Natural variation of nutrient homeostasis among laboratory and field strains in *Chlamydomonas reinhardtii*

Sara M. Esteves[1], Alice Jadoul[1], Fabrizio Iacono[2], Marie Schloesser[1], Bernard Bosman[3], Monique Carnol[3], Tom Druet[4], Pierre Cardol[2], Marc Hanikenne[1]

[1] InBioS-PhytoSystems, Translational Plant Biology, University of Liège, Belgium;

[2] InBioS-PhytoSystems, Genetics and Physiology of Microalgae, University of Liège, Belgium;

[3] InBioS-PhytoSystems, Laboratory of Plant and Microbial Ecology, University of Liège, Belgium

[4] Medical Genomics-Unit of Animal Genomics (GIGA), University of Liège, Belgium

Corresponding author

Marc Hanikenne, marc.hanikenne@uliege.be

University of Liège

InBioS - PhytoSystems

Quartier de la Vallée, 1

Chemin de la Vallée, 4 - Bât B22

B4000 Liège

Belgium

Tel: +32-4-3663844

Running title: Natural variation in Chlamydomonas

Highlights

Extensive genetic variation has been identified within the model microalga *Chlamydomonas reinhardtii*. We describe how this variation influences the response to nutrient deficiency, highlighting differences between natural and lab strains.

Abstract

Natural variation among individuals and populations exists in all species, playing key roles in response to environmental stress and adaptation. Micro- and macro-nutrients have a wide range of functions in photosynthetic organisms and mineral nutrition plays thus a sizable role in biomass production. To maintain nutrient concentrations inside the cell within physiological limits and prevent the detrimental effects of deficiency or excess, complex homeostatic networks have evolved in photosynthetic cells. The microalga *Chlamydomonas reinhardtii* (Chlamydomonas) is a unicellular eukaryotic model for studying such mechanisms. In this work, twenty-four Chlamydomonas strains, comprising field isolates and laboratory strains, were examined for intraspecific differences in nutrient homeostasis. Growth and mineral content were quantified in mixotrophy, as full nutrition control, and compared to autotrophy and 9 deficiency conditions for macronutrients (-Ca, -Mg, -N, -P, -S) and micronutrients (-Cu, -Fe, -Mn, -Zn). Growth differences among strains were relatively limited. However, similar growth was accompanied by highly divergent mineral accumulation among strains. The expression of nutrient status marker genes and photosynthesis were scored in pairs of contrasting field strains, revealing distinct transcriptional regulation and nutrient requirements. Leveraging this natural variation should enable a better understanding of nutrient homeostasis in Chlamydomonas.

Keywords

Chlamydomonas reinhardtii, ionome, natural variation, nutrient deficiency, nutrient homeostasis, photosynthesis, iron, manganese

Introduction

Photosynthetic organisms require both macro- and micro-nutrients in sufficient amount to sustain growth and complete their life cycle. Outside an adequate supply range, they cannot maintain homeostasis, leading to nutrient deficiency or toxicity (Römheld, 2011). Nevertheless, photosynthetic organisms have colonized very diverse environments, both in soil and water, characterized by extreme variations of nutrient supply, and therefore present a high diversity of nutrient homeostatic networks (Huang and Salt, 2016). For instance, four distinct iron (Fe) uptake machineries, of diverse evolutionary origins, are found, and in many cases co-exist, in algae (Blaby-Haas et al. 2017). Complex and tightly regulated mechanisms evolved to deal with inadequate nutrient availability, such as exclusion or hyperaccumulation to manage metal excess (Hanikenne and Nouet, 2011; Krämer, 2010), or inducible high affinity uptake, sparing and recycling systems to cope with nutrient deficiencies (Miramar *et al.*, 2003; Marschner, 2011; Saito *et al.*, 2011; Gutu *et al.*, 2011).

Species display more or less wide ecological ranges with variation among and within their populations allowing colonization and subsistence in diverse environments, thanks to phenotypic plasticity or local adaptation (VanWallendael *et al.*, 2022). Hence, different strategies to maintain nutrient homeostasis are reflected in the ionome (defined by Lahner et al., 2003; and Salt et al., 2008 as the mineral nutrient and trace element composition), which can be used to distinguish strains/accessions thanks to characteristic ionomic profiles (Buescher *et al.*, 2010; Atwell *et al.*, 2010; Baxter *et al.*, 2012). Considering natural variation within species not only provides a broader picture of a species behaviour but is instrumental for the identification of new genes and ecologically relevant alleles as it allows the study of quantitative (continuous) traits while the use of mutants is more suited for qualitative (discrete) trait analysis (Alonso-Blanco and Koornneef, 2000; Weigel, 2012), increasing the power of model organisms (Gasch *et al.*, 2016). Examining natural variation of the ionome in *Arabidopsis thaliana* (Arabidopsis) (Huang and Salt, 2016; Pita-Barbosa *et al.*, 2019) has for instance allowed identifying key genes involved in mineral nutrition or metal tolerance (Atwell et al., 2010; Baxter et al., 2008; Chao et al., 2012). In that respect selecting crop varieties with high nutrient-use efficiency is an important goal for breeders (Bhatt *et al.*, 2020; Thiébaut and Hanikenne, 2022).

Chlamydomonas reinhardtii (Chlamydomonas) is a single-cell biflagellate green microalga used as a model to dissect fundamental biological processes (Harris, 2001; Salomé and Merchant, 2019; Sasso et al., 2018). Chlamydomonas is a reference in the study of photosynthesis (e.g., Dent *et al.*, 2001; Wakao *et al.*, 2021), and related processes such as light

perception (e.g., Choudhary *et al.*, 2019; Hart and Gardner, 2021), CO_2 concentration (Moroney and Ynalvez, 2007; e.g., Fei *et al.*, 2022) or biofuel production (Rupprecht, 2009; e.g., Fakhimi and Tavakoli, 2019), but also of the cell cycle (e.g., Ma *et al.*, 2021), mobility (Shih *et al.*, 2013) or multicellularity (e.g., Bernardes *et al.*, 2021). It also serves as model for nutrition-related studies, including, nitrogen, sulfur, phosphate (Calatrava *et al.*, 2017; Saroussi *et al.*, 2017; Plouviez *et al.*, 2022) as well metal homeostasis (Hanikenne, 2003; Merchant *et al.*, 2006; Blaby-Haas and Merchant, 2017).

So far, research into the Chlamydomonas biology has essentially focused on a set of commonly-used laboratory strains, related to the strains originally isolated by Smith in the 1940's and 50's (Smith, 1946; Smith and Regnery, 1950; Harris, 2009), hereafter referred to as laboratory strains. It was recently shown that the genomes of these laboratory strains contain highly conserved segments alternating with a mosaic of segments made of two alternate haplotypes with 2% sequence divergence and that they display phenotypic diversity (Gallaher *et al.*, 2015). Additional strains displaying even higher (up to 3%) and randomly distributed genetic variation in their genomes (Flowers *et al.*, 2015) were later isolated, mostly in North America, but were so far only marginally used in experimental approaches into the biology of Chlamydomonas (Harris, 2009). Although they have been conserved for a certain time at the Chlamydomonas Resource Center or other repositories, these highly divergent field isolates (Flowers et al. 2015) will hereafter be referred as natural strains to distinguish them from laboratory strains derived from the Smith original isolates.

Intraspecific variation in cellular zinc (Zn), copper (Cu), manganese (Mn) and Fe contents for 4 Chlamydomonas laboratory strains grown mixotrophically in Tris-Acetate-Phosphate (TAP) medium was reported (Kropat et al., 2011), whereas variation of growth, cell size and chlorophyll content in a partially overlapping set of 5 laboratory strains upon varying Fe supply was also described (Gallaher *et al.*, 2015). Nonetheless, nutrient homeostasis research in Chlamydomonas mostly relied on the study of a handful of strains and mutants (Pittman *et al.*, 2009; Ibuot *et al.*, 2017; Hui *et al.*, 2022; Kochoni *et al.*, 2022). Here, we aimed to uncover the extent of intraspecific variation in nutrient homeostasis in Chlamydomonas and to assess how biased or incomplete our view of homeostasis mechanisms in Chlamydomonas may be from the characterization of only a few commonly-used laboratory strains. In particular, we: (i) explored the variation of growth and ionome profile within a panel of 14 natural and 10 laboratory Chlamydomonas strains grown mixotrophically in TAP, photoautotrophically in Tris-Minimal-Phosphate (TMP) medium as well as in 9 TAP media deficient for single nutrients (calcium, magnesium, nitrogen, phosphorus, sulphur, copper, iron, manganese and

zinc), (ii) assessed the partitioning of this variation among laboratory and natural strains, and (iii) scoring as phenotypic traits the expression of nutrient deficiency marker genes, examined the distinct molecular strategies deployed to manage nutrient deficiency in selected pairs of contrasting natural strains.

Materials and Methods

Chlamydomonas strains

Twenty-four *Chlamydomonas reinhardtii* strains were acquired from the Chlamydomonas Resource Center and SAG Culture Collection of Algae as described in **Table 1**. Strains were maintained on 1.5% plant agar (Duchefa Biochemie B.V.) Tris-Acetate-Phosphate (TAP) medium (Gorman and Levine, 1965) under 100 $\mu E.m^{-2}.s^{-1}$ continuous light.

Culture conditions

TAP medium was used as control and base for all mineral deficiency media. All TAP media contained 1 $mL.L^{-1}$ of glacial acetate and the final solution was titrated to pH 7.0 with HCl. TMP medium was prepared as described by Gorman and Levine (1965). The nine single deficiency media were prepared as described in **Table 2**. N, P, S and Fe deficiency conditions were based on previous work by Figueroa-Torres et al. (2017), Barreiro et al. (2013), Laurinavichene et al. (2002) and Glaesener et al. (2013), respectively; and the other deficiency conditions were determined in preliminary experiments.

For each experiment, freshly growing cells on TAP agar plates were used to initiate pre-cultures in TAP liquid medium. After two-days, the optical density at 750 nm (OD_{750nm}) was measured, as a proxy to estimate biomass, using a spectrophotometer (GENESYS™ 20 Visible Spectrophotometer, Thermo Scientific™). These pre-cultures were then used to inoculate 35 mL of media (TAP, TMP or TAP with a single mineral deficiency), to a final concentration of approximately 10^4 $cells.mL^{-1}$. The OD_{750nm} was then measured from day 3 until day 7 to follow growth (**Supplementary Figure S1**) (Thiriet-Rupert *et al.*, 2021). Considering the diversity of strains (with varying cell size and aggregation, Gallaher et al. 2015, Flowers et al. 2015) and growth conditions used in the study, optical density at 750 nm, as a proxy to estimate biomass, was selected to measure growth.

To minimize contamination by trace amounts of micronutrients (Cu, Fe, Mn, Zn), all glassware was rinsed with HCl, plasticware was preferred and, if not possible, the material was rinsed with 1 M EDTA. All assays including all 24 Chlamydomonas strains were conducted in

two independent experiments, with two independent cultures each. All assays focusing on pairs of Chlamydomonas strains were conducted with three biological replicates.

