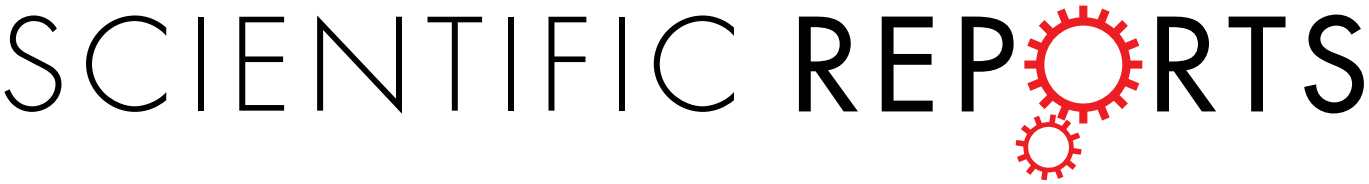

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High-density genetic map and identification of QTLs for responses to temperature and salinity stresses in the model brown alga *Ectocarpus*

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Deciphering the genetic architecture of adaptation of brown algae to environmental stresses such as temperature and salinity is of evolutionary as well as of practical interest. The filamentous brown alga *Ectocarpus* sp. is a model for the brown algae and its genome has been sequenced. As sessile organisms, brown algae need to be capable of resisting the various abiotic stressors that act in the intertidal zone (e.g. osmotic pressure, temperature, salinity, UV radiation) and previous studies have shown that an important proportion of the expressed genes is regulated in response to hyposaline, hypersaline or oxidative stress conditions. Using the double digest RAD sequencing method, we constructed a dense genetic map with 3,588 SNP markers and identified 39 QTLs for growth-related traits and their plasticity under different temperature and salinity conditions (tolerance to high temperature and low salinity). GO enrichment tests within QTL intervals highlighted membrane transport processes such as ion transporters. Our study represents a significant step towards deciphering the genetic basis of adaptation of *Ectocarpus* sp. to stress conditions and provides a substantial resource to the increasing list of tools generated for the species.

Brown algae (Phaeophyceae) represent one of only five eukaryotic lineages that have evolved complex multicellularity independently. They are very distantly related to other major lineages such as animals or green plants[1,2]. These seaweeds constitute the dominant vegetation in the intertidal and subtidal zone of coastal ecosystems. They display a large variability of morphologies and some brown algae, such as kelps, may form extensive forests that offer suitable habitats for numerous species and hence support ocean biodiversity[3,4]. Brown algae have a wide range of uses as food, cosmetics or fertilizers and there is increasing attention on new biotechnological applications such as biofuels or cell-wall polysaccharides[5–7]. Despite their evolutionary, ecological and economic importance, many aspects of the biology of brown algae remain poorly understood. In particular, the range of phenotypic variation of such morphologically simple organisms, as well as the sources of this variation, their impact on fitness and the limits they may impose on adaptive responses to environmental conditions are largely unknown.

A large number of seaweed species are able to colonize the intertidal zone, and some of these species live only in this habitat. The intertidal zone is often characterized by the presence of multiple stresses including high temperature, low or high salinity, extreme irradiation with sunlight, wave mechanical force, desiccation and osmotic shocks due to tidal emersion-immersion cycles. There is evidence that the vertical distribution of organisms is related to their ability to tolerate different types of stress. This is particularly the case in the intertidal zone, where the difference between tolerant and susceptible species lies in the extent to which they can recover photosynthesis

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when re-immersed in seawater[8]. Coastal habitats may present other stresses, such as low salinity in estuarine environments, high concentrations of heavy metals and other chemical pollutants near ports, mines or coastal cities, spatial temporal variations in upwelling and other coastal oceanographic processes[9,10]. Adding to this complexity, variations in environmental conditions and their impact can even be observed at the microhabitat scale[11]. Remarkably, a number of seaweed species are able to cope with these strong environmental gradients. From an evolutionary point of view, two mechanisms can explain this tolerance to abiotic stressors: either evolution towards a generalist, all-purpose phenotype by increasing phenotypic plasticity, or local adaptation to specific sets of environmental conditions. It is however difficult to determine the relative importance of phenotypic plasticity and local adaptation, respectively, in determining differences among populations[12]. Plasticity may mimic local adaptation resulting in differences between populations that are not due to genetic factors[13]. While an extensive literature is available on the range of phenotypic plasticity in responses of seaweeds to both biotic and abiotic factors[14], the genetic basis of their responses to environmental heterogeneity remains poorly explored. Several mechanisms involved in the responses to stressors such as heat, salinity and dehydration have been shown to be conserved among land plant taxa, including the presence of common actors such as reactive oxygen species (ROS), ion fluxes, activation of kinases and a cascade of reactions leading to the expression of transcription factors[15–17]. However, the extent to which mechanisms of stress tolerance known for those taxa are used by brown algae is largely unknown. Although some aspects might be conserved[18,19], other important processes are likely to be found only in brown algae because of their unique evolutionary history. To understand the stress tolerance features characteristic of this group, it is therefore essential to study this phenomenon using brown algal models rather than extrapolating information from distant models in other phylogenetic groups. Such knowledge is of evolutionary as well as of applied interest, as novel biomolecules and metabolic pathways may be discovered. Furthermore, with the current expansion of the algal aquaculture sector, brown algae need to be well characterized for future domestication and selective breeding to provide the industry with optimized strains.

