

# Evidence of concurrent local adaptation and high phenotypic plasticity in a polar microeukaryote

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## Summary

Here we investigated whether there is evidence of local adaptation in strains of an ancestrally marine dinoflagellate to the lacustrine environment they now inhabit (optimal genotypes) and/or if they have evolved phenotypic plasticity (a range of phenotypes). Eleven strains of *Polarella glacialis* were isolated and cultured from three different environments: the polar seas, a hyposaline and a hypersaline Antarctic lake. Local adaptation was tested by comparing growth rates of lacustrine and marine strains at their own and reciprocal site conditions. To determine phenotypic plasticity, we measured the reaction norm for salinity. We found evidence of both, limited local adaptation and higher phenotypic plasticity in lacustrine strains when compared with marine ancestors. At extreme high salinities, local lake strains outperformed other strains, and at extreme low salinities, strains from the hyposaline lake outperformed all other strains. The data suggest that lake populations may have evolved higher phenotypic plasticity in the lake habitats compared with the sea, presumably due to the high temporal variability in salinity in the lacustrine systems. Moreover, the interval of salinity tolerance differed between strains from the hyposaline and hypersaline lakes, indicating local adaptation promoted by different salinity.

## Introduction

Evolutionary processes fine-tune species to their environments (Pelletier *et al.*, 2009). In particular, the process of local adaptation is of fundamental importance and typically involves the generation of an optimal phenotype, in the absence of other forces or constraints (Kawecki and Ebert, 2004). As a result, resident genotypes (individuals) would on average have a higher relative fitness in their local habitats than individuals from foreign habitats (Pigliucci, 2001; DeWitt and Scheiner, 2004). This scenario typically occurs in homogeneous environments. However, in environmentally heterogeneous habitats, adaptive phenotypic plasticity may evolve instead. This means that multiple phenotypes can be produced from a single genotype when exposed to different conditions (Bradshaw, 1965). Provided that there are no costs of phenotypic plasticity, phenotypic rather than genetic differentiation would evolve in heterogeneous environments (Kawecki and Ebert, 2004). However, in many instances, there are substantial costs to plasticity, and if there is enough genetic variation, plasticity will not evolve (DeWitt *et al.*, 1998).

Local adaptation and phenotypic plasticity have mostly been studied in multicellular organisms. However, to date, it is unknown how important both processes are in unicellular eukaryotes (protists). Recently, a handful of studies have explored this topic and provide evidence of high phenotypic plasticity (Finlay *et al.*, 2006; Krenek *et al.*, 2012) as well as of local adaptation (Boenigk *et al.*, 2006; 2007; Weisse *et al.*, 2011). Moreover, in marine systems, genetic and ecological differentiation (rather than plasticity) have been shown for both cyanobacteria (Kashtan *et al.*, 2014) and bacteria (Shapiro *et al.*, 2012). These studies reveal subpopulations within the same (marine) environment that appear to have adapted to different environmental conditions through natural selection.

Protists have some characteristics that set them apart from multicellular organisms. To start with, there are contradictions with regards to the extent of their gene flow. Restricted gene flow is considered a prerequisite for local adaptation, and when gene flow is high, populations cannot differentiate. So far, there is evidence suggesting both high and low gene flow in different protist taxa (Foissner, 1999; Fenchel and Finlay, 2004; Ryneerson

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and Armbrust, 2004; Godhe and Härnström, 2010; Rengefors *et al.*, 2012; Rodriguez-Martinez *et al.*, 2012). Second, genetic theory predicts that the potential to adapt decreases linearly with decreasing effective population size (Hill, 1982). This suggests that free-living aquatic protists, which often have population sizes in the range of  $10^4$ – $10^6$  individuals  $L^{-1}$  should have a high potential for local adaptive evolution similar to plants with large populations (Leimu and Fischer, 2008). The large genetic diversity stored in these huge microbial populations could prevent the emergence of phenotypic plasticity. The presence of hundreds of ecologically differentiated subpopulations in the marine cyanobacterium *Prochlorococcus*, which also has a huge effective population size (Kashtan *et al.*, 2014), supports this scenario. Nevertheless, the habitats of free-living protists may favour phenotypic plasticity. Free-living protists are found in all types of habitats, and some of those, such as lakes, have high environmental variability (both seasonally and spatially). This high environmental heterogeneity may favour adaptive phenotypic plasticity, and thus counteract local adaptation. In summary, the interplay of external forces (e.g. environmental variability) as well as organismal and population characteristics (e.g. genomic variation, gene flow) should define whether protists tune to their environments by local adaptation or phenotypic plasticity.

