Variation in Thermal Tolerance of the Giant Kelp's Gametophytes: Suitability of Habitat, Population Quality or Local Adaptation?

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The giant kelp *Macrocystis pyrifera* is a cosmopolitan species of cold-temperate coasts. Its South-American distribution ranges from Peru to Cape Horn and Argentina, encompassing a considerable temperature gradient, from 3 to 20°C. Temperature is known to strongly affect survival, growth and reproduction of many kelp species, and ongoing global warming is already eroding their range distribution. Their response to thermal variability was shown to vary among genetically differentiated regions and populations, suggesting a possible adaptive divergence in thermal tolerance traits. This study aimed at testing the existence of local adaptation in the giant kelp, in regions separated by up to 4000km and strong thermal divergence. Two complementary experiments mimicked reciprocal transplants through a common garden approach, each habitat being represented by a given temperature corresponding to the regional average of the sampled populations. Several proxies of fitness were measured in the haploid stage of the kelp, and sympatric *versus* allopatric conditions (i.e. individuals at the temperature of their region of origin *versus* in a different temperature and *versus* individuals from other regions in that temperature) were compared. Additionally, a heat wave at 24°C was applied to measure the tolerance limits of these gametophytes. A significant interaction between experimental temperature and region of origin revealed that temperature tolerance varied among regions. However, depending on the fitness parameter measured, high latitude populations from the sub-Antarctic region were not always less heat resilient than populations from the warmer region of Peru. Even at 24°C, a temperature that is exceptionally reached in the southernmost part of the kelp's natural habitat, all the gametophytes survived, although with strong differences in other traits among regions and populations within regions. This large range of temperature tolerance supports the idea of kelp gametophytes being a resistant stage. Finally, local adaptation *sensu stricto* was not detected. Fertility was more influenced by the geographic origin than by temperature, with possible effects of marginal conditions

at the extremes of the distribution range. The latter results also suggest that stochastic dynamics such as genetic drift restricts adaptive processes in some populations of *M. pyrifera*.

Keywords: phenotypic plasticity, latitudinal gradient, common garden experiment, heat tolerance, local adaptation

INTRODUCTION

Kelp forests are distributed in most cold-temperate coasts. Even though they can be found in inter-tropical regions (e.g. Peru, Brazil, among others), they are restricted to specific areas where temperature do not exceed 22-24°C, such as in deep water (>60m) or upwelling centers. In approximately a third of their worldwide distribution, kelp forests are declining (Krumhansl et al., 2016). Several drivers explain this dynamic of distribution ranges, including overharvesting in some regions but the effect of warming became the major focus of attention, with an increasing number of studies focusing on the low physiological tolerance to temperature increases (Lind and Konar, 2017; Park et al., 2017; Gonzalez et al., 2018; Nepper-Davidsen et al., 2019; Borlongan et al., 2019; Hollarsmith et al., 2020, see review by Smale, 2020). Low latitude populations seem particularly affected (Wernberg et al., 2010; Filbee-Dexter et al., 2020). While most of these studies highlight the sensitivity to heat waves (Smale and Wernberg, 2013; Wernberg, 2021), differences among regions suggest some adaptive divergence in thermal tolerance across the distribution range (King et al., 2019; Liesner et al., 2020a; Liesner et al., 2020b; Diehl and Bischof, 2021). Ecological niche models predict major range shifts towards higher latitudes when including parameters of physiological responses to environmental stressors (Assis et al., 2017; Franco et al., 2018; Martinez et al., 2018). However, the capacity to evolve adaptations to changing environments is generally not considered (Valladares et al., 2014). Locally adapted populations could theoretically experience fitness reductions in a rapidly changing environment, and could decline as rapidly as maladapted populations (Cotto et al., 2017). On the other hand, locally adapted populations could also export favorable alleles to neighboring populations and thus slow down the range shift due to warming (Bush et al., 2016). It has been shown that kelp populations with reduced genetic diversity are less resilient to heat waves and may go extinct (Wernberg et al., 2018), suggesting a lack of genetic variance underlying thermal tolerance traits is actually restricting the evolution of thermal adaptations in these populations.

How thermal adaptations could evolve in kelps? On the one side, the heterogeneity of selective pressures experienced across the distribution range, due for instance to the latitudinal variability in coastal temperatures, is expected to stimulate the evolution of phenotypic traits providing increased fitness in local/regional habitats. The underlying evolutionary process and the resulting pattern correspond to local adaptation (Williams, 1966; see **Box 1**). Local adaptation emerges if the action of selection is stronger than other evolutionary forces. Notably, local adaptation seems more likely in large populations for which the intensity of drift is weak and does not prevent the action of

selection (Eckert et al., 2008). On the contrary, by promoting an effective gene flow among populations and habitats, dispersal may limit population divergence and therefore counteract the process of local adaptation (Endler, 1973; Slatkin, 1973; Hendry et al., 2001). Migration may also generate maladaptation, because introgression of foreign alleles can be disadvantageous for locally adapted populations (Pujol and Pannell, 2008). The equilibrium between these evolutionary forces and its effect on the process of local adaptation has been widely discussed (e.g. Lenormand, 2002; Blanquart and Gandon, 2011; Blanquart et al., 2012; Blanquart and Gandon, 2014). For example, when the migration rate is very low, local genetic drift may reduce the additive genetic variance and eventually fix a reduced number of genotypes, therefore hampering local adaptation process. In this context, a moderate migration may speed up the process of adaptive divergence by allowing favorable alleles to be introduced in local populations (Blanquart and Gandon, 2011). Most kelps have limited dispersal capacities, due to the predominance of spore dispersal, suggesting therefore that population divergence may evolve under an equilibrium between selection and drift. Another layer of complexity corresponds to the heteromorphic haploid-diploid life cycle typical of kelps, which deeply influences population dynamics and evolutionary trajectories (Valero et al., 1992; Otto and Gerstein, 2008; Gerstein and Otto, 2009). The diploid individual, i.e. the sporophyte, is large and constitutes the actual kelp forests. It produces spores after meiosis, which gives rise to a microscopic filamentous haploid stage, the gametophyte. Each sporophyte is able to release tens to hundreds of thousands of spores, which represent a myriad of new genetic combinations on which selection can act in the gametophyte stage. Moreover, because of the absence of dominance effects during the haploid phase, deleterious mutations are expected to be rapidly purged, and selection for beneficial alleles is expected to be more efficient, both mechanisms theoretically favoring the process of local adaptation. However, kelp gametophytes are often depicted as a resistance stage (e.g. Ladah and Zertuche-González, 2007), suggesting they produce an all-purpose phenotype relatively insensitive to environmental variability. The question of selection efficiency in this peculiar haploid and microscopic stage is largely unaddressed in kelps.

We aimed to explore this question with the giant kelp *Macrocystis pyrifera* as a biological model. The giant kelp is the largest brown algae that grows up to 50m long and forms marine forests (Steneck et al., 2002) that host a considerable biodiversity (Graham et al., 2007). This kelp has a wide distribution: present in both hemispheres, it ranges from Alaska to Mexico, from Peru to Argentina and the Falkland Islands. It is also found in isolated regions such as South Africa, south Australia and New Zealand and in most of the Sub-Antarctic islands (Graham et al.,
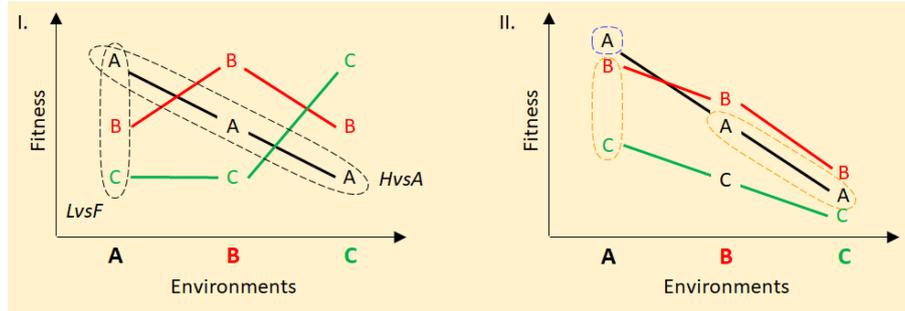
Box 1 | What is local adaptation and how should it be detected.