Ectocarpus sp., a small filamentous alga, has been established as the model organism for the brown algae, inducing the generation of several genetic and genomic resources such as a fully sequenced genome[1], a genetic map[20] and transcriptomic data[19,21]. Previously named *Ectocarpus siliculosus*, it has recently become clear that this taxa corresponds to a separate species that has not been described previously[22] and we therefore refer to the species provisionally as *Ectocarpus* sp. 1c[23], simplified as *Ectocarpus* sp. in the text below. As with many other members of the brown algal group, this species alternates between a haploid (gametophyte) and a diploid (sporophyte) phase, and thus its life cycle involves sequential development of two successive complex organisms[24]. A recent study in the *Ectocarpus* group has shown that haploid and diploid organisms also exhibit clear niche partitioning. However, mainly asexual populations also exhibit similar habitat usage[25]. Although brown algae are mainly marine species, rare cases of adaptation to fresh water have been reported[26]. An *Ectocarpus* species, *Ectocarpus subulatus*, is able to live in both marine and freshwater, suggesting extreme physiological plasticity[23,27–29]. Freshwater colonization is a rare event that is thought to induce rapid evolutionary radiations[30]. The colonization event involving this brown alga is still under investigation but it does not appear to have involved extensive radiation, as only about 1% of these species have colonized freshwater[31]. Recent analyses showed that the transition to low salinity has been accompanied by fundamental morphological, transcriptomic and metabolomic changes but might be still reversible[31], suggesting high phenotypic plasticity rather than local adaptation. This transition is also dependent on the host interactions with its complex bacterial communities[29]. Although *E. subulatus* is a different species, such host-bacterial interactions may also play a role in the adaptation of *Ectocarpus* sp. to its environment (although this aspect has not been investigated in the study presented here).

Analysis of the *Ectocarpus* sp. genome suggested that this species has evolved effective mechanisms to survive its harsh intertidal environment such as a complex photosynthetic system and a large number of ion channels[1]. These characteristics may have contributed to its ability to colonize freshwater environments. However, even with the current improved assembly and annotation of the *Ectocarpus* genome, about 39% of the predicted genes lack functional annotations based on available databases[32]. Hence, an important proportion of the genes in the genome do not have a predicted function and, therefore, approaches such as detection of quantitative trait loci (QTLs) and analyses of polymorphism of candidate genes[33] are important to explore the genetic basis of adaptation in this species.

QTL analysis provides insights into the genetic determinism of traits and is an important aid for marker assisted selection[34,35]. QTL mapping has been used successfully to improve our understanding of the genetic basis of traits in land plants[36] and in animals, including marine animals[37], but remains rarely investigated in algae[38]. With recent advances in sequencing technology, molecular markers such as single nucleotide polymorphisms (SNPs) can now be identified in abundance at continually decreasing cost, even in complex genomes. Methods such as Restriction site Associated DNA (RAD) sequencing allow the complexity of the genome to be reduced in order to produce high confidence SNPs cost effectively for various applications[39,40].

In our study, we generated 3,588 RAD-seq-based SNP markers to construct a dense genetic map for *Ectocarpus* sp. and used it to identify QTLs for growth-related traits and their plasticity under different temperature and salinity conditions. Several candidate QTLs for high temperature and low salinity tolerance were detected and GO (Gene Ontology) enrichment tests within these QTLs contributed to our understanding of the ability of *Ectocarpus* sp. to adapt to high temperature or low salinity.

Results

Identification of phenotypic traits under temperature and salinity stress. To study the genetic basis of growth under temperature and salinity stresses, we grew a family of 89 *Ectocarpus* sp. haploid progeny, together with their two parental strains (six clones for each strain) for 12 days in growth cabinets under three temperature (13 °C as control, 26 °C and 28 °C; hereafter T13, T26 and T28) and three salinity conditions (34‰ as control, 20‰ and 15‰; hereafter S34, S20 and S15). Temperature and salinity stresses were applied

