Supporting Information

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SI Materials and Methods

Biological Material. Three of the *Ectocarpus* strains used in this study were offspring of a field sporophyte collected in 1988 in San Juan de Marcona, Peru (Ec 17). These strains were Ec 32 (wild-type male), Ec 25 (wild-type female), and Ec 137 (male individual carrying the spontaneous *immediate upright* mutation; ref. 1). Ec 32 is the strain for which a complete genome sequence is available (2). The *immediate upright* mutation was introgressed into a female background by crossing with Ec 25 and isolating meiotic offspring from the resulting sporophyte Ec 372 (1). The female *imm* strain was designated Ec 419. The *ouroboros* mutant strain (Ec 494) was produced by UV mutagenesis of strain Ec 32 (see below). Ec 494 was back-crossed with Ec 25, resulting in sporophytes heterozygous for *oro*, which in turn produced a generation of gametophytes that carried *oro.* These gametophytes should have had a reduced level of any additional substitutions introduced during the mutagenesis. These gametophytes were either female (e.g., Ec 560) or male. One of the male strains (Ec 561) was used for a cross with a genetically more distantly related female strain [Ec 568, meiotic offspring of a field sporophyte (Ec 721) from Arica, northern Chile (3)]. Note that offspring derived by asexual, clonal propagation (e.g., via mitospores or parthenogenetic regeneration of gametes) were considered to be identical to their parents and retained the same strain number. For further details, see Table S6.

Cultivation, crossing, raising of sporophytes from zygotes, isolation of meiotic families, and sexing of gametophyte strains were carried out as described (3, 4). Day length was 10-h light: 14-h dark. For the study of germination, spores or gametes were allowed to settle on coverslips. Zygotes were produced and isolated as described by Peters et al. (4). The sex of gametophytes was determined by microscopic observation of zygote formation in hanging-drop preparations (5) in which the strains to be tested had been combined with fertile thalli of male and female reference strains. Genetic analysis of meiotic offspring followed (6).

Photopolarization Tests. Gametophytes and sporophytes were grown at low density in 5-cm (7-8 mL) Petri dishes under unidirectional white light. The orientation of germination was scored ($n > 300$) according to four quadrants (i.e., toward the light, away from the light, or in one of the two quadrants perpendicular to the light). Algae that germinated into the quadrant away from the light were scored as exhibiting negative phototrophy.

Microarray Analysis of mRNA Abundances. The microarray used to evaluate mRNA abundance in wild-type and mutant algae has been described (7). The microarray is based on 17,119 contig and singleton sequences derived from 90,637 EST sequences that have been mapped to 10,600 of the 16,256 genes identified in the *Ectocarpus* genome (2).

To prepare material for the microarray analysis, the *imm* and *oro* mutants were crossed, and one of the resulting diploid sporophytes was used to produce a segregating population of 269 gametophytes. Parthenotes were derived from these gametophytes by gamete germination and were classified into four groups based on morphological phenotype during early development: wild-type, *imm* mutants, *oro* mutants, and *imm oro* double mutants. RNA was extracted in triplicate (three biological replicates) from 12 bulked segregants from each of these groups and from wild-type gametophyte material (Ec 32). The parthenotes were allowed to fully develop vegetatively before harvesting, with many individuals carrying plurilocular zoidangia (i.e., either sporangia or gametangia depending on the life cycle generation). Partheno-sporophytes were harvested before they produced unilocular sporangia. Bulked segregatants were used to minimize variation due to unlinked polymorphic loci that may have been segregating in the population. RNA was extracted from ~300 mg (wet weight) of tissue following a modified version (1) of the protocol described by Apt et al. (8). Briefly, this protocol involved extraction with a cetyltrimethylammonium bromide (CTAB)-based buffer and subsequent phenol-chloroform purification, LiCl-precipitation, and DNase digestion (Turbo DNase, Ambion) steps. RNA quality and concentration was then analyzed on 1.5% agarose gel stained with ethidium bromide and a NanoDrop ND-1000 spectrophotometer (NanoDrop Products).

