here were from an artificial selection experiment with *Chlorella sorokiniana* [37]. Here, the term “ancestral strain” refers to the original genotype used to initialize the experimental evolution project. “Evolved populations” refer to populations propagated during the laboratory evolution experiment, whereas “evolved isolates” refer to single-colony isolates obtained from evolved populations and used for phenotypic assays in the present study. The term “culture” is used to describe a laboratory growth unit. In this earlier study, serial batch algal cultures founded by the ancestral genotype were propagated under either benign (25°C) or cold (15°C) conditions. Parallel evolution lines were set up and were subject to four types of population-level selection: random selection, high-biomass selection, high-lipid selection, or rotational selection that alternated between the high-biomass and high-lipid targets.

This earlier study involved 384 algal cultures (2 temperatures × 4 selection regimes × 4 replicate evolution lines × 12 algal cultures per evolution line). After approximately 40 generations of laboratory evolution, algal populations showed increased cell yield and lipid production at their selection temperatures, though no difference was observed among the four artificial selection regimes in either cell yield or lipid production [37]. In the present study, 48 algal isolates were obtained from each of the two evolution environments (15℃ or 25°C). Specifically, three algal cultures with the greatest cell yield within each evolution line were chosen, and one single evolved isolate was obtained from each of the chosen algal cultures.

Each algal culture was grown in 70 mL BG11 liquid medium, in 100 mL transparent centrifuge tubes with lids loosened. Algal cultures were placed in incubators with a light: dark cycle of 12h: 12h, and light intensity was ~ 57 μmol photons m^{-2} s^{-1} (GXZ-380A; Ningbo Jiangnan Instrument Factory, China). Cultures were shaken manually once every day, and their positions in the incubator were rotated to reduce environmental heterogeneity. Following completion of the artificial selection experiment, evolved populations were streaked onto BG11 agar plates for single-colony isolation. Plates were incubated at the corresponding evolution temperatures (15°C or 25°C) for 10–20 days, and single isolates were obtained by picking up individual colonies that were then propagated in BG11 liquid medium. The resulting cultures were then used for reciprocal transplant assays, and samples of those cultures were also cryopreserved at − 80°C using the GeneArt® Cryopreservation Kit for Algae (Thermo Fisher Scientific, Waltham, MA, USA) for long-term storage.

2.2 Reciprocal transplant assays

All the 96 evolved isolates (48 from the 15°C evolution treatment and 48 from the 25°C evolution treatment), together with the ancestral strain (*C. sorokiniana* strain FACHB-24), were assayed for growth performance and lipid content at both 15°C and 25°C. The ancestral strain was cultured and measured simultaneously with evolved isolates under the same reciprocal transplant conditions. Prior to the artificial selection experiment, the ancestral strain was maintained at 25°C. Each algal culture was reconditioned and then grown for two cycles of 14-day batch culture growth, the first cycle for acclimation, and the second for phenotypic measurements. We ensured that the initial cell density of both growth cycles was ~ 0.3×10^6 cells mL^{-1}, by measuring optical density (OD) to estimate cell densities of the inoculum cultures and adjusting the inoculum volume.

Cell densities were estimated by daily measurement of optical density during the reciprocal transplant assays. Specifically, 200 μL of each culture was loaded into a 96-well microplate, and OD_{750} was measured using a microplate reader (Bio Tek Synergy H1). Each culture was assayed in triplicate. The OD_{750} values were converted to the cell density, cell density (10^7 cells mL^{-1}) = $4.230 \times OD_{750} + 0.039$ (R^2 =0.997). This conversion relationship was previously established by our research group using the same *Chlorella sorokiniana* experimental material and the same cultivation system as those used in the present study[37]. Briefly, serial dilutions of algal cultures were prepared, and their cell densities were determined microscopically while the corresponding OD_{750} values were measured using a microplate reader. Thus,

the calibration applied here was system-specific rather than transferred from a different algal strain or cultivation system. Our observation suggested that algal cultures did not reach their stationary growth phase during the assays (Fig. S1a). Thus we calculated the specific growth rate index [38] for each culture based on cell densities at day 1 and 14 during the reciprocal transplant assays, $\mu = \frac{\ln(C_1/C_0)}{(t_1-t_0)}$, where μ ($d^{-1}$) was the specific growth rate, and C_1 and C_0 were cell densities at day 14 and day 1, respectively. This index was used to quantify cumulative population growth performance during the assay period. Chlorophyll a (Chl-a) content was measured at the end of the assay cultivation as an additional biomass-related indicator. The detailed extraction procedure and calculation method are described in the Supplementary Information.