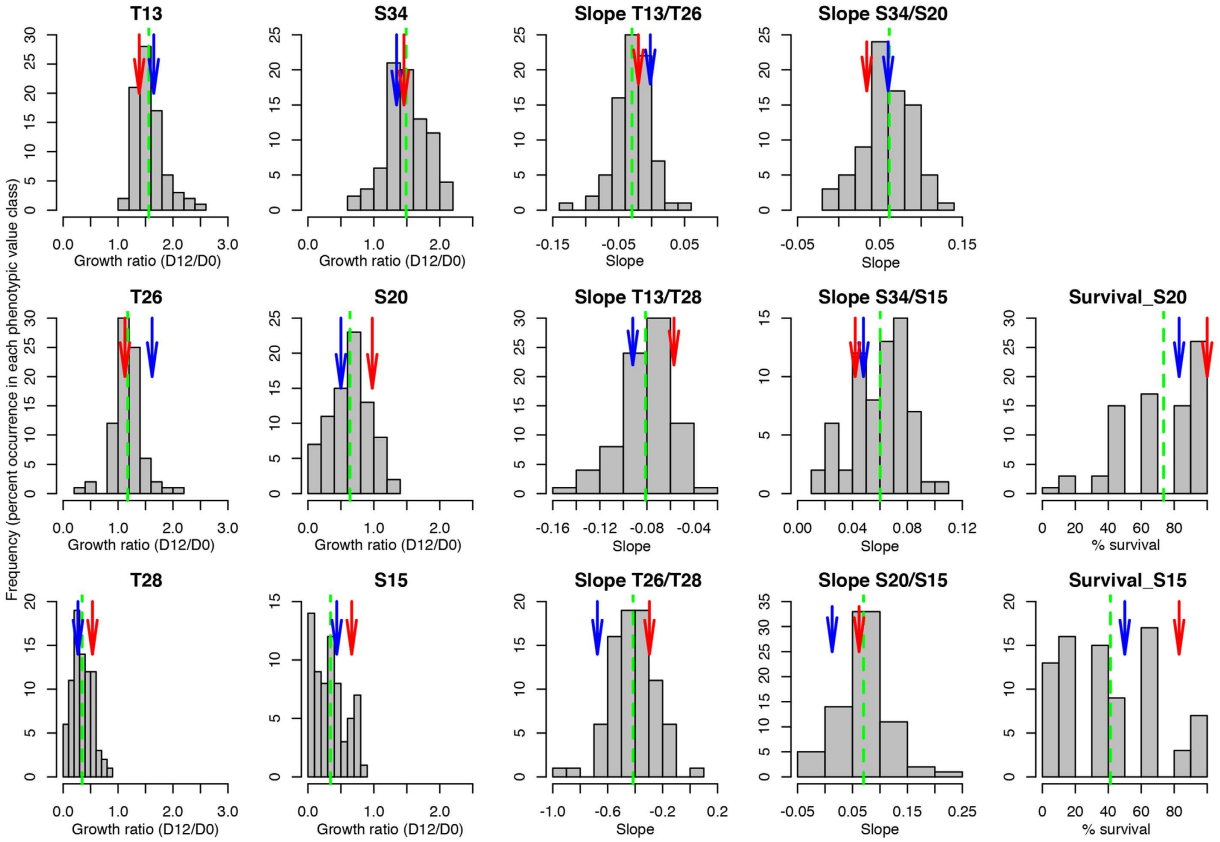

Figure 1. Distributions of the progeny BLUPs of the studied traits. T13, T26 and T28 correspond to growth rate phenotypes for temperatures 13 °C, 26 °C and 28 °C respectively. S34, S20 and S15 correspond to growth rate phenotypes for 34‰ salinity, 20‰ and 15‰ respectively. Plasticity traits are represented by a letter followed by a number and a slash, then another letter and number. For example, S34/S20 corresponds to plasticity represented by slope of the reaction norm between salinity 34‰ condition and salinity 20‰ condition. Surv_S15 and Surv_S20 correspond to survival trait for conditions salinity 15‰ and salinity 20‰. Frequency distributions of the progeny BLUPs of the analyzed traits; blue arrows indicate mean values for the male parent and red arrows indicate mean values for the female parent; the green dashed line represents the mean value for the progeny population; "Slope" represents the slope values of the reaction norm between two conditions indicated at the top of each graph; "% survival" represents the proportion of survival among replicates of each progeny for salinity conditions 15‰ and 20‰.

under normal, control salinity (34‰) and temperature (13 °C) conditions, respectively. An initial series of test experiments aimed at selecting optimal temperature and salinity conditions for QTL detection had shown that 30 °C and 10‰ salinity were extreme conditions for the mapping family, leading to high mortality. We therefore selected our stress conditions so that they had significant impacts on the growth phenotype of the offspring without killing a high proportion of individuals (we considered up to 30% of mortality as acceptable, i.e. none of the conditions should induce a complete loss of more than 26 progeny).

Growth, expressed as a ratio of the thallus area on day 12 to the thallus area on day 0, varied significantly across the studied progeny under all three salinity conditions (p-value between 4×10^{-4} and 7×10^{-16}, ANOVA) but only under two of the three temperature conditions (p-value = 0.005 for 26 °C, p–value $< 1 \times 10^{-10}$ for 28 °C and p = 0.2 for 13 °C, ANOVA). A significant sex-dependent difference was observed under 15‰ salinity (adjusted p-value Tukey comparison test: 3×10^{-7}) with males showing more growth than females (growth ratio of 0.47 vs 0.30 respectively).

The impact of the applied stresses was clearly visible through their effects on the distribution of the traits, with a reduced growth rate for the stress conditions and a much lower population mean for the growth ratio (Fig. 1). We observed substantial variance in all traits across the progeny, with the fastest growers having growth ratios 2 to 5-fold larger than the slowest growers. Differences between the mean values for each parent were more limited.

Transgressive individuals (i.e. displaying more extreme phenotypes than the parental strains) were observed in the mapping family under almost all conditions (Fig. 1).