Double-stranded cDNA was synthesized and amplified with the SMART cDNA synthesis kit (Clontech) and the M-MuLV reverse transcriptase (Finnzymes) starting from 100 ng of total RNA. The PCR amplification was optimized for each sample so that the minimum number of cycles necessary to produce good-quality cDNA (between 19 and 27 depending on the sample) was used. After phenol:chloroform:isoamyl alcohol (25:24:1) extraction and ethanol precipitation, the cDNA was resuspended in 14 μL of water. cDNA samples used for microarray hybridizations were required to fulfill the following quality criteria: concentration > 250 μg l-1, A260/280 ≥ 1.7, A260/230 ≥ 1.5, median size ≥ 400 bp. Hybridizations were carried out as described (7), and statistical analysis was carried out using the Statistical Analysis of Microarrays (SAM) method (9) in the TIGR MeV package (Version 3.1). Gene Ontology (GO) annotation was used to assign genes to functional categories (http://www.geneontology.org). To identify general trends, the GO annotation was converted into GO slim annotation.

To carry out a global cluster analysis, a one-way ANOVA test (with *P* values adjusted using the Benjamini Hochberg method) was carried out to identify genes that exhibited significant variances in transcript abundance across the five sample classes. The 4,046 genes identified by this test were then grouped into four clusters of 1,352; 1,262; 1,032; and 400 genes using uncentered Pearson correlation distance and Ward linkage. These clusters included 230, 207, 136, and 56 genes with GO annotations, respectively. To identify overrepresented or underrepresented categories of gene function, the GO annotations were converted to GO slim annotations (using GOslim_pir; http://www.geneontology.org/GO.slims.shtml) and, for each cluster, the abundance of each category was compared with its abundance in the genome as a whole.

qRT-PCR Analysis of mRNA Abundances. Total RNA was extracted from three biological replicates of wild-type, *imm* mutant, *oro* mutant, and *oro imm* double mutant parthenotes and from wild-type gametophytes using a protocol adapted from ref. 8. The samples used for qRT-PCR analysis were not the same as those analyzed in the microarray experiment. Contaminating genomic DNA was eliminated by DNase treatment by using the TURBO DNA-free kit (Applied Biosystems). The concentration and quality of the RNA was determined by spectrophotometry and agarose gel electrophoresis. Between 0.2 and 2.0 μg of total RNA was reverse-transcribed to produce cDNA using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Real-time qRT-PCR was performed on RNA from pooled samples (10 different strains for each phenotype, in triplicate).

Primer pairs were designed using the Primer3 software (http://frodo.wi.mit.edu/primer3/) so that the amplified fragment corresponded to the 3′UTR region or the most 3′ exon of the gene

being analyzed (Table S7). Primer parameters were as follows: amplicon size 80–180 bp, primer length between 18 and 24 nucleotides (optimum 20 nucleotides), Tm between 59 °C and 61 °C (optimum 60 °C), %GC between 30% and 70% (optimum 50%). The specificity of the primers was tested both by comparing with the *Ectocarpus* genome and cDNA database sequence using Blast and by carrying out *in silico* PCR simulations using the e-PCR program (10).

qRT-PCR was carried out using the ABsolute QPCR SYBR Green ROX Mix (ThermoScientific) in a Chromo4 thermocycler (Bio-Rad Laboratories), and data were analyzed with the Opticon monitor 3 software (Bio-Rad Laboratories). The specificity of amplification was checked using a dissociation curve. The amplification efficiency was tested using a genomic dilution series and was always between 90% and 110%. To allow quantification, a standard curve was established for each gene by using a range of dilutions of *Ectocarpus* genomic DNA (between 80 and 199,600 copies), and expression level was normalized against the *EF1α* reference gene (11). Two technical replicates were carried out for the standard curves and three technical replicates for the samples. The data shown correspond to mean ± SE for three biological replicates.