The goal of our study was to determine whether a planktonic protist (dinoflagellate) species has evolved high phenotypic plasticity and/or local adaptation as a response to a historical environmental transition from a more homogeneous to a range of heterogeneous habitats with different selective regimes. The species investigated here, *Polarella glacialis*, is a planktonic phototrophic species with a bipolar distribution (Montresor *et al.*, 2003) which often dominates the planktonic community in the polar seas (Stoecker *et al.*, 2000), sea ice (Thomson *et al.*, 2006) and in some Antarctic saline lakes (Vestfold Hills) (Rengefors *et al.*, 2008). The species is presumably haploid as other dinoflagellates and propagates by asexual cell division in the water column. It forms cysts (Montresor *et al.*, 1999), likely formed following sexual reproduction (Pfiester and Anderson, 1987), which serve as resting stages during the winter (Rengefors *et al.*, 2008) and presumably also serve as dispersal propagules. Although ribosomal SSU (18S) sequences are identical for Arctic and Antarctic strains (Rengefors *et al.*, 2008; Logares *et al.*, 2009a), suggesting high dispersal ability, this gene is inadequate for analysing whether there is currently ongoing long distance dispersal. Fine-scale population genetic markers instead suggest that the populations of *P. glacialis* in the Vestfold Hills are differentiated (Logares *et al.*, 2009a) from marine populations.

The Antarctic lakes inhabited by *P. glacialis* differ from each other and the ocean environment. In the marine environment, salinity is usually stable around 35 parts per thousand (ppt); however, in the sea ice, the ice pockets may vary considerably in salinity. The lakes in which *P. glacialis* are found differ greatly in salinity, from half of seawater (SW) salinity up to twice SW salinity. Salinity is one of the major abiotic factors that affect the distribution of populations and species (Lee and Bell, 1999). The transition between fresh and marine waters is a major obstacle for colonization in both macroorganisms and microorganisms, including dinoflagellates (Logares *et al.*, 2007a; 2009b).

The saline lakes of the Vestfold Hills represent a natural experiment for exploring microbial evolutionary processes. These were isolated from the sea by isostatic rebound following deglaciation periods and changes in sea level (e.g. Zwartz *et al.*, 1998; Gibson *et al.*, 2009). During the formation process, marine microbes became progressively isolated in the new waterbodies. Today, most saline lakes are closed or endorheic basins with inflow from snow melt and no outflow. Due to variation in the amount of evaporation, meltwater and marine ingress, lakes have developed different salinities. In addition, fluctuations occur within the lakes throughout the year with lower salinity occurring in conjunction with ice melt, especially in surface waters. There are also annual variations related to the level of inflow. Thus, ancestrally marine microbes were exposed progressively to drastically new environmental conditions in the lakes, which should have promoted local adaptation. In particular, *P. glacialis* from the lakes would experience a variable environment both within and between the lakes, potentially selecting for the evolution of phenotypic plasticity in terms of salinity tolerance. *Polarella glacialis* is expected to have 15–20 asexual generations during one growth season, meaning that each generation may match their phenotype against the present environmental conditions.

We tested for evidence of local adaptation in *P. glacialis* by comparing the performance (growth rate) of 11 strains at their own and reciprocal site conditions (with regards to salinity) in a common garden experiment. Growth rate is often the only fitness-related trait that can be easily measured in microorganisms (Belotte *et al.*, 2003). We hypothesized that strains from a hypersaline lake would grow best at high salinities, strains from a hyposaline lake at salinities below SW, while marine strains would grow best at SW salinities. Phenotypic plasticity was examined by testing the reaction norm of growth rate to salinity for each strain. A reaction norm is defined as the pattern of phenotypic expression of a single genotype across a range of environments, in this case salinity. We expected a wider salinity reaction norm in lake strains than in marine strains, which would be adapted to a more stable salinity environment.

## Results

### All strains were genetically different

Sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA confirmed that all 11 strains (all clonal but one, PGRS-6) in the experiment belonged to *P. glacialis* (see Table 1 for origin). This confirms previous results that show identical 18S and almost identical ITS sequences (Rengefors *et al.*, 2008; Logares *et al.*, 2009a) of these strains. The ITS sequences from all strains differed only at one position out of the 543 base pairs that were aligned (see Table 1 for GenBank accession numbers, alignment available at dryad.org following acceptance). Due to only one single ambiguous/variable site, no phylogenetic tree was constructed for these sequences. All lake strains had a C in position 62, while the marine strains either had a G or were ambiguous (G/C) (CCMP strains; alignment available on dryad.org following acceptance). Amplified fragment length polymorphism (AFLP) genotyping, a proxy of whole genome variation, using 409 scored loci confirmed that all strains in the experiment differed genetically (data available at dryad.org). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Jaccard distance tree illustrates that genotypes differ, but does not indicate that strains cluster by habitat (Fig. 1).