Lipid content of each culture at the end of the reciprocal transplant assays was quantified using the sulpho-phospho-vanillin (SPV) colorimetric method [39]. For each assay culture, aliquot of 14 mL (for 15°C-assayed samples) or 5 mL (for 25°C-assayed samples, to ensure the absorbance fell within the linear range of the standard curve) was centrifuged at 3,176 g for 10 minutes (4°C), and the supernatant was discarded. Next, 0.5 mL of concentrated (98%) sulfuric acid was added, and the mixture was heated at 90°C for 10 minutes, followed by cooling in an ice bath for 5 minutes. Subsequently, 3 mL of freshly prepared phospho-vanillin reagent (1.2 g L^{-1}, stored in the dark until use) was added. The reaction was carried out in a shaker at 37°C (200 rpm) for 10 min. Finally, 200 μL of the reaction mixture was transferred to a 96-well microplate, and optical absorbance was measured at 530 nm. Lipid content was estimated based on the following standard curve: lipid (mg) = 0.069 × OD_{530} − 0.001 (R^2 =0.993) [37]; then lipid content per unit of volume of culture was calculated (lipid yield, μg mL^{-1}). Each sample was measured in triplicate. The calibration curve established in Lu et al. [37] was linear over a lipid concentration range of 0.01–0.20 mg (corresponding to OD_{530} = 0.085–2.962). All samples analyzed in the present study fell within this range. The conversion equation used for lipid quantification was established and validated previously using the same SPV assay protocol, lipid standard, and spectrophotometric measurements [37]. All experimental procedures in the present study were identical to those used for calibration curve establishment, and this validated equation was applied for lipid concentration calculation. Lipid content per cell (pg $cell^{-1}$) was calculated as the ratio of lipid yield to cell density for each culture.

The magnitude of evolutionary changes, referred to as “selection response”, was calculated as log response ratio (LnRR, logarithm of the ratio of evolved isolates relative to the ancestral strain) in each trait for every algal isolate at each assay temperature.

2.3 Data analysis

Data were analyzed in the R environment (R 4.4.2). The difference in phenotypic traits between the evolved isolates and the ancestral strain was examined using one-sample *t* tests or Wilcoxon tests, depending on whether the assumptions of normal distribution were met. Linear mixed-effects models were used for the effects of experimental evolution temperature and assay temperature on phenotypic

traits, where evolved isolate ID was a random factor. The significance of explanatory variable effects was evaluated using the “Anova” function from the “car” package [40]. Multiple comparisons based on the linear mixed-effects model were carried out using the “multcomp” package [41].

3. Results

3.1 Cold-evolved algae produced more lipid

Algal isolates from the 15℃ experimental evolution temperature had slightly, though significantly, higher specific growth rate compared with those from 25℃, regardless of assay temperature (Fig. 1a; Table S1; linear mixed-effects model, evolution temperature, $F_{1,94}$ = 33.26, P < 0.001; assay temperature, $F_{1,94}$ = 405.04, P < 0.001; interaction, $F_{1,94}$ = 1.99, P = 0.162). Consistent with the specific growth rate results, Chlorophyll a (Chl-a) content, measured as an additional biomass-related indicator, showed qualitatively similar patterns among treatments (Fig. S1; Table S1).

Lipid content per cell was much higher in the 15℃-evolution, relative to 25℃-evolution algae; and this was particularly true under the 25℃ assay conditions (Fig. 1b; Table S1; evolution temperature, $F_{1,94}$ = 107.72, P < 0.001; assay temperature, $F_{1,94}$ = 97.50, P < 0.001; interaction, $F_{1,94}$ = 37.00, P < 0.001). The significant interaction reflected the fact that the difference between the two evolution temperatures was considerably larger at 25°C than at 15°C assay temperature. Specifically, lipid content per cell of the 15℃-evolution algae was 1.65-fold of that of 25℃-evolution algae when assayed at 15℃, and 2.56-fold when assayed at 25℃ (Table S1).