1. Peters AF, et al. (2008) Life-cycle-generation-specific developmental processes are modified in the *immediate upright* mutant of the brown alga *Ectocarpus siliculosus*. *Development* 135:1503–1512.
2. Cock JM, et al. (2010) The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* 465:617–621.
3. Peters AF, et al. (2010) Genetic diversity of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Peru and northern Chile, the area of origin of the genome-sequenced strain. *New Phytol* 188:30–41.
4. Peters AF, Scornet D, Müller DG, Kloareg B, Cock JM (2004) Inheritance of organelles in artificial hybrids of the isogamous multicellular chromist alga *Ectocarpus siliculosus* (Phaeophyceae). *Eur J Phycol* 39:235–242.
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8. Apt KE, Clendennen SK, Powers DA, Grossman AR (1995) The gene family encoding the fucoxanthin chlorophyll proteins from the brown alga *Macrocystis pyrifera*. *Mol Gen Genet* 246:455–464.
9. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98:5116–5121.
10. Schuler GD (1997) Sequence mapping by electronic PCR. *Genome Res* 7:541–550.
11. Le Bail A, et al. (2008) Normalisation genes for expression analyses in the brown alga model *Ectocarpus siliculosus*. *BMC Mol Biol* 9:75.

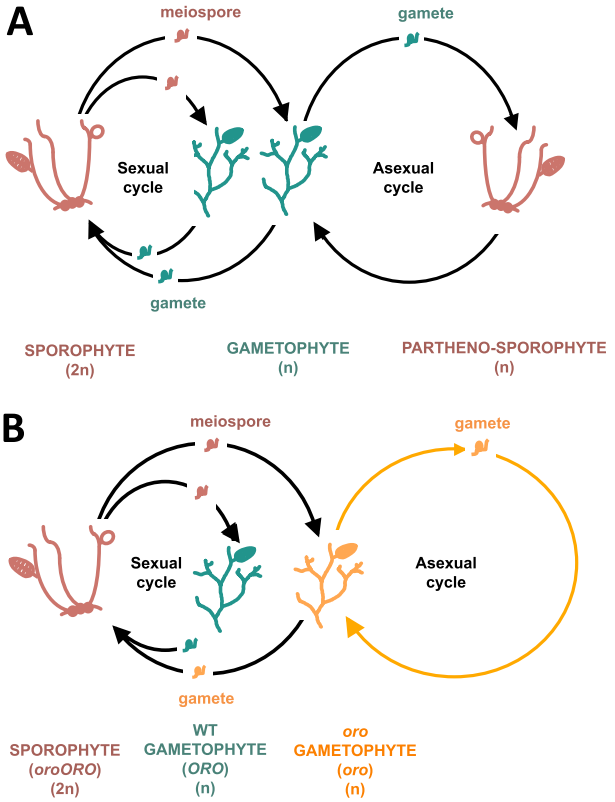

Fig. S1. Comparison of life cycle transitions in wild-type *Ectocarpus* (*A*) and in the *oro* mutant (*B*).

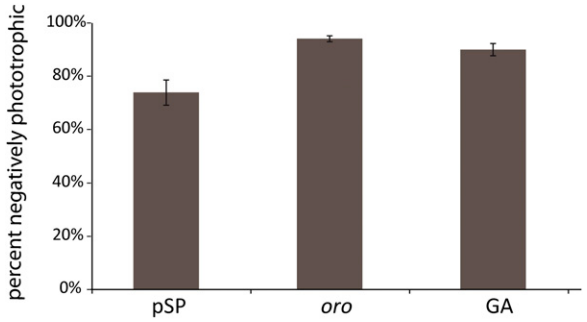

Fig. S2. Photopolarization of *oro* and wild-type *Ectocarpus* germlings in response to unidirectional light. The negative phototropic responses of wild-type gametophytes and of *oro* parthenotes were significantly more marked that those of the wild-type sporophytes. No significant difference was found between the responses of *oro* and wild-type gametophytes. pSP, wild-type partheno-sporophyte; GA, wild-type gametophyte. Error bars show SDs.