ICP-AES analysis

At day 4 of the cultures, samples for ICP-AES were prepared based on the protocol by Thiriet-Rupert et al.(2021). Briefly, 10 mL of culture were harvested in metal-free tubes and centrifuged at 2000 x *g* for 5 min to remove the media. Cells were resuspended with 5 mL of 5 mM EDTA (pH 7.0) and centrifuged at 2000 x *g* for 5 min, this step was repeated twice. The pellet was then washed with 5 mL distilled water. After centrifugation at 2000 x *g* for 5 min, 3 mL of >65% (v/v) nitric acid were added to the pellet, then stored at 4°C for 2 days for digestion. Before analysis, distilled water was used to bring the final volume to 10 mL and 200 µL of >65% (v/v) nitric were added to each sample.

The elements Ca, Cu, Fe, potassium (K), sodium (Na), Mg, Mn, P and Zn were quantified using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Vista AX CCD Simultaneous ICP-AES, Varian). The results were normalized using the OD_{750nm}.

RNA extraction and qRT-PCR

Total RNA was extracted using an adapted genomic DNA extraction protocol followed by selective RNA precipitation with LiCl (Loppes and Radoux, 2001; Newman et al., 1990).

At day 4 of the cultures, 10^7 cells were harvested and centrifuged at 2000 x *g* for 5 min. The pellet was frozen with liquid N_2 and kept at -80°C until extraction. Extraction was initiated by resuspending the cells in lysis buffer [2% SDS (w/v), 400 mM NaCl, 40 mM EDTA, 100mM Tris HCl pH 8.0], adding phenol/chloroform/isoamyl alcohol (25:24:1) and incubating 5 minutes with agitation. The aqueous phase was separated by 5 min centrifugation at 15000 x *g* and re-extracted with chloroform/isoamyl alcohol (24:1). Total RNAs were precipitated overnight at 4 °C in 8 M LiCl and collected by a 5 min centrifugation at 15000 x *g*. Pelleted total RNAs were then washed with 70% ethanol, centrifuged, and diluted in RNase free H_2O. DNA contaminations were removed using the DNase Max kit (Qiagen) following the manufacturer's instructions. Total RNA concentration was determined spectrophotometrically using a NanoDrop 2000 (Thermo Scientific) and the RNA quality was confirmed using agarose gel electrophoresis. Complementary DNA (cDNA) synthesis was performed from 1 µg of total RNAs using the RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific). Quantitative reverse transcription-PCR (qRT-PCR) was performed using the QuantStudio™ 5

system (Thermo Fisher Scientific) and the Takyon™ Low ROX Probe 2X MasterMix dTTP blue (Eurogentec). Each reaction contained the 8 µL master mix, 125 nM of each primer (**Supplementary Table S1**) and 4 µl of 50x-diluted cDNA and was performed in triplicate. The reaction conditions were as follows: (i) pre-PCR at 50 °C for 2 min, then 95 °C for 10 min, (ii) 40 cycles of PCR at 95 °C for 15 s and 60 °C for 15 s, (iii) melting curve at 95 °C for 15 s, 60 °C for 15 s and 95 °C for 15 s. Primers were designed ensuring that they annealed in non-polymorphic sequences between strains to be compared and are described in **Supplementary Table S1.**

Gene expression was calculated using the qbase+ software, version 3.2 (Biogazelle, Zwijnaarde, Belgium) (Hellemans *et al.*, 2008), by the $2^{-\Delta\Delta CT}$ method using *CBLP* and *RPL13* as reference genes and normalized to one of the replicates (Livak and Schmittgen, 2001; Nouet *et al.*, 2015; Thiriet-Rupert *et al.*, 2021). The stability of the reference genes in our experimental conditions was confirmed during the normalization step embedded in qBase (M = 0.212, CV = 0.074). Statistical analysis using one-way ANOVA was performed using the software's statistics wizard.

Photosynthesis and pigment analysis

100 µL of 4-day old cultures were transferred to a white 96-well plate and dark adapted for 10 min (Kalaji *et al.*, 2014). Time-resolved chlorophyll fluorescence was then measured using a SpeedZen 200 fluorescence imaging system (Johnson *et al.*, 2009) equipped with red LEDs (650-670nm) for actinic and saturating lighting, and blue LEDs (450-470 nm) as fluorescence detection lights. PSII variable fluorescence was monitored through a saturation curve composed of 7 light-steps (25, 50, 91, 130, 270, 420, 600 µmol of quanta m^{-2} s^{-1}) of 4 minutes each (one saturating pulse of 220ms every minute). Fv/Fm was calculated on dark-adapted samples as "Fm-Fs/Fm", while rETR was calculated as "(Fm'-Fs'/Fm')*light intensity" and non-photochemical quenching (NPQ) was calculated was calculated as "Fm-Fm'/Fm' " the last two pulses at each light-step were averaged. The saturation curve was followed by 23 minutes of dark and a final pulse was used to monitor the photoinhibitory effect of the actinic light on the Fv/Fm (Fv/Fm recovery).

For pigment quantification, 1 mL of 4-day old culture was centrifuged at 2000 x *g* for 5 min and the lipophilic pigments were extracted using 1 mL of methanol. The supernatant was recovered after centrifuging the samples at 2000 x *g* for 5 min and the absorbance was measured at 470 nm, 652 nm and 665 nm. Chlorophyll *a* and *b*, carotenoid concentrations were calculated as described in Wellburn (1994).

Data analysis and representation

Unless otherwise stated, data treatment was performed using RStudio IDE for R (RStudio Team, 2021; R Core Team, 2022). Data was organized using tidyverse *v1.3.1* (Wickham *et al.*, 2019), reshape2 *v1.4.4* (Wickham, 2007), summarized using Rmisc *v1.5* (Hope, 2013) and statistical analyses (Kruskal-Wallis and pairwise Dunn tests) were performed using the FSA *v0.6.1* (Ogle *et al.*, 2021) and rcompanion *v2.4.1* (Mangiafico, 2021) packages.

Data was then plotted using the packages ggpubr *v0.4.0* (Kassambara, 2020) or ComplexHeatmap *v2.8.0* (Gu *et al.*, 2016) with custom-made colour scales using circlize *v0.4.13* (Gu *et al.*, 2014). Plots were exported in vector image format using the svglite *v2.0.0* (Wickham *et al.*, 2021) package.

Results

Growth and ionome variation in mixotrophy and autotrophy

The 24 laboratory (L) and natural (N) strains (**Table 1**) were initially grown in liquid medium in mixotrophy (TAP) and photoautotrophy (TMP). Optical density was recorded (OD_{750nm}, as a proxy to estimate biomass) from day 3 to 7 of culture, with an intermediary sampling point at day 4 for element profiling (**Supplementary Figure S1, Supplementary Dataset S1**). Final optical density (day 7) in TAP medium was overall 4 times higher than in TMP, possibly to CO_2 limitation in these growth conditions (**Figure 1A-B, Supplementary Datasets S1-S3**). Optical density of the least growing strain in TAP [(N) CC-1374] was approximately 5x higher than growth of the least growing strain in TMP [(L) CC-5325]. Three laboratory strains (CC-5325, CC-125 and CC-3348) were among the 3 least growing strains in TMP medium while the 3 least growing strains in TAP were natural strains (CC-1374, CC-2343, CC-2935) (**Figure 1A**). In contrast, a laboratory strain (CC-1418) was one of the most growing strains in both TAP and TMP. Overall, the range of optical density variation at day 4, i.e., the range between the least and the most growing strains, among laboratory strains was larger in TMP (3.3x) than in TAP (1.5x), whereas the opposite was observed for natural strains, with a 1.64x range in TMP and a 2.7x range in TAP (**Supplementary Dataset S2**).In TAP, copper (Cu) accumulation was the most variable trait, with a 12x range between the most and the least accumulating strains, whereas iron (Fe) accumulation was the least variable trait (2x) among micronutrients (**Figure 2A**, **Supplementary Datasets S4 and S5**). Among macronutrients, calcium (Ca) accumulation displayed the widest variation (9x) upon growth in

TAP, whereas phosphorus (P) accumulation was the least variable (2x). (**Figure 2A**). Variation of Cu and Fe accumulation was larger in natural strains whereas Ca and P accumulation varied more in laboratory strains (**Supplementary Dataset S5**).

In TMP, the accumulation of all elements, with the stark exception of Cu, was higher than in TAP (**Figure 2B**), with average Ca accumulation being the most increased (2.5x). The reduction in Cu content might result from decreased cellular Cu requirement, as respiration is decreased during photoautotrophic growth (Kropat *et al.*, 2015). Similar to TAP, Cu accumulation was the most variable trait (11x), together with Ca accumulation (9x), and Fe accumulation varied the least (3x).

Growth and ionome variation under nutrient deficiency

The strains were next grown for 7 days in TAP liquid medium either fully (-Ca, -Mn, -Zn) or partially (-Cu, -Fe, -Mg, -S, -P and -N) deprived of a mineral nutrient (**Table 2**). Depending on the element, using partial or full depletion allowed finding a balance between inducing a deficiency response while at the same time maintaining growth. These differences in treatments determined different intensities of nutrient deficiency and, for the most part, may explain the differences in growth observed among deficiency conditions (**Figure 3A, Supplementary Dataset S1**).

All nutrient deficiency treatments negatively impacted median growth of the strain panel at day 4, with the exception of -Zn (**Figure 3A**). However, after 7 days of culture, all treatments resulted in lower average optical density comparatively to the TAP full nutrition control: this reduction ranged from 0.9x for -Zn to 0.3x for -Mn (**Supplementary Figure S2**). Chlamydomonas cells contain similar concentrations of micronutrients (Merchant et al., 2006 and **Figure 2A**), but Mn deficiency (no Mn added in the medium, **Table 2**) had a much stronger impact on growth than Zn deficiency (no Zn added in the medium as well, **Table 2**), for instance. Both treatments also had a very different effects on residual Zn or Mn accumulation in the cells, with 0.2x and 0.02x reductions, respectively (**Figure 4, Supplementary Dataset S6**). TAP medium was suggested to contain Zn in excess (Merchant et al. 2006), and Zn may be carried over in cells from the pre-culture. It was previously shown that 2 consecutive transfers to medium without Zn were required to fully inhibit Chlamydomonas growth (Hong-Hermesdorf et al. 2014). Chlamydomonas might as well be able to take up trace Zn contaminants present in the chemicals and acid-washed vessels used to prepare the TAP medium (Merchant and Helmann, 2012).

Laboratory strains overall tolerated nutrient deficiencies better than natural strains, representing ~70% of the top 3 most growing strains across all conditions. In contrast, natural strains represented ~63% of the bottom 3 least growing strains (**Figure 3B, Supplementary Dataset S5**). The most growing laboratory strain in TAP [(L) CC-1418] was also among the 3 most growing strains under -Cu, -Fe, -Mg, -P and -Zn, whereas in contrast it was the strain the most impacted by -Ca. Two other laboratory strains were among the 3 most growing strains for multiple deficiency treatments: (L) CC-410 (-Cu, -Mg, -P and -Zn), and (L) CC-1010 (-Ca, -Fe and -S). On the other hand, the strains most often among the least growing strains were from both backgrounds: (N) CC-1374 (-Cu, -Mg, -Mn, -P, -S, -Zn), (L) CC-125 (-Mg, -N, -S), (L) CC-1690 (-Cu, -N, -S) and (N) CC-2343 (-Ca, -Fe, -P). However, while (N) CC-1374 and (N) CC-2343 were already among the least growing strains in TAP, the (L) CC-125 and (L) CC-1690 strains were in the top 3 most growing strains in -Ca and -Zn, respectively. Overall, significant growth differences between natural (N) and laboratory (L) strains were observed in 6 out of 11 media, including TAP and TMP **(Supplementary Figure S3**).