Local adaptation is an evolutionary process resulting from the balance between selection and other forces, leading to an increased average fitness of individuals in the habitat from which they originate. A spatially heterogeneous environment will therefore promote the differentiation of populations occupying different habitats, by evolving different adaptations to each local condition. Testing local adaptation commonly involves reciprocal transplant experiments, yet the statistical treatment can address different predictions from this process. The Local versus Foreign test (**LvsF**; Leimu and Fischer, 2008) assumes individuals born in environment A will perform better than individuals immigrating from environment B, because the latter are expected to bear adaptations to environment B that are not suitable in environment A. Figure I. below illustrates the case with 3 environments, where individuals in their local environment always perform better than other individuals in that same environment (i.e. comparisons are made "vertically", that is among individuals from different origins for each specific environmental condition). The Home versus Away test (**HvsA**; Kawecki and Ebert, 2004) assumes individuals will perform better in their native environment than in any other environment, because the traits that evolved in response to selection from environment A are not suitable in other environments. In Figure I., comparisons of average performance of individuals issued from the same population or region are made "horizontally", i.e. among habitats.

When populations are highly connected by migration, the average fitness of a population may be reduced because some of the individuals born there have their parents bearing adaptations from other environments. This process leads to a homogenization of the distribution of trait diversity among populations, erasing the differences in both **LvsF** and **HvsA** comparisons. Eventually, populations with a strong immigration load, such as think populations, may become maladapted to their local environment due to an overrepresentation of individuals bearing adaptations to other environments (i.e. both **LvsF** and **HvsA** will show lower performance of locals).

LvsF and **HvsA** approaches can be restricted by the intrinsic quality of both the environment and the population: a poor or stressful environment may affect every individual whatever their origin; a population with high inbreeding depression or mutational load may perform poorly in any environment. In the case illustrated in Figure II, environment A systematically outperforms environment C, and individuals C bear the lowest fitness whatever the environment. Individuals A are locally adapted according to **LvsF** and **HvsA**, while C individuals are maladapted. However, B individuals are locally adapted according to **LvsF** but not **HvsA** as we can see their performance is highest in environment A, as it is for any individual. This complex pattern arises because individual performance is the result of both environmental and population quality, and the efficiency of selection in driving adaptations. More broadly, under the **LvsF** contrast, it is difficult to distinguish the effects of adaptive divergence and the quality of focal populations on the average performance of individuals. Similarly, the **HvsA** test confounds the effects of local adaptation and environmental quality. To disentangle these multiple factors, we consider an array of populations (i.e. a metapopulation) distributed in different environments (following Blanquart et al., 2013). It becomes possible to compare different populations from each environment, in order to assess the effects of environment and population qualities, using a classical two-ways ANOVA. When the interaction is statistically significant, local adaptation can be assessed by comparing Sympatric combinations (i.e. the average performance of populations of a same native environment in that environmental condition) with all Allopatric combinations (i.e. including the Foreign and Away definitions). Blanquart et al. (2013) showed that this approach is a general framework that allows testing for local adaptation *sensu stricto* in a metapopulation model of adaptive divergence. Moreover, it increases the statistical power for detecting local adaptation because it integrates **LvsF** and **HvsA** approaches after removing the effects of environment and population variability. In Figure II., average fitness of populations A in environment A (blue dotted circle) represents the sympatric condition, while both the fitness of other populations in environment A and of populations A in other environments (orange dotted ellipses) represent the allopatric condition.

2007). Along the coast of Chile and Peru, its large distribution encompasses the 3 recognized biogeographic discontinuities (Camus, 2001): the Peruvian province north of 30°S (mean temperatures around 18°C, with low seasonality but large inter-annual variability); the Magellan province south of 42°S (mean temperatures around 8°C, with high seasonality but only marginal inter-annual variability); the Intermediate Region along Central Chile (where both seasonality and inter-annual variability are modest, and a mean temperature around 12°C). This distribution therefore encompasses a significant environmental heterogeneity (**Figure 1**). Yet, it is not homogeneously occupied. For instance, populations from northern Chile and Peru are sparse and isolated (Vásquez et al., 2010), and correspond to the northern edge for the South American distribution. In contrast, the species is conspicuous between 30°S and the sub-Antarctic region of South America, although a patchy distribution is observed in

some fjords and sounds of Patagonia and the Magellan region (personal observations). It was hypothesized that such a wide distribution is the result of both the global ecological success of *M. pyrifera* due to its large physiological and morphological variability (Graham et al., 2007) and its ability to disperse over large distances through rafting of fertile individuals (Macaya et al., 2005, Rothaüsler et al., 2011, Thiel and Haye, 2006; Tala et al., 2016). Yet, the origin of its phenotypic variability, i.e plasticity or local adaptation, has never been clearly evaluated. Some discontinuities in the spatial genetic structure are matched by phenotypic divergence among populations when sharp environmental discontinuities are observed, suggesting the existence of environmental restrictions to effective dispersal at scales<30km (Camus et al., 2018). Previous studies further support this hypothesis by showing that spatial genetic diversity is strongly structured by habitat discontinuities (i.e. islands,

long sandy beaches; Alberto et al., 2010) and environmental gradients (i.e. sea surface temperature, light extinction with depth; Alberto et al., 2011; Johansson et al., 2015). These small-scale, environmentally driven population differentiation at both the genetic and the phenotypic diversities strongly suggest adaptive divergence occurs along the distribution range of the species.

With its wide geographic range and the extent of ecological variability met by its populations, *Macrocystis pyrifera* appears to be an ideal candidate species to tackle several questions around local adaptation. Does the large and environmentally heterogeneous distribution range promote local adaptation? Are low latitude populations located at the edge of the South American distribution range experiencing maladaptation due to extreme thermal conditions? At what spatial scale do populations diverge in their response to environmental heterogeneity? These questions are relevant to understand how the giant kelp copes with such environmental variability along its wide distribution. In this context, we aimed at examining the regional variance of thermal tolerance of the giant kelp and testing for local adaptation through two experiments. While they differed in their choice of experimental units, both were based on a common-garden approach simulating reciprocal transplantations of individuals among habitats, and were performed under laboratory-controlled conditions. Experiment 1 aimed at maximizing the number of populations sampled per region, following Blanquart et al. (2013), to maximize the statistical power of the sympatric-allopatric comparison of the local adaptation test (see **Box 1**). In order to better assess the variation among populations within regions, experiment 2 was performed with fewer populations but a higher number of individuals per population.

FIGURE 1 | Distribution of the sampling locations within the three biogeographic regions. All sites (except LVL* and ALG*) were included in experiment 1. Experiment 2 included all populations indicated on the left of the map. The colored gradation indicates the latitudinal gradient of coastal temperatures. MP, Magellan Province (average temperature of 8°C); CG, Corcovado Gulf (average temperature of 12°C); IR, Intermediate Region (average temperature of 12°C) and PP, Peruvian Province (average temperature of 18°C).