### High phenotypic plasticity and significant local adaptation

*Polarella glacialis* strains showed growth over a wide salinity range, ranging from 4 to 70 ppt (Table 2). Unfortunately, some of the growth rate data could not be transformed to conform to homogeneous variances. However, the differences in variances were small (less than two times) and thus we proceeded with a two-way analysis of variance (ANOVA) but with a more conservative *P*-value ( $P < 0.01$ ). The analyses yielded significant differences among the three groups of strains (hyposaline Lake Abraxas,

hypersaline Ekho Lake, polar sea) and between different salinity levels ( $P < 0.001$ , ANOVA). Phenotypic plasticity differed among strains from the three habitats. Strains from low salinity Lake Abraxas had the widest plasticity to salinity with growth from 4 to 70 ppt, while strains from high salinity Ekho Lake grew from 18 to 70 ppt (note though that treatment with salinity 61 failed) (Table 2, Fig. 2). Strains from the marine habitat had different ranges of salinity tolerance, but were overall less plastic and none were able to grow at salinities above 53 ppt (Table 2, Fig. 2). The strain-specific reaction norms show that the optimum growth rates under these experimental conditions varied among the strains, but were for the most part highest between 35 and 53 ppt (Table 2, Fig. 2).

There was a pattern of higher fitness of local versus foreign strains in extreme salinities (Fig. 3B), but not in the more moderate salinities (Fig. 3A). At low salinity (18 ppt), lake strains performed better than marine strains, although there was a significant difference only between Ekho and marine strains ( $P < 0.05$ , one-way ANOVA, Tukey test). At high salinity level (53 ppt), the low salinity strains from Lake Abraxas performed significantly better than both marine foreign strains and local high salinity strains (Fig. 3A,  $P < 0.01$ , one-way ANOVA, Tukey test). At marine salinity, however, there was no difference between local and foreign strains. The test of local adaptation at the extreme salinities showed clear evidence of local adaptation (Fig. 3B). At the low salinity level (4 ppt), the local strains (Abraxas) performed much better than both sets of foreign strains, which did not grow at all. At high salinity (70 ppt), both sets of lake strains had higher growth rates than the marine strains, which did not grow ( $P < 0.05$ , one-way ANOVA), and differed significantly from each other, with the local strains (Ekho Lake) outperforming foreign lake strains.

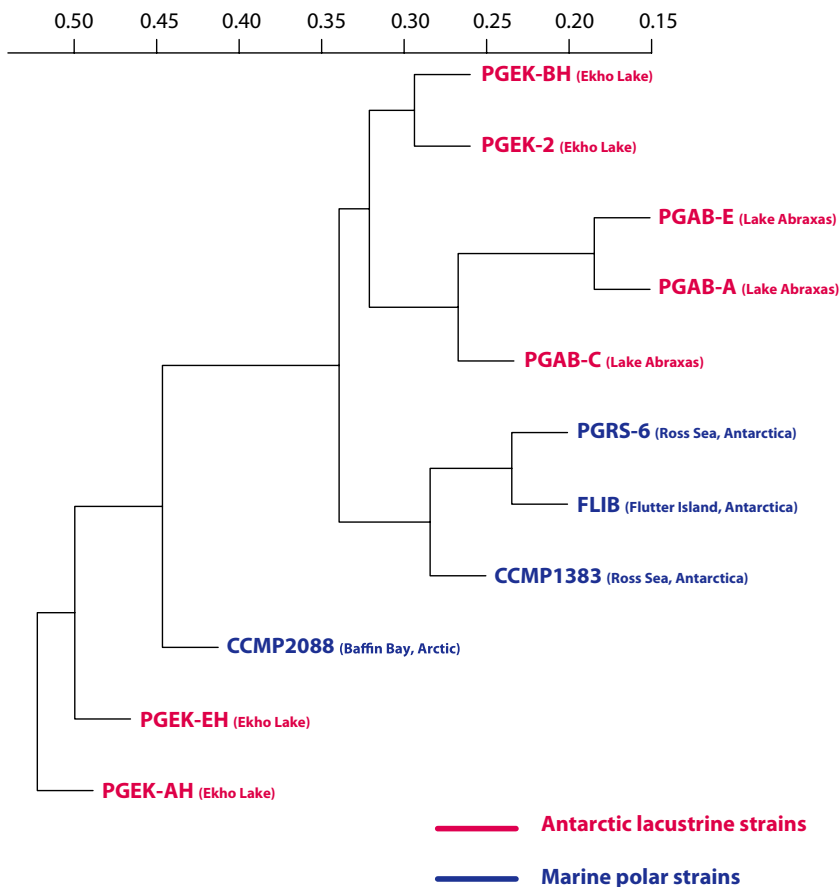
## Discussion

Microorganisms are extremely numerous and diverse, yet they are largely understudied by evolutionary biologists

**Table 1.** Strain denomination, collection site and year, and GenBank accession numbers for partial rRNA sequences.

Strains	Collection site	Coordinates	Salinity	Elevation (m. a.s.l.)	Year	SSU	ITS
PGAB-A	Lake Abraxas	68°29'20"S, 78°17'30"E	18	13	2005		EU445323
PGAB-C	Lake Abraxas	68°29'20"S, 78°17'30"E	18	13	2005		EU445324
PGAB-E	Lake Abraxas	68°29'20"S, 78°17'30"E	18	13	2005	EF434276	EU445325
PGEK-AH	Ekho Lake	68°31'16"S, 78°16'12"E	40	-2	2005		EU445326
PGEK-BH	Ekho Lake	68°31'16"S, 78°16'12"E	40	-2	2005	EF434277	EU445327
PGEK-EH	Ekho Lake	68°31'16"S, 78°16'12"E	40	-2	2005		EU445328
PGEK-2	Ekho Lake	68°31'16"S, 78°16'12"E	40	-2	2005		KC852878*
CCMP 2088	Baffin Bay, Arctic	74°29'N, 78°35'W	35		1998	EF434275	EU445332
CCMP 1383	Ross Sea	77°50'S, 166°30'E	35		1991	EF417317	EU445333
PGRS-6	Ross Sea	77°50'S, 166°30'E	35		1997		KC852879*
FLIB	Flutter Island, Davis	68°33'S, 77°50'E	35		1997		KC852880*

The asterisk indicates new sequences from this study.



