Table S1. Databases used as sequence and annotation sources. Species names are provided along with descriptions and database names, appropriate references and web links (URLs). We recruited representatives from five lineages of microalgae: (*i*) green algae, including the picophytoplankton *Micromonas* sp. RCC299 (Worden *et al.*, 2009); the free-living, small-genome alga *Ostreococcus tauri* (Palenik *et al.*, 2007); the virus-harboring alga *Chlorella variabilis* NC64A (Blanc *et al.*, 2010a); and the laboratory model alga *Chlamydomonas reinhardtii* (Merchant *et al.*, 2007); (*ii*) red algae, including a primitive alga with a simple cellular architecture, *Cyanidioschyzon merolae* (Matsuzaki *et al.*, 2004); (*iii*) diatoms, including the marine pennate diatom *Phaeodactylum tricornutum* (Bowler *et al.*, 2008) and the centric diatom *Thalassiosira pseudonana* (Armbrust *et al.*, 2004); (*iv*) brown algae, including the marine *Ectocarpus siliculosus* (Cock *et al.*, 2010); and (*v*) Eustigmatophyta, a diverse Stramenopile group that evolved between the brown algae and diatoms, represented by *N. oceanica*.

Species	Database	Description	URL	Reference
C. merolae	*Cyanidioschyzon merolae* genome	*Cyanidioschyzon merolae* Genome Project	http://merolae.biol.s.u-tokyo.ac.jp	(Nozaki *et al.*, 2007)
P. tricornutum	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/Phatr2/Phatr2.home.html	(Bowler *et al.*, 2008)
T. pseudonana	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/thaps1/thaps1.home.html	(Armbrust *et al.*, 2004)
F. cylindrus	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/Fracy1/Fracy1.home.html	
N. oceanica	EnergyAlgaeDB	A functional genomics database for energy microalgae	http://www.bioenergychina.org:8989/	(Wang *et al.*, 2013)
E. siliculosus	Orcae	Online Resource for Community Annotation of Eukaryotes	http://bioinformatics.psb.ugent.be/orcae/	(Cock *et al.*, 2010)
O. tauri	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/Ostta4/Ostta4.home.html	(Palenik *et al.*, 2007)
***Micromonas* sp. RCC299**	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/MicpuN3/MicpuN3.home.html	(Worden *et al.*, 2009)
C. variabilis	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/ChlNC64A_1/ChlNC64A_1.home.html	(Blanc *et al.*, 2010b)

NC64A				
***C. subellipsoidea* C-169**	JGI	DOE Joint Genome Institute	http://genome.jgi.doe.gov/Coc_C169_1/Coc_C169_1.home.html	(Blanc *et al.*, 2012)
C. reinhardtii	JGI	DOE Joint Genome Institute	http://genome.jgi-psf.org/Chlre4/Chlre4.home.html	(Merchant *et al.*, 2007)
V. carteri	Phytozome	Phytozome	http://genome.jgi-psf.org/Volca1/Volca1.home.html	(Prochnik *et al.*, 2010)
A. thaliana	TAIR	The *Arabidopsis* Information Resource	http://www.arabidopsis.org/	(Initiative, 2000; Garcia-Hernandez *et al.*, 2002)

Table S2. The ratio of the rate of nonsynonymous substitutions (*Kn*) to the rate of synonymous substitutions (*Ks*) of hydroxyl-methyl-glutaryl-CoA synthase (HMGS). A *Kn/Ks* ratio of 1 is assumed to indicate neutrality, *Kn/Ks* > 1 is a signature of positive selection at the amino-acid level, and *Kn/Ks* < 1 is indicative of purifying selection.

Species	*Kn/Ks*	*Kn*	*Ks*
Halobacterium sp. DL1	0.951	0.412	0.434
Saccharomyces cerevisiae	0.651	0.315	0.484
Cyanidioschyzon merolae	0.460	0.281	0.612
Phaeodactylum tricornutum	0.386	0.200	0.519
Thalassiosira pseudonana	0.296	0.112	0.380
Fragilariopsis cylindrus	0.185	0.136	0.735
Nannochloropsis oceanica	0.437	0.255	0.583
Nannochloropsis salina	0.537	0.231	0.430
Chlorella variabilis NC64A	0.489	0.160	0.327
Coccomyxa subellipsoidea C-169	0.453	0.150	0.332
Chlamydomonas reinhardtii	0.436	0.118	0.270
Volvox carteri	0.311	0.088	0.283
Pinus sylvestris	0.181	0.034	0.190
Arabidopsis thaliana	0.168	0.048	0.287
Solanum lycopersicum	0.119	0.042	0.353
Taxus x media	0.259	0.039	0.150
Homo sapiens	0.305	0.236	0.772