MATERIAL AND METHODS

Sampling and Isolation of Gametophytes

Twenty-one sampling sites were selected among the three biogeographic provinces of the Chilean coast (**Figure 1**). For each sampling site, a 200m-transect parallel to the coast (whenever possible) was made. Every 50 meters, one healthy and reproductive blade was collected from two different sporophytes. Such strategy allowed the collection of gametophyte progeny from 10 sporophytes per site. Reproductive blades were washed with fresh water and a 2-4cm^2 piece of fertile tissue was placed for 2h in 50mL-tubes filled with sterile seawater for spore release. Tissue was then removed from the tube and spores were allowed to settle on glass slides. The spores developed into the gametophytic phase as they were kept in standardized culture conditions of 12/12 day/night, approximately 10 photons.m^{-2}.s^{-1}. of red light to avoid gamete production. Each gametophyte progeny was stored at 12°C. Individual gametophytes were isolated after their differentiation into males and females and grown individually under vegetative growth conditions (red light) with weekly change of sterile SFC-enriched seawater (Correa et al., 1988). This collection of gametophytes represents the basal material used for the two independent experiments.
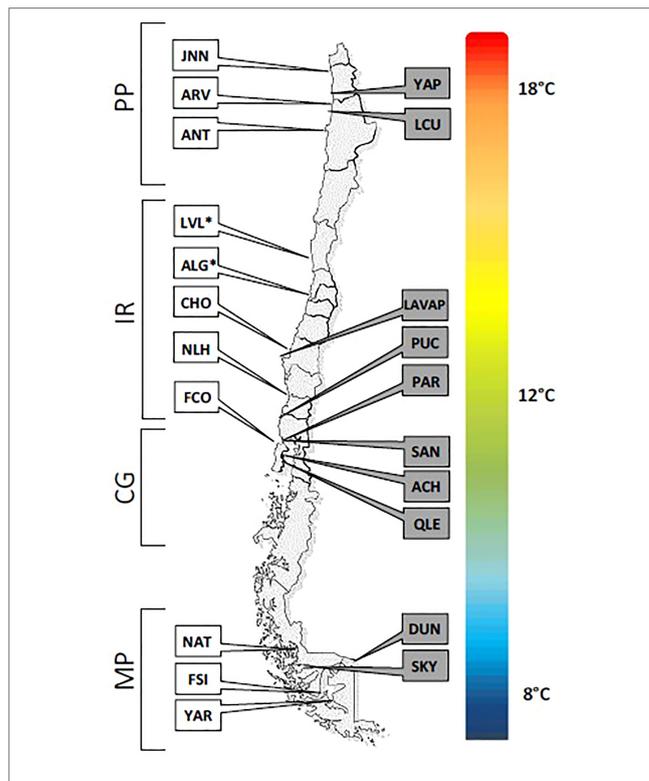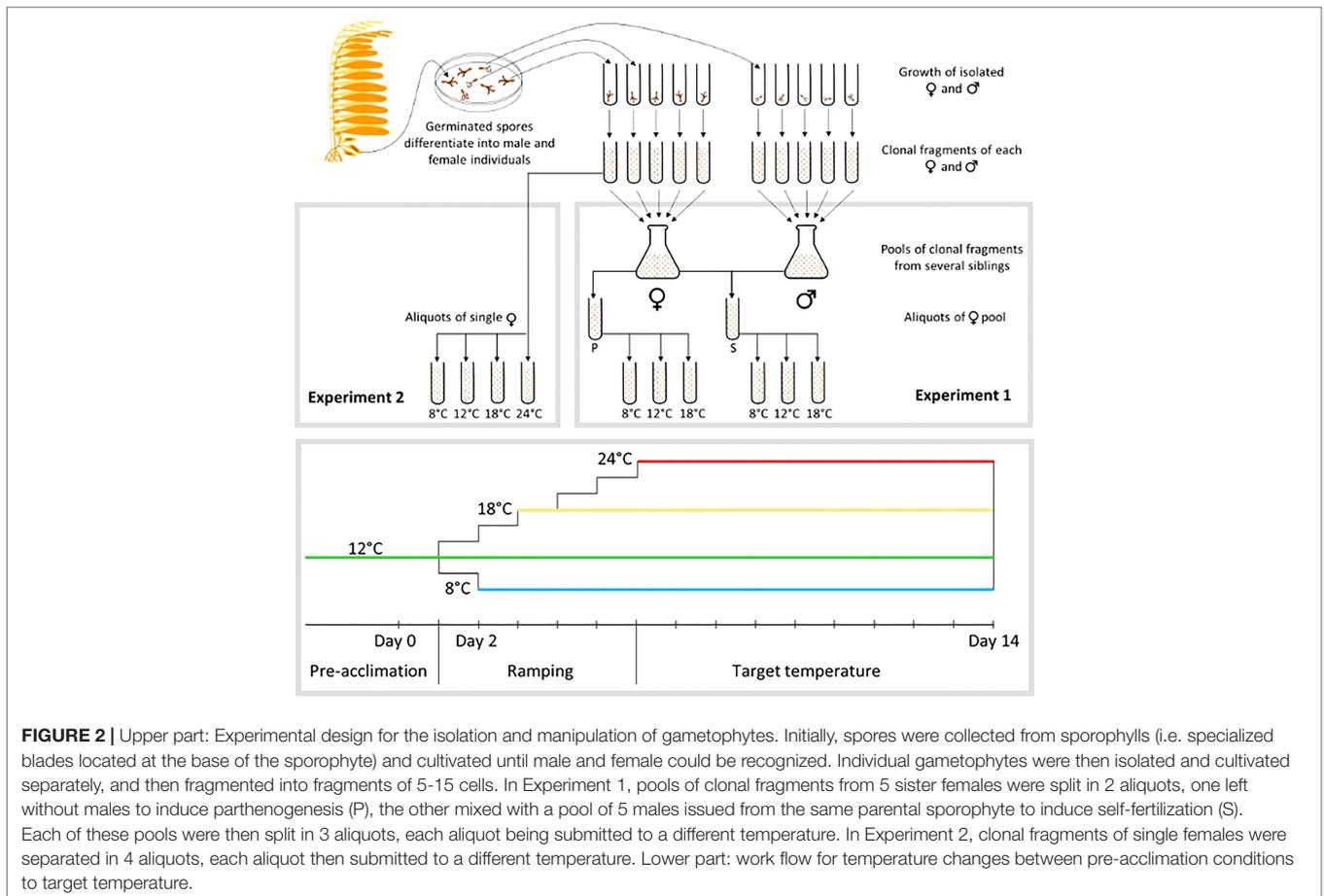
Experimental Designs

The two common garden experiments consisted in submitting gametophytes of different origins to different temperatures (e.g. 8°C, 12°C and 18°C for Magellan Province, MP, Intermediate Region, IR, and Peruvian Province, PP, respectively), each corresponding to the average of each of the 3 sampled regions. In addition, a heat wave was simulated by submitting the gametophytes of Experiment 2 (see below) to 24°C. Such a temperature is largely above the usual coastal temperatures in all the distribution range, but can occasionally be reached in the PP during austral summers. This treatment aimed at evaluating the limit of thermal tolerance of gametophytes. We expected that the northernmost populations would show a better tolerance to this unusually high temperature than populations of IR and MP, which are never exposed to this environmental condition. Before starting the experiments, gametophytes were acclimated at white light for 5 days to activate growth and fertility. Each gametophyte was fragmented in order to obtain hundreds of clones of similar size (**Figure 2**). These clones were then distributed among the different aliquots, each of which including at least 50 clonal fragments, and cultivated for 14 days at the experimental temperatures. Each of these aliquots were considered as

experimental units. The change of temperature from the standard culture condition (i.e. at 12°C) to the experimental temperature was performed smoothly by ramping up or down by 2°C every day until reaching the target experimental temperature (**Figure 2**). Besides temperature, all treatments were kept at identical culture conditions: light intensity was 25-27 photons. m^{-2}.s^{-1}, a photoperiod of 12:12 h (light:dark), and SFC-enriched sterile seawater.