Average mineral accumulation levels in the strain panel were either increased (31% of all observations) or decreased (69% of all observations) by nutrient deficiencies, indicative of the tight interconnections among nutrient homeostasis networks (**Figure 4, Supplementary Datasets S4 and S7**). In particular, the elements whose supply was limited in the medium all displayed on average a strongly reduced accumulation in the strains, ranging from a 0.3x reduction for Mg to a 0.02x for Cu, indicating that the deficiency treatments were effective. Whereas average growth reduction ranged between 1x and 0.2x, the impact of nutrient deficiencies on element accumulation was massive ranging from 0.3x to 1.3x for Mg to 0.02x to 8.5x for Mn compared to TAP. Across all conditions and strains, Fe accumulation again appeared the most tightly regulated, as in TAP, whereas the most variable elements were other micronutrients, Cu, Mn and Zn.

Most striking was the global effect of Ca deficiency on Mn accumulation which on average increased by 8.5x across the 24-strain panel compared to TAP. Cu was strongly accumulated upon Ca (1.4x), Fe (2.8x) and Zn (2.9x) deficiencies. Zn displayed higher accumulation upon Mn (2x), Ca (2.2x) and Fe (2.3x) deficiencies, and conversely Fe accumulated more in cells upon Cu (1.4x) and Zn (1.5x) deficiencies.

Compared to TAP, Ca accumulation was negatively affected in a similar fashion by Ca, but also Fe and Mn deficiencies (~0.2x), and even more so by Cu, Mg and P deficiencies (<0.05x) (**Figure 4**). P was the second most negatively affected element, with strong accumulation reduction upon Cu and Mg deficiencies (~0.1x).

Ionome variation at the strain level under nutrient deficiency

Hierarchical clustering of the element profiling data was next conducted to further assess the determinants of the ionomic variation in Chlamydomonas (**Supplementary Figure S4, Supplementary Dataset S8**). It is remarkable that the growth conditions had a bigger impact on the clustering than the strain origin, lab or natural. This for instance contrasts with (i) the footprint of local adaptation in the metal hyperaccumulator *Arabidopsis halleri*, where geographic origin has more impact on the ionome and transcriptome than the exposure to Zn or cadmium (Cd) excess (Schvartzman *et al.*, 2018; Corso *et al.*, 2018, 2021) or (ii) a number of ionomic traits in Arabidopsis (Campos et al. 2021). In Chlamydomonas, the treatment clusters seldom contained strains grown in other conditions. Mn deficiency was a noticeable exception, with strains distributing in two distant clusters, independently of their lab or natural origin, whereas strains exposed to N deficiency were the most dispersed among clusters (**Supplementary Figure S4**).

A more detailed analysis of data clustering was then conducted for individual growth conditions. It showed that, even in TAP, very distinct element accumulation patterns were observed among strains **(Figure 5A)**. Most clusters grouped both lab and natural strains, suggesting again that strain origin was not the most discriminating factor. The (L) CC-3348 strain displayed the most divergent ionome profile, marked by high Fe, Zn, K and Cu, but low Mn, accumulation **(Figure 5A)**.

Upon Mn deficiency **(Figure 5B)**, 10 strains, 4 of which laboratory strains, accumulated Mn below detection levels and, for 3 of those, K could not be detected either. The effect of Fe deficiency on the strain ionomes displayed a general trend: an overaccumulation of Cu, Zn and Na coupled with an under accumulation of Mg, Fe and Ca **(Figure 5 C)**, with the exception of strain (L) CC-3348, which under accumulated all elements.

Clustering in the other deficiency media showed the same blend of natural and laboratory strains, and how the accumulation of some elements was key for clustering **(Supplementary Figure S5)**. Briefly, in TMP, two laboratory strains (CC-5325 and CC-1010) were an exception with increased Cu accumulation opposite to the lower accumulation observed in all other strains (**Figure 1G**). Although it varied in amplitude (40x), Mn accumulation in -Ca condition was a conserved trait in all strains, with the exception of strain CC-2342 **(Supplementary Figure S5 E)**. Overall the -N, -Zn and -S conditions had the lowest impact on the ionomes **(Supplementary Figure S5 B, H, D)**, whereas the -P, -Mg and -Cu conditions had a strong

negative effect on the accumulation of most elements in cells **(Supplementary Figure S5 C, F, G)**.

At the end of this analysis, pairs of divergent natural strains were selected for a deeper exploration of the variation of the Mn and Fe deficiency responses (by scoring expression of nutrient status marker genes and photosynthetic parameters), as these conditions had a strong impact in the ionome phenotypes and natural strains were so far much less examined than laboratory strains (**Figure 5B-C**).

Manganese deficiency response in two natural strains

Two natural strains with contrasting ionome phenotypes upon Mn deficiency were selected: (N) CC-2290, which accumulated Mn and K below detection levels, and (N) CC-1373, which accumulated those two elements **(Figure 5B)**. In this condition, the strains also differed in their accumulation of Fe (1.5x), Cu (5.1x), Mg (7.3x), Zn (13x) and P (33x), all those elements being more accumulated in CC-1373 (**Supplementary Figure S6, Supplementary Dataset S9**).

NRAMP1 (*Natural Resistance-Associated Macrophage Protein 1*), a gene strongly induced by Mn deficiency (Allen *et al.*, 2007*b*), whose product was suggested to transport Mn in yeast (Rosakis and Köster, 2005) and to be a Mn assimilation transporter in Chlamydomonas (Allen *et al.*, 2007*b*), was constitutively lower expressed (4x) in TAP in strain CC-2290 (**Figure 6A**). *NRAMP1* moreover failed to be induced by Mn deficiency in strain CC-2290, which resulted in a 125x lower expression than in strain CC-1373 **(Figure 6A)**. Similarly, the expression of *MTP4* (*Metal Tolerance Protein 4*) was lower in CC-2290 in TAP and was not induced by Mn deficiency, compared to CC-1373 (8x induction) (**Figure 6A**). *MTP4* was previously shown to be Mn-deficiency inducible in a laboratory strain and encodes an Mn-transporting MTP, possibly involved in Mn internal storage (Allen et al. 2007b; Ibuot et al., 2020).

Allen et al. (2007*b*) showed that Mn deficiency in the CC-425 lab strain resulted in reduced Fe accumulation, and in the transcriptional induction of genes encoding the Fe uptake machinery in Chlamydomonas (Hanikenne *et al.*, 2009; for review, see Blaby-Haas and Merchant, 2012, 2017). In the natural strain CC-1373, two components of this machinery [*FRE1* (*Ferric Reductase 1*) and *FOX1* (*Ferroxidase 1*)], used as Fe deficiency marker genes, were upregulated by Mn deficiency. As for *NRAMP1* and *MTP4*, the Mn-responsiveness of these genes was abolished in strain CC-2290 **(Figure 6A)**. Albeit less marked, a similar expression profile was observed for the *IRT1* (*Iron-Regulated Transporter 1*) gene, involved

391 in an alternative Fe^{2+} uptake pathway than *FOX1* (Hanikenne et al. 2009; Blaby-Haas and
392 Merchant, 2012, 2017), among the two strains **(Figure 6A)**, resulting in a ~20x lower
393 expression of the gene in strain CC-2290 compared to CC-1373 upon Mn deficiency.
394 Chlamydomonas possesses two superoxide dismutase (SOD) isozymes: a Mn
395 containing (MnSOD) in the mitochondria, and a Fe containing (FeSOD) in the chloroplast
396 (Kitayama *et al.*, 1999). The MnSOD is encoded by 5 genes (*MSD1-5*). *MSD3* is the isoform
397 most induced transcriptionally by both Mn and Fe deficiency and MnSOD activity was reported
398 to be reduced under Mn deficiency (Allen *et al.*, 2007*b*). This loss in activity is not
399 compensated by an increased expression or activity of the plastid FeSOD (FSD1) in the CC-
400 425 laboratory strain (Allen *et al.*, 2007*b*). In strain CC-1373, the expression of both *MSD3*
401 and *FSD1* was strongly induced by Mn deficiency **(Figure 6A)**, suggesting a compensation
402 mechanism replacing MnSOD by FeSOD, in contrast to CC-425. *MSD3* was constitutively
403 highly expressed in both TAP and -Mn conditions and *FSD1* was down-regulated by -Mn in
404 strain CC-2290.
405 The two natural strains further differed in their Cu and Zn accumulation levels upon
406 Mn deficiency (**Figure 5B**), and marker genes for the Cu and Zn nutritional status were
407 investigated. The *CTR2* gene, encoding a high affinity Cu transporter located at the plasma
408 membrane and responsive to Cu (Castruita et al., 2011; Page et al., 2009), was significantly
409 induced in strain CC-1373 by Mn deficiency, unlike in strain CC-2290. In contrast, the
410 expression of *ZRT1* and *ZRT3*, two Zn-nutrition responsive transporters (Malasarn *et al.*, 2013),
411 was unaltered by Mn deficiency, although *ZRT1* was significantly overexpressed in CC-1373
412 compared to CC-2290 **(Figure 6A)**.
413 Mn deficiency is known to lead to secondary P deficiency and to the up-regulation of a
414 number of P transporters in the CC-425 laboratory strain (Allen et al., 2007b). This
415 transcriptional regulation was however suggested to be independent of the P deprivation
416 regulatory network (Allen *et al.*, 2007*b*). Natural strains CC-1373 and CC-2290 displayed
417 opposite P accumulation profiles: in strain CC-1373, P accumulation was low on TAP and
418 slightly increased (1.1x) upon Mn deficiency (**Figure 5A-B**) whereas it was high on TAP and
419 decreased (0.03x) upon Mn deficiency in strain CC-2290 (**Figure 5A-B**). The P accumulation
420 profiles were essentially mirrored by the expression of *PTB2*, a P-nutrition responsive
421 phosphate transporter-encoding gene (Moseley *et al.*, 2006; Grossman and Aksoy, 2018),
422 whose expression was induced by Mn deficiency in CC-1373 and was constitutively high in
423 CC-2290 (**Figure 6A**).

Mn is essential for photosynthesis: it is a key component of the oxygen-evolving complex (OEC– Mn_4CaO_5), thanks to its multiple oxidation states, which allows the catalysis of water deprotonation and electron transfer through the photosynthetic chain (Merchant and Sawaya, 2005; Shen, 2015). Mn-deficiency is known to impact photosynthesis at the photosystem II (PSII) level (Teichler-Zallen, 1969; Allen *et al.*, 2007*b*). Therefore, some photosynthetic parameters were evaluated in the two strains: (i) the maximum quantum yield of photosystem II (PSII) photochemistry (Fv/Fm), (ii) the relative electron transfer rate of photosystem II (rETR) at different light intensities, (iii) non photochemical quenching (NPQ) at different light intensities, and (iv) photoinhibition measured from Fv/Fm recovery in the dark after the exposure to actinic light (reviewed in Murchie and Lawson, 2013). In TAP, both strains exhibited similar Fv/Fm and rETR upon low light exposure (**Figure 6B, C, Supplementary Dataset S10)**. This was reflected by similar OD_{750nm} for CC-2290 and CC-1373 achieved at day 4 (Figure 1A). In contrast, upon high light exposure in TAP, rETR values were lower and NPQ values higher in CC-2290 (**Figures 5C, D**). Under Mn deficiency at all light intensities, CC-2290 was more impacted than CC-1373 (**Figures 5C**), with lower rETR values, which is consistent with the stronger growth reduction observed for CC-2290 in these conditions. Altogether, these observations point to a donor-side PSII limitation at the level of the OEC because of low intracellular [Mn] in CC-2290. In addition, a lower chla/b ratio was observed in CC-2290 under Mn deficiency, which suggested a higher PSII:PSI stoichiometry, as previously observed in pea (Chow *et al.*, 1990), or a larger chlb-rich PSII antenna size in CC-2290 (**Supplementary Figure S7C**).