**Fig. 1.** Jaccard distance tree based on AFLP analyses (409 loci) showing that strain genotypes differ, and that three of the marine strains, and five of the lacustrine strains cluster, but that there is no consistent clustering with habitat origin.

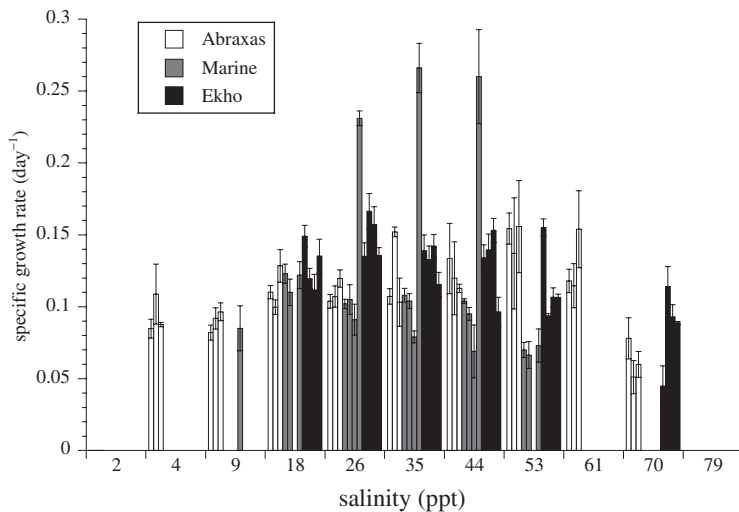
compared with multicellular organisms (Gerstein and Moore, 2011). Because they differ fundamentally from larger organisms when it comes to body size, population size and reproduction (mainly asexual), knowledge is limited regarding the evolution of local adaptation and phenotypic plasticity in microbial populations. We investigated the aquatic protist *P. glacialis* (dinoflagellate) that

has a bipolar distribution and is found in habitats with a wide salinity range. We found evidence of higher phenotypic plasticity in strains isolated from saline lakes in contrast to a more moderate plasticity in the marine environment. We also found evidence of local adaptation, as different ranges of plasticity corresponded with habitats of different salinity.

**Table 2.** Summary of salinity reaction norms experiment for all strains.

Strains	Starting (native) salinity (ppt)	Growth rate native salinity ( $\text{day}^{-1}$ )	Max growth rate ( $\text{day}^{-1}$ )	Growth range (salinity, ppt)
PGAB-A	18	0.11	0.15	4–70
PGAB-C	18	0.10	0.14	4–70
PGAB-E	18	0.13	0.15	4–70
PGEK-AH	35	0.13	0.17	18–70
PGEK-BH	35	0.14	0.16	18–70
PGEK-EH	35	0.11	0.14	18–70
PGEK-2	35	0.14	0.15	18–70
CCMP 2088	35	0.10	0.11	18–53
CCMP 1383	35	0.11	0.12	18–53
PGRS-6	35	0.27	0.27	4–53
FLIB	35	0.08	0.09	26–44

Specific growth rates (division per day) of strains at native salinity conditions, maximum growth rate and range of salinity tolerance [in ppt (parts per thousand)].