Experiment 1 used 3 to 6 populations per region, and considered the Intermediate Region as 2 different units: the Corcovado Gulf and the open coast (19 populations in total, see **Figure 1** for details). These 2 sub-regions experience the same average temperature but the populations of *M. pyrifera* are strongly differentiated both genetically and phenotypically (Camus et al., 2018). Due to logistical limitations, the design of experiment 1 was at the expense of the number of sporophytes per population (1 to 3; **Table S1**). For each sporophyte progeny, 5 female and 5 male gametophytes were selected, fragmented and pooled by sex (**Figure 2**). Males were merely used as gamete donors to assess female fecundity, defined as the proportion of egg cells successfully fertilized at day 14. The female pools were subdivided in two replicates. One was kept without males to measure survival and physiological vigor. The other was mixed with their respective male sibs' pool to induce self-fertilization

and measure fertility and fecundity. The density of fragments per pool of females alone was defined so that the bottom of the well (1cm diameter) was uniformly covered. In the mixed pools, 5 fragments per individual gametophyte (either male or female) were selected to build the pool, so that the reproductive investment and success could be standardized. Each of these pools was done in triplicate, i.e. cloned replicates of the same isolated gametophytes, and placed at the different temperatures (**Figure 2**). The survival rate was quantified as the ratio of the number of living female gametophytes at day 14 to their initial number by direct counting under a stereoscopic microscope. Fertility was estimated as the proportion of living clonal fragments bearing reproductive structures at day 6, and fecundity was defined as the proportion of egg cells successfully fertilized at day 14. Proxies of physiological vigor were estimated through the parameter of photosynthetic efficiency (Fv/Fm) at days 2 and 14, using a pulsed amplitude-modulated fluorometer (Diving PAM, Hansatech; following Krause and Weis, 1984; Juneau and Popovic, 1999).

Experiment 2 used up to 8 sporophytes per population (**Table S1**) and 3 to 5 populations per region (see **Figure 1** for further details). This experiment was performed on five sister females per sporophyte. Each of these five female gametophytes were cloned separately to obtain hundreds of

FIGURE 2 | Upper part: Experimental design for the isolation and manipulation of gametophytes. Initially, spores were collected from sporophylls (i.e. specialized blades located at the base of the sporophyte) and cultivated until male and female could be recognized. Individual gametophytes were then isolated and cultivated separately, and then fragmented into fragments of 5-15 cells. In Experiment 1, pools of clonal fragments from 5 sister females were split in 2 aliquots, one left without males to induce parthenogenesis (P), the other mixed with a pool of 5 males issued from the same parental sporophyte to induce self-fertilization (S). Each of these pools were then split in 3 aliquots, each aliquot being submitted to a different temperature. In Experiment 2, clonal fragments of single females were separated in 4 aliquots, each aliquot then submitted to a different temperature. Lower part: work flow for temperature changes between pre-acclimation conditions to target temperature.

fragments (**Figure 2**), then acclimated under white light and to the temperature of the region of origin. At least 50 clones per female gametophytes were exposed to the different experimental temperatures, i.e. at 8, 12, 18 and 24°C (**Figure 2**). Growth multiplication factor was estimated using a subsample of 100μL of a homogenized suspension of female's fragments stained with 50 μL of neutral red solution (concentration of 0.01 g/L). This colorant stains living cells, which can be distinguished from dead cells. Four pictures per sample (in different areas of the well) were taken under microscope at 20x magnification, to obtain an average count at days 2 and 14. A batch-script (Java v 1.8) was run under the software ImageJ (http://imagej.net), to count living and dead cells. Growth multiplication factor (GMF) was estimated as the ratio of living cells at day 14 over day 2. The proxy of physiological vigor was chlorophyll production (following Krause and Weis, 1984). Briefly, chlorophyll a concentration was measured with an AquaFluor (Turner Designs), and normalized with the estimate of living females to produce an estimation of the quantity of Chlorophyll a per living cell. The relative chlorophyll a production was estimated as the ratio between day 14 and day 2. Fertility was estimated from these same images by detecting eggs in females based on a circularity parameter: $circ = 4\pi \cdot Surface/Perimeter^2$. *circ* ranges from 0 to 1 (i.e. perfect circle), and a threshold-value of 0.8 considered the cell circular enough to be an egg (as opposed to the rod-shaped vegetative cells of the gametophyte).

Data Analysis

Our approach was based on the Sympatric versus Allopatric (SvsA) test recommended by Blanquart et al. (2013). This test aims at analyzing the variance of fitness among habitats and populations of origin, as well as their interaction (see **Box 1**). By considering the temperature homogeneous at the scale of the region, habitats were thus summarized by the average regional temperature (fixed in laboratory, and noted TEXP hereafter). Experiments performed here slightly differed from a strict transplant design (**Tables S2 A**, **B**), as the region was considered as the evolutionary unit (i.e. the habitat was considered homogeneous within region) while populations were replicated samples within regions. This implied that local adaptation was tested through the interaction between the regional temperature and region of origin, and required to introduce the populations as a random factor nested within regions. For both experiment, the linear mixed model on fitness proxies (W) was thus defined as follow:

W ~ TEXP + Region + Population[Region] + (TEXP x Region) + (TEXP x Population[Region])

The importance of the random effects of populations was estimated using a likelihood-ratio test. To this aim, a submodel including only fixed effects was designed (W ~ TEXP + Region + (TEXP x Region)). The log-likelihood of each model was estimated, and the significance of the ratio [logL(submodel)]/ [logL(full model)] was tested against a χ^2-distribution. Tukey's

HSD *post hoc* tests were then performed, to detail the effect of fixed factors (i.e. region and TEXP). When the interaction (TEXP x Region) was significant, the SvsA comparison, which is embedded in the interaction, was tested in a second phase. Its F-ratio was calculated over the interaction (TEXP x Region), and its significance was assessed against the F-distribution (F, df1 = 1; df2 = df interaction). The obtained p< 0.05 indicates whether or not sympatric region-temperature combinations perform better than allopatric ones (**Box 1**), which case was considered as evidence of local adaptation *sensu stricto* (Blanquart et al., 2013).

A second test of local adaptation was performed, according to the definition "Local versus Foreign" (Kawecki and Ebert, 2004). The pairwise comparison of the fitness of local versus foreign gametophytes was estimated at a given experimental temperature, through the assessment of the effect size Hedge's d, calculated as:

$$g^* = \alpha \frac{\overline{X}_L - \overline{X}_F}{s^*}$$

where $\overline{X}_L$ and $\overline{X}_F$ are the mean fitness of respectively local and foreign gametophytes under the considered experimental condition, and α is a corrective term for biases due to sampling size (n_L and n_F for local and foreign populations or regions respectively). Here, we used α =1 because our interest was in the sign (i.e. negative or positive) of Hedge's g-values. The pooled standard deviation s^* is estimated by:

$$s^* = \sqrt{\frac{(n_L - 1)s_L^2 + (n_F - 1)s_F^2}{n_L + n_F - 2}}$$

With s_L^2 and s_F^2 the sample variance of fitness for respectively local of foreign groups. This method was detailed in the meta-analysis of Leimu and Fischer (2008). All other things being equal (i.e without deme and/or habitat quality effects) positive values of g^* indicate a fitness advantage of local over foreign individuals. On the opposite, negative values could indicate maladaptation. However, according to Leimu and Fischer (2008), a mixed result of positive and negative g-values leads to the rejection of the hypothesis of local adaptation or maladaptation.

The response of fitness proxies to the simulated heat wave (TEXP = 24°C, experiment 2) was analyzed through an ANOVA performed on the following linear mixed model:

W = Region + Population[Region]

The same likelihood-ratio approach was then used to assess the effect of populations as a random factor nested in regions. These analyses were performed using the functions *lm* and *lmer* (package *lme4*) in R 3.4.4. Survival rates (experiment 1) were previously transformed to $sin^{-1}(\sqrt{x})$, to fit normality expectations.