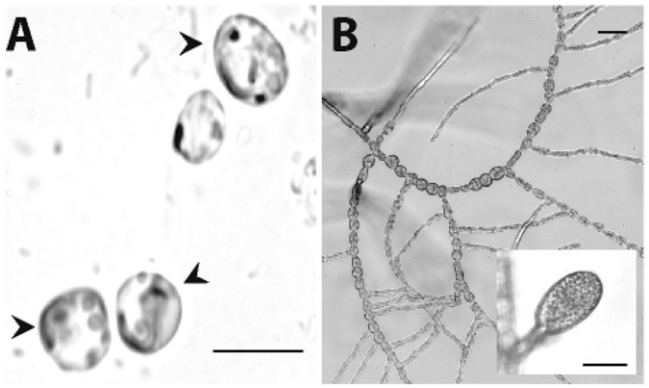

Fig. S3. Sporophyte produced by crossing an *oro* and a wild-type strain. (*A*) Fusion of gametes produces zygotes (arrowheads). (*B*) Zygotes grow into diploid sporophytes, which develop unilocular sporangia (*Inset*) where meiosis takes place. (Scale bars: 10 μm.)

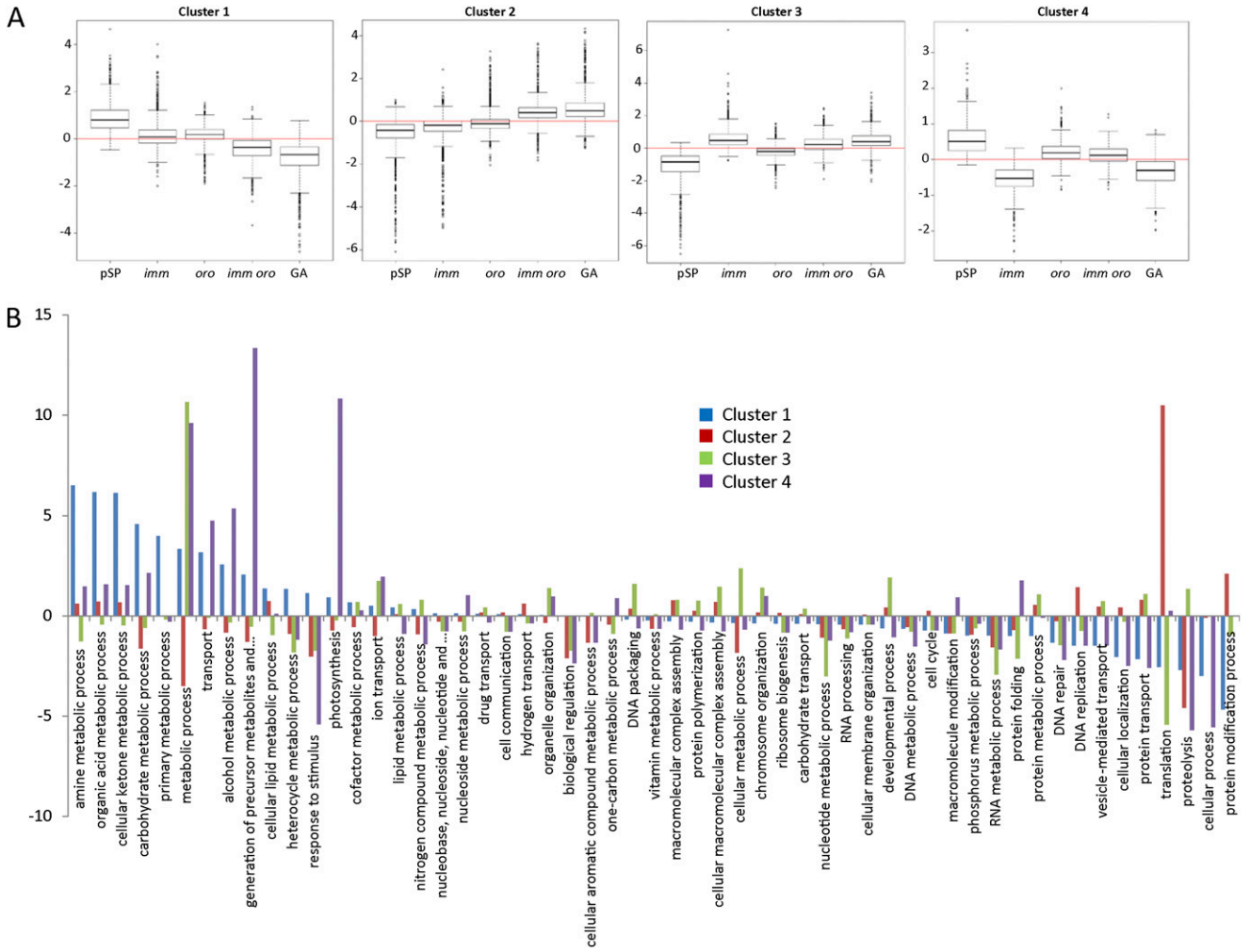

Fig. S4. Cluster analysis of the data from the microarray analysis of transcript abundance in sporophyte, gametophyte, and mutant individuals. (*A*) Box plots showing the trends in transcript abundance for four clusters of genes that had been identified as being differentially expressed by ANOVA test (see *SI Materials and Methods*). Clusters 1–4 contain 1,352, 1,262, 1,032, and 400 genes, respectively. Log2 transcript abundances for each gene are presented as the hybridization signal divided by a reference value corresponding to the median hybridization signal for that gene across all of the samples analyzed. (*B*) Percentage abundance of genes with specific GO slim assignments within each cluster relative to the abundance of genes with the same GO slim assignment in the genome as a whole. Only GO slim categories that represent at least 0.3% of the total in the genome as a whole are shown. pSP, wild-type partheno-sporophyte; GA, wild-type gametophyte.