Table S3. Comparison of sequence identity and conserved domains of hydroxyl-methyl-glutaryl-CoA synthase (HMGS) proteins between algal species and *A. thaliana*. Microalgal HMGS orthologs were BLASTed with *A. thaliana* HMGS. Abbreviations: *C. merolae* (Cm), *P. tricornutum* (Pt), *T. pseudonana* (Tp), *F. cylindrus* (Fc), *N. oceanica* (No), *E. siliculosus* (Es), *Micromonas* sp. RCC299 (Mi), *O. tauri* (Ot), *C. variabilis* NC64A (Cv), *C. subellipsoidea* C-169 (Cs), *C. reinhardtii* (Cr) and *V. carteri* (Vc).

Protein ID	Max Identity	Query cover	E value	Conserved domain(s)	Pfam ID
Cm.CMM189C	42%	98%	8e-101	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Tp.260768	44%	96%	6e-116	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Pt.16649	43%	97%	2e-109	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Pt.33278	31%	46%	3e-12	HMG_CoA_synt_N	PF01154
Fc.185767	44%	97%	2e-104	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Fc.199578	43%	97%	9e-106	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
No.g249	50%	98%	3e-121	HMG_CoA_synt_N, HMG_CoA_synt_C, ketoacyl-synt	PF01154, PF08540 and PF00109
Ns. 4043	56%	98%	1e-119	HMG_CoA_synt_N, HMG_CoA_synt_C, ketoacyl-synt	PF01154, PF08540 and PF00109
Es.Esi0335_0012	44%	95%	9e-113	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Es.Esi0131_0082	40%	99%	2e-107	HMG_CoA_synt_N, HMG_CoA_synt_C, ketoacyl-synt	PF01154, PF08540 and PF00109
Cs.27385	50%	97%	2e-134	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Cv.138158	41%	97%	1e-119	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Cr.118594	37%	84%	2e-40	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540
Vc.59473	38%	97%	7e-94	HMG_CoA_synt_N and HMG_CoA_synt_C	PF01154 and PF08540

Table S4. List of sterol biosynthetic enzymes in *A. thaliana*. Protein names, descriptions, accession numbers and references are provided.