TABLE 1 | Best fit model of the analysis of the variance observed in fitness proxies in the experiment 1, according to the methodology recommended by Blanquart et al. (2013).

	Survival			Fertility			Fecundity			Photosynthetic efficiency		
Model						W ~ TEXP + Region + (TEXP x Region)						
L-ratio	1,783 p = 0.182			-1,14E⁻¹³ p = 1			0,644 p = 0.422			8,68 p = 0.003		
ANOVA on submodel	Df	Sum. Sq	Mean. F (*p-value*) Sq	Df	Sum. Sq	Mean. F (*p-value*) Sq	Df	Sum. Sq	Mean. F (*p-value*) Sq	Df	Sum. Sq	Mean. F (*p-value*) Sq
TEXP	2	6,411	3,205 **19.333 (<0.001)**	2	0,059	0,03 2.203 (0.11)	2	0,392	0,196 **10.039 (0.001)**	2	0,036	0,018 **7.73 (<0.001)**
Region	3	3,389	1.130 **6.814 (<0.001)**	3	0,331	0,11 **8.193 (<0.001)**	3	0,213	0,071 3.648 (0.01)	3	0,043	0,014 **6.109 (<0.001)**
TEXP x Region	6	0,637	0,106 0.640 (0.70)	6	0,11	0,018 1.366 (0.24)	6	0,186	0,031 1.589 (0.15)	6	0,008	0,001 0.596 (0.73)
Residuals	100	16,58	0,166 0.000	91	1,227	0,013	106	2,068	0,02	108	0,254	0,002

*TEXP, experimental temperature; MP, Magellan Province; CG, Corcovado Gulf; IR, Intermediate Region; PP, Peruvian Province. P-values for the F-statistics are provided in brackets (in bold when significant). Details of analysis, including the fixed effects, are provided in **Supplementary Material (Table S3)**.*

RESULTS

Experiment 1

For all fitness proxies surveyed in this experiment (i.e. survival, fertility, fecundity and photosynthetic efficiency), either the effect of the experimental temperature (TEXP) and/or region of origin were strongly significant (p< 0.01; **Table 1**). Survival rates ranged between 61 and 100% at 8°C and 12°C (**Figure 3**), but dropped between 6 and 62% at 18°C, indicating a strong negative impact of the northern temperature on survival. Fecundity appeared largely higher at the intermediate temperature (i.e 12°C; fecundity > 20%; **Figure 3**) than at 8 and 18°C (i.e. between 0 and 11%), except for females from the Magellan Province which maintained a fecundity close to 0. Photosynthetic efficiency varied slightly, and higher values were reached at 8°C (**Figure 3**). The effect of the region of origin appeared strongly significant for each of the four proxies (p< 0.01; **Table 1**). This was mainly explained by the gametophytes from Corcovado Gulf, which showed lower survival rates but higher fertility than any other region (**Table S4**). Fertility of gametophytes from Peruvian Province was close to 0 whatever the experimental temperature, while fecundity was among the highest at 12 and 18°C. Pairwise comparisons among treatments are provided in **Table S4** (Tukey's HSD tests).

The factor "population of origin", nested within regions, did not significantly contribute to the total variance of the proxies, except for the photosynthetic efficiency – the less variable proxy we used (L-ratio = 8.68, p = 0.003; **Table 1**, and **Table S3** for details about mixed linear models). For the three other proxies, the full models failed to reach convergence (see REML criterion of convergence, **Table 1**) - likely because of too low sampling size within population relative to the high variance of these proxies in our experimental data. The interaction between experimental temperature and region of origin was significant for none of the four proxies, suggesting no evidence of local adaptation.

The distributions of Hedge's d at the regional scale did not reveal clear patterns (**Figure S1**), as the results varied depending on the considered fitness proxy. All g* values for the survival rate and photosynthetic activity of MP versus others regions were positive. Yet, fertility and fecundity responded in the opposite way, i.e. all g*-values were negative. The test performed for the others regions produced both positive and negative g* values, and therefore did not support the hypothesis of local adaptation.

Experiment 2

In this experiment, the number of parental sporophytes per population was sufficient for the likelihood approach to converge, and a significant effect of populations-within-region was detected for growth and fertility, but not for physiological vigor (i.e. chlorophyll

TABLE 2 | Best fit model of analysis of the variance observed in fitness proxies in the experiment 2, according to the methodology recommended by Blanquart et al. (2013).

	Growth				Fertility				Physiological Vigor			
Model							W ~ TEXP + Region + (TEXP x Region)					
L-ratio	11,79	p< 0.001			7,95E+00	p = 0.005			-1,71	p = 1		
ANOVA on submodel	Df	Sum. Sq	Mean. Sq	F (*p-value*)	Df	Sum. Sq	Mean. Sq	F (*p-value*)	Df	Sum. Sq	Mean. Sq	F (*p-value*)
TEXP	2	11,91	5,955	2.057 (0.131)	2	9,4E⁻⁰⁹	4,7E⁻⁰⁹	2.203 (0.113)	2	4,043	2,022	**4.836 (0.009)**
Region	2	10,07	5,037	1.740 (0.179)	2	2,0E⁻⁰⁸	1,0E⁻⁰⁸	**4.760 (0.010)**	2	1,44	0,72	1.723 (0.182)
TEXP x Region	4	21,39	5,348	1.847 (0.122)	4	5,7E⁻⁰⁹	1,4E⁻⁰⁹	0.663 (0.618)	4	6,111	1,528	**3.655 (0.006)**
Residuals	177	512,35	2,895		179	3,8E⁻⁰⁷	2,1E⁻⁰⁹		179	74,819	0,418	

*TEXP, experimental temperature; MP, Magellan Province; CG, Corcovado Gulf; IR, Intermediate Region; PP, Peruvian Province. P-values for the F-statistics are provided in brackets (in bold when significant). Details of analysis, including the fixed effects, are provided in **Supplementary Material (Table S5)**.*
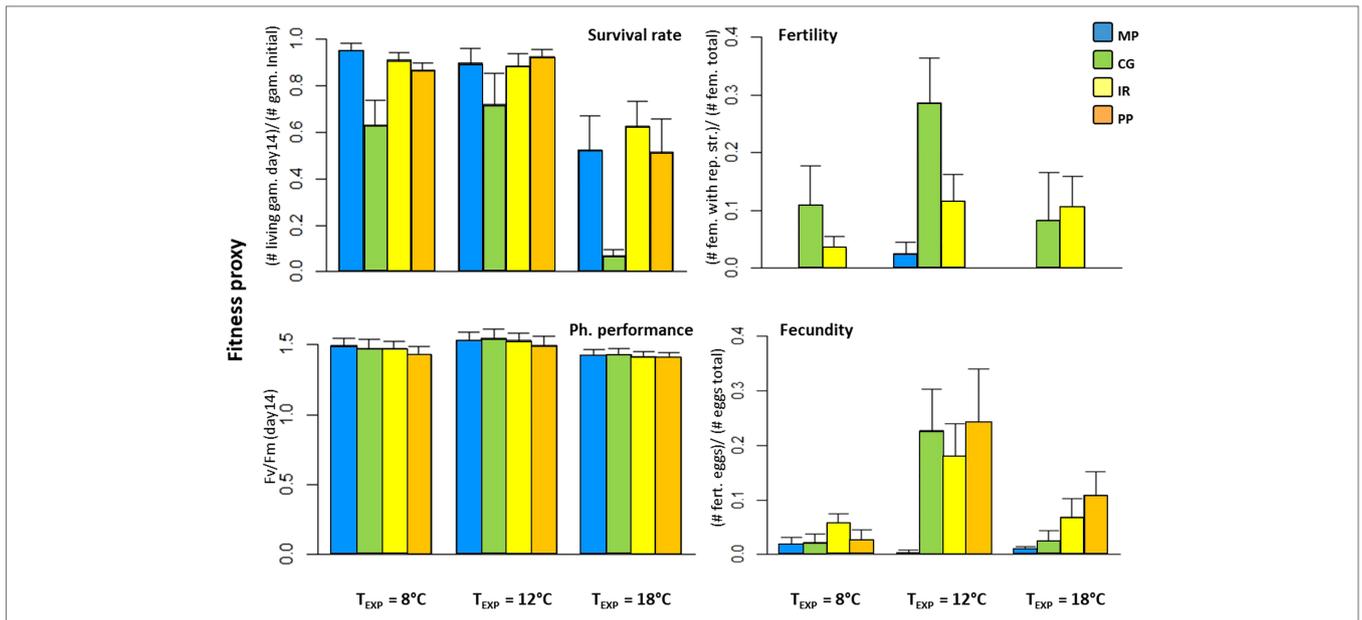
FIGURE 3 | Mean fitness of gametophytes from the four regions included in experiment 1, after 14 days under experimental conditions. Related results of ANOVA and Tukey's HSD tests were consigned in **Table 1** and **Table S4**, respectively. For the definitions of PP, IR, CG and MP, see **Figure 1**. The survival rate was quantified as the ratio of the number of living gametophytes at the day 14 to the initial number of gametophytes. Fertility was estimated as the ratio of living females bearing reproductive structures at day 6 to the total number of females. Fecundity was defined as the ratio of egg cells successfully fertilized at day 14 to the total number of eggs. Proxies of physiological vigor were estimated through the parameter Fv/Fm at day 14. Note: no interaction TEXP x Region was significant.