Iron deficiency response in two natural strains

Fe deficiency resulted in variation of Fe accumulation among natural strains (**Figure 5C, Supplementary Dataset S8**) but had even more impact on the accumulation of other elements (**Figure 5C**) and on optical density (**Supplementary Figure S2**). Two natural strains, CC-2343 and CC-4414, differing for P (1.9x), Zn (2.6x), Mn (3.3x) and Cu (4x) accumulation (**Supplementary Figure S8, Supplementary Dataset S11)**, as well as growth (1.8x), upon Fe deficiency were further characterized.

The response of Chlamydomonas laboratory strains to reduced Fe supply in the medium has been characterized in details, distinguishing two stages: (i) a deficiency response, when Fe supply is reduced but is more or less sufficient to sustain growth essentially thanks to the induction of the Fe uptake pathways (*FOX1*/*FTR1* on one side and *IRT1*/*IRT2* on the other side); and (ii) a limitation response, when Fe supply is so low that cells are strongly affected

and trigger massive reorganisation of the photosynthetic apparatus to spare Fe (La Fontaine *et al.*, 2002; Moseley *et al.*, 2002; Merchant *et al.*, 2006; Allen *et al.*, 2007*a*; Naumann *et al.*, 2007; Urzica *et al.*, 2012; Page *et al.*, 2012). In this study, Fe supply was reduced to 1.8 µM Fe, corresponding to the deficiency stage described above (**Table 2**).

Components of the Fe uptake machinery were differentially expressed in the two natural strains (**Figure 7A**). *FOX1* was less expressed in TAP and displayed lower induction by Fe deficiency in strain CC-2343, having a higher Fe accumulation in both conditions (**Figure 5A, C**), compared to strain CC-4414. *FRE1* and *IRT1* were in contrast expressed at similar levels and strongly induced by Fe deficiency in both strains (**Figure 7A**). All 3 genes were previously reported to be strongly induced by Fe deficiency in the CC-425 and CC-4532 laboratory strains (Allen *et al.*, 2007*b*,*a*; Glaesener *et al.*, 2013; Urzica *et al.*, 2013).

Similarly, the *CTR2* and *CYC6* (*Cytochrome* c_6) genes were up-regulated by Fe deficiency in strain CC-4414 only (**Figure 7A**). As mentioned above, CTR2 is involved in Cu uptake, whereas *CYC6* encodes an heme-containing cytochrome, which substitutes for plastocyanin under Cu deficiency enabling maintenance of photosynthesis in these conditions (Kropat et al., 2015; Merchant, 1998; Merchant and Bogorad, 1986). The *CTR2* and *CYC6* induction by Fe deficiency in strain CC-4414 suggests a higher Cu requirement and Cu sparing, through plastocyanin replacement, in the strain, possibly to support the strong induction of the FOX1 multicopper oxidase (Kropat *et al.*, 2015). It however did not fully compensate this increased Cu demand, as the CC-4414 strain accumulated less Cu (0.25x) than strain CC-2373 upon Fe deficiency (**Figure 5C**).

NRAMP1 expression was similar between the two strains in TAP (**Figure 7A**), consistent with their similar Mn accumulation (**Figure 5A**). *NRAMP1* was previously shown to respond to Mn but not Fe deficiency in Chlamydomonas laboratory strains (Allen *et al.*, 2007*b*; Urzica *et al.*, 2012). This does not exclude that NRAMP1 can transport other metal cations than Mn (Chang *et al.*, 2020), including Fe, as its distantly related NRAMP1 plant homologs (Hanikenne *et al.*, 2005; Blaby-Haas and Merchant, 2012), which are involved in both Mn and Fe homeostasis (Cailliatte *et al.*, 2010; Castaings *et al.*, 2016; Chen *et al.*, 2019). In our conditions, Fe-deficiency led to reduced (0.2x) Mn accumulation in strain CC-4414, which was not compensated by increased expression of *NRAMP1*, in agreement with previous reports in lab strains (Allen et al., 2007b). In contrast, in strain CC-2343, Fe deficiency had a positive effect on Mn nutrition, with higher Mn accumulation (1.1x) accompanied by decreased *NRAMP1* expression. Fe deficiency induced the *IRT1* and *IRT2* genes (Figure 7A, Blaby-Haas

and Merchant, 2012; Urzica et al., 2012), whose products might be responsible for increased Mn uptake (Dubeaux *et al.*, 2018).

Chlamydomonas cells experiencing Fe deficiency are more susceptible to oxidative damage (Naumann *et al.*, 2007) and the transcriptional response to Fe deficiency involves a large set of oxidative stress response genes (Urzica *et al.*, 2012). In laboratory strains, strong induction of *MSD3* (encoding a chloroplast and mitochondria localized MnSOD), at the transcript, protein and activity levels, has been reported upon Fe deficiency (Allen *et al.*, 2007*b*; Urzica *et al.*, 2012; Page *et al.*, 2012). FeSOD, also chloroplast-localized, encoded by *FSD1*, has a more complex fate. It is not transcriptionally regulated by Fe deficiency. However, in these conditions, the FSD1 protein is initially degraded, before *de novo* synthesis and Fe re-acquirement enabled by an Fe sparing machinery triggered at the expense of other Fe-containing proteins (Allen *et al.*, 2007*b*; Urzica *et al.*, 2012; Page *et al.*, 2012). The CC-2343 and CC-4414 natural strains deployed distinct strategies to deal with the Fe deficiency-induced oxidative stress: CC-2343 induced *FSD1* expression whereas CC-4414 induced *MSD3* as described in laboratory strains (**Figure 7A**).

Fv/Fm and rETR were reduced upon Fe deficiency in both CC-2343 and CC-4414 natural strains **(Figure 7B-C, Supplementary Dataset S12)**, indicating impaired photosynthesis. However, the photosynthetic apparatus of strain CC-4414 was more impacted upon Fe deficiency as indicated by lower maximum rETR, lower NPQ at most light intensities, and a lower Fv/Fm recovery. In Chlamydomonas laboratory strains, a major target of Fe deficiency is the Fe-rich PSI complex, which is subject to reduction and remodelling, as well as other Fe-requiring photosystem proteins, with the purpose of sparing Fe and prioritizing respiration (Moseley *et al.*, 2002; Yadavalli *et al.*, 2012; Page *et al.*, 2012). Despite these preventive measures, electron flow between PSII and PSI was shown to be disturbed, leading to ROS generation, which primarily targets PSII (Naumann *et al.*, 2007). Here, strain CC-4414 displayed an increased chla/b ratio upon Fe deficiency, indicative of an increased PSI:PSII ratio (Chow *et al.*, 1990), which may suggest either (i) a lower capacity to recycle and remodel PSI in these conditions and/or (ii) a higher sensitivity of PSII to ROS. The higher NPQ observed in CC-4414 upon Fe deficiency, at least at some light intensities (**Figure 7D, Supplementary Dataset S12**), may support the latter hypothesis. Chlorosis was reported to be not visible at early Fe-deficiency stages (Moseley *et al.*, 2002; Terauchi *et al.*, 2010; Urzica *et al.*, 2012). At day 4, both CC-2273 and CC-4414 cell culture appeared lighter green **(Supplementary Figure**

S9A), which may be linked to the decrease in chlorophyll/carotenoid ratio in Fe-deficiency **(Supplementary Figure S9D)**.

Discussion

Variation of the ionome largely exceeds variation of growth in Chlamydomonas

In this study, we describe the large extent of phenotypic variation that exists in a panel of 24 Chlamydomonas strains, in line with the important genetic variation previously reported among laboratory and natural strains (Flowers *et al.*, 2015; Gallaher *et al.*, 2015). This variation was evident in both mixo- and photoautotrophy, as well as upon deficiency of essential macro- and microelements. Growth variation was observed in all tested conditions, with in several cases partitioning of this variation among natural and laboratory strains. Strikingly, much larger variation was observed at the ionome level. If the relative abundance of the micro- and macroelements reported here agrees with previous observations in 5 wild-type and 2 mutant strains in varied growth conditions, the range of accumulation variation among strains in a single medium exceeds by far what has been previously reported in Chlamydomonas (Merchant *et al.*, 2006). This variation is quite extensive in comparison to other model organisms (e.g., Atwell *et al.*, 2010; Yu *et al.*, 2012; Baxter *et al.*, 2012) or to field-collected taxonomically diverse plant species (Watanabe et. al., 2007). It is much larger than ionome variation reported in roots or leaves of Arabidopsis accessions grown in hydroponics or on soil (Baxter *et al.*, 2012). For instance, the range of Cu accumulation in roots of Arabidopsis accessions was reported to be ~3x (Baxter *et al.*, 2012), whereas it is ~12x in Chlamydomonas (**Figure 2A**). The extent of variation observed among Chlamydomonas strains is comparable to variation observed between mutant and wild-type yeast strains (Yu *et al.*, 2012). Higher impact of growth conditions on the ionome vs biomass production suggests that, among strains, distinct ionome equilibrium (i.e. distinct homeostatic networks) are able to support growth. This homeostatic flexibility is not similar for all elements. Among micronutrients, accumulation of Fe, which is both essential and, as a redox active ion, highly toxic in excess (Marschner, 2011), is the most tightly controlled, with the narrower range among Chlamydomonas strains (**Figures 2 and 4**). In contrast, Cu, although essential and highly toxic as well in excess (Marschner, 2011), displays a surprisingly much wider range of accumulation (**Figures 2 and 4**), raising the question about how such low or high accumulation is handled by the cells. This likely reflects the capacity of the strains to uptake, efficiently use and/or store nutrients (see below).

Interactions between nutrients also vary among Chlamydomonas strains

Ionome variation among Chlamydomonas strains does not only concern individual element accumulation but also interactions between elements (**Figures 2 and 4**). Such interactions take place for several reasons. Fe, Zn, Mn and Cu interactions are at least in part due to shared or interacting uptake pathways (Allen et al., 2007b; la Fontaine et al., 2002; Malasarn et al., 2013), as exemplified in a detailed analyses of pairs of strains (Figures 6, 7 and 8). Interactions between Ca, S and micronutrients at least in part result also from their colocalization in acidocalcisomes, lysosome-derived structures storing metals in excess for future use in Chlamydomonas (Tsednee et al., 2019; Schmollinger et al., 2021). As Mg is chelated by rRNA and ATP, the largest cytoplasmic reservoirs of Pi and Mg, an interaction between P and Mg homeostasis is expected and coo-regulation mechanisms have been described (e.g., Bruna *et al.*, 2021).