Symbol	Description	Accession	Reference
AACT1	acetoacetyl-CoA thiolase	AT5G47720	(Jin *et al.*, 2012)
AACT2	acetoacetyl-CoA thiolase	AT5G48230	(Jin *et al.*, 2012)
HMGS	hydroxymethylglutaryl-CoA synthase	AT4G11820	(Montamat *et al.*, 1995)
HMGR1	hydroxy-3-methylglutaryl coenzyme A reductase	AT1G76490	(Enjuto *et al.*, 1994; Nieto *et al.*, 2009)
HMGR2	hydroxy-3-methylglutaryl coenzyme A reductase	AT2G17370	(Enjuto *et al.*, 1994; Lumbreras *et al.*, 1995)
MVK	mevalonate kinase	AT5G27450	(Riou *et al.*, 1994)
PMK	phosphomevalonate kinase	AT1G31910	(Phillips *et al.*, 2008)
MPDC1	mevalonate diphosphate decarboxylase	AT2G38700	(Cordier *et al.*, 1999)
MPDC2	mevalonate diphosphate decarboxylase	AT3G54250	(Cordier *et al.*, 1999)
DXS1	1-deoxy-D-xylulose 5-phosphate synthase 1	AT4G15560	(Phillips *et al.*, 2008)
DXS2	1-deoxy-D-xylulose 5-phosphate synthase 2	AT3G21500	(Carretero-Paulet *et al.*, 2013)
DXS3	1-deoxy-D-xylulose 5-phosphate synthase 3	AT5G11380	(Phillips *et al.*, 2008)
DXR	1-deoxy-d-xylulose 5-phosphate reductoisomerase	AT5G62790	(Lichtenthaler, 1999; Schwender *et al.*, 1999)
CMS	4-diphosphocytidyl-2C-methyl-D-erythritol synthase	AT2G02500	(Rohdich *et al.*, 2000)
CMK	4-(cytidine 5’-diphospho)-2-C-methyl-D-erythritol kinase	AT2G26930	(Hsieh & Goodman, 2005)
MDS	2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	AT1G63970	(Hsieh & Goodman, 2005)
HDS	hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase	AT5G60600	(Seemann *et al.*, 2005)
HDR	4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase	AT4G34350	(Guevara-García *et al.*, 2005)
IPI1	isopentenyl diphosphate:dimethylallyl diphosphate isomerase	AT3G02780	(Campbell *et al.*, 1998)
IPI2	isopentenyl diphosphate:dimethylallyl diphosphate isomerase	AT5G16440	(Campbell *et al.*, 1998)
GPS	geranyl diphosphate synthase	AT2G34630	(Bouvier *et al.*, 2000)
FPS1	farnesyl diphosphate synthase	AT4G17190	(Closa *et al.*, 2010)
FPS2	farnesyl diphosphate synthase	AT5G47770	(Closa *et al.*, 2010)
GGPPS	geranylgeranyl pyrophosphate synthase	AT1G49530	(Okada *et al.*, 2000)
SQS	squalene synthase 1	AT4G34640	(Nakashima *et al.*, 1995)
SQE	squalene epoxidase	AT1G58440	(Rasbery *et al.*, 2007)
CAS	cycloartenol synthase 1	AT2G07050	(Babiychuk *et al.*, 2008)
LAS	lanosterol synthase 1	AT3G45130	(Ohyama *et al.*, 2009)
SMO1-1	sterol 4-alpha-methyl oxidase 1-1	AT4G12110	(Darnet *et al.*, 2001)
SMO2	sterol 4-alpha-methyl oxidase 2	AT2G29390	(Darnet *et al.*, 2001)
SMO1	sterol 4-alpha-methyl oxidase 1	AT1G07420	(Darnet *et al.*, 2001)
CPI1	cyclopropyl isomerase	AT5G50375	(Lovato *et al.*, 2000)

FK	sterol C-14 reductase (Fackel)	AT3G52940	(Schrick *et al.*, 2004)
CYP51	cytochrome P450 51	AT1G11680	(Kim *et al.*, 2005; Kim *et al.*, 2010)
HYD1	C-8 sterol isomerase (Hydra)	AT1G20050	(Souter *et al.*, 2002)
SMT1	sterol methyltransferase 1	AT5G13710	(Carland *et al.*, 2010)
SMT2	sterol methyltransferase 2	AT1G20330	(Carland *et al.*, 2010)
SMT3	sterol methyltransferase 3	AT1G76090	(Carland *et al.*, 2010)
DWF7	delta7 sterol C-5 desaturation (Dwarf7)	AT3G02580	(Choe *et al.*, 1999b)
DWF7-2	delta7 sterol C-5 desaturation (Dwarf7-2)	AT3G02590	(Choe *et al.*, 1999b)
DWF5	sterol delta7 reductase (Dwarf5)	AT1G50430	(Lecain *et al.*, 1996)
DWF1	(24)-sterol reductase (Dwarf1)	AT3G19820	(Klahre *et al.*, 1998; Choe *et al.*, 1999a)
DET2	steroid-5-alpha-reductase (De-etiolated)	AT2G38050	(Chory *et al.*, 1991; Fujioka *et al.*, 1997)

Table S5. Primers used in these experiments.

