GO ID	Term	Annotated	Significant	Expected	Rank in classic	p-value classic
GO:0015698	inorganic anion transport	3	2	0.15	1	0.007
GO:0006820	anion transport	4	2	0.2	2	0.014
GO:0015031	protein transport	22	4	1.1	3	0.02
GO:0008104	protein localization	24	4	1.2	5	0.027
GO:0006396	RNA processing	6	2	0.3	8	0.032
GO:0006886	intracellular protein transport	17	3	0.85	9	0.048
GO:0006605	protein targeting	8	2	0.4	12	0.056
GO:0072594	establishment of protein localization	8	2	0.4	13	0.056
GO:1902580	single-organism cellular localization	9	2	0.45	15	0.07
GO:0006811	ion transport	21	3	1.05	16	0.082
GO:0016482	cytoplasmic transport	11	2	0.55	17	0.1
GO:0006810	transport	103	8	5.14	22	0.128
GO:0051234	establishment of localization	104	8	5.19	23	0.133
GO:0009148	pyrimidine nucleoside triphosphate biosynthesis	3	1	0.15	25	0.142
GO:0043094	cellular metabolic compound salvage	3	1	0.15	26	0.142
GO:0007005	mitochondrion organization	3	1	0.15	24	0.142
GO:0044743	intracellular protein transmembrane importation	3	1	0.15	27	0.142
GO:0009147	pyrimidine nucleoside triphosphate metabolism	3	1	0.15	28	0.142
GO:0017038	protein import	3	1	0.15	29	0.142
GO:0051179	localization	106	8	5.29	32	0.144

Table 5. List of the top 20 GO terms identified by the test for GO term enrichment in genes located within the QTL intervals identified in this study. See materials and methods for details.

between individuals within populations, for reasons that are still to be fully understood[57]. In our study, recombination rates varied along and between the different *Ectocarpus* sp. LGs (average rate per LG between 11.11 cM/Mb for LG28 and 17.93 cM/Mb for LG4), at a 1 Mb scale. Although no clear differential patterns between the LGs were observed, this result should be considered with caution for several reasons. First, resolution of cross-based estimates of recombination rates like those we provide here depends on the number of recombination events occurring in a generation, and the generation of the family analyzed here involved only one meiosis per individual. Second, because the scale at which the recombination rates are examined may influence the detected relationships between recombination and genomic features and, consequently, patterns at coarse scales may not accurately reflect relationships at finer scales[58].

Our experimental setup allowed us to detect 38 QTLs for growth-related traits and their plasticity under different temperature and salinity conditions and 1 QTL for sex. Eighteen of the 28 LGs contained QTLs for these traits. Although several of the QTLs had large confidence intervals, some showed relatively small ones. For example, the QTLs for T28 on LG2 and LG18 had small confidence intervals, which contained respectively 41 and 6 annotated or predicted genes. There was only one case of co-localization of QTLs (for temperature and salinity on LG2 for traits T28 and S15). The rarity of co-localization of QTLs was in agreement with the low to moderate correlation between traits, with most correlations being statistically non-significant (Table 5). More co-localizations were observed when survival and plasticity traits were considered. In three cases (LG1, LG2 and LG26), plasticity QTLs co-localized with main traits. These co-localizations could be due to the presence of genes that control metabolic pathways that influence multiple traits or to the presence of pleiotropic genes.

Phenotypic plasticity is one of the strategies used by organisms to cope with variability in their environment but its genetic basis is often poorly documented[59]. Plasticity often involves altering gene expression and physiology in response to environmental cues[60]. The QTL approach developed here could allow evolutionary questions such as the evolution of the reaction norm as a response to the changing environment to be addressed. The detection of plasticity QTLs in *Ectocarpus* sp. indicates that factors controlling plasticity in this species are likely to be genetically-based[41]. We also identified several antagonistic QTLs (i.e. QTLs with alleles that have opposing effects). It is thought that, in such cases, trait differences may have arisen through drift and/or through selection that fluctuates in direction[46,61]. Antagonistic QTLs are also considered to be able to generate transgressive segregants[46], which is in accordance with our results.