The combination of 15℃-evolution algae and 25℃ culture conditions gave the greatest population-level lipid production (Fig. 1c; Table S1; evolution temperature, $F_{1,94}$ = 107.84, P < 0.001; assay temperature, $F_{1,94}$ = 159.33, P < 0.001; interaction, $F_{1,94}$ = 58.19, P < 0.001). Specifically, the lipid yield of the 15℃-evolution algae was 2.21-fold of that of 25℃-evolution algae when assayed at 15℃, and 3.43-fold when assayed at 25℃ (Table S1). Because lipid content was quantified using the SPV colorimetric assay, these measurements represent total lipid content. Thus, the differences reported here should be interpreted as differences in total lipid accumulation and population-level lipid yield.

3.2 Evolutionary responses: evolution versus non-evolution environments

Selection response values (log response ratio) were calculated as standardized measures of evolutionary changes relative to the ancestral strain. For specific growth rate, all the 15℃-evolution algal isolates showed positive selection response values in both 15°C and 25°C assay conditions; and a majority (41 out of 48) of the 25℃-evolution isolates did so (Fig. 2a). Compared with the 25℃-evolution isolates, the 15℃-evolution ones had greater mean values of selection response in both assay environments (Fig. 2a; Table S3; evolution temperature, $F_{1,94}$ = 27.39, P < 0.001; assay temperature, $F_{1,94}$ = 666.76, P < 0.001; interaction, $F_{1,94}$ = 5.67, P = 0.019).

For the lipid content per cell trait, 47 out of 48 isolates of the 15℃-evolution environment had positive selection response values in both 15°C and 25°C assay conditions; and 24 out of 48 isolates from the 25℃-evolution environment did so (Fig. 2b). Compared with the 25℃-evolution isolates, the 15℃-evolution ones had greater mean values of selection response in both assay environments (Fig. 2b; Table S3; evolution temperature, $F_{1,94}$ = 108.33, P < 0.001; assay temperature, $F_{1,94}$ = 0.56, P = 0.455; interaction, $F_{1,94}$ = 6.91, P = 0.010).

For the population-level lipid yield, all isolates of the 15℃-evolution environment had positive selection response values in both 15°C and 25°C assay conditions; and 30 out of 48 isolates from the 25℃-evolution environment did so (Fig. 2c). Compared with the 25℃-evolution isolates, the 15℃-evolution ones had greater mean values of selection response in both assay environments (Fig. 2c; Table S3; evolution temperature, $F_{1,94}$ = 119.04, P < 0.001; assay temperature, $F_{1,94}$ = 38.45, P < 0.001; interaction, $F_{1,94}$ = 2.56, P = 0.113).

3.3 Evolutionary responses: association between growth rate and lipid content per cell

Under the 15°C assay conditions, almost all (47 out of 48) of the 15°C-evolution isolates exhibited positive selection response values in both specific growth rate and lipid content per cell; and over three-fourths (37 out of 48) of the 25°C-evolution isolates did so (Fig. 3a).

When assayed at 25°C, all of the 15°C-evolution isolates showed positive selection response values in both specific growth rate and lipid content per cell; and 28 out of 48 isolates from the 25°C evolution environment did so (Fig. 3b). Among the 25℃-evolution isolates, 18 isolates showed a trade-off between the two traits: increased growth rate was accompanied with decreased lipid content per cell; and 2 isolates showed decreased growth rate and increased lipid content per cell (Fig. 3b).

3.4 Evolutionary responses: association between growth rate and lipid yield

Under the 15°C assay conditions, all of the 15°C-evolution isolates exhibited positive selection response values in both specific growth rate and population-level lipid yield; and approximately 85% (41 out of 48) of the 25°C-evolution isolates did so (Fig. 4a).