production rate; **Table 2**; **Table S5** for details about mixed models). The effects of TEXP and region of origin were significant for some fitness proxies (physiological vigor and fertility respectively; p< 0.01; **Table 2**) but neither was significant for growth multiplication factor. The highest physiological vigor was reached at TEXP = 18°C, while the highest fertility was observed in gametophytes from the Intermediate Region (**Figure 4**; **Table S6**). Interestingly,

the Growth multiplication factors were largely homogeneous among treatments, as neither the TEXP, nor the Region and nor their interaction were significant. An uneven distribution of fitness proxies among populations was observed (**Figure 4**), some of them being substantially "fitter" than others, notably populations FCO and NLH for Intermediate Region and ARV and ANT for Peruvian Province, which outperformed the others at all
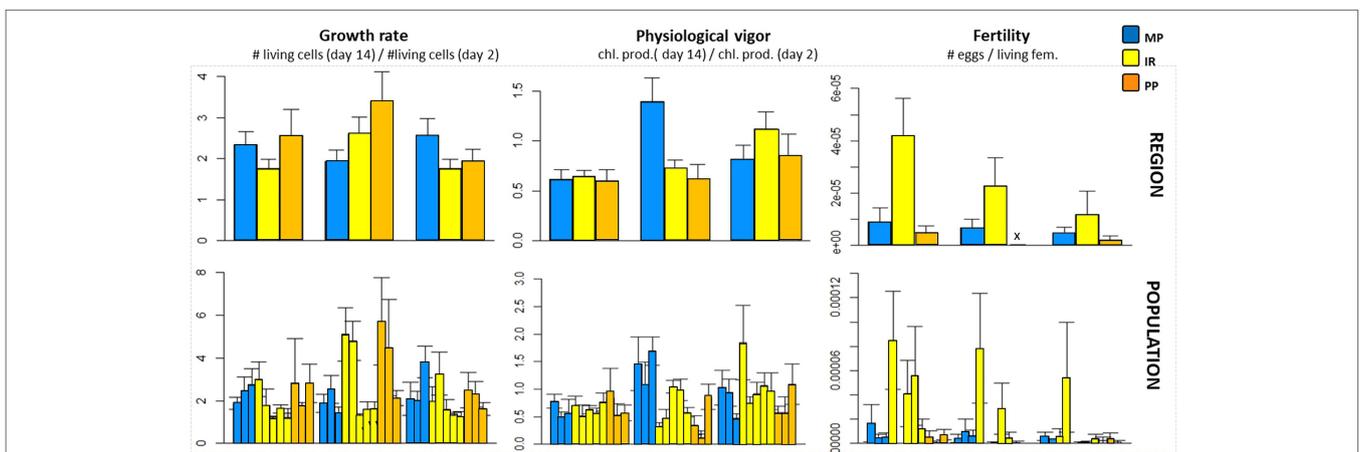

FIGURE 4 | Mean fitness of gametophytes included in experiment 2 after 14 days under experimental conditions. Above, the mean fitness was estimated for each region (regional scale, **Table 2** and **Table S6** for ANOVA and Tukey's HSD tests). Below, the same experimental outputs were presented at the population-scale. For the definitions of PP, IR, CG and MP, see **Figure 1**. Populations' order from left to right on the x-axis corresponds to south-to-north in **Figure 1**. Growth rate was estimated as the ratio of living cells at day 14 over the day 2. The physiological vigor was estimated as the ratio of chlorophyll a production between day 14 and day 2. Fertility was estimated by counting the number of eggs per living female. The interaction TEXP x Region was only significant for the physiological vigor.

TABLE 3 | Best fit model of analysis of the variance observed in fitness proxies in the Heat Wave experiment.

	Growth				Fertility				Physiological Vigor			
Model					**W ~ Region**							
L-ratio	0	p = 1			1,10E-01	p = 0.73			0	p = 1		
ANOVA on submodel	Df	Sum. Sq	Mean. Sq	F (*p-value*)	Df	Sum. Sq	Mean. Sq	F (*p-value*)	Df	Sum. Sq	Mean. Sq	F (*p-value*)
Region	2	8,63	4,316	0.729 (0.487)	2	1,2E⁻⁰⁹	5,85E⁻¹⁰	1.8882 (0.160)	2	18,821	9,411	**11.625 (<0.001)**
Residuals	60	355,21	5.920		60	1,9E⁻⁰⁸	3,10E⁻¹⁰		60	48,57	0.810	

*TEXP, experimental temperature; MP, Magellan Province; CG, Corcovado Gulf; IR, Intermediate Region; PP, Peruvian Province. P-values for the F-statistics are provided in brackets (in bold when significant). Details of analysis, including the fixed effects, are provided in **Supplementary Material (Table S7)**.*

experimental temperatures. The interaction [TEXP x Region] was significant for the physiological vigor. Yet, neither the SA contrast ($F_{(1,4)} = 0.69$; p = 0.45), nor the examination of the Tukey's test (**Table S6**) revealed local adaptation *sensu stricto*. Finally, the distribution of Hedge's d was in line with the former results (**Figure S2**), as they were uniformly distributed on both sides of y = 0 (**Figure S2**).

Heat Wave

Regardless of the region of origin, the ability of gametophytes to grow was affected by the experimental temperature of 24°C with GMF generally centered around 1 (**Figure 5**), indicating that most gametophytes neither grew nor lost biomass due to cell death. This trend was independent of the region of origin ($F_{(2,60)} = 0.73$; p = 0.49; **Table 3**). The factor "population of origin" was not significant (L-ratio = 0, p = 1, **Table 3** and **Table S7**), yet the pool of gametophytes from Antofagasta (ANT) were a noticeable exception, with GMF > 2.5 (**Figure 5**). The physiological vigor was well explained by the region of origin

($F_{(2;\ 60)}$ = 11.63; p<0.001; **Table 3**), with the lowest values observed in the Magellan Province. In fact, gametophytes from this region were the most impacted by the heatwave as they showed the lowest values for the 3 fitness proxies. Contrary to our hypothesis of local adaptation, both physiological vigor and fertility were higher for gametophytes from the Intermediate Region than those from the Peruvian Province, the only region where such high temperature can be reached (**Table 3** and **Table S8** for Tukey's tests).

