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Chris Bowler, Bethany D. Jenkins, Tatiana A. Rynearson, and et al

The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes

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Diatoms are photosynthetic secondary endosymbionts found throughout marine and freshwater environments, and are believed to be responsible for around one-fifth of the primary productivity on Earth^{1,2}. The genome sequence of the marine centric diatom *Thalassiosira pseudonana* was recently reported, revealing a wealth of information about diatom biology^{3–5}. Here we report the complete genome sequence of the pennate diatom *Phaeodactylum tricorutum* and compare it with that of *T. pseudonana* to clarify evolutionary origins, functional significance and ubiquity of these features throughout diatoms. In spite of the fact that the pennate and centric lineages have only been diverging for 90 million years, their genome structures are dramatically different and a substantial fraction of genes (~40%) are not shared by these representatives of the two lineages. Analysis of molecular divergence compared with yeasts and metazoans reveals rapid rates of gene diversification in diatoms. Contributing factors

include selective gene family expansions, differential losses and gains of genes and introns, and differential mobilization of transposable elements. Most significantly, we document the presence of hundreds of genes from bacteria. More than 300 of these gene transfers are found in both diatoms, attesting to their ancient origins, and many are likely to provide novel possibilities for metabolite management and for perception of environmental signals. These findings go a long way towards explaining the incredible diversity and success of the diatoms in contemporary oceans.

The sequenced diatoms represent two of the major classes of diatoms—the bi/multipolar centrics (Mediophyceae), to which *T. pseudonana* belongs, and the pennates (Bacillariophyceae), to which *P. tricorutum* belongs (Supplementary Fig. 1). The earliest fossil deposit from centrics is 180 million years (Myr) old and that from pennates is 90 Myr old^{6,7}. Although being the youngest, the pennates are by far the most diversified, and they are major components of both

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Table 1 | Major features of the *P. tricornutum* and *T. pseudonana* genomes

	<i>P. tricornutum</i>	<i>T. pseudonana</i>
Genome size	27.4 Mb	32.4 Mb
Predicted genes	10,402	11,776
Core genes*	3,523	4,332
Diatom-specific genes*	1,328	1,407
Unique genes*	4,366	3,912
Introns	8,169	17,880
Introns per gene	0.79	1.52
Long-terminal-repeat retrotransposon content	5.8%	1.1%

* Different classes of genes were assigned by comparing the *P. tricornutum* and *T. pseudonana* predicted proteomes with those from two plants, three green algae, one red alga, three metazoans, two fungi and ten other chromalveolates (see Supplementary Information) by all-against-all BLASTP using an expected cut-off value of 10^{-5} . Core genes were defined as being present in representatives from all these eukaryotic groups, diatom-specific genes were present in both of the diatoms but not elsewhere, and unique genes were only found in one of the two diatoms. The different numbers of diatom-specific genes in the two diatoms is a consequence of species-specific gene duplication events.

pelagic and benthic habitats⁷. They display a range of features, including their bilateral symmetry, that distinguish them from centric species. For example, they have amoeboid isogametes, by contrast with the motile sperm and oogamy observed in centric species; they are major biofoulers; they include toxic species; and they generally respond most strongly to mesoscale iron fertilization^{7,8}. Furthermore, members of the raphid pennate clade can glide actively along surfaces.

The completed *P. tricornutum* genome is approximately 27.4 megabases (Mb) in size, which is slightly smaller than *T. pseudonana* (32.4 Mb), and *P. tricornutum* is predicted to contain fewer genes (10,402 as opposed to 11,776; Table 1, Supplementary Fig. 2). Gene identification and functional analysis was facilitated by the availability of more than 130,000 expressed sequence tags (ESTs) generated from cells grown under 16 different conditions. In total, 86% of gene predictions had EST support (Supplementary Table 1).

P. tricornutum shares 57% of its genes with *T. pseudonana* (see Supplementary Information for criteria used), of which 1,328 are absent from other sequenced eukaryotes (Table 1). The molecular divergence between the two diatoms was assessed by examining the percentage amino acid identity of 4,267 orthologous gene pairs (Table 2, Fig. 1). We found an average identity of 54.9% between diatom orthologues, in comparison with approximately 43% between the diatoms and a more distantly related heterokont, the non-photosynthetic oomycete *Phytophthora sojae*. This agrees with the predicted ancient separation (around 700 Myr ago) of these lineages^{9,10}. The divergence between the two diatoms is similar to what is observed between *Saccharomyces cerevisiae* and the related yeast *Kluyveromyces lactis*, and about halfway between the *Homo sapiens*/*Takifugu rubripes* (pufferfish) divergence and the *H. sapiens*/*Ciona intestinalis* (sea squirt) divergence (Table 2, Fig. 1). The more rapid evolutionary rates of diatoms compared with other organismal groups (for example, the fish–mammal divergence probably occurred in the Proterozoic era earlier than 550 Myr ago¹¹) is consistent with