The most striking observation is the massive Mn accumulation in -Ca condition, with on average 8.5x more than in TAP control conditions (**Figure 4**). Ca-Mn interactions in acidocalcisomes have been described previously in Chlamydomonas (Tsednee *et al.*, 2019). Several transmembrane transporters [e.g., CAX (Cation Exchanger) or BICAT (bivalent cation transporter)] involved in the transport of both Ca and Mn have been identified in plants, including for the import of these elements into the chloroplast, as well as other compartments, where Mn may also replace Ca at Ca-binding-sites (reviewed in He *et al.*, 2021).

A number of nutrient interactions observed in this study were reported before in individual Chlamydomonas strains, such as an increased Cu and Fe accumulation upon Zn deficiency described for CC-4532 (Malasarn *et al.*, 2013) or reduced P accumulation in Mn deficiency reported for strain CC-425 (Allen *et al.*, 2007*b*) suggesting that, although subjected to important variation, the direction of these interactions is conserved among strains. In contrast, on average across the 24 strains, Mn concentrations decreased upon Zn deficiency and Fe concentrations increased upon Mn deficiency while opposite trends were observed in individual strains by Malasarn et al. (2013) and Allen et al. (2007*b*), respectively, suggesting that nutrient interactions can also occur in opposite directions among strains.

Mn and Fe deficiencies trigger divergent transcriptional responses and ionome outcomes in two natural strains

Dissecting the molecular mechanisms underlying ionomic differences in selected pairs of natural strains exposed to Mn or Fe deficiency, respectively, revealed the distinct strategies of the strains to manage nutrient deficiency (**Figure 8**).

In both cases, the transcriptional regulation of marker genes contributed to the interactions between Mn and Fe, Zn or Cu homeostasis, which have been extensively documented in both flowering plants (Socha and Guerinot, 2014; Ricachenevsky *et al.*, 2018; Hanikenne *et al.*, 2020) and Chlamydomonas (Allen et al., 2007b; Blaby-Haas and Merchant, 2017; Castruita et al., 2011; Hanikenne et al., 2009, 2005; Urzica et al., 2012), mainly because these nutrients share transporters and chelators.

The contrasted expression patterns of Mn-, Fe-, Cu- and Zn-responsive genes, encoding metal transporters, in the CC-1373 and CC-2290 strains are likely to account for the contrasted Mn (*NRAMP1, MTP4*), as well as Fe (*FRE1*, *FOX1*), Cu (*CTR2*) and Zn (*ZRT1*) accumulation levels in control and/or in Mn deficiency conditions (**Figure 5B**). Differential expression between the two strains does not only concern genes encoding functions in metal uptake at the plasma membrane, but also reflects different intracellular storage (*MTP4*) and metal usage (*FSD1*/*MSD3*) strategies. Some of the transporters may influence the accumulation of several elements. Hence, *NRAMP1* has been reported to also transport Zn (Chang *et al.*, 2020). Given the low selectivity for metal cations, including Mn and Zn, of the IRT1 homolog in Arabidopsis (Vert *et al.*, 2001; Dubeaux *et al.*, 2018), it may directly contribute to Mn and Zn uptake. The reduced Cd accumulation in an *irt1* Chlamydomonas mutant (Thiriet-Rupert *et al.*, 2021) indeed suggests that IRT1 may also transport divalent metal cations other than Fe. Noticeably, despite some major differences of *NRAMP1, FRE1* or *FOX1* gene expression in TAP, there was only relatively minor variation of Fe (1.5x), Mn (1.04x, not significant), and Zn (1.2x, not significant) accumulation between strains CC-2290 and CC-1373 (**Supplementary Figure S6**). It is also interesting to note that, in Chlamydomonas, Fe-deficiency response elements are for instance found in the promoter of genes encoding Mn transporters (e.g., MTP4, Fei et al., 2009). The diversity of the molecular response to Mn deficiency among two natural strains highlights that these interactions can take different forms within the species, suggesting possible divergence in Mn sensing and regulatory mechanisms.

Similarly, Fe accumulation and Fe-responsive gene expression patterns (*FOX1*, *IRT1*), together with oxidative stress management (*FSD1, MSD3*) and photosynthetic responses (**Figure 7**), altogether suggest that the CC-2343 and CC-4414 natural strains handle Fe homeostasis distinctly, with strain CC-4414 experiencing a stronger Fe deficiency possibly stemming from less efficient Fe uptake, reduced Fe sparing capacity and/or higher Fe requirements, even on TAP, which in turn impacts the accumulation and use of other metals, such as Cu, Mn and Zn. That such variation of Fe homeostasis existed in Chlamydomonas was

previously shown (Gallaher *et al.*, 2015). We provide here some molecular understanding of the mechanisms underlying such variation.

Conclusion

These detailed analyses in selected natural strains highlight differences in metal requirements as well as major differences in the transcriptional regulation of metal homeostasis genes, suggesting that distinct metal sensing and signalling may also take place among strains. In conclusion, our study underlines the extent of phenotypic variation within the Chlamydomonas species and leveraging this natural variation should enable a better understanding of nutrient homeostasis mechanisms in Chlamydomonas and in photosynthetic organisms.

Supplementary data

Supplementary Table S1. Deficiency marker gene description and qRT-PCR primers.

Supplementary Figure S1. Schematic representation of the experimental design.

Supplementary Figure S2. Variation of the impact of mineral deficiencies on growth of 24 Chlamydomonas strains.

Supplementary Figure S3. Variation of the impact of nutrient deficiencies on growth of laboratory vs natural Chlamydomonas strains.

Supplementary Figure S4. Clustering of 24 Chlamydomonas strains exposed to the 10 nutrient deficiencies based on their ionome profiles.

Supplementary Figure S5. Clustering of 24 Chlamydomonas strains based on their ionome profiles.

Supplementary Figure S6. Comparison of the nutrient concentrations in the CC-1373 and CC-2290 natural Chlamydomonas strains upon Mn deficiency.

Supplementary Figure S7. Variation of the pigment composition in response to Mn deficiency between the CC-1373 and CC-2290 Chlamydomonas natural strains.

Supplementary Figure S8. Comparison of the nutrient concentrations in the CC-2343 and CC-4414 natural Chlamydomonas strains upon Fe deficiency.

Supplementary Figure S9. Variation of the pigment composition in response to Fe deficiency between the CC-2343 and CC-4414 Chlamydomonas natural strains.

Supplementary Dataset S1. Data of the growth curves of each of the 24 Chlamydomonas strains.

Supplementary Dataset S2. Descriptive statistics of day 4 optical density measurements in all tested media.

Supplementary Dataset S3. Descriptive statistics of the growth curves in all tested media.

Supplementary Dataset S4. Data of the ionome of each of the 24 Chlamydomonas strains.

Supplementary Dataset S5. Descriptive statistics of the ionome.

Supplementary Dataset S6. Top and bottom 3 strains for all measured parameters at day 4.

Supplementary Dataset S7. Descriptive statistics of the relative nutrient concentrations under nutrient deficiency.

Supplementary Dataset S8. Heatmap dataset.

Supplementary Dataset S9. Strains CC-1373 and CC-2290 nutrient concentrations in TAP and -Mn.

Supplementary Dataset S10. Statistic differences for rETR and NPQ for strains CC-1373 and CC-2290 in TAP and -Mn.

Supplementary Dataset S11. Strains CC-2343 and CC-4414 nutrient concentrations in TAP and -Fe.

Supplementary Dataset S12. Statistic differences for rETR and NPQ for strains CC-2343 and CC-4414 in TAP and -Fe.

Acknowledgements

We thank A. Degueldre, R. Houet, and S. Banneux for technical support, as well as F. Bouché, A. Caccamo and F. Vega de Luna for helpful discussions.

Author contributions

M.H., P.C. and T.D. conceived and directed the research. M.H., S.M.E., A.J., F.I. and P.C. designed experiments. S.M.E, A.J., B.B., M.S., F.I., P.C., B.B. and M.C. performed experiments. S.M.E., M.H., F.I. and P.C. analysed and interpreted the data. S.M.E. and M.H. generated all Figures and Supporting Information material. S.M.E. and M.H. wrote the manuscript, with comments of all authors.

Conflict of Interest

The authors have no conflicts to declare.

Funding statement

Funding was provided by the University of Liège (ARC GreenMagic to M.H., P.C. and T.D.) and by the "Fonds de la Recherche Scientifique-FNRS" (MIS-F.4511.16, PDR-T0120.18, PDR-T.0104.22 to M.H.). F.I. was supported by the FRIA. P.C and T.D. are Senior Research Associate and Research Director of the F.R.S.-FNRS, respectively.

Data availability statement

Data to support the findings of this study are openly available in **Supplementary Datasets S1-S12**.

References

Allen MD, Del Campo JA, Kropat J, Merchant SS. 2007*a*. *FEA1*, *FEA2*, and *FRE1*, encoding two homologous secreted proteins and a candidate ferrireductase, are expressed coordinately with *FOX1* and *FTR1* in iron-deficient *Chlamydomonas reinhardtii*. Eukaryotic Cell **6**, 1841–1852.

Allen MD, Kropat J, Tottey S, Del Campo JA, Merchant SS. 2007*b*. Manganese deficiency in Chlamydomonas results in loss of photosystem II and MnSOD function, sensitivity to peroxides, and secondary phosphorus and iron deficiency. Plant Physiology **143**, 263–277.

Alonso-Blanco C, Koornneef M. 2000. Naturally occurring variation in Arabidopsis: an underexploited resource for plant genetics. Trends in Plant Science **5**, 22–29.

Atwell S, Huang YS, Vilhjálmsson BJ, *et al.* 2010. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. Nature **465**, 627–631.

Barreiro A, Hairston NG. 2013. The influence of resource limitation on the allelopathic effect of *Chlamydomonas reinhardtii* on other unicellular freshwater planktonic organisms. Journal of Plankton Research **35**, 1339–1344.

Baxter I, Hermans C, Lahner B, Yakubova E, Tikhonova M, Verbruggen N, Chao D yin, Salt DE. 2012. Biodiversity of mineral nutrient and trace element accumulation in *Arabidopsis thaliana*. PLoS ONE **7**, e35121.

Baxter IR, Vitek O, Lahner B, Muthukumar B, Borghi M, Morrissey J, Guerinot M Lou, Salt DE. 2008. The leaf ionome as a multivariable system to detect a plant's physiological status. Proceedings of the National Academy of Sciences, USA **105**, 12081–12086.

Bernardes JP, John U, Woltermann N, Valiadi M, Hermann RJ, Becks L. 2021. The evolution of convex trade-offs enables the transition towards multicellularity. Nature Communications **12**, 1–9.

Bhatt R, Hossain A, Sharma P. 2020. Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: A review. Open Agriculture **5**, 176–186.

Blaby-Haas CE, Merchant SS. 2012. The ins and outs of algal metal transport. Biochimica et Biophysica Acta - Molecular Cell Research **1823**, 1531–1552.

Blaby-Haas CE, Merchant SS. 2017. Regulating cellular trace metal economy in algae. Current Opinion in Plant Biology **39**, 88–96.

Bruna RE, Kendra CG, Groisman EA, Pontes MH. 2021. Limitation of phosphate assimilation maintains cytoplasmic magnesium homeostasis. Proceedings of the National Academy of Sciences, USA **118**, e2021370118.