Epistasis is considered to be an important genetic basis of complex phenotypes[62,63]. More than one trait was involved in all the 21 cases of epistatic interactions detected in this study, suggesting pleiotropy not only for single loci but also for epistatic effects. Among those 21 epistatic interactions, we observed only six cases where one of the markers had a main QTL effect on the trait. This suggests that epistasis is an important mechanism for the control of the response to stress in *Ectocarpus* sp., at least when growth ratio is used as a proxy for fitness.

Few QTL × sex interactions were identified in our study. These interactions were sex-antagonistic since the effects of the QTL alleles were opposite for the two sexes. Such interactions indicate that the effect of a QTL is different between the two sexes and this can be important in evolutionary as well as in breeding contexts. Evolutionary arguments implicate QTL × sex interactions in the maintenance of variation for complex traits[64]. From a biological point of view, QTL × sex interactions can be explained by invoking interactions between genes with different effects in different sexual environments (considering sexes as different environments and hence

QTL × sex interactions to be QTL × environment interactions)[65]. However, we cannot exclude the possibility that the observed QTL × sex interactions observed in our study may just reflect the different origins of the parental strains and their specific trait inheritance, since we cannot separate the effects of their sex and their origin. The detection of main effects, epistasis and sexually-dimorphic QTLs suggest complex genetic regulation of growth under stress conditions.

A GO enrichment test identified six terms significantly enriched in putative genes lying in the QTL intervals, among which, four were linked to ion and protein transport processes. Of the 562 putative genes in the QTL intervals, 102 were also identified as being significantly differentially expressed between low and high salinity by Dittami *et al.*[31] (Supplementary Dataset), but more interestingly, the authors also observed an accumulation of intracellular ions at different levels between freshwater and marine strains, suggesting an important role for ion transport in the adaptation to low salinities. The list of putative stress-related genes corresponding to the QTL intervals identified in the current study should be treated with caution because there are uncertainties linked to positions of QTLs and because growth is a complex phenotype integrating numerous metabolic pathways. Taking into account these limitations, the data presented here is however in accordance both with the suggested importance of intracellular ion composition and osmotic balance in adaptation to stresses such as low salinity and with the large number of ion channels observed in the genome of the species[1,31]. Furthermore, transport processes such as ion transport are well studied in land plants and have been shown to be involved in the response to osmotic stresses[16,66]. In seagrasses, several genes have been also shown to respond to elevated sea surface temperatures[67] or to be under selection along the salinity gradient of the intertidal shore[68].

In our study, we built a high-density genetic map using ddRAD-seq approaches and detected several QTLs for growth rate under high temperature and low salinity stresses as well as related plasticity and survival traits. GO enrichment tests suggested a role for ion transport genes among the main loci controlling the QTLs. This study represents a significant step towards deciphering the genetic architecture of adaptation of *Ectocarpus* sp. to stress conditions and, furthermore, adds a substantial resource to the increasing list of resources generated for the species. It also opens new perspectives in population genomics of adaptation in brown algal species. As mentioned in the introduction, brown algae have also been shown to display important interactions with complex bacterial communities, which influence their ability to respond to environmental stressors and this aspect makes the picture more complex. Several studies have shown that algal-bacterial associations are beneficial for the algal hosts, increasing their fitness[69,70] and some brown algal species such as *Ectocarpus fasciculatus* and *Pylaiella littoralis* are absolutely dependent on their associated bacteria[71,72]. These complex interactions were not included in this study but should be examined in future studies in order to have a more complete understanding of the adaptation of *Ectocarpus* sp. to its stressful environments.