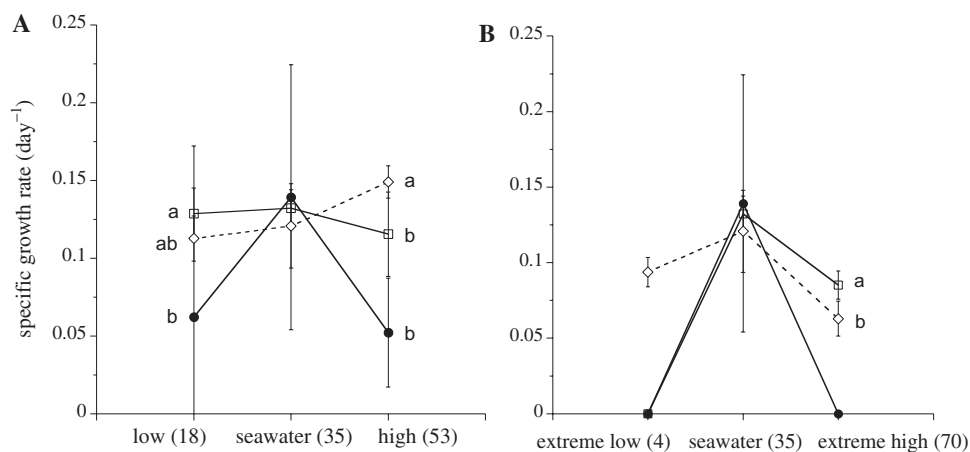


**Fig. 2.** Phenotypic plasticity (measured as specific growth rate) in response to salinity (in ppt) of strains from different habitats. Strains from hyposaline Lake Abraxas shown by white columns, strains from the marine environment by grey bars and strains from hypersaline Lake Ekho with black bars. Note the wide salinity ranges of lake strains in contrast to marine strains, and that strains from the hyposaline lake grow at lower salinities than strains from marine and hypersaline populations. Bars show means for three replicates per strain. Error bars indicate standard deviation. Both treatment (salinity) and population show significant differences (ANOVA,  $P < 0.01$ ).

### High phenotypic plasticity

*Polarella glacialis* showed growth over an exceptionally wide salinity range from slightly brackish (salinity of 4 ppt) to twice the salinity of SW (salinity of 70 ppt). Although many dinoflagellates are able to grow at a range of salinities, *P. glacialis* is extreme. For example, several brackish Baltic Sea species (Kremp *et al.*, 2005; Logares *et al.*, 2007b) can grow at salinities down to 0–3 ppt and up to 30 ppt, and most marine species have a range between 10 ppt (Lim and Ogata, 2005) and 45–50 ppt (e.g. Sullivan and Andersen, 2001; Magana and Villareal, 2006; Philips *et al.*, 2006). The heterotrophic flagellate *Oxyrrhis marina*, which is phylogenetically basal to the dinoflagellates, has a slightly wider range. This species

grew best at 25–30 ppt but some strains showed growth at 55 ppt (Lowe *et al.*, 2005). Because our reaction norm tests were standardized to a limited time period (approximately 3–4 weeks, see *Experimental procedure*) to be able to compare strains, it is possible that some strains could have even wider salinity tolerances if they were left to acclimate for longer. Moreover, since the test was aimed at comparing strains, we made no adjustments for differences in ion balance, pH or alkalinity to mimic the natural conditions. Thus, it is possible that higher growth rates and higher tolerance in salinity could be found in nature. Also, during periods of clear ice, light levels can be much higher than the light intensity we used, which also likely would affect maximum growth rates. Nevertheless, all growth rates were measured when cells were in



**Fig. 3.** Test of local adaptation of strains from the hyposaline lake Abraxas, the hypersaline lake Ekho Lake and marine strains in 'foreign' and 'local' environments represented as (A) low salinity equivalent to Lake Abraxas (18 ppt), seawater salinity (35 ppt) and high salinity equivalent to Ekho Lake (53 ppt) and (B) extreme low salinity in Abraxas (4 ppt), seawater salinity (35 ppt) and extreme high salinity in Ekho Lake (70 ppt). Fitness is measured as mean specific growth rate ( $n = 3$ ), error bars show standard deviation of four strains per lake (open diamonds for Lake Abraxas, open squares for Ekho Lake) and three strains for the marine habitat (filled circles). Small letters (a and b) indicate statistical difference (one-way ANOVA, Tukey test,  $P < 0.05$ ) between strains.

exponential phase, much below maximum cell concentrations which can reach 300 000 cells ml<sup>-1</sup> during stationary phase.

The salinity reaction norm exhibited by *P. glacialis* differed among strains isolated from the three habitats ( $P < 0.001$ , ANOVA). The marine strains on average had the most narrow salinity tolerance and did not grow at salinities above 53 or below 18 ppt. The lake strains had much wider ranges, especially those from the Lake Abraxas population. These salinity ranges corresponded well with the habitats that the strains originated from, in that strains from the hypersaline lakes were able to grow at the highest salinities, and the strains from the hyposaline lake were able to grow at the lowest salinities.

The marine strains, which most likely represent the ancestral population, are for the most part exposed to SW, which varies little around 35 ppt. However, *P. glacialis* is common in sea ice communities, for example in Prydz Bay off the Vestfold Hills (Gibson, 1999), where it experiences a very wide range of salinity and is thus pre-adapted to short-term salinity variation. The lake strains, in contrast, have been exposed to temporal differences in salinity, both annually and over geological time. Lake Abraxas has a mixolimnion salinity that ranges between 16 and 18 ppt (Perriss and Laybourn-Parry, 1997), but during ice melt, the upper surface waters where dinoflagellates accumulate may become as low as 5 ppt. Ekho Lake, in contrast, has a mixolimnion that ranges from 50 ppt near the surface increasing to 180 ppt close to the chemocline (Gibson, 1999), but the water at the immediate surface may drop to 5 ppt during ice melt.