Table S1. Phenotypic analysis of families of gametophytes derived from individual unilocular sporangia from sporophytes heterozygous at the *ORO* locus

Unilocular sporangium	No, of individuals*	No. of wild-type individuals	No. of *oro* individuals
1	24	10	14
2	25	13	12
3	26	10	16
4	24	11	13
5	25	13	12
6	26	15	11
7	19	11	8
8	25	8	17
9	22	5	17
10	21	13	8
11	25	14	11
12	25	13	12
13	25	12	13
14	27	14	13
Total	339	162	177

*Number of individuals scored per unilocular sporangium.

Table S2. Determination of the sex of families of *oro* gametophytes derived from individual unilocular sporangia from sporophytes heterozygous at the *ORO* locus

Unilocular sporangium	No, of *oro* females	No. of *oro* males
1	3	4
2	1	6
3	3	5
4	4	2
5	5	2
6	1	4
7	5	4
8	4	7
9	3	4
10	0	3
11	2	1
12	2	6
Total	33	48

Table S3. Phenotypic analysis of families of gametophytes derived from individual unilocular sporangia from sporophytes heterozygous for both the *oro* and *imm* mutations

Unilocular sporangium	No. of WT individuals	No. of *oro* individuals	No. of *imm* individuals	No. of *oro imm* individuals
1	6	8	1	1
2	8	2	0	10
3	2	3	1	3
4	9	6	1	4
5	4	8	4	0
6	6	3	3	7
7	4	6	5	5
8	4	7	2	5
9	1	8	10	0
10	9	3	8	0
11	4	6	2	6
12	1	3	5	10
13	5	4	5	6
14	3	3	4	7
15	4	3	2	4
16	4	2	2	7
Total	75	76	60	79

The heterozygous sporophytes were derived by crossing two gametophytes carrying respectively the *imm* and *oro* mutations.