Materials and Methods

Mapping family. The mapping family was generated by crossing the male *Ectocarpus* sp. strain Ec 32 (whose genome has been fully sequenced) with a compatible female strain, Ec 568. See Heesch *et al.*[20] for a detailed description of the production of the family. Briefly, a single F1 hybrid sporophyte isolated from the cross (Ec 569) was used to produce a family of sibling haploid gametophytes, each individual being derived from a separate meiotic event. Gametes from each individual were then allowed to germinate parthenogenetically to produce haploid partheno-sporophytes. Eighty-nine progeny of this haploid family, plus haploid partheno-sporophytes derived from the parental gametophytes, were cultivated for the experiments.

Culture conditions and phenotyping. For both the temperature and salinity stress experiments, preliminary tests were carried out to determine conditions that resulted in phenotypic differences without causing extreme mortality. Three temperature conditions were selected: 13 °C (control condition) and two higher stress temperatures, 26 °C and 28 °C. Similarly, three salinity conditions were also selected: 34‰ (normal sea salinity) as the control and two low salinity conditions, 20‰ and 15‰. For each strain, 6 replicates were used and individuals were randomly distributed in 6-well plates containing 6 ml of autoclaved, Provasoli-enriched seawater. Natural seawater was used for temperature experiments (salinity is stable at around 33.5‰ to 34.5‰) while artificial seawater was used for salinity experiments. Note that differences might exist between natural and artificial seawater regarding nutrients. Experiments were carried out in growth chambers and the positions of the plates were permutated daily to reduce microenvironment effects. Light conditions were 12 h/12 h day/night (~2000 lux light intensity) and the temperature in the growth chamber was set to 13 °C for the salinity experiment. The plates were maintained in the growth chambers for 12 days. Each individual was photographed under a binocular microscope before and after the experiment and thallus area was measured using the program ImageJ. Growth was estimated as the ratio of the thallus area on day 12 to the thallus area on day 0. By treating different experimental conditions as different environments, we determined reaction norms of the mapping progeny and used the slopes of these reaction norms as plasticity traits. The relation of these plasticity traits to specific environmental variations was indicated by giving the two environmental conditions separated by a slash mark (e.g.: S34/S20 is the plasticity trait between salinity conditions 34‰ and 20‰).

DNA extraction and generation of RAD-seq data. For DNA extraction, the haploid family together with the parental lines were grown in a growth chamber in sterile seawater medium for 6 weeks under a 12-hour light photoperiod and 13 °C. As the cultures were not fully axenic, 5 ml of a mix of antibiotics (9 mg/ml of Penicillin G, 4.5 mg/ml of Streptomycin and 0.9 mg/ml of chloramphenicol) were added to each liter of seawater used for the culture to reduce the bacterial load. Harvested individuals were frozen and lyophilized, and DNA was extracted using the NucleoSpin® 96 Plant II kit (Macherey-Nagel GmbH & Co. KG, Germany), according to the manufacturer's instructions. DNA quality was checked on agarose gels and the quantity measured by PicoGreen® (Fisher Scientific).

Preparation of the double digest RAD library (ddRAD-seq) was carried out following Peterson *et al.*[40]. The genomic DNA was digested with the restriction enzymes *Pst*I and *Hha*I (New England Biolabs, https://www.neb.com/), which had been selected based on *in silico* digestion simulations, and adapters were ligated using the T4 DNA ligase (New England Biolabs, https://www.neb.com/). After pooling the samples which had been individually barcoded with a unique adapter and cleaning with AMPure XP beads (Beckman Coulter Genomics), size selection (between 150 and 600 bp) was carried out with a Pippin-Prep kit (Sage Science, Beverly, MA, USA). A PCR amplification was then carried out using the Q5® hot Start High-Fidelity DNA polymerase kit (New England Biolabs) to increase the concentration of the libraries and to add Illumina flowcell annealing sequences, multiplexing indices and sequencing primer annealing regions, to all fragments. Compared with the original protocol, the barcodes were modified by adding an extra nucleotide, extending to six nucleotides (see Supplementary Dataset). Both an Agilent® 2100 Bioanalyzer® (Agilent Technologies) and qPCR were used to quantify the libraries generated from each individual. Libraries with distinct multiplexing indices were then combined in equimolar ratios to compose a final pool of libraries for sequencing. An Illumina Hiseq 2500 platform (Rapid Run Mode) was used to generate a total of 470 million (48.5 Go) high quality (Q30 = 82%) 100 bp paired-end sequence reads at a cluster density of 850 K/mm^2 (HiSeq Rapid SBS Kit v2, 200 cycles). As a test, a subset of the library was also sequenced with Illumina MiSeq, providing 93 million additional reads (MiSeq Reagent Kit v3, 150 cycles) The raw sequence data in fastq format are stored in the Sequence Read Archive (SRA) at National Center for Biotechnology Information (NCBI) and can be accessed at NCBI homepage (https://www.ncbi.nlm.nih.gov/; bioproject accession PRJNA371840 or study accession SRP099384).