During their formation, the lakes have undergone considerable changes in salinity. The history of the lakes is complex, some may have pre-dated the Last Glacial Maximum (LGM) 18 000 to 20 000 years before present (Gibson *et al.*, 2009) (e.g. Lake Abraxas) while others, such as Ekho Lake, were formed after the LGM by isolation from the sea through isostatic rebound following deglaciation periods and changes in sea level (e.g. Zwartz *et al.*, 1998; Gibson *et al.*, 2009). Due to variation in the amount of evaporation, meltwater and periodic marine ingress, lakes have developed different salinities. The sedimentary record suggests that during the LGM, there was little productivity within Lake Abraxas due to thick ice cover, but at the end of the LGM, the lake would have been readily colonized by dinoflagellate cysts from the nearby marine environment.

Since the studied lakes have highly variable environments in terms of salinity, this implies so called fine-grained conditions that are expected to give rise to high phenotypic plasticity. According to the grain size model for the evolution of plasticity, organisms are predicted to evolve plasticity in fine-grained conditions (Levins, 1968). Fine-grained conditions are characterized by heterogene-

ous habitats, while coarse-grained environments are those in which the organisms experience a homogeneous environment. Our results support this hypothesis, in that the marine strain of *P. glacialis* had a relatively narrow salinity tolerance, while the lacustrine strains possessed a wide tolerance.

#### *Simultaneous wide phenotypic plasticity and local adaptation*

Given the high phenotypic plasticity in salinity tolerance for *P. glacialis*, it was unclear whether local adaptation would occur in lacustrine strains. However, our results point to specialization to local conditions in terms of the width and horizontal shifts of ranges of salinity tolerance. When the strains are grouped into lake and marine strains, it becomes clear that the lake strains outperform the marine strains in extreme environments (high and low salinity). Furthermore, the data showed wider phenotypic plasticity in the lake strains compared with the marine strains, with a specialization in tolerance to the local salinity environment. However, the strict criteria of 'locals' outperforming 'foreign' strains in the local environment was not satisfied. The likely reason for this is that local adaptation was not achieved by the evolution of single (and different) optimal phenotypes in different lakes. Instead, selection seems to have favoured the evolution of plastic strains with different growth optima in different salinities. Nevertheless, these conclusions must be viewed with caution, as our sample size is limited to two lakes and three marine sites, and with a total of 11 strains. The major problem with small sample sizes is usually that effects are harder to detect, i.e. a risk of false negatives. Here, however, we found significant differences in the groups of strains despite small sample sizes. Screening of more strains would obviously be desirable, but will need future sampling and culturing efforts in the polar regions.

Local adaptation to salinity conditions has been studied in numerous multicellular species including microscopic ones such as rotifers. Alcantara-Rodriguez and colleagues (2012) showed local adaptation in the rotifer belonging to the *Brachionus plicatilis* complex. They found that strains from low salinity lakes were adapted to low salinity but found no differences between strains from higher salinities, as was the case in our study. Salinity is a major selective force affecting biogeographic patterns and genetic divergence, as the differences in ionic conditions imposes different requirements on the cellular machinery. In fact, the freshwater–marine boundary prevents frequent cross colonizations not only in multicellular organisms but also in unicellular organisms including protist (Logares *et al.*, 2007a; 2009b).

There is evidence from other studies of both large intraspecific phenotypical differences and adaptations to

habitats. For example, the chrysophyte *Spumella* sp. showed both genetic clustering and temperature growth optima that corresponded to the temperature regimes of their habitats (Boenigk *et al.*, 2007). Similarly, there was evidence of temperature and pH adaptation in ciliates and a flagellate (Gächter and Weisse, 2006; Weisse *et al.*, 2011), and significant diversity in temperature reaction norms in a freshwater diatom (Gsell *et al.*, 2012). Krenek and colleagues (2012) found a wide thermal tolerance in ciliate *Paramecium caudatum* and some evidence of local adaptation in strains from less variable tropical lakes, but not evidence of local adaptation to thermal gradients in strains from temperate lakes.

Since the *P. glacialis* strains were maintained in the lab during many generations, previous to the start of the experiments (5 years  $\geq$  approximately 300 generations) evolution during this time cannot be ruled out. Changes in clonal cultures are often observed when cultures are maintained for very long time periods, with for instance selection to pH tolerance (Berge *et al.*, 2012). All our cultures (except PGRS-6) were clonal, and consequently any evolution must be the result of mutations or sexual reproduction among identical cells. We cannot rule out sexual events within cultures, as these are common in dinoflagellates, but also did not observe resting cysts which typically form following sexual reproduction. However, all lake cultures were kept at constant salinity during the entire culture time. Marine and Ekho strains were kept at a salinity of 35 and Abraxas strains at about 9 ppt; therefore, evolution in the lab cannot explain the results obtained in the current experiment.

## Conclusions

We have evidence indicating that the dinoflagellate *P. glacialis* has evolved a very high phenotypic plasticity, likely in response to the variability in lake salinity (both historically and on an annual basis), rather than evolving several different optimal phenotypes adapted to different salinities. Yet, the significant differences in the reaction norms in growth rate appeared tuned to the three habitat types, pointing to local adaptation.