Buescher E, Achberger T, Amusan I, *et al.* 2010. Natural Genetic Variation in Selected Populations of *Arabidopsis thaliana* Is Associated with Ionomic Differences. PLoS ONE **5**, 11081.

Cailliatte R, Schikora A, Briat JF, Mari S, Curie C. 2010. High-Affinity Manganese Uptake by the Metal Transporter NRAMP1 Is Essential for Arabidopsis Growth in Low Manganese Conditions. The Plant Cell **22**, 904–917.

Calatrava V, Chamizo-Ampudia A, Sanz-Luque E, Ocaña-Calahorro F, Llamas A, Fernandez E, Galvan A. 2017. How Chlamydomonas handles nitrate and the nitric oxide cycle. Journal of Experimental Botany **68**, 2593–2602.

Castaings L, Caquot A, Loubet S, Curie C. 2016. The high-affinity metal Transporters NRAMP1 and IRT1 Team up to Take up Iron under Sufficient Metal Provision. Scientific Reports **6**, 37222.

Castruita M, Casero D, Karpowicz SJ, *et al.* 2011. Systems Biology Approach in Chlamydomonas Reveals Connections between Copper Nutrition and Multiple Metabolic Steps. The Plant Cell **23**, 1273–1292.

Chang P, Yin H, Imanaka T, Igarashi Y, Li N, Luo F. 2020. The metal transporter CrNRAMP1 is involved in zinc and cobalt transports in *Chlamydomonas reinhardtii*. Biochemical and Biophysical Research Communications **523**, 880–886.

Chao DY, Silva A, Baxter I, Huang YS, Nordborg M, Danku J, Lahner B, Yakubova E, Salt DE. 2012. Genome-Wide Association Studies Identify Heavy Metal ATPase3 as the Primary Determinant of Natural Variation in Leaf Cadmium in *Arabidopsis thaliana*. PLOS Genetics **8**, e1002923.

Chen HM, Wang YM, Yang HL, Zeng QY, Liu YJ. 2019. NRAMP1 promotes iron uptake at the late stage of iron deficiency in poplars. Tree Physiology **39**, 1235–1250.

Choudhary SK, Baskaran A, Sharma P. 2019. Reentrant Efficiency of Phototaxis in *Chlamydomonas reinhardtii* Cells. Biophysical Journal **117**, 1508–1513.

Chow WS, Melis A, Anderson JM. 1990. Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proceedings of the National Academy of Sciences, USA **87**, 7502–7506.

Corso M, An X, Jones CY, Gonzalez-Doblas V, Schvartzman MS, Malkowski E, Willats WGT, Hanikenne M, Verbruggen N. 2021. Adaptation of *Arabidopsis halleri* to extreme metal pollution through limited metal accumulation involves changes in cell wall composition and metal homeostasis. New Phytologist **230**, 669–682.

Corso M, Schvartzman MS, Guzzo F, Souard F, Malkowski E, Hanikenne M, Verbruggen N. 2018. Contrasting cadmium resistance strategies in two metallicolous populations of *Arabidopsis halleri*. New Phytologist **218**, 283–297.

Dent RM, Han M, Niyogi KK. 2001. Functional genomics of plant photosynthesis in the fast lane using *Chlamydomonas reinhardtii*. Trends in Plant Science **6**, 364–371.

Dubeaux G, Neveu J, Zelazny E, Vert G. 2018. Metal Sensing by the IRT1 Transporter-Receptor Orchestrates Its Own Degradation and Plant Metal Nutrition. Molecular Cell **69**, 953-964.e5.

Fakhimi N, Tavakoli O. 2019. Improving hydrogen production using co-cultivation of bacteria with *Chlamydomonas reinhardtii* microalga. Materials Science for Energy Technologies **2**, 1–7.

Fei X, Eriksson M, Yang J, Deng X. 2009. An Fe deficiency responsive element with a core sequence of TGGCA regulates the expression of FEA1 in *Chlamydomonas reinharditii*. Journal of Biochemistry **146**, 157–166.

Fei C, Wilson AT, Mangan NM, Wingreen NS, Jonikas MC. 2022. Modelling the pyrenoid-based CO_2-concentrating mechanism provides insights into its operating principles and a roadmap for its engineering into crops. Nature Plants 2022 8:5 **8**, 583–595.

Figueroa-Torres GM, Pittman JK, Theodoropoulos C. 2017. Kinetic modelling of starch and lipid formation during mixotrophic, nutrient-limited microalgal growth. Bioresource Technology **241**, 868–878.

Flowers JM, Hazzouri KM, Pham GM, *et al.* 2015. Whole-Genome Resequencing Reveals Extensive Natural Variation in the Model Green Alga *Chlamydomonas reinhardtii*. The Plant Cell **27**, 2353–2369.

La Fontaine S, Quinn JM, Nakamoto SS, Dudley Page M, Göhre V, Moseley JL, Kropat J, Merchant S. 2002. Copper-dependent iron assimilation pathway in the model photosynthetic eukaryote *Chlamydomonas reinhardtii*. Eukaryotic Cell **1**, 736–757.

Gallaher SD, Fitz-Gibbon ST, Glaesener AG, Pellegrini M, Merchanta SS, Merchant SS. 2015. Chlamydomonas Genome Resource for Laboratory Strains Reveals a Mosaic of Sequence Variation, Identifies True Strain Histories, and Enables Strain-Specific Studies. The Plant Cell **27**, 2335–2352.

Gasch AP, Payseur BA, Pool JE. 2016. The Power of Natural Variation for Model Organism Biology. Trends in Genetics **32**, 147–154.

Glaesener AG, Merchant SS, Blaby-Haas CE. 2013. Iron economy in *Chlamydomonas reinhardtii*. Frontiers in Plant Science **4**, 337.

Gorman DS, Levine RP. 1965. Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences, USA **54**, 1665–1669.

Grossman AR, Aksoy M. 2018. Algae in a Phosphorus-Limited Landscape. Annual Plant Reviews **48**, 337–374. doi.org/10.1002/9781118958841.ch12

Gu Z, Eils R, Schlesner M. 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics **32**, 2847-2849.

Gu Z, Gu L, Eils R, Schlesner M, Brors B. 2014. circlize implements and enhances circular visualization in R. Bioinformatics **30**, 2811–2812.

Gutu A, Alvey RM, Bashour S, Zingg D, Kehoe DM. 2011. Sulfate-Driven Elemental Sparing Is Regulated at the Transcriptional and Posttranscriptional Levels in a Filamentous Cyanobacterium. Journal of Bacteriology **193**, 1449.

Hanikenne M. 2003. *Chlamydomonas reinhardtii* as a eukaryotic photosynthetic model for studies of heavy metal homeostasis and tolerance. New Phytologist **159**, 331–340.

Hanikenne M, Esteves SM, Fanara S, Rouached H. 2020. Coordinated homeostasis of essential mineral nutrients: a focus on iron. Journal of Experimental Botany **72**, 2136-2153.

Hanikenne M, Merchant SS, Hamel P. 2009. Transition Metal Nutrition: A Balance Between Deficiency and Toxicity. The Chlamydomonas Sourcebook 3-Vol set.333–399.

Hanikenne M, Motte P, Wu MCS, Wang T, Loppes R, Matagne RF. 2005. A mitochondrial half-size ABC transporter is involved in cadmium tolerance in *Chlamydomonas reinhardtii*. Plant, Cell and Environment **28**, 863–873.

Hanikenne M, Nouet C. 2011. Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. Current Opinion in Plant Biology **14**, 252–259.

Harris EH. 2001. Chlamydomonas as a model organism. Annual Review of Plant Biology **52**, 363–406.

Harris EH. 2009. The Genus Chlamydomonas. The Chlamydomonas Sourcebook. Elsevier, 1–24.

Hart JE, Gardner KH. 2021. Lighting the way: Recent insights into the structure and regulation of phototropin blue light receptors. Journal of Biological Chemistry **296**, 100594.

He J, Rössner N, Hoang MTT, Alejandro S, Peiter E. 2021. Transport, functions, and interaction of calcium and manganese in plant organellar compartments. Plant Physiology **187**, 1940–1972.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. 2008. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biology **8**, 1–14.

Hope RM. 2013. Rmisc: Rmisc: Ryan Miscellaneous.

Huang XY, Salt DEE. 2016. Plant Ionomics: From Elemental Profiling to Environmental Adaptation. Molecular Plant **9**, 787–797.

Hui C, Schmollinger S, Strenkert D, Holbrook K, Montgomery HR, Chen S, Nelson HM, Weber PK, Merchant SS. 2022. Simple steps to enable reproducibility: culture conditions affecting Chlamydomonas growth and elemental composition. The Plant Journal **111**, 995-1014.

Ibuot A, Dean AP, McIntosh OA, Pittman JK. 2017. Metal bioremediation by CrMTP4 over-expressing *Chlamydomonas reinhardtii* in comparison to natural wastewater-tolerant microalgae strains. Algal Research **24**, 89–96.

Ibuot A, Dean AP, Pittman JK. 2020. Multi-genomic analysis of the cation diffusion facilitator transporters from algae. Metallomics **12**, 617–630.

Johnson X, Vandystadt G, Bujaldon S, Wollman FA, Dubois R, Roussel P, Alric J, Béal D. 2009. A new setup for in vivo fluorescence imaging of photosynthetic activity. Photosynthesis research **102**, 85–93.

Kalaji HM, Schansker G, Ladle RJ, *et al.* 2014. Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues. Photosynthesis Research **122**, 121–158.

Kassambara A. 2020. ggpubr: 'ggplot2' Based Publication Ready Plots. https://cran.r-project.org/package=ggpubr

Kitayama K, Kitayama M, Osafune T, Togasaki RK. 1999. Subcellular localization of iron and manganese superoxide dismutase in *Chlamydomonas reinhardtii* (Chlorophyceae). Journal of Phycology **35**, 136–142.

Kochoni E, Aharchaou I, Ohlund L, Rosabal M, Sleno L, Fortin C. 2022. New insights in copper handling strategies in the green alga *Chlamydomonas* reinhardtii under low-iron condition. Metallomics **14**, 33.

Krämer U. 2010. Metal Hyperaccumulation in Plants. Annual Review of Plant Biology **61**, 517–534.

Kropat J, Gallaher SD, Urzica EI, Nakamoto SS, Strenkert D, Tottey S, Mason AZ, Merchant SS. 2015. Copper economy in Chlamydomonas: Prioritized allocation and eallocation of copper to respiration vs. Photosynthesis. Proceedings of the National Academy of Sciences, USA **112**, 2644–2651.

Kropat J, Hong-Hermesdorf A, Casero D, Ent P, Castruita M, Pellegrini M, Merchant SS, Malasarn D. 2011. A revised mineral nutrient supplement increases biomass and growth rate in *Chlamydomonas reinhardtii*. Plant Journal **66**, 770–780.

Lahner B, Gong J, Mahmoudian M, *et al.* 2003. Genomic scale profiling of nutrient and trace elements in *Arabidopsis thaliana*. Nature Biotechnology **21**, 1215-1221.

Laurinavichene T V., Tolstygina I V., Galiulina RR, Ghirardi ML, Seibert M, Tsygankov AA. 2002. Dilution methods to deprive *Chlamydomonas reinhardtii* cultures of sulfur for subsequent hydrogen photoproduction. International Journal of Hydrogen Energy **27**, 1245–1249.