By treating different experimental conditions as different environments, we observed significant genotype by environment (G × E) interactions (genotype × salinity p-value = 2×10^{-6} and genotype × temperature p-value = 4×10^{-4}), which is potentially indicative of a genetic basis of variation of phenotypic plasticity[41,42]. Reaction norms of the progeny for temperature and salinity variations were also analyzed. The results

Trait	Mean	Min	Max	V_g	CV_g	H^2 (*s.e.*)
S34	1.49	0.67	2.16	0.039	0.13	0.42 (0.11)
S20	0.64	0.024	1.3	0.054	0.36	0.78 (0.04)
S15	0.37	0.008	0.9	0.033	0.49	0.78 (0.05)
T13	1.56	1.17	2.42	0.014	0.08	0.20 (0.14)
T26	1.18	0.4	2.07	0.024	0.13	0.36 (0.14)
T28	0.34	0.03	0.86	0.019	0.41	0.62 (0.08)
Surv_S20	—	—	—	1.02	—	0.65 (0.09)
Surv_S15	—	—	—	1.73	—	0.76 (0.06)
Salinity	1	0.008	2.16	0.009	0.09	0.31 (0.19)
Temperature	1.03	0.03	2.42	0.002	0.04	0.1 (0.18)
Survival	—	—	—	1.42	—	0.72 (0.05)

Table 1. Trait statistics. T13, T26 and T28 correspond to growth rate phenotypes for temperatures 13 °C, 26 °C and 28 °C respectively. S34, S20 and S15 correspond to growth rate phenotypes for 34‰ salinity, 20‰ and 15‰ respectively. Surv_S15 and Surv_S20 correspond to survival trait for conditions salinity 15‰ and salinity 20‰. Salinity, Temperature and Survival correspond to the joint analysis of the three salinity conditions, the three temperature conditions and the two survival conditions respectively. V_g and CV_g corresponds to genetic variances and their coefficients of variation (CV_g expressed as $\sqrt{V_g}$/trait mean), H^2 (*s.e.*) corresponds to broad-sense heritabilities and their standard errors estimated via jackknife resampling. Min and Max are based on average values for individuals and not the raw replicate values.

showed that several reaction norms had different slopes and therefore crossed, suggesting significant G × E (Supplementary Fig. S1). We therefore used the slopes of these reaction norms as plasticity traits. There were three temperature plasticity traits (T13/T26, T13/T28 and T26/T28) and three salinity plasticity traits (S34/S20, S34/S15 and S20/S15, Fig. 1).

In total, 15 traits were tested: 3 for temperature, 3 for salinity, 3 for temperature plasticity, 3 for salinity plasticity, 2 for survival, plus sex (Fig. 1).

Broad-sense heritability estimates for growth related-traits and survival varied between 0.20 and 0.78 for individual traits and between 0.10 and 0.72 when combining all conditions of temperature and salinity (Table 1).

Construction of a ddRAD-seq-based genetic map. Barcoded ddRAD libraries were constructed for the two parents and their 89 progeny using the enzymes *Pst*I and *Hha*I and were pooled for sequencing. Of the initial total of 470 million reads, the Process_radtags module of the Stacks pipeline together with additional cleaning steps retained 339 million reads. The Ref_map process in Stacks generated 183,336 consensus sequences from this sequence data, of which 181,655 were unique, and the Genotypes program identified 11,740 putative markers within these regions. After application of a series of filters (see materials and methods), 6,275 of the 11,740 markers identified by Stacks were retained. During the filtering, nine individuals (out of the 89 progeny) with excessive missing genotypes were removed.

For the construction of the genetic map, distorted markers that did not show the expected 1:1 segregation pattern (at a threshold of 0.05%) were also filtered out. When two or more markers exhibited similar segregation patterns (based on a similarity of $\geq$0.95), only one of the markers was retained. Markers with suspect linkages were also filtered out. Finally, a total of 3,588 markers and 80 individuals were used for the construction of the genetic map. Maps obtained with R/qtl and JoinMap using this data were compared and found to be very similar.

The 3,588 SNPs were distributed over 28 linkage groups (LGs), with a minimum logarithm of the odd (LOD) score of 4.0. The total length of the map was 2,585.7 centimorgans (cM), which accounted for 98.35% of the estimated genome (Ge) length (Ge = 2629 cM). The average spacing between two adjacent markers was 0.7 cM and the largest gap was 20.3 cM (on LG28). The lengths of the 28 LGs ranged from 41.8 cM with 54 markers to 152.3 with 217 markers (Table 2, Supplementary Fig. S2 and Supplementary Dataset).

Considering the 214 Mbp genome size of *Ectocarpus* sp.[1], the estimated global recombination rate was 12.28 cM/Mb. The average recombination rate per LG varied between 11.11 cM/Mb for LG28 and 17.93 cM/Mb for LG4. At a 1 Mbp scale along the different LGs, the recombination rate varied considerably with broad patterns of decreases and increases (Supplementary Fig. S3). However, there were no substantial differences between the LGs.