Table S4. Phenotypic analysis of families of gametophytes derived by back-crossing a gametophyte carrying both the *imm* and *oro* mutations with a wild-type strain

Unilocular sporangium	No. of individuals*	No. of *oro* individuals	No. of *imm* individuals	No. of *imm oro* individuals	No. of wild-type individuals
1	21	6	5	6	4
2	16	5	3	3	5
3	16	3	3	4	6
4	14	6	2	3	3
5	26	9	3	9	5
6	17	3	3	5	6
7	16	5	6	2	3
Total	126	37	25	32	32

*Number of individuals scored per unilocular sporangium.

Table S5. Fold differences in transcript abundance relative to the wild-type sporophyte, as measured by qRT-PCR

Locus ID	Predicted function	Fold change expression (log2)			
		imm/pSP	*oro*/pSP	*imm oro*/pSP	GA/pSP
Esi0009_0052	3′5′-Cyclic nucleotide phosphodiesterase domain	1.76	2.32	1.92	1.97
Esi0049_0053	Adhesin-like protein	5.13**	5.81**	3.88**	3.68**
Esi0068_0016	Swi/SNF domain protein	−1.49	0.41	0.37	2.10
Esi0074_0057	Homeodomain-like	0.01	2.13	2.18*	6.12*
Esi0095_0057	Transcription factor (Myb and SANT domains)	−2.74*	−3.23*	−2.93*	−4.55*
Esi0203_0032	Argonaut	−1.63**	−1.46**	−1.89**	−0.97**
Esi0245_0009	*imm* down-regulated 17	−2.22	−4.82	−3.94	−7.31*
Esi0292_0018	SMAD/FHA domain protein	−1.37	−1.38	−0.90	−0.35
Esi0308_0025	Serine/threonine-protein kinase	4.61**	7.18**	9.36**	10.59**
Esi0556_0008	Putative cell wall structural protein	2.50	10.46**	11.33**	13.20**

Fold changes, calculated using log2 values, are the average of three independent biological replicates. Significant differences (ANOVA) with respect to transcript abundance in wild-type sporophytes are indicated. *$P < 0.05$; **$P < 0.01$. GA, wild-type gametophyte; *imm*, imm mutant; *oro*, oro mutant; pSP, wild-type partheno-sporophyte.

Table S6. *Ectocarpus* strains used in this study

Name	Generation, sex	Life history phenotype	Genotype	Description	Origin	Year	CCAP strain code
Ec17	SP	Wild type	*ORO ORO*	Field isolate	San Juan de Marcona, Peru	1988	CCAP 1310/193
Ec25	GA f	Wild type	*ORO*	Meiotic offspring from Ec17	Laboratory	2002	CCAP 1310/3
Ec32	GA m	Wild type	*ORO*	Meiotic offspring from Ec17	Laboratory	2002	CCAP 1310/4
Ec137	GA m	*imm*	*imm*	Meiotic offspring from Ec17, carrying imm mutation	Laboratory	2002	CCAP 1310/319
Ec197	SP	Wild type	*imm IMM oro ORO*	Cross 597f imm+oro x 32m	Laboratory, this work	2009	
Ec372	SP	Wild type	*IMM imm*	Cross 25f wt x 137m imm	Laboratory	2003	CCAP 1310/320
Ec400	GA m	no data	No data	Male reference strain for sex test by crossing	Laboratory	2002	CCAP 1310/329
Ec410	GA f	no data	No data	Female reference strain for sex test by crossing	Laboratory	2002	CCAP 1310/330
Ec419	GA f	*imm*	*imm ORO*	Female gametophyte carrying the *imm* mutation, wild type for *oro*	Laboratory	2003	CCAP1310/321
Ec494	GA m	*oro*	*oro*	UV-mutagenized Ec32, life-history mutant *"ouroboros"*	Laboratory, this work	2005	
Ec568	GA f	Wild type	*ORO*	Meiotic offspring from Ec721	Laboratory	2006	CCAP 1310/334
Ec555	SP	Wild type	*ORO oro*	Cross 25f wt x 494m oro	Laboratory, this work	2005	
Ec556	SP	Wild type	*ORO oro*	Cross 25f wt x 494m oro	Laboratory, this work	2005	
Ec557	SP	Wild type	*ORO oro*	Cross 25f wt x 494m oro	Laboratory, this work	2005	
Ec560	GA f	*oro*	*oro*	Meiotic offspring from Ec557	Laboratory, this work	2005	
Ec561	GA m	*oro*	*oro*	Meiotic offspring from Ec556	Laboratory, this work	2005	
Ec702	SP	Wild type	*ORO oro*	Cross 568f wt x 561m oro	Laboratory, this work	2007	
Ec566	SP	Wild type	*imm IMM oro ORO*	Cross 419f imm x 494m oro	Laboratory, this work	2006	
Ec581-9	GA m	oro	*oro oro*	Cross of 560f o x 494m o	Laboratory, this work	2006	
Ec592	GA	Wild type	*IMM ORO*	Meiotic offspring from 566	Laboratory, this work	2007	
Ec594	GA	*imm*	*imm ORO*	Meiotic offspring from 566	Laboratory, this work	2007	
Ec597	GA f	*imm oro*	*imm oro*	Meiotic offspring from 566	Laboratory, this work	2007	
Ec600	GA	oro	*IMM oro*	Meiotic offspring from 566	Laboratory, this work	2007	
Ec721	SP	Wild type	*ORO ORO*	Field isolate	Arica, Chile	2006	