## Experimental procedure

### Algal cultures

Cultures of *P. glacialis* used in this study are listed in Table 1. Clonal isolates were established in the Antarctic summer of 2004/2005 from the mixolimnia of meromictic lakes in the Vestfold Hills, Antarctica (68° S, 78° E). Lake Abraxas is a hyposaline lake with the upper mixolimnion waters having a salinity of 14–17 ppt and Ekho Lake is a hypersaline lake with a mixolimnion salinity averaging 50 ppt but ranging up to 180 ppt, increasing with depth towards the chemocline.

Single cells were isolated manually into sterile-filtered lake water spiked with f/2 medium nutrients (Guillard and Rytter, 1962) to establish clonal cultures. The cultures were later transferred to f/2 medium based on sterile-filtered and autoclaved SW from the Øre Sound between Sweden and Denmark. The cultures were maintained at half strength SW salinity (~ 18 ppt) (Abraxas strains PGAB-A, -E, -C) and full strength SW salinity (~ 35 ppt) (Ekho strains PGEK-AH, -BH, -EH). Marine strains were obtained from several sources. Strain PGRS-6 was isolated from the Ross Sea, Antarctica. Although the latter was not clonal, we included it in the experiment, fully aware that it could behave differently from the other strains. Strain FLIB was isolated from fast ice brine (upper 50 cm) at a site near Flutter Island, adjacent to the Vestfold Hills. CCMP strains were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton. Strain CCMP 1383 was originally isolated from the McMurdo Sound, Ross Sea, Antarctica. Strain CCMP 2088 was isolated from Baffin Bay, Arctic Sea. The lake and CCMP strains were identified by scanning electron microscopy and analysis of complete SSU rRNA sequences (Rengefors *et al.*, 2008) and later the entire ribosomal ITS region (Logares *et al.*, 2009a). Additionally, for this study, we sequenced the ribosomal ITS region of the strains PGEK-2, PGRS-6, FLIB (GenBank accession numbers in Table 1).

### Genotyping of strains

Strains were genotyped using AFLP in order to confirm that strains were genetically different. DNA was extracted using a cetyltrimethylammonium bromide protocol based on Dempster and colleagues (1999). Only samples of high DNA quality, i.e. with a 260/280 ratio of 2.0, were used for downstream analyses. The DNA samples were stored at -80°C. AFLPs were run in triplicates from one extraction and included one set of negatives. We followed the fluorescein protocol based on Vos and colleagues (1995) using the six primer combinations used in Logares and colleagues (2009a). The strains PGEK-2, PGRS-6 and FLIB had not been processed previously. For full protocol, see Logares and colleagues (2009a). AFLP fragments were analysed using a capillary sequencer with a 1000 bp MapMarker. Peak heights were calculated and binned in GeneMapper and transferred to AFLPScore (Whitlock *et al.*, 2008) for normalizing, scoring and error rate calculations. AFLPdat (Elrich, 2006) was used to transfer input files. Clustering was made using a UPGMA dendrogram based on Jaccard's distances using the R package Vegan (R-Development-Core-Team, 2008).

### Salinity reaction norms

The experiment was set up to investigate the growth rates of strains at different salinities to determine the salinity reaction norms and to perform a test of local adaptation. Salinity tolerance limits were defined as the salinity before the salinity level where growth ceased. Temperature and light conditions remained the same (see below). Each strain was initially grown at a salinity level close to its ambient salinity at the time of isolation (see above). Salinity was increased or decreased in a stepwise manner. To increase salinity, we added molecular grade NaCl after autoclaving the SW. To dilute salinity,

filtered SW was diluted with deionized Milli-Q water and subsequently autoclaved. Sterile-filtered nutrients, vitamins and metals were added after autoclaving. The salinities of the media were checked with a conductivity/salinity meter. Salinity was decreased stepwise by 9 ppt down to a salinity of 9. Below 9 ppt, salinity was decreased by half for every step, i.e. to 4.5, 2.25 and 1.125 ppt. Salinity was correspondingly increased from 35 ppt (100% SW salinity) to salinities of 44, 53, 61, 70, 79 and 88 ppt (Fig. 1) corresponding to 125%, 150%, 175%, 200%, 225% and 250% of SW salinity. Since the purpose of the study was to compare strains at different treatments, rather than determine absolute salinity tolerance and maximum growth rates, we accepted the fact that the ion composition may have differed among salinity treatments.

Each of the 11 isolates was grown in its 'ambient' salinity at which it had been maintained (18 or 35 ppt) in a total volume of 100 ml in Nunc Flasks until the cell concentration was approximately 100 000 cells ml<sup>-1</sup>, which is the mid-exponential phase. Each strain was then inoculated into the same salinity (35 or 18 ppt), to higher salinity (44 or 26 ppt) and to lower salinity (26 or 9 ppt). Each strain and treatment was grown in four replicate flasks with a final starting concentration of 20 000 cells ml<sup>-1</sup>. Dilution effects of salinity due to carry-over during transfer were accounted for, and thus the salinities above represent the final salinity in the new batch.