When assayed at 25°C, all of the 15°C-evolution isolates showed positive selection response values in both specific growth rate and population-level lipid yield; and 35 out of 48 isolates from the 25°C evolution environment did so (Fig. 4b). Among the 25℃-evolution isolates, 11 isolates showed a trade-off between the two traits: increased growth rate was accompanied with decreased population-level lipid yield; and 2 isolates showed decreased growth rate and increased lipid yield (Fig. 4b).

4. Discussion

Lipid accumulation within cells may be favored under cold conditions [14, 22–26]. Previous studies have primarily described physiological responses to cold exposure, rather than evolutionary changes resulting from prolonged selection under cold conditions. This raises the question of whether long-term evolution under cold conditions can enhance lipid accumulation and whether the resulting phenotype can also be expressed if transferred to a warmer environment. In our ancestral *Chlorella sorokiniana* strain, short-term physiological response to a mildly cold temperature (15℃) does not cause higher lipid content per cell (Table S1). However, laboratory evolution at this temperature did lead to an improvement in this trait (Fig. 2b & Fig. 3a). Crucially, those evolved algal isolates showed extremely high lipid production when grown at a warm temperature (25℃). Consequently, the "unnatural" combination of cold-evolved algae and warm culture conditions gave the greatest lipid production, which provides a potentially useful strategy for improving lipid production without sacrificing growth.

Growth rate is typically suppressed at cold temperatures, and rapid growth was presumably the major trait that had been selected for in our 15℃-evolution algae. The underlying mechanisms may include faster resource uptake or greater resource storage capacity [42]. Importantly, we used a batch culture approach in the evolution experiment, that is, cultures underwent serial dilutions. Therefore, rapid response to the nutrient-replete conditions after each cycle of dilution may have contributed to fitness gain in this system [43, 44]. Greater lipid reserves within cells may thus be a response correlated with selection for rapid growth under cold, resource-fluctuating conditions. However, the absolute expression of this evolved trait remained dependent on the contemporary assay temperature. While the 15°C-evolved isolates contained more lipid content per cell than the 25°C-evolved isolates at both assay temperatures, their lipid content per cell was substantially higher when assayed at 25°C than at 15°C. Specifically, the lipid content per cell of the 15°C-evolved isolates was 1.65-fold that of the 25°C-evolved isolates at 15°C, increasing to 2.56-fold at 25°C. This pattern may reflect a genotype-by-environment interaction. This interpretation is further supported by the significant interaction between evolution temperature and assay temperature for lipid content per cell (Tables S2 and S4). Evolution at 15°C may have resulted in changes in carbon acquisition, carbon storage capacity, or carbon allocation toward lipid reserves, whereas the low assay temperature may have limited the realization of this evolved capacity by potentially constraining photosynthesis, enzymatic activity, and metabolic flux. After transfer to 25°C, these physiological constraints were relaxed, allowing the cold-evolved isolates to more fully express their enhanced lipid-accumulation capacity. Thus, cold evolution appears to have increased the capacity for lipid production, whereas warm cultivation favored the phenotypic expression of that evolved capacity. Because photosynthesis, carbon allocation, and lipid-biosynthesis pathways were not directly measured, these mechanistic explanations remain hypotheses that require further physiological and molecular investigation. The persistence of the enhanced lipid phenotype after the acclimation cycle suggests that the observed differences were not solely attributable to an immediate physiological response to temperature transfer. In this study, phenotypic measurements were conducted after an initial acclimation cycle followed by a second cultivation cycle under the same assay conditions. The maintenance of higher lipid accumulation in cold-evolved isolates after this acclimation period indicates that the phenotype persisted beyond an acute response to environmental transfer. However, the

molecular basis of this persistence cannot be resolved from phenotypic measurements alone and may involve genetic, epigenetic, or other inherited regulatory mechanisms.

Our results seem to contradict the local adaptation view: organisms native to an environment usually perform better than those from the other environment [45]. Here, our algae from the 15℃ experimental evolution environment showed slightly faster growth rate than those from 25℃, regardless of assay conditions (Fig. 1a). The absence of a growth advantage for the 25℃-evolved isolates in their home environment, challenges the simplicity of these expected patterns. The mechanisms underlying this pattern remain unclear. One possible explanation is that evolutionary adaptation at the warmer temperature involved ecological processes such as interference competition that may have constrained the evolution of growth rate [46, 47]. Our assays of the growth performance of monocultures reflect only the intrinsic growth characteristics rather than fitness under competitive or other ecological conditions. Whether the observed growth advantage of the 15°C-evolved isolates extends to other components of fitness or to competitive environments remains to be tested. Nonetheless, organisms' intrinsic growth performance is of relevance to industrial applications such as biofuel production, which are typically based on growing algal monocultures.

The lack of local adaptation suggested by our results may be contingent on our experimental evolution and assay temperatures. For example, our cold-evolved algae may show fitness cost if grown under very hot conditions, where protein thermal stability would be a major determinant of fitness [48]. In natural environments, the evolution of algae might have been driven by short-term selection of extreme environmental conditions. During periods of very cold conditions, microalgae must adjust membrane lipid composition and upregulate the expression of antifreeze proteins [49, 50], whereas during high-temperature periods, they must enhance the production of heat shock proteins (HSPs) [51] and activate antioxidant defense systems [52].

The positive correlation between selection response in lipid content per cell and growth rate observed here may also be "unnatural". High growth rate and high lipid content per cell can hardly be achieved simultaneously [26]. This contradiction is also a difficult problem in the research field of microalgal lipid production [9]. Organisms are faced with multiple challenges in natural environments, including changing abiotic conditions, competitors, predators and parasites. In particular, the antagonist species interactions may be more intense in warmer environments [53, 54]. Adaptation to those complex conditions may decouple the correlation between lipid storage and fast growth, and facilitate the optimization of adaptive phenotypes through alternative means, such as enhancing stress resistance or defense mechanisms [55]. This decoupling could arise through genetic trade-offs, where alleles conferring advantages in dealing with biotic antagonists (e.g., investment in defense compounds) impose energetic costs that constrain maximal growth rates [55, 56]. Our results therefore highlight the potential of experimental evolution to explore phenotypic combinations that may be difficult to obtain through conventional cultivation or short-term physiological manipulation.

Importantly, the primary contribution of the present study is to demonstrate a proof-of-concept strategy for improving the growth–lipid relationship through environmental evolutionary training, rather than to generate strains that are already optimized for direct industrial deployment. Translating this strategy toward industrial application will require a second stage of development focused on producing and validating strains under production-relevant conditions. Future studies could conduct cold evolutionary training in controlled bench-scale photobioreactors or other small bioreactor systems, where light intensity, nutrient supply, mixing, aeration, carbon availability, and dilution regimes can be systematically optimized to better approximate industrial cultivation. Long-term propagation and stability assays will also be important for determining whether the enhanced phenotype can be maintained across repeated cultivation cycles and under continuous or semi-continuous operation. In parallel, more comprehensive product characterization should quantify not only total lipid production but also lipid composition and quality, including fatty acid profiling using fatty acid methyl ester (FAME) analysis coupled with gas chromatography–mass spectrometry (GC-MS), particularly when evaluating suitability for biodiesel applications. Direct cell counting, time-resolved measurements of nutrients and biomass, and improved quantification of cellular and population-level lipid production would further strengthen process evaluation. Finally, genomic, epigenomic, transcriptomic, and functional analyses could be integrated with these production-oriented experiments to identify the mechanisms associated with desirable phenotypes and guide subsequent strain improvement. Together, these approaches would provide a pathway from the evolutionary strategy demonstrated here toward the development of robust algal materials suitable for industrial production.

Overall, our findings demonstrate the potential of environmental evolutionary training as a strategy for improving the growth–lipid relationship in microalgae. This study provides a proof of concept for subsequent strain and process optimization toward biotechnological applications.

5. Conclusion

Laboratory evolution under controlled environmental conditions can generate algal phenotypes with altered growth and lipid accumulation. Our study specifically showed that laboratory evolution of algal at a mildly cold temperature could result in a lipid-overproducing trait, and this trait may be maintained under warm culture conditions. The combination of cold-evolved algae and warm culture conditions therefore gave the highest levels of lipid yield. Notably, the enhanced lipid accumulation was not accompanied by a substantial reduction in specific growth rate under the tested conditions, suggesting that improved lipid production can be achieved without a pronounced trade-off in growth performance. These findings highlight laboratory evolution as a promising approach to improving microalgal lipid production, while further studies are needed to elucidate the underlying mechanisms and evaluate its potential under industrial cultivation conditions.

Declarations

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Data availability The raw data presented in this study are publicly available. The original contributions presented in this study are included in the article.

Ethics approval and consent to participate Not applicable.

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Figures

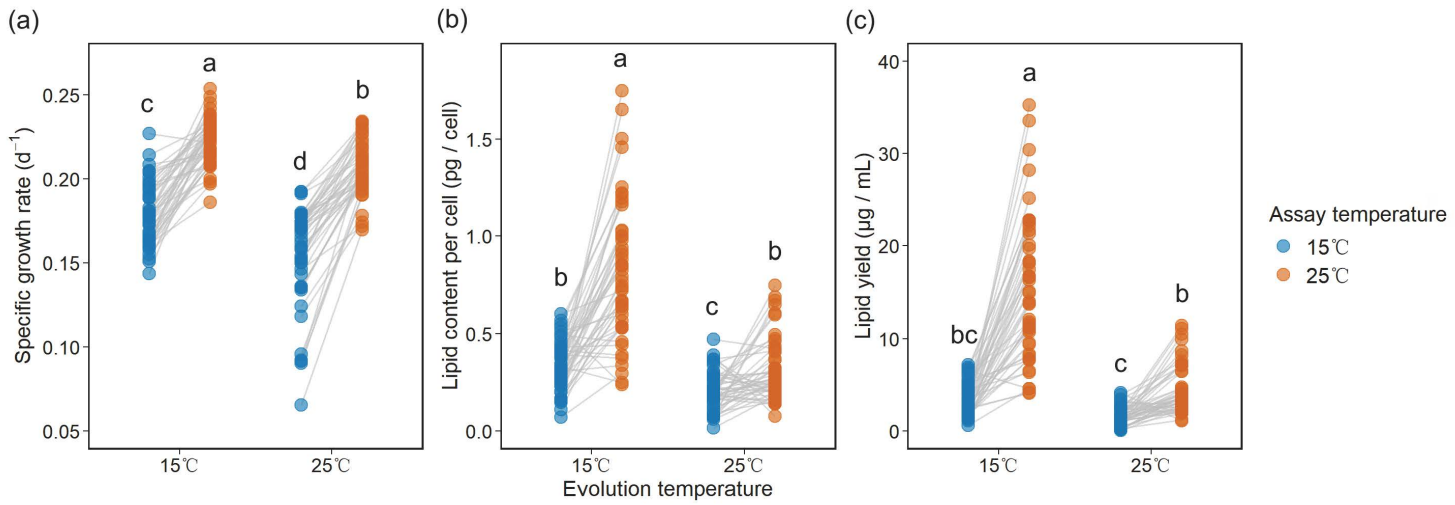


Figure 1

Specific growth rate (a), lipid content per cell (b), and population-level lipid yield (c) of the algal isolates. Each line represented one individual algal isolate. Multiple comparisons based on the linear mixed-effects model were carried out using the “multcomp” package. Different letters indicate significant differences in the mean phenotypic traits between two comparison groups.

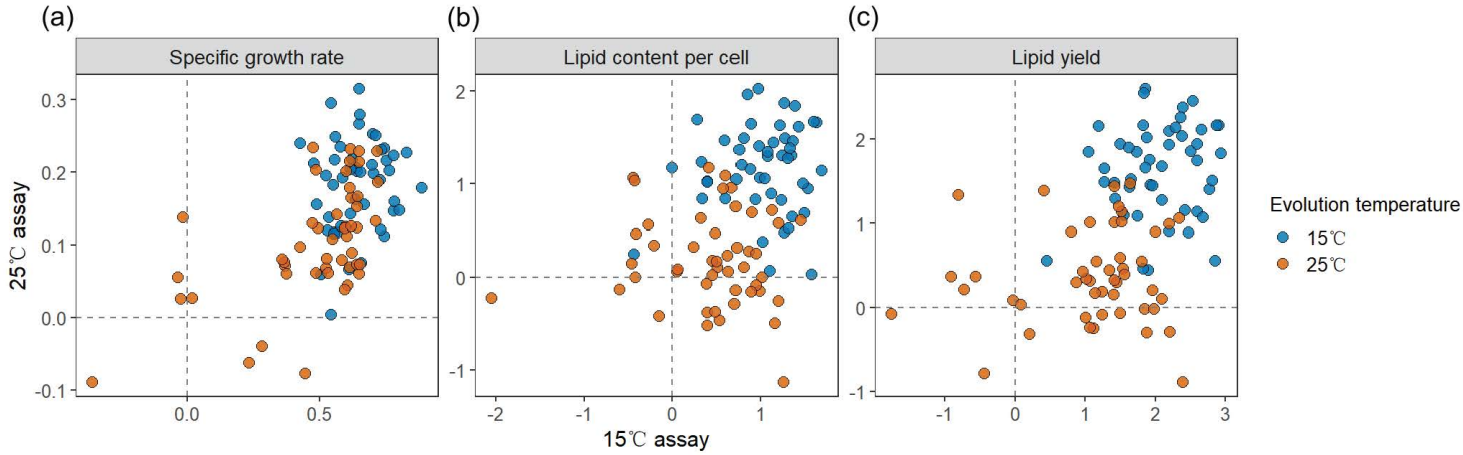


Figure 2

The correlation of selection responses for specific growth rate (a), lipid content per cell (b) and population-level lipid yield (c) between 15 °C and 25 °C assay conditions, calculated as the logarithm of the ratio of evolved to ancestral isolates (LnRR). Each point represents an algal isolate. The horizontal and vertical dashed lines through zero indicated the null hypothesis that evolved algal isolates did not differ from the ancestral strain.

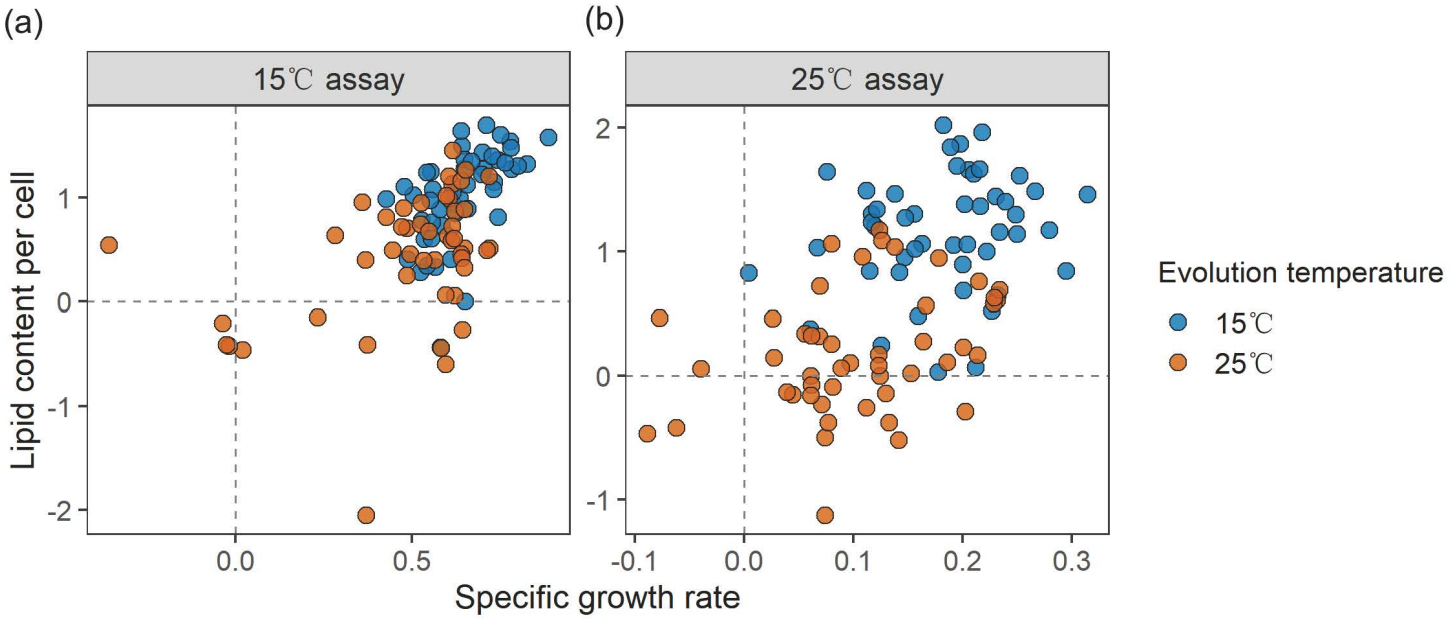


Figure 3

The correlation of selection responsesin specific growth rate and lipid content per cell assayed at 15 °C (a) and 25 °C (b), calculated as the logarithm of the ratio of evolved to ancestral strains (LnRR). Each point represents an algal isolate. The horizontal and vertical dashed lines through zero indicated the null hypothesis that evolved algal isolates did not differ from the ancestral strain.

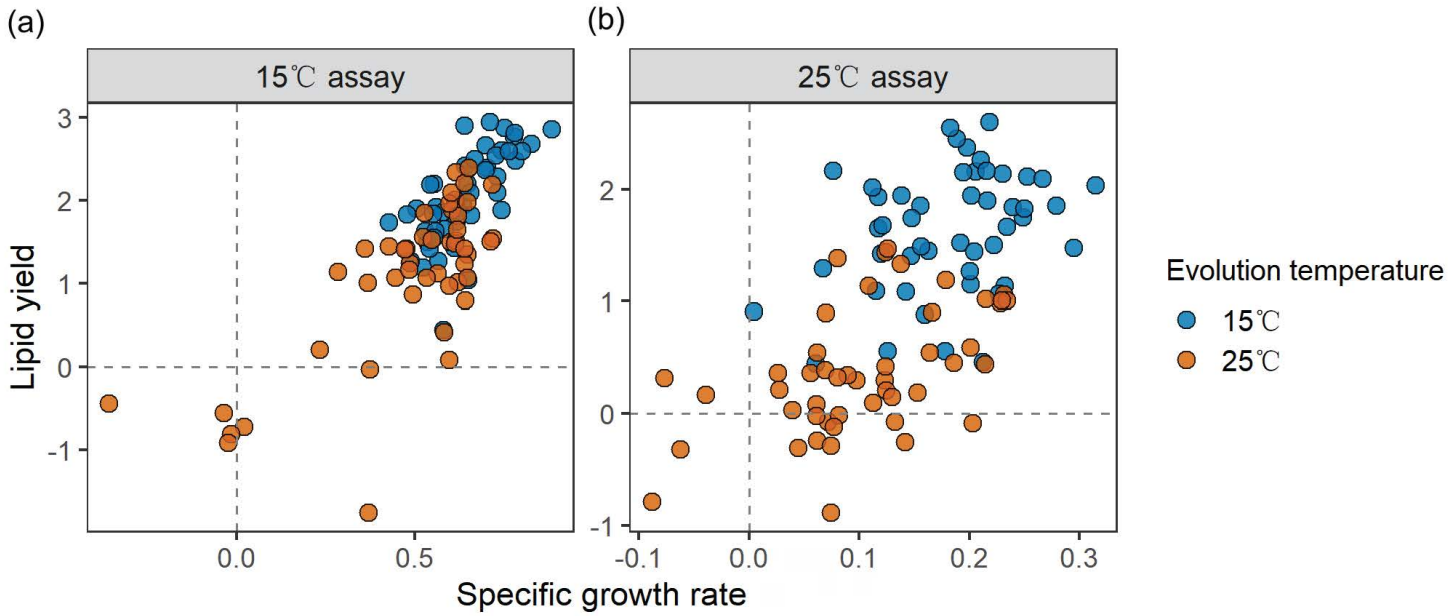


Figure 4

The correlation of selection responsesin specific growth rate and population-level lipid yield assayed at 15 °C (a) and 25 °C (b), calculated as the logarithm of the ratio of evolved to ancestral strains (LnRR).

Each point represents an algal isolate. The horizontal and vertical dashed lines through zero indicated the null hypothesis that evolved algal isolates did not differ from the ancestral strain.

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