Analysis of ddRAD-seq data. The ddRAD-seq sequencing data was analyzed using the Stacks pipeline (version 1.40)[73]. Raw sequence reads were checked for intact barcodes and the restriction enzyme sites. A sliding window of 25% of the length of a read was set to check sequence quality and reads with <90% base call accuracy were discarded. Because Stacks removes whole reads when adapter contamination is detected, we first used the program Cutadapt (version 1.8.3) to check for the presence of adapters and cut only adapter sequences. Paired-end sequencing of shorter fragments generated overlapping reads that were treated with the program PEAR (version 0.9.10)[74]. PEAR identifies paired-end non-overlapping reads and generates a single consensus sequence from overlapping read pairs. These single consensus sequences were added to the singleton rem1 and rem2 sequences produced by Stacks for a unique group of singleton sequences. Paired-end reads as well as singletons were then trimmed to 95 bp with the program TRIMMOMATIC (version 0.32)[75]. The paired-end sequences and the singletons were then aligned to the *Ectocarpus* reference genome[1] using Bowtie 2 (version 0.12.9)[76]. Output aligned .sam files were subsequently imported in to the Stacks pipeline and the final Genotypes program exported haplotypes encoded as genotypes at the different loci.

Construction of the genetic map. After Stacks analysis, genotypes were exported in a generic format that was imported into Excel for filtering. As we used a haploid population, only aa/bb segregation was expected. All non-expected segregation patterns, as well as loci with log likelihood values below −30, were removed. Loci with over 25% of missing data were also removed. In the finale matrix (see Supplementary Dataset), A genotypes derived from the male parent and B genotypes derived from the female parent. The R package R/qtl (version 1.39–5)[77] was used for the construction of the genetic map, in conjunction with either R/ASMap (version 0.4–7) or JoinMap® version 4.1. At this stage, individuals with more than 40% of missing genotypes were removed. Further filtering was done within the mapping programs. Markers that showed significant segregation distortion ($\leq$5%) were removed. Recombination fraction estimates were used to correct erroneous genotypes or switched alleles. Markers were also filtered based on their similarity, on whether they were located on the same contig and based on suspect linkage patterns. In JoinMap, the maximum likelihood mapping algorithm was applied.

Estimated genome (Ge) length was obtained by adding 2 s (s being the average spacing between markers along the LGs) to the length of each LG to account for chromosome ends beyond the terminal markers of each LG[78]. The map coverage was then estimated as the ratio between the observed map length and Ge.

The R/xoi package (version 0.66–9) was used to obtain a smoothed estimate of the recombination rate along the LGs, in 1 Mbp sliding windows.

Statistical analyses of phenotypic data. Statistical treatments were done in R. BLUPs were obtained by fitting several linear mixed models (LMM) with the R package lme4 (version 1.1–12) and selecting the optimal model with AIC:

$$Y_{ij} = \mu + G_i + R_j + \varepsilon_{ij} \quad (1)$$

where Y_{ij} represents the value of the trait under investigation for the genotype i at replicate j, μ the general mean of the trait, G_i the random effect of the genotype i, R_j the random effect of the replicate j and ε_{ij} the random residual effect.