All strains were grown at 3°C, at a light intensity of 20 µmol photons m<sup>-2</sup> s<sup>-1</sup> (cool white fluorescent), and a light : dark cycle of 14:10 h. These conditions were chosen for the common garden experiment as they represent a natural environment likely experienced by the species in nature, rather than optimal growth conditions. The light level represents a typical under-ice lake light climate that is very variable and is often at or below 20 µmol photons m<sup>-2</sup> s<sup>-1</sup> (light data from Rengefors *et al.*, 2008). Since cells grew exponentially at these light levels and corresponded to growth rates in the literature (Buma *et al.*, 2006; Zheng *et al.*, 2012), we assumed that they were not severely light limited. Strain CCMP 2088 had the same mean growth rate (0.10 day<sup>-1</sup>, Table 2) as when grown at 70 µmol photons m<sup>-2</sup> s<sup>-1</sup> in Zheng and colleagues (2012), indicating that light was not limiting in our experiments. Buma and colleagues (2006) observed a range of growth rate from 0.11 to 0.223 day<sup>-1</sup> when light was increased from 18 to 200 µmol photons m<sup>-2</sup> s<sup>-1</sup> in a *P. glacialis* strain, but these differences were not significant; moreover, our fastest growing strain grew at 0.27 day<sup>-1</sup> (Table 2). Sampling for cell counts commenced after 1 week, and subsequently every 4 days on a total of four occasions. Strains were sampled (on ice, in a sterile hood) taking 1.5 ml aliquots for flow cytometry counts and 0.5 ml for Lugol's solution (for microscopical checking of cells). Flow cytometry samples were fixed with 81 µl formaldehyde and Lugol's samples with 7 µl Lugol's solution. After the fourth sampling cell, concentration was checked. For treatments that had not grown, further increments/decreases in salinity were stopped. Otherwise the cultures were allowed to grow to 100 000 cells (still exponential phase) or were diluted down to 100 000 cells ml<sup>-1</sup> and the strains were either inoculated into higher or lower salinity according to the scheme above until cultures no longer showed growth.

Cell numbers were counted with a Becton Dickinson FACSort flow cytometer based on chlorophyll auto-

fluorescence using the FL3 detector. Cell counts were measured at medium speed (35 µl min<sup>-1</sup>) for 2, 3 or 4 min. Cell concentrations were determined and the specific growth rates were calculated during the exponential growth phase using 3–4 sampling occasions. Cell concentrations were ln-transformed and plotted against time, and the slope of the regression gave the specific growth rate defined as Eq. (1):

$$\text{Specific growth rate: } \mu = (\ln N_1 - \ln N_2) / (t_1 - t_2) \quad (1)$$

where  $N_1$  and  $N_2$  are the initial and final densities and  $t_1$  and  $t_2$  are the initial and final times.

If the specific growth rate was less or equal to 0, it was considered that growth had ceased and the experiment was ended for that particular strain. Mean specific growth rate was subsequently calculated per strain and salinity. A two-way ANOVA was performed to determine if salinity and strain group (Abraxas, Ekho, marine strains) had a significant effect on growth rates (dependent variable) (IBM SPSS Statistics v.22 for Macintosh). As part of the data set did not satisfy the Levene's test for homogeneous variances, a stricter  $P$ -value cut-off was used ( $P < 0.01$ ) for significance.

#### Local adaptation test

Local adaptation was tested in the strict sense by comparing the performance (specific growth rate) of 'local' and 'foreign' strains at their own and reciprocal site salinity conditions. Data were used from the experiment above. The SW habitat was considered as the treatment with a salinity of 35 ppt and with 'local' strains including all four marine strains. Lake Abraxas was represented by the treatment where salinity conditions were 18 ppt and included all strains from Lake Abraxas as 'local'. Ekho Lake was represented by the salinity treatment 53 ppt, and 'local' strains were all strains isolated from Ekho Lake. A second test was also performed using more extreme salinities for the hypersaline (max 70 ppt) and hyposaline habitat (min 4 ppt), based on actual salinities measured for these lakes (Ekho Lake and Abraxas respectively). The mean specific growth rates were plotted as a local strains × habitat interaction. The plots also allowed evaluation of performance at 'home' and 'away' of the strains. However, the 'local' versus 'foreign' criterion was used as a diagnostic of local adaptation. This criterion emphasizes the comparison between populations (in our case strains) within each habitat: in each habitat, the local strains are expected to show higher fitness than strains from other habitats (Kawecki and Ebert, 2004). Strains were grouped according to origin (Abraxas, Ekho and marine) with specific growth rate as the dependent variable. Data for the five salinities were tested separately for homogeneity of variances. One-way ANOVAs were performed separately for each of the three salinities representing 'local salinities' for each of the two local adaptation tests described above. A Tukey post-hoc test was used where needed. All tests were run using R (R-Development-Core-Team, 2008).

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#### Data accessibility

DNA sequence GenBank accession numbers: KC852878-80. AFLP and growth rate data and alignments are available at <http://datadryad.org> doi:10.5061/dryad.07b2d.

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