Identification of QTLs for responses to temperature and salinity stresses. Combination of genotyping and phenotyping data allowed the identification of a total of 39 QTLs, with between 0 and 10 QTLs detected per trait across a total of 15 traits tested. The highest number of QTLs (10) was observed for the plasticity trait S34/S20. No QTLs were detected for the traits T13 and S34. Each QTL is associated among others with the observed phenotypic variance (R^2) and an estimated additive effect (a) that it accounts for. The detected QTLs explained 6% to 34% of the observed phenotypic variance, depending on the trait (Table 3). In some cases, QTLs for different traits mapped approximately to the same genomic region (Fig. 2). For all the temperature and salinity traits (i.e. growth ratios), and for the plasticity traits, there were both cases where the allele transmitted by the male parent was favorable (increasing the growth ratio) and cases where it was unfavorable (decreasing the

LG	Number of markers	LG length (cM)	Average spacing between markers (cM)	Maximum spacing between markers (cM)
1	217	152.3	0.7	11.2
2	194	96.5	0.5	5.3
3	202	90.4	0.4	8.5
4	180	116.7	0.7	7.6
5	168	117.9	0.7	6.7
6	171	110.4	0.6	8.8
7	170	113.4	0.7	9.3
8	152	88.7	0.6	6.3
9	155	87.8	0.6	7.6
10	138	92	0.7	8
11	120	92	0.8	10
12	127	112.4	0.9	8.9
13	124	92.6	0.8	7.6
14	124	96.7	0.8	6.3
15	119	83.1	0.7	7.8
16	106	80.7	0.8	12.8
17	111	64.9	0.6	7.6
18	116	74.8	0.7	11.5
19	107	78.5	0.7	8.7
20	112	81.8	0.7	10.1
21	87	128.9	1.5	12.8
22	103	71.4	0.7	18.3
23	91	72.4	0.8	8.8
24	90	71.6	0.8	8.8
25	96	71.9	0.8	7
26	79	112.5	1.4	16.9
27	75	91.5	1.2	19.4
28	54	41.8	0.8	20.3
overall	**3588**	**2585.7**	**0.7**	**20.3**

Table 2. Linkage group statistics for the genetic map.

growth ratio), indicating antagonistic QTLs (see values of additive effect *a* in Table 3). In contrast, for the two survival traits, the male allele was always unfavorable (lowering the survival value).

The sex of each individual was determined using PCR primers based on the coding sequence of the sex-linked gene Esi0068_0003[21,43]. Using this characteristic as a binary trait, a major sex-associated locus was mapped to LG13. This result confirmed the position of the previously identified sex-determining region[21].

Twenty-one significant epistatic interactions were detected, involving 38 unique linkage map positions. Among these interactions, only six involved previously detected QTL positions (Supplementary Table S1). For example, in the case of trait T28, the result of epistasis between LG1 and LG14 (Supplementary Fig. S4) showed that the locus on LG1 (at 6 cM) had an effect only in the presence of the B genotype (A and B genotypes derived from the male and female parents, respectively) at the locus on LG14 (at 64 cM). In the presence of the A genotype at the locus on LG14, no significant difference was observed between the growth ratio of individuals carrying the A genotype and those carrying the B genotype at the locus on LG1.

We also detected five significant QTL × sex interactions, where the effect of the locus was different for the two sexes, representing potential sexually antagonistic alleles (Supplementary Fig. S5).

Gene ontology enrichment test. A genome-wide list of candidate genes was constructed by retaining all loci in the 1.5-LOD support confidence intervals of the complete set of QTLs. This list of 562 genes included 263 genes that had associated GO terms. In the re-annotated genome of *Ectocarpus*[32], 17,418 genes were identified among which 10,688 had predicted functions and 7,383 had associated GO terms. We built a reference list, consisting of genes located in 10 kb windows around the SNPs localized on the genetic map. This gave a list of 2,710 genes among which, 1,156 had associated GO terms (Supplementary Dataset). A GO enrichment test was carried out by comparing the candidate list of 263 genes which had associated GO terms with the reference list of 1,156 genes also having associated GO terms. Six terms were significantly enriched in the candidate gene list compared with the reference at $p = 0.05$, with one term having a p-value < 0.01. Four of those 6 terms were linked to transport processes, the most significant being anion transport (Table 4, Supplementary Fig. S6 and Supplementary Dataset).