Broad-sense heritability of individual traits was calculated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_\varepsilon^2}{J}} \quad (2)$$

where σ_G^2 is the genetic variance, σ_ε^2 the environmental variance and J the number of replicates.

Some strains did not survive at salinities of 15‰ and 20‰ and hence we introduced a binomial survival trait. The survival traits were analyzed by fitting generalized linear mixed-effects models (GLMM) and using $\frac{\pi^2}{3}$ as an approximate to the environmental variance as suggested by Nakagawa and Schielzeth[79].

The different temperature, salinity or survival conditions were also combined as a single phenotype (temperature, salinity or survival). Variances and broad-sense heritabilities were obtained by fitting LMMs or GLMMs and selecting the optimal model:

$$Y_{ijk} = \mu + G_i + R_j + E_k + GE_{ik} + \varepsilon_{ijk} \quad (3)$$

where E_k is the fixed effect of environment (different conditions of temperature, salinity or survival) and G_{ik} the random interaction effect between genotype *i* and environment *k*. The sex of each strain was included in the data. Broad-sense heritability of individual traits was calculated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{K} + \frac{\sigma_\varepsilon^2}{JK}} \quad (4)$$

where σ_{GE}^2 is the G × E interaction variance, *K* the number of environments and *J* the number of replicates.

QTL mapping. QTL mapping was performed using the R package R/qtl[77] as well as MapQTL® version 5. In R/qtl, the scanone function was used to perform interval mapping with the Haley-Knott regression method, including sex as a cofactor. 5% significance thresholds of the LOD scores were obtained by 5,000 permutations. In MapQTL, composite interval mapping was performed with the MQM method. The method uses markers as cofactors in an approximate multiple-QTL model with additive and dominant gene actions. Cofactors were selected in a first round as loci with LOD ≥2.5 after a simple interval mapping. Then this list was extended with markers on a given linkage group selected every 10 or 20 cM and optimized via the automatic cofactor selection method. The method uses a backward elimination procedure to determine which markers show a significant association and which do not, and ultimately produces a list of significant cofactor markers. For survival traits and sex, the "binary" or the "nonparametric" models were applied. BLUP values were used for QTL mapping. For QTL confidence intervals, 1.5-LOD supports were used. Mapchart 2.3[80] was used to draw the QTL on the linkage map.

QTL × sex interactions were detected by using "sex" as a covariate. QTL × QTL additive epistatic interactions were detected by the scantwo function of R/qtl, performed by the Haley-Knott regression. Four models were used for this: a full model where the two QTLs are allowed to interact, an additive model where the QTLs are assumed to act additively, a single-QTL model, which corresponds to the result of the single-QTL genome scan and a null model assuming no QTL[81].

Gene ontology enrichment test. Taking advantage of the fact that the genome of the species has been sequenced and carefully annotated, we tested GO enrichment among the putative genes within the QTL intervals. For that, we made two lists: a candidate list consisting of all loci in the 1.5-LOD support confidence intervals of the QTLs, based on the sequenced reference genome and a reference list including all the putative genes in the mapped scaffolds, refining the search only on a 10 Kb window around each SNP on the genetic map. Only loci with clear GO identifications were retained from the two lists. The candidate list was then compared to the reference list using the R/topGO package (version 2.26.0), which is designed to facilitate semi-automated enrichment analysis for GO terms. The test was based on gene count and used the "classic" algorithm with the "Fisher" statistic.

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Author Contributions

S.M.C., J.M.C., M.V. and P.B. conceived the study, managed its organization and edited the draft versions of the manuscript. K.A. performed research (all the different steps), analyzed data and wrote the first draft of the manuscript, F.L. performed research (preliminary tests and phenotyping of the mapping family), S.M. performed research (the ddRAD-seq experiment), G.M. analyzed data for his master's internship, S.F. edited the manuscript, S.M.C. and A.C. analyzed data. All authors discussed the results, commented on the manuscript, read and approved the final manuscript.

Additional Information

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