Livak KJ, Schmittgen TD. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods **25**, 402–408.

Loppes R, Radoux M. 2001. Identification of short promoter regions involved in the transcriptional expression of the nitrate reductase gene in *Chlamydomonas reinhardtii*. Plant Molecular Biology **45**, 215–227.

Ma F, Salomé PA, Merchant SS, Pellegrini M. 2021. Single-cell RNA sequencing of batch Chlamydomonas cultures reveals heterogeneity in their diurnal cycle phase. The Plant Cell **33**, 1042–1057.

Malasarn D, Kropat J, Hsieh SI, Finazzi G, Casero D, Loo JA, Pellegrini M, Wollman FA, Merchant SS. 2013. Zinc deficiency impacts CO_2 Assimilation and disrupts copper homeostasis in *Chlamydomonas reinhardtii*. Journal of Biological Chemistry **288**, 10672–10683.

Mangiafico S. 2021. rcompanion: Functions to Support Extension Education Program Evaluation. https://cran.r-project.org/package=rcompanion

Marschner H (Ed.). 2011. *Marschner's Mineral Nutrition of Higher Plants*. Academic Press.

Merchant SS, Allen MD, Kropat J, Moseley JL, Long JC, Tottey S, Terauchi AM. 2006. Between a rock and a hard place: Trace element nutrition in Chlamydomonas. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research **1763**, 578–594.

Merchant SS, Helmann JD. 2012. Elemental Economy: microbial strategies for optimizing growth in the face of nutrient limitation. Advances in Microbial Physiology **60**, 91–210.

Merchant S, Sawaya MR. 2005. The Light Reactions: A Guide to Recent Acquisitions for the Picture Gallery. The Plant Cell **17**, 648–663.

Miramar DM, Inda LA, Saraiva LM, Peleato ML. 2003. Plastocyanin/cytochrome c 6 interchange in *Scenedesmus vacuolatus*. Journal of Plant Physiology **160**, 1483–1486.

Moroney J V., Ynalvez RA. 2007. Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*. Eukaryotic Cell **6**, 1251–1259.

Moseley JL, Allinger T, Herzog S, Hoerth P, Wehinger E, Merchant S, Hippler M. 2002. Adaptation to Fe-deficiency requires remodeling of the photosynthetic apparatus. The EMBO Journal **21**, 6709–20.

Moseley JL, Chang C-W, Grossman AR. 2006. Genome-Based Approaches to Understanding Phosphorus Deprivation Responses and PSR1 Control in *Chlamydomonas reinhardtii*. Eukaryotic Cell **5**, 26–44.

Murchie EH, Lawson T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. Journal of Experimental Botany **64**, 3983–3998.

Naumann B, Busch A, Allmer J, Ostendorf E, Zeller M, Kirchhoff H, Hippler M. 2007. Comparative quantitative proteomics to investigate the remodeling of bioenergetic pathways under iron deficiency in *Chlamydomonas reinhardtii*. Proteomics **7**, 3964–3979.

Newman SM, Boynton JE, Gillham NW, Randolph-Anderson BL, Johnson AM, Harris EH. 1990. Transformation of Chloroplast Ribosomal RNA Genes in Chlamydomonas: Molecular and Genetic Characterization of Integration Events. Genetics **126**, 875-888.

Nouet C, Charlier JB, Carnol M, Bosman B, Farnir F, Motte P, Hanikenne M. 2015. Functional analysis of the three HMA4 copies of the metal hyperaccumulator *Arabidopsis halleri*. Journal of Experimental Botany **66**, 5783–5795.

Ogle DH, Doll JC, Wheeler P, Dinno A. 2021. FSA: Fisheries Stock Analysis. https://github.com/droglenc/FSA

Page MD, Allen MD, Kropat J, Urzica EI, Karpowicz SJ, Hsieh SI, Loo JA, Merchant SS. 2012. Fe Sparing and Fe Recycling Contribute to Increased Superoxide Dismutase Capacity in Iron-Starved *Chlamydomonas reinhardtii*. The Plant Cell **24**, 2649–2665.

Page MD, Kropat J, Hamel PP, Merchant SS. 2009. Two Chlamydomonas CTR copper transporters with a novel cys-met motif are localized to the plasma membrane and function in copper assimilation. The Plant Cell **21**, 928–943.

Pita-Barbosa A, Ricachenevsky FK, Flis PM. 2019. One "OMICS" to integrate them all: ionomics as a result of plant genetics, physiology and evolution. Theoretical and Experimental Plant Physiology **31**, 71–89.

Pittman JK, Edmond C, Sunderland PA, Bray CM. 2009. A Cation-regulated and Proton Gradient-dependent Cation Transporter from *Chlamydomonas reinhardtii* Has a Role in Calcium and Sodium Homeostasis. Journal of Biological Chemistry **284**, 525–533.

Plouviez M, Oliveira da Rocha CS, Guieysse B. 2022. Intracellular polyphosphate is a P reserve in *Chlamydomonas reinhardtii*. Algal Research **66**, 102779.

R Core Team. 2022. R: A Language and Environment for Statistical Computing. https://www.r-project.org/

Ricachenevsky FK, de Araújo Junior AT, Fett JP, Sperotto RA. 2018. You shall not pass: Root vacuoles as a symplastic checkpoint for metal translocation to shoots and possible application to grain nutritional quality. Frontiers in Plant Science **9**, 412.

Römheld V. 2011. Diagnosis of Deficiency and Toxicity of Nutrients. Marschner's Mineral Nutrition of Higher Plants: Third Edition. Elsevier Inc., 299–312.

Rosakis A, Köster W. 2005. Divalent metal transport in the green microalga Chlamydomonas reinhardtii is mediated by a protein similar to prokaryotic Nramp homologues. Biometals **18**, 107–120.

RStudio Team. 2021. RStudio: Integrated Development Environment for R.

Rupprecht J. 2009. From systems biology to fuel-*Chlamydomonas reinhardtii* as a model for a systems biology approach to improve biohydrogen production. Journal of Biotechnology **142**, 10–20.

Saito MA, Bertrand EM, Dutkiewicz S, Bulygin V V., Moran DM, Monteiro FM, Follows MJ, Valois FW, Waterbury JB. 2011. Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocosphaera watsonii*. Proceedings of the National Academy of Sciences, USA **108**, 2184–2189.

Salomé PA, Merchant SS. 2019. A Series of Fortunate Events: Introducing Chlamydomonas as a Reference Organism. The Plant Cell **31**, 1682–1707.

Salt DE, Baxter I, Lahner B. 2008. Ionomics and the study of the plant ionome. Annual Review of Plant Biology **59**, 709–733.

Saroussi S, Sanz-Luque E, Kim RG, Grossman AR. 2017. Nutrient scavenging and energy management: acclimation responses in nitrogen and sulfur deprived Chlamydomonas. Current Opinion in Plant Biology **39**, 114–122.

Sasso S, Stibor H, Mittag M, Grossman AR. 2018. The natural history of model organisms: From molecular manipulation of domesticated *Chlamydomonas reinhardtii* to survival in nature. eLife **7**, e39233.

Schvartzman MS, Corso M, Fataftah N, Scheepers M, Ecile Nouet C, Bosman B, Carnol M, Motte P, Verbruggen N, Hanikenne M. 2018. Adaptation to high zinc depends on distinct mechanisms in metallicolous populations of *Arabidopsis halleri*. New Phytologist **218**, 269–282.

Shen J-R. 2015. The Structure of Photosystem II and the Mechanism of Water Oxidation in Photosynthesis PSII: photosystem II. Annu. Rev. Plant Biol **66**, 23–48.

Shih SM, Engel BD, Kocabas F, Bilyard T, Gennerich A, Marshall WF, Yildiz A. 2013. Intraflagellar transport drives flagellar surface motility. eLife **2**, e00744.

Smith GM. 1946. The Nature of Sexuality in Chlamydomonas. American Journal of Botany **33**, 625–630.

Smith GM, Regnery DC. 1950. Inheritance of Sexuality in *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences, USA **36**, 246–248.

Socha AL, Guerinot M Lou. 2014. Mn-euvering manganese: The role of transporter gene family members in manganese uptake and mobilization in plants. Frontiers in Plant Science **5**, 106.

Teichler-Zallen D. 1969. The Effect of Manganese on Chloroplast Structure and Photosynthetic Ability of *Chlamydomonas reinhardtii*. Plant Physiology **44**, 701–710.

Terauchi AM, Peers G, Kobayashi MC, Niyogi KK, Merchant SS. 2010. Trophic status of *Chlamydomonas reinhardtii* influences the impact of iron deficiency on photosynthesis. Photosynthesis Research **105**, 39–49.

Thiébaut N, Hanikenne M. 2022. Zinc deficiency responses: bridging the gap between Arabidopsis and dicotyledonous crops. Journal of Experimental Botany **73**, 1699–1716.

Thiriet-Rupert S, Gain G, Jadoul A, Vigneron A, Bosman B, Carnol M, Motte P, Cardol P, Nouet C, Hanikenne M. 2021. Long-term acclimation to cadmium exposure reveals extensive phenotypic plasticity in Chlamydomonas. Plant Physiology **187**, 1653–1678.

Tsednee M, Castruita M, Salomé PA, *et al.* 2019. Manganese co-localizes with calcium and phosphorus in Chlamydomonas acidocalcisomes and is mobilized in manganese-deficient conditions. Journal of Biological Chemistry **294**, 17626–17641.

Urzica EI, Casero D, Yamasaki H, *et al.* 2012. Systems and Trans-system level analysis identifies conserved iron deficiency responses in the plant lineage. The Plant Cell **24**, 3921–3948.

Urzica EI, Vieler A, Hong-Hermesdorf A, Page MD, Casero D, Gallaher SD, Kropat J, Pellegrini M, Benning C, Merchant SS. 2013. Remodeling of membrane lipids in iron-starved Chlamydomonas. Journal of Biological Chemistry **288**, 30246–58.

VanWallendael A, Lowry DB, Hamilton JA. 2022. One hundred years into the study of ecotypes, new advances are being made through large-scale field experiments in perennial plant systems. Current Opinion in Plant Biology **66**, 102152.

Vert G, Briat JF, Curie C. 2001. Arabidopsis IRT2 gene encodes a root-periphery iron transporter. The Plant Journal **26**, 181–189.

Wakao S, Shih PM, Guan K, *et al.* 2021. Discovery of photosynthesis genes through whole-genome sequencing of acetate-requiring mutants of *Chlamydomonas reinhardtii*. PLOS Genetics **17**, e1009725.

Weigel D. 2012. Natural Variation in Arabidopsis: From Molecular Genetics to Ecological Genomics. Plant Physiology **158**, 2–22.

Wellburn AR. 1994. The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. Journal of Plant Physiology **144**, 307–313.

Wickham H. 2007. Reshaping Data with the {reshape} Package. Journal of Statistical Software **21**, 1–20.

Wickham H, Averick M, Bryan J, *et al.* 2019. Welcome to the {tidyverse}. Journal of Open Source Software **4**, 1686.

Wickham H, Henry L, Pedersen TL, Luciani TJ, Decorde M, Lise V. 2021. svglite: An 'SVG' Graphics Device. https://cran.r-project.org/package=svglite

Yadavalli V, Jolley CC, Malleda C, Thangaraj B, Fromme P, Subramanyam R. 2012. Alteration of Proteins and Pigments Influence the Function of Photosystem I under Iron Deficiency from *Chlamydomonas reinhardtii*. PLOS ONE **7**, e35084.