	Trait	LG	Peak (cM)	LOD	R^2 (%)	a	1.5LOD Left	1.5LOD Right	Method
Salinity	S15	2	60	3.83	23	0.110	49	64	RQTL_Scanone
		6	52.686	4.97	10	0.077	50	52.7	MapQTL_MQM
		23	12.585	3.96	9.4	−0.048	7.6	12.6	MapQTL_MQM
	S20	1	54.123	5.81	11.2	−0.075	53	55	MapQTL_MQM
		6	63.964	4.62	9.6	0.068	62	64	MapQTL_MQM
		21	74.591	4.4	10.7	0.117	70.7	76	MapQTL_MQM
		24	71.624	4.89	10.2	−0.099	68.6	71.6	MapQTL_MQM
		26	36.69	3.38	6.8	−0.090	31.4	36.7	MapQTL_MQM
		26	56.356	3.2	6.4	0.090	51.4	56.3	MapQTL_MQM
	S20/S15	7	74.093	4.18	27	−0.033	68.4	86	MapQTL_MQM
	S34/S15	2	60.456	9.43	32.1	−0.029	60	64	MapQTL_MQM
		7	109.287	3.83	11	−0.007	108	113	MapQTL_MQM
		22	47.593	4.1	10.6	0.016	44	47.6	MapQTL_MQM
		22	58.867	3.53	9.7	−0.015	50	59	MapQTL_MQM
	S34/S20	1	13.812	3.95	5.8	−0.008	11.2	13.9	MapQTL_MQM
		1	54.123	15.83	33.6	0.020	54	56	MapQTL_MQM
		4	70.291	7.9	13.3	0.018	65	70.3	MapQTL_MQM
		4	83.254	8.62	15.2	−0.022	82.3	83.3	MapQTL_MQM
		11	30.17	7.61	12.7	0.015	28.8	30.2	MapQTL_MQM
		16	0	4.93	8	0.011	0	0	MapQTL_MQM
		16	21.773	6.43	10.4	−0.013	10.8	21.8	MapQTL_MQM
		26	9.778	5.63	8.5	−0.012	8.6	11.5	MapQTL_MQM
		26	56.356	6.61	10.5	−0.016	50.5	56.4	MapQTL_MQM
		26	80.621	6.39	9.6	0.012	74.4	80.7	MapQTL_MQM
	Survival_S15	23	17	3.75	5.6	−0.010	7	37.7	RQTL_Scanone
	Survival_S20	11	91.953	6.48	13	−10.408	91.9	92.3	MapQTL_MQM
		19	50.116	3.52	6.3	−7.211	45.5	50.4	MapQTL_MQM
		22	37.558	4.6	6.7	−13.560	36.5	41	MapQTL_MQM
Temperature	T26	9	32.541	4.18	19.5	0.188	31	34	MapQTL_MQM
	T28	2	57.891	9.54	21.3	0.058	57.5	57.9	MapQTL_MQM
		4	17.573	3.65	6.8	−0.040	11.3	18.6	MapQTL_MQM
		4	91.605	13.35	33.6	0.081	91.6	94.3	MapQTL_MQM
		14	0	5.8	11.4	−0.045	0	0	MapQTL_MQM
		18	15.042	4.83	9.3	0.037	15	15.1	MapQTL_MQM
		24	37.7	3.72	7	0.014	33	63	RQTL_Scanone
	T13/T26	5	99.023	3.65	13.4	−0.010	94	101	MapQTL_MQM
	T13/T28	26	111.259	3.26	12.7	0.012	90	112	MapQTL_MQM
	T26/T28	6	63.964	4.63	18.4	0.069	63.9	64.1	MapQTL_MQM
	Sex	13	40.1	52.1	75	0.500	39	41	RQTL_Scanone

Table 3. QTL mapping results. T13, T26 and T28 correspond to growth rate phenotypes for temperatures 13 °C, 26 °C and 28 °C respectively. S34, S20 and S15 correspond to growth rate phenotypes for 34‰ salinity, 20‰ and 15‰ salinity respectively. Plasticity traits are represented by a letter followed by a number and a slash, then another letter and number. For example, S34/S20 corresponds to plasticity represented by slope of the reaction norm between the two conditions salinity 34‰ condition and salinity 20‰ condition. Surv_S15 and Surv_S20 correspond to survival trait for conditions salinity 15‰ and salinity 20‰. In the method column, MapQTL_MQM means that the QTL was detected by both MapQTL and R/qtl, RQTL_Scanone means that the QTL was only detected by R/qtl. a: estimated additive effect = (mu_A − mu_B)/2. Mu_A: the estimated mean of the distribution of the quantitative trait associated with the "a" genotype which is the genotype of the male parent. R^2: the percentage of the variance explained for by the QTL. 1.5LOD_Left and 1.5LOD_Right represent left and right limits of the 1.5-LOD support interval.

Discussion

Growth under different temperature and salinity conditions showed a distribution with the presence of transgressive segregants among the offspring in almost all cases, even when there was very little difference between the parents. Transgressive individuals may have characteristics allowing them to occupy new ecological habitats or to perform more effectively in already occupied environments[44]. These transgressive segregants are likely to be the result of complementary action of different alleles of genes from the male and female parent[45,46] and their