DISCUSSION

This study focused on the haploid stage, *i.e.* the gametophyte, of the giant kelp *Macrocystis pyrifera*, isolated from different biogeographic regions and covering a large latitudinal range. The physiological state of these gametophytes and the fitness proxies (i.e. growth, survival, fertility and fecundity) varied according to the experimental temperature and the region of origin. This kind
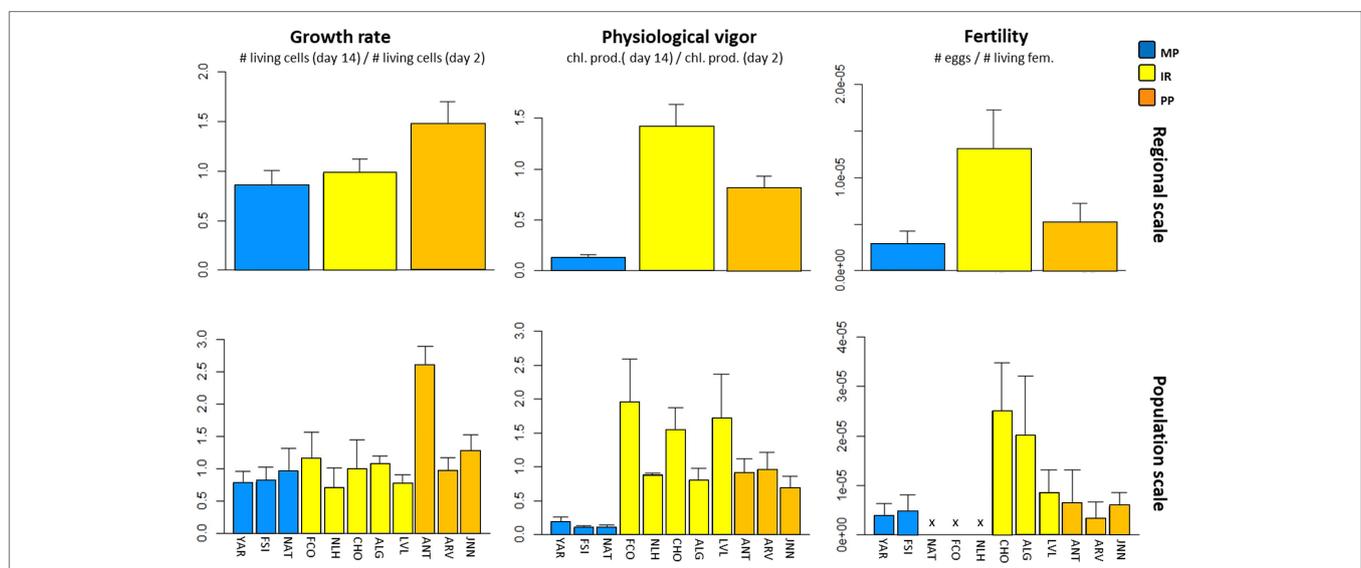

FIGURE 5 | Distribution of fitness of gametophytes facing a simulated heat wave (TEXP = 24°C). This test was performed during experiment 2. Above, the mean fitness was estimated for each region (regional scale, see **Table 3** and **Table S8** for ANOVA and Tukey's HSD tests). For the definitions of PP, IR, CG and MP, and locations see **Figure 1**. Growth rate was estimated as the ratio of living cells at day 14 over the day 2. The physiological vigor, corresponding to the relative chlorophyll a production, was estimated as the ratio between day 14 and day 2. Fertility was estimated by counting the number of eggs per living female.

of result has been observed in a number of recent studies on kelps thermal tolerance (King et al., 2019; Liesner et al., 2020a; Liesner et al., 2020b; Martins et al., 2020). The divergence in thermal tolerance among populations suggests the existence of a genetic background for these traits. Yet, this is not direct evidence of an adaptive divergence leading to local adaptation. Indeed, no evidence of local adaptation *sensu stricto* was detected by neither Blanquart's method nor Leimu and Fisher's test. This latter is considered as highly conservative (Blanquart et al., 2013; Bucharova et al., 2017), as it often leads to rejection of the paradigm "local is best". Indeed, according to this LvsF-definition, each local experimental unit must perform better than foreign ones. When increasing the sampling effort, the odds that some foreign individuals perform better than locals is also increased. Moreover, this test can detect local adaptation only in situations where population quality effects are much reduced. Indeed, differences in population quality may increase the pooled standard deviation (noted $s*$ in the material and methods section), which alters the value of Hedge's d. Thus, the local versus foreign test alone cannot finely explain the observed variations of fitness in our study, as the effect of deme quality was proved to be large. Since Blanquart's method revealed that fitness proxies varied according to genetic (*i.e.* among regions or among populations) and environmental (experimental temperature) sources of variation, rather than the sympatric vs allopatric contrast, the variability of Hedge's d is thus mainly imputable to the variability of regional and population quality. Hereafter, we discuss the putative ecological and evolutionary causes and consequences of this variability, in the light of the recommendation of J. Hereford (2009) claiming that "studies that do not demonstrate local adaptation should not be looked on as failed experiments, but as demonstrations that populations may be prevented from reaching adaptive optima".

The large thermal tolerance of gametophytes represents the first likely explanation, as they were able to survive most experimental treatments, including the heat wave. Surviving gametophytes invested in vegetative growth in both experiments, to the detriment of reproductive functions (notably fertility was reduced at high temperatures, 18°C or above). Such trade-offs among fitness traits (e.g. survival, growth and reproduction) in response to stressful temperature was shown in other kelps (e.g. Oppliger et al., 2012 for *Lessonia nigrescens*), other seaweeds (e.g. Araújo et al., 2015 for *Ascophyllum* sp.; Chu et al., 2011 for *Sargassum* sp.) and number of terrestrial plants (reviewed by Hereford, 2009). This phenomenon was observed in wild gametophyte stands and ends once suitable environmental conditions are restored for successful sexual reproduction (Dayton, 1985; Hoffmann and Santelices, 1991; Edwards, 2000; Carney and Edwards, 2010; Delebecq et al., 2016). In this context, gametophyte survival at 24°C, even for those collected in the Magellan Province where temperatures rarely overpass 10°C, suggests the gametophytes are acting as a resting stage (Ladah et al., 1999) like a seed bank (Edwards, 2000). The ability of kelp gametophytes to persist for years under laboratory conditions have never been clearly observed in the wild. While previous estimations proposed

that gametophytes can survive for a few weeks (Deysher and Dean, 1986; Hoffmann and Camus, 1989; Reed et al., 1997), more recent work suggests some gametophytes could persist in the field during several months (Carney et al., 2013; Robuchon et al., 2014), enough to survive the warm season. Kelp demographic turnover may be ensured by two possible mechanisms: either gametophytes survive during warm seasons until colder conditions are met to complete mating and restore the diploid phase, or spores are released during the cold season to ensure a rapid development of the gametophyte stage. In any case, successful sexual reproduction is likely to occur mainly when temperature is below 18°C. Such condition is met during austral winter (June-August) and during the upwelling season (spring and early summer; Thiel et al., 2007).