Primer	Oligonucleotide Sequence 5'–3'	Product Size (bp)
HMGS-g249-F	CTTCAGATGCCTCATCGTGC	
HMGS-g249-R	TCTTCCCTAGATATGGGTCCGT	225
GPS-g8423-F	CAGGCAGGCGTGTTGAGC	
GPS-g8423-R	GGTTGCGGCGTGGTAGTC	143
IPI-g7649-F	AAGCCAATCAAAGCCATCTCC	
IPI-g7649-R	CATCGTCGCCAAGTTCTGC	208
DXS-g6105-F	GAGATTGCGACGGAAGATAGG	
DXS-g6105-R	CGCTGCATAAACGTGCTGTA	203
DXR-g3685-F	ATGAGCAGCCTCGTACAAAATAT	
DXR-g3685-R	CAGCAAGATGCGTGGGTCG	153
CMS-g4346-F	CCCAAACCGAATGAGGAAA	
CMS-g4346-R	CCAGCAAGAAGCACAACAGAG	252
CMK-g9877-F	CGCCTTTGGGGACACTTT	
CMK-g9877-R	GAGTTGGTTGGCTGCGAATA	92
MDS-g3364-F	TCTGCTGCCCACTCCGACG	
MDS-g3364-R	CACATTCCCAATTTTGTACCCTC	192
HDS-g5186-F	GGACGGATTAGGAGACGGG	
HDS-g5186-R	GCTGGGGCACGAGACAAA	133
HDR-g6910-F	CGTCGCATCATTCAATCGC	
HDR-g6910-R	CCCCAACAGAACCCGTAGC	185
SQS-g8550-F	AATGGCAACGGTTATAGGGAC	
SQS-g8550-R	AACTCGCAACGCACAGAAGA	123
FK-g6702-F	AGCCTAAGCAGATCAAGGTGAAGA	
FK-g6702-R	CGTAGTTTGGGCAGCAGTAGTAAA	174
CYP51-g4528-F	TGTCTTTACCGTCAACCTTTTCA	
CYP51-g4528-R	TCGGGTCACTGTCATACACTACTC	164
SMT1-g7669-F	AGCCAAGATCACAGGTGTAACCA	
SMT1-g7669-R	AACGAGCAGTCGGGAAACG	138
SMT2-g7986-F	CAGTCCTTTCACTTTGCTCCTCG	
SMT2-g7986-R	CATGCCTGGCTTCAACCCTAA	105
DWF7-g9730-F	CTTTTGACTTACCTTCGGGACTTG	
DWF7-g9730-R	GGGCTAACTGAATCTGATCCTTGA	166

DWF5-g9012-F	GAGCGTGACCCTGCAACTCG	
DWF5-g9012-R	CCAGGCAGCCCCAACAAA	119
DWF1-g2385-F	CAAGTTTGGCGGGTTTGTCA	
DWF1-g2385-R	CCCAGTCCTCTGCCTTTCGA	131
PSY-g6239-F	CGAGAAGAAGAAAGGGAGACGG	
PSY-g6239-R	CAGATGAAGCGACCAAGAGGAC	171
ACS-g5570-F	TCCCTACGCCCTGTCGCT	
ACS-g5570-R	CGCAAAATGTATTCCGTCTCG	137
ACS-g1468-F	GGGTGGAGGAGGTGATTGTG	
ACS-g1468-R	GGCGAAAGTGGCTTCAGTG	146
ACC-g7511-F	CGAGAACCTGCCGTTGATG	
ACC-g7511-R	GGCGGGATGTAGACGAAGAC	147
ACC-g1005-F	GGAAAGCAGGCAAGGTCG	
ACC-g1005-R	GATCCGTCGGGCAGAAGA	278
ACLY-g8770-F	GAACATCAAGGAGATTAGCCAGAG	
ACLY-g8770-R	GCGTGTAGCTCGTTCGTCAAG	93
ME-g2252-F	AGGGATGAACTACGAAATGGC	
ME-g2252-R	TGGTGGGGTTGGACAAGG	278
ME-g3463-F	TAAAATCGGCAGCAGCAATG	
ME-g3463-R	AGGGGTTCCTACAGATGTCCC	65
FAS I-g927-F	TGAAGCCCCATTGGTAGTCG	
FAS I-g927-R	GACCTAAGTCCCGCAAAATCTC	296
FAE-g5258-F	TGAGTTCCACCAAGTCTTCCAA	
FAE-g5258-R	ACCCGCATCGCTCCAATC	267
FAE-g6504-F	CTTCTCAGAGGCGGCACAA	
FAE-g6504-R	GAGCGTCAGATTCCAGTAGGC	253
FAD-g1678-F	CACAGACTTCATCTACAAGCATCC	
FAD-g1678-R	TCCACCTTATTTTCCCGCATA	282
FAD-g7957-F	GAGAAATGCTTCCAGAAATCCC	
FAD-g7957-R	GCGTGGCAAATGTGACCG	248

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