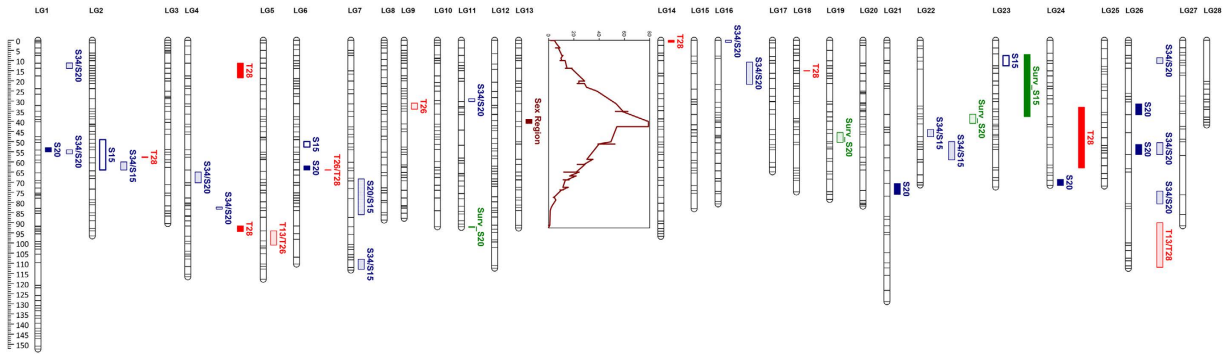

Figure 2. Genetic map showing the localization of detected QTLs for temperature, salinity and their associated plasticity and survival traits. Blue color indicates salinity and its plasticity traits, red indicates temperature and associate plasticity and green indicates survival traits. The brown color on LG13 represents the sex-determining region and the QTL LOD score curve from a Kruskal-Wallis non-parametric test in MapQTL. T13, T26 and T28 correspond to growth rate phenotypes for temperatures 13 °C, 26 °C and 28 °C respectively. S34, S20 and S15 correspond to growth rate phenotypes for 34‰, 20‰ and 15‰ salinity, respectively. Plasticity traits are represented by a letter followed by a number and a slash, then another letter and number. For example, S34/S20 corresponds to plasticity represented by the slope of the reaction norm between 34‰ and 20‰ salinity. Surv_S15 and Surv_S20 correspond to survival traits for 15‰ and 20‰ salinity, respectively.

	T13	T26	T28	S34	S20
T26	0.095				
T28	−0.017	0.003			
S34	0.038	0.217*	−0.05		
S20	−0.2	−0.26*	0.163	0.025	
S15	0.149	0.061	0.202	0.024	0.531****

Table 4. Pearson's correlation coefficients between main studied traits. See Table 2 for trait details. *Significant at 0.05. ****Significance <0.0001.

presence provides the potential for improvement of the traits using selective breeding or as a result of natural selection.

The two parents of the mapping family originated from the west coast of South America, between the south of Peru and the north of Chile, representing about 2,000 km of coastal ecosystem where seasonal variation in seawater temperature is high (between 13 °C in winter and 25 °C in summer on average, Fig. 3). The parental strains are therefore presumably adapted to living in a changing environment. The annual average temperature of the original location of the male parent is lower than that of the female parent (Fig. 3). And it is noteworthy that it was only at the highest temperature that the female parent performed better than the male one. This may be explained by the mean temperatures at the parental locations, but since 28 °C is not a common mean temperature encountered there, this is rather speculative. Nevertheless, their occurrence in rockpools or low tides during summer months can expose these *Ectocarpus* strains to very high temperatures. Furthermore, the average seasonal temperature of seawater in the distribution zone of the species can increase by 4 °C to 10 °C during *El Niño*-Southern Oscillation events[47].

The distributions of growth ratios varied little at 13 °C and 26 °C but significantly decreased at 28 °C. Even at 28 °C, the majority of the offspring could maintain non-null growth. A temperature of 30 °C may exceed the tolerance of this family as observed during preliminary tests, at least under our laboratory conditions, but it is possible that the response of the population to extreme temperatures would show some differences under field conditions compared to the observations in laboratory conditions, particularly because several stressors act in synergy in the wild.

For salinity stress, the female parent always performed better than the male parent. Typical salinity of seawater in the distribution range of the species is 34–36‰. However, our data showed that several strains were able to maintain active growth even at 15‰. Although most of the species of the *Ectocarpus* genera are predominantly marine, some strains (e.g. *E. subulatus* isolates) are tolerant to low salinity (i.e. occur in estuaries) or are even adapted to a freshwater environment[31,48]. The maintenance of a capacity to respond to large variations in water salinity in the extremely arid region of the Atacama Desert, where there are no estuaries, is likely a result of an adaptive evolution in other regions of the coast. Interestingly, this low salinity tolerance in *Ectocarpus* species may explain why there are cases of colonization of freshwater environments, albeit rare. Dittami *et al.*[31] suggested that, while high stress tolerance and plasticity may be prerequisites for the colonization of freshwater, genomic alterations are likely to have occurred and these alterations would have produced permanent changes in metabolite profiles to stabilize the transition.

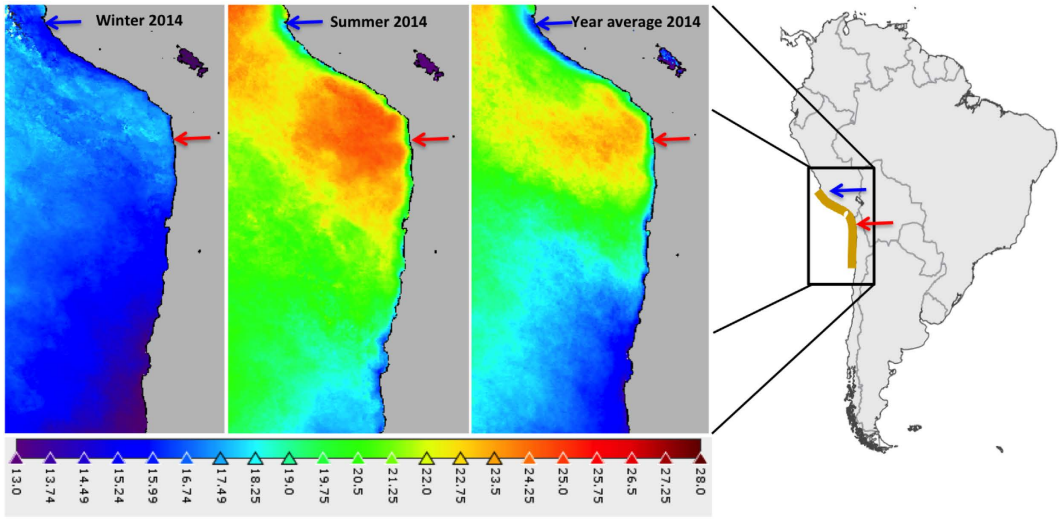

Figure 3. Sea surface temperature (sst) in the distribution range of *Ectocarpus* sp. for year 2014. The blue arrow represents the original location of the male parent (San Juan de Marcona, Peru) and the red arrow represents the original location of the female parent (Arica, Chile). Created with the SeaWiFS Data Analysis System (SeaDAS) visualization and data processing software version 7.2, using seasonal (winter and summer) and annual MODIS sst data from 2014: http://seadas.gsfc.nasa.gov/installers/; http://oceandata.sci.gsfc.nasa.gov/MODIS-Aqua/Mapped. NASA Goddard Space Flight Center, Ocean Biology Processing Group; (2014): Sea-viewing Wide Field-of-view Sensor (SeaWiFS) Ocean Color Data, NASA OB.DAAC, Greenbelt, MD, USA. http://doi.org/10.5067/ORBVIEW-2/SEAWIFS_OC.2014.0. Accessed 2015/05/11. Maintained by NASA Ocean Biology Distibuted Active Archive Center (OB.DAAC), Goddard Space Flight Center, Greenbelt, MD, USA.

Broad-sense heritability estimates were low to moderate but increased with the degree of stress for the environmental conditions we tested (from 0.20 to 0.78). The heritability estimates were lower when temperature and salinity stress were combined (0.10 and 0.31). It is well known that estimated heritability of a trait can vary either with environmental conditions or between populations. Hoffmann and Merilä[49] summarized hypotheses to explain the relationship between environmental effects and heritability. It is possible that phenotypic variations between genotypes would be difficult to detect unless resources become limiting, regardless of the history of selection[50]. In contrast, unfavorable conditions could also decrease the importance of observed genetic variation if increased environmental variance reduces the adaptive value of individual genotypic combinations. This might apply to our study, when we combined stress conditions for temperature and for salinity, hence increasing the overall environmental heterogeneity. Furthermore, although we were able to replicate genotypes in this studied species, traits are nonetheless measured with some error and this might lead to an underestimate of heritability if phenotypic variance is higher under unfavorable conditions[51].

With 3,588 SNP markers, the genetic map described here is considerably denser than the previously published map for *Ectocarpus* sp., which was based on 406 microsatellite markers[20]. Overall, the distribution of the markers on the 28 LG was homogenous. The 28 LGs detected here are coherent with cytogenetic studies carried out using European strains of *E. siliculosus* that indicated an approximate number of 25 chromosomes[52].

Assignation of the SNP markers to sequence scaffolds allowed 500 of the 1,561 scaffolds of the genome sequence to be anchored onto linkage groups, significantly improving the large-scale assembly of the genome[32]. Integration of this new information along with the data from the microsatellite-based linkage map[20] has allowed a total of 531 scaffolds to be anchored onto linkage groups, corresponding to 177 Mbp of sequence data and 90.5% of the total genome. The improved genetic map will represent an important resource for future genetic and comparative genomic studies using *Ectocarpus* sp. as a model species.

The quality of a genetic map is influenced by the density of the markers used and the size of the mapping family. As far as marker density is concerned, next generation sequencing technologies represent a means to tremendously improve the generation of genetic markers and the ddRAD sequencing approach used in this study produced enough markers to cover the major part of the genome. However, the mapping family is a "pseudo F2" haploid population where each individual is derived from a separate but unique meiosis. This, plus the relatively small size of the family, constitute factors that may limit the power of QTL detection[53].

We estimated a global recombination rate of 12.28 cM/Mb for *Ectocarpus* sp., an estimate that is higher than values obtained for most terrestrial plant species[54]. If we examine data available for *Saccharina japonica*[55,56], the only other brown algal species for which both a genetic map and a complete genome sequence currently exist, the global recombination rate is 4.93 cM/Mb. This value is more similar to those observed for angiosperms. The genome size of *Saccharina japonica* is 537 cM[56], which is 2.5 times larger than that of *Ectocarpus* sp. These estimates are in accordance with the observation of negative correlation between global recombination rate and genome size, pointing to a putative role of LTR retrotransposons[54]. More estimates are needed for additional brown algae to provide improved insights into global recombination rates in this group. In any case, recombination is a very complex feature because it is known to vary substantially across eukaryote genomes and even