CCAP, Culture Collections of Algae and Protozoa (marine) reference number, Dunstaffnage Marine Laboratory, Oban, Scotland; GA, gametophyte; *imm*, *immediate upright* mutation; *oro*, *ouroboros* mutation; SP, sporophyte.

Table S7. Oligonucleotides used for the qRT-PCR analysis

Locus_ID	Sequence	Amplicon size, bp
Esi0009_0052		
3′UTR	AAGCGGGTTTACCGAGTGTT	149
3′UTR	GCGCCTCCATTTCTTTCTCT	
Esi0010_0199		
Last exon	AGGAGAAGACGGCACGATT	92
Last exon	GGCCATTCCCAAAGTCCT	
Esi0049_0053		
3′UTR	ATCGTGTTCAGTCGGATGG	139
3′UTR	GAGCAGTCTCAGGACCAACAA	
Esi0068_0016		
Exon 4	CTCCCGGAAACAACAATGAA	95
Exon 4	GTCTGACCGCGCTTGATAAC	
Esi0074_0057		
3′UTR	GGGTTAAAGGGACAGCAACA	125
3′UTR	CAGCGAGGGAGGTGATTAGA	
Esi0095_0057		
3′UTR	ATCAGGCTGAGGTGGTGTTT	111
3′UTR	ACCCGGAATATCGACAGGTT	
Esi0138_0012		
3′UTR	CAGCTACGTGTCGATCTTGG	121
3′UTR	GATGGATGTCAGAGAGGCAGA	
Esi0203_0032		
3′UTR	GCCGATGTCTGTCTTGTTTG	141
3′UTR	TCTAGCCTGCCTGTTCGTTT	
Esi0245_0009		
3′UTR	GAACAACGACCTCCGTAACC	132
3′UTR	GCCGCAACCATGAAGTAATC	
Esi0292_0018		
3′UTR	AGCCTTGTTTGGTACGTGGA	110
3′UTR	CAACCCGCCAAAGATAGATG	
Esi0308_0025		
Last exon	CTCAACCAAGGCTGTGACC	132
3′UTR	CGGTTCCTCATCTTGGTACTCT	
Esi0445_0006		
3′UTR	GATGGCGGGAACTACAACA	134
3′UTR	CAACAGACCGCACCAAATAC	
Esi0556_0008		
3′UTR	GTATCTGGCGACTGGATGCT	101
3′UTR	CGACGGAAACCCAGGTAAA	
Esi0387_0021 (EsEF1α)		
Last exon	CAAGTCCGTCGAGAAGAAGG	147
3′UTR	CCAGCAACACCACAATGTCT	

Locus_ID, *Ectocarpus* gene model.

Other Supporting Information Files

Dataset S1 (XLSX)