Considering the large distribution range, we hypothesize that selection on gametophytes does not promote local adaptation but instead favors all-purpose phenotypes, allowing them to cope with the diverse array of environmental conditions within the range. This would be the case if a stabilizing selection is acting on a large spatial scale (Price et al., 2003). While dispersal through rafting of mature sporophytes is probably too rare to ensure an effective gene flow among populations, it allows the transport of individuals for several hundred (Thiel and Haye, 2006), and up to several thousands of kilometers (Macaya and Zuccarello, 2010). The subsequent release of spores then may occur in a very distinct habitat than the origin of the parental rafting sporophyte. Under such a scenario, the resistance and plasticity of gametophytes appears as a necessary property to ensure successful recruitment. However, to what extent selection for rare long-distance dispersal and successful recruitment could prevent specialization to local, or even regional, environmental conditions is difficult to predict. This hypothesis questions the weight of the parental effects on the fitness of the migrant progeny (defined as the part of the phenotype of descendants explained by the phenotype and/or the environment of parental sporophytes, Mousseau and Fox, 1998; Wolf and Wade, 2009), as they may potentially affect the success of recruitment. Transgenerational effects of temperature have been observed in kelps: the thermal tolerance of juvenile sporophytes was influenced by the temperature at which the gametophytes were grown and reproduced in *Laminaria digitata* (Liesner et al., 2020b). However, such inherited epigenetic variation from the sporophyte to the gametophyte stage is less likely as i. spores are small and their nutrient content is very low; ii. after germination, the young gametophytes are physiologically independent; iii. recent studies suggest that, at least in plants, the epigenetic modifications that are environmentally induced showed a limited meiotic stability (Paszkowski and Grossniklaus, 2011; Wibowo et al., 2016), suggesting that meiotic spores may not be an adequate carrier of the maternal effect on the gametophytic stage; iv. brown algae seem to lack the capacity to methylate DNA (Vigneau and Borg, 2021). We therefore might expect an overall low incidence of parental effects. Besides a reduced impact on estimations of fitness during our experiments, the phenotype of immigrant gametophyte would consequently depend on its own plasticity.

The results of the two experiments also highlighted that low temperature may restrain fertility and fecundity, while survival rate and the physiological state remained globally stable. Therefore, the range of optimal temperature for reproduction in *M. pyrifera* seems to be restricted to few degrees around 12°C. In fact, regional temperatures tend to be seasonally variable, and may range between 14 and 20°C in the Peruvian Province and between 3 and 10°C in the Magellan Province. In this context, the choice of fixed experimental temperatures at the regional average (8°C for MP and 18°C PP) may have forced the gametophytes to express their plasticity in the form of a resistance state, yet in detriment to the detection of LA (if existing). This is a difficulty inherent to laboratory-controlled experiments, which are oversimplifications of the environmental factors involved, usually disregarding the interaction among them. This being said, several features of the reproductive strategy of *M. pyrifera* and dispersal capacity of its spores may also prevent the reach of a local adaptive optimum. One of these relates to the likely effect of genetic drift. In their meta-analysis about local adaptations in plants, Leimu and Fisher (2008) showed that LA is usually not detected in populations of<1000 fertile individuals. This agreed with the classical theoretical expectation that increased stochasticity (through reduced effective size) diminishes the efficiency of selection. The effective size of kelp populations is generally not known, but might be relatively small in the case of *M. pyrifera*. The species is distributed along the shoreline, in stands that rarely exceed a few meters in width (Vásquez et al., 2010). The production of meiotic spores occurs on specialized blades located at the base of the individual, close to the substrate where the hydrodynamics is reduced by the large standing biomass. Most of the spores settle within a few hours after release. This morphological specificity, as well as the short duration of the spores' swimming phase, greatly restrict spore dispersal and effective gene flow within and among kelp stands. Population genetic estimate of mean dispersal distance ranges between 0.5 to 3 km, and is strongly determined by coastal current velocity (Alberto et al., 2010). Additionally, a temporal survey across 11 years of Californian kelp populations showed that their stochastic dynamics was mitigated by the presence of persistent neighbor stands, further suggesting dispersal was relevant only at local scales (Castorani et al., 2015). Equivalent temporal survey is not available for the Chilean Coast, but fluctuating demography is expected in the Peruvian Province, where El Niño events are known to cause strong kelp mortality (Thiel et al., 2007). Considered together, these elements suggest low neighborhood and effective sizes of giant kelp's populations. In this context, migration-selection equilibrium is potentially an unreachable state because of the dominant effect of genetic drift. Stochasticity may be particularly strong at range edge where a reduced averaged fitness is expected due to marginality. Such mechanism explained the particularly poor thermal tolerance of populations of *L. digitata* located at warm range limits (Liesner et al., 2020a). Our two experiments included populations from Peruvian and Magellan Provinces, close to the edge of the distribution range of *M. pyrifera*. Marginality was taken into account by the factor "region of origin" (i.e. the "deme quality"

sensu Blanquart et al., 2013), and was expected to confer a bad quality caused by stochastic fixation of deleterious mutations (Willi et al., 2006). Several results were in line with this expectation. Indeed, gametophytes from these provinces had low fertility compared to those from the Intermediate Region. Increased investment in vegetative growth of the gametophyte, uneven sex ratio and meiotic alterations or apomixis have been observed in other kelps at their range margins (Oppliger et al., 2011; Oppliger et al., 2012; Oppliger et al., 2014). Such changes in reproductive strategies are expected to further reduce effective size (Hoffmann and Blows, 1994) and should slow down the selective processes acting on the standing variability (Glemin and Ronfort, 2013). Altogether, the combination of stressful environmental conditions that lower fitness and the harsh genetic context (e.g. stochastic fluctuations of deleterious alleles and inbreeding) may strongly reduce adaptability of marginal populations (Willi et al., 2006).

Adaptive optima to regional temperatures seem to be hardly reached in *M. pyrifera* gametophytic populations. We proposed two possible explanations that are non-mutually exclusive. One likely restriction stems from the effect of stochasticity driving the dynamics and evolutionary trajectories of the giant kelp's populations. In parallel, selection may favor generalist gametophytic phenotypes over specialized ones to local habitat, as a consequence of selection in unpredictable environmental conditions. As the product of meiosis, the gametophyte population holds a large diversity of haploid genotypes expressing different thermal tolerance traits and reproductive strategies on which selection can act. With potentially reduced mutational load and nutrient requirements, and a strong phenotypic plasticity or wide physiological tolerance, this haploid stage is able to transmit most of the standing genetic variance of thermal tolerance traits to the next diploid generation. Therefore, tackling the question of local adaptation requires further experiments on the diploid sporophyte to complete the puzzle of the actual evolutionary trajectory in this heteromorphic haploid-diploid species. So far, we conclude that the large distribution range of this cosmopolitan species is better explained by its capacity to colonize distant and diverse habitats, than by the adaptation to local conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.802535/full#supplementary-material

Supplementary Table 1 | Details about sampling effort and the number of parental sporophytes that provided the gametophytes included in our experiments.

Supplementary Table 2 | Experimental design and structure of our dataset, indicating which are sympatric or allopatric combinations.

Supplementary Table 3 | Details about the mixed linear models used to analyze data from the experiment 1.

Supplementary Table 4 | Pairwise comparisons among treatments from the experiment 1 (*post hoc* Tukey's HSD tests), performed for significant fixed effects (see).

Supplementary Table 5 | Details about the mixed linear models used to analyze data from the experiment 2.

Supplementary Table 6 | Pairwise comparisons among treatments from the experiment 2 (*post hoc* Tukey's HSD test), performed for significant fixed effects (see).

Supplementary Table 7 | Details about the mixed linear models used to analyze data from the experiment of Heat Wave.

Supplementary Table 8 | Pairwise comparisons among treatments from the experiment of Heat Wave (*post hoc* Tukey's HSD test), performed for significant fixed effects (see).

Supplementary Figure 1 | Hedge's d distribution for testing the hypothesis of "local is best", performed with data from Experiment 1 at the regional scale. The null hypothesis (i.e. no local adaptation) cannot be rejected as both negative (maladaptation) and positive (local adaptation) d-values were encountered for each proxy.

Supplementary Figure 2 | Hedge's d distribution for testing the hypothesis of "local is best", performed with data from Experiment 2 at the population scale. The null hypothesis (i.e. no local adaptation) cannot be rejected as both negative (maladaptation) and positive (local adaptation) d-values were encountered for each proxy.

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