Yu D, Danku JMC, Baxter I, Kim S, Vatamaniuk OK, Vitek O, Ouzzani M, Salt DE. 2012. High-resolution genome-wide scan of genes, gene-networks and cellular systems impacting the yeast ionome. BMC Genomics **13**.

Figure legends

Figure 1. Growth variation among 24 Chlamydomonas strains grown in mixotrophy and autotrophy. **(A-B)** Growth, measured as optical density at 750 nm (OD_{750nm}) from day 3 to day 7 of culture, in TAP **(A)** and TMP **(B)**. Each coloured dot represents the mean ± SD for each strain and the colouring follows the same order as the colour key (bottom). The laboratory strains (L) are coloured in a yellow-dark red scale and the natural strains (N) are coloured in green-violet scale. **(A-B)** The boxes represent the 1st quartile, median and 3rd quartile of the data for all strains, and the whiskers extend from the median ± 1.5 interquartile range, whereas outliers are represented by black dots. Values are from 2 independent experiments, with 2 independent cultures each.

Figure 2. Ionome variation of 24 Chlamydomonas strains in mixotrophy and autotrophy. (**A**) Ionome profiling (ICP-AES) at day 4 of culture in TAP. Elements are ordered from left to right by growing median concentrations in cells (µmol. OD^{-1}). **(B)** Ionome profiling at day 4 of culture in TMP. The values are relative to TAP (100%, noted with a dashed line). **(A-B)** The boxes represent the 1st quartile, median and 3rd quartile of the data for all strains, and the whiskers extend from the median ± 1.5 interquartile range, whereas outliers are represented by black dots. Values are from 2 independent experiments, with 2 independent cultures each.

Figure 3. Variation of the impact of nutrient deficiencies on growth of 24 Chlamydomonas strains. **(A)** Growth, measured as optical density at 750 nm (OD_{750nm}), at day 4 of culture on TAP (mixotrophy, control), TMP and TAP with single deficiencies for 5 macroelements (-Ca, -Mg, -N, -P, -S) or 4 microelements (-Cu, -Fe, -Mn, -Zn). The boxes represent the 1st quartile, median and 3rd quartile of the raw data, and the whiskers extend from the median ± 1.5 interquartile range whereas outliers are represented by black dots. Each coloured dot represents the mean ± SD for each strain. Values are from 2 independent experiments, with 2 independent cultures each. The laboratory strains (L) are coloured in a yellow-dark red scale and the natural strains (N) are coloured in green-violet. The strains are ordered according to the colour key. OD_{750nm} average values for each treatment were compared with the values of the TAP control condition using the Wilcoxon test, and significant differences are indicated with *: p <= 0.05, **: p <= 0.01, ***: p <= 0.001 and ****: p <= 0.0001. **(B)** Ranking of the strain growth from the most (1) to the least (24) performing. Rectangles are color-coded as in (**A**).

Figure 4. Variation of the impact of mineral deficiencies on the ionome profile of 24 Chlamydomonas strains. Samples were analysed by ICP-AES at day 4 of culture on TAP (mixotrophy, control) and on TAP with single deficiencies of 5 macroelements (-Ca, -Mg, -N, -P, -S) or 4 microelements (-Cu, -Fe, -Mn, -Zn). The concentrations of the 9 quantified elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn) are represented relatively to their concentrations in TAP (100%, noted with a dashed line). The boxes represent the 1st quartile, median and 3rd quartile of the raw data, and the whiskers extend from the median ± 1.5 interquartile range whereas outliers are represented by black dots. Coloured dots represent the relative average element concentrations per treatment: macronutrient deficiencies (-Ca, -Mg, -N, -N, -P, -S) in blue scale and micronutrient deficiencies (-Cu, -Fe, -Mn, -Zn) in red scale. The entire dataset is presented in the right panel, and a focus on the relative concentrations ranging 0%-300% relative to the control is shown in the left panel. Values are from 2 independent experiments, with 2 independent cultures each.

Figure 5. Clustering of 24 Chlamydomonas strains based on their ionome profiles. The concentrations of 9 elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn) were measured by ICP-AES in samples collected at day 4 of culture in TAP (mixotrophy, control) **(A)** or in TAP with Mn **(B)** or Fe **(C)** deficiencies. Concentration values are provided as mean values from 2 independent experiments, with 2 independent cultures each. **(A)** TAP. The average values per strain were centred on the average values across the 24 strains and scaled around this average, per column (values are provided in Dataset S11). Central values are in white. **(B-C)** Mn and Fe deficiencies, respectively. The average concentrations are relative to the TAP condition. For easier representation, null values were replaced by 10^{-4}. The data was scaled using a $\log_{10}$ transformation and the control value, represented as 2 (100% = 10^2 %), is coloured in white. Dendrograms represent the Euclidean distance clustered by complete linkage. Strain origin [natural (N) or laboratory (L)] is shown between brackets. Boxed strains were used for detailed pairwise comparisons.

Figure 6. Variation of the nutrient marker gene and photosynthetic responses to Mn deficiency between the CC-1373 and CC-2290 Chlamydomonas natural strains. Samples were collected at day 4 of culture in TAP and in TAP -Mn media. **(A)** Relative transcript levels of the *NRAMP1*, *MTP4*, *FRE1*, *FOX1*, *IRT1*, *PTB2*, *MSD3*, *FSD1*, *CTR2*, *ZRT1* and *ZRT3* genes in the strains CC-1373 and CC-2290. Values are means ± SE of 3 technical PCR replicates

performed on samples from 2 to 3 biological replicates for each strain and condition. The expression levels are relative the *CbLp* and *RPL13* housekeeping genes and to the average of the 3 technical replicates of the first biological replicate of strain CC-1373 in TAP medium (1 unit, noted with a dashed line). The Y-axis is transformed using a pseudo-logarithmic scale, starting at 0.001 (unlabelled tick mark). Statistically different (Dunn test, $p<0.05$) expression levels are represented with different letters. **(B)** Maximum quantum yield of photosystem II (Fv/Fm) of dark-adapted samples before (initial, dark grey) and 23 minutes after (recovery, light grey) the saturation curve measurement. **(C)** Relative electron transport (rETR) upon exposure to increasing light intensity (0-600 µmol photons.m^{-2}.s^{-1}). (D) Non-photochemical quenching (NPQ) upon exposure to increasing light intensity (0-600 µmol photons.m^{-2}.s^{-1}) followed by darkness (Dark). (**B-D**) Values are means ± SD (n=3). Non-parametric pairwise multiple comparisons were performed using Dunn's test and the different grouping letter were attributed when statistical differences were found. Small letters in (**B**) refer to the initial Fv/Fm while the capital letters refer to the recovery. Statistics for rETR and NPQ data can be found in **Dataset S9**.

Figure 7. Variation of the nutrient marker gene and photosynthetic responses to Fe deficiency among the CC-2343 and CC-4414 Chlamydomonas natural strains. Samples were collected at day 4 of culture in TAP and in TAP -Fe media. **(A)** Relative transcript levels of the *FRE1*, *FOX1*, *IRT1*, *NRAMP1*, *CTR2*, CYC6, *FSD1*, *MSD3*, *ZRT1* and *ZRT3* genes in strains CC-2343 and CC-4414. Values are means ± SE of 3 technical PCR replicates performed on samples from 2 to 3 biological replicates for each strain and condition. The expression levels are relative the *CbLp* and *RPL13* housekeeping genes and to the average of the 3 technical replicates of the first biological replicate of the strain CC-4414 in TAP medium (1, noted with a dashed line). The Y-axis is scaled using a pseudo-logarithmic scale, starting at 0.001 (unlabelled tick mark). Statistically different (Dunn test, $p<0.05$) expression levels are represented with different letters. **(B)** Maximum quantum yield of photosystem II (Fv/Fm) of dark- (initial, dark grey) and light-adapted cells (recovery, light grey). **(C)** Relative electron transport (rETR) of the strains exposed to an increasing light intensity (0-600 µmol photons.m^{-2}.s^{-1}). (D) Non-photochemical quenching (NPQ) upon exposure to increasing light intensity (0-600 µmol photons.m^{-2}.s^{-1}) followed by darkness (Dark). (**B-D**) Values are means ± SD (n=3). Non-parametric pairwise multiple comparisons were performed using Dunn's test and the different grouping letter were attributed when statistical differences were found. Small letters in (**B**)

refer to the initial Fv/Fm while the capital letters refer to the recovery. Statistics for rETR and NPQ can be found in **Dataset S11**.

Figure 8. Summary scheme of marker gene expression and photosynthesis variations among selected pairs of natural strains. (**A**-**B**) Strains CC-1373 and CC-2290 in control condition (**A**) and under -Mn (**B**). (**C**-**D**) Strains CC-4414 and CC-2343 in control condition (**C**) and under -Fe (**D**). The colored shapes indicate different gene markers (metal transporters represented with an arrow), color-coded according to the associated metal (yellow: iron, blue: copper, pink: manganese, grey: phosphate, red: zinc. Hexagons represent the Fv/Fm after dark adaptation (black) and after 23 minutes of saturating light (white). Stars represent the average optical density (OD at 750nm) at day 4 of culture.

Table 1. Panel of 24 Chlamydomonas strains.

Name	Common Name
CC-124[1]	137c
CC-125[1]	137c
CC-410[1]	137c
CC-503[1]	cw92
CC-1009[1]	UTEX 89
CC-1010[1]	UTEX 90
CC-1373[1]	SAG 54.72
CC-1374[1]	SAG 77.81]
CC-1418[1]	SAG 18.79
CC-1690[1]	21 gr
CC-1952[1]	S1 C5
CC-2290[1]	S1 D2
CC-2342[1]	Jarvik #6
CC-2343[1]	Jarvik #224
CC-2344[1]	Jarvik #356
CC-2931[1]	-
CC-2935[1]	-
CC-2936[1]	-
CC-2937[1]	-
CC-2938[1]	-
CC-3348[1]	SAG 73.72, C8
CC-4414[1]	DN2
CC-5325[1]	cw15 (Jonikas CMJ030-JR397)
SAG 11-31[2]	-

The strains were obtained from the Chlamydomonas Resource Center (http://www.chlamycollection.org)[1] or the Göttingen University Culture Collection of Algae (https://sagdb.uni-goettingen.de)[2] in 2017. The 10 laboratory strains are underlined. CC: Chlamydomonas Center; SAG: Sammlung von Algenkulturen der Universität Göttingen.

Table 2. Composition of the single element deficiency media.

Nutrients	Conditions	TAP Control (µM)	Deficiency (µM)	Ratios
Macronutrients				
Acetate	TMP	17,416	0	0
Calcium	-Ca	387.6	0	0
Magnesium	-Mg	405.7	5.4	$^1/_{75}$
Nitrogen	-N	7,478	747.9	$^1/_{10}$
Sulphur	-S	506.5	56.6	$^1/_9$
Phosphate	-P	1,000	100	$^1/_{10}$
Micronutrients				
Iron	-Fe	17.95	1.8	$^1/_{10}$
Copper	-Cu	6.29	0.006	$^1/_{1000}$
Manganese	-Mn	25.6	0	0
Zinc	-Zn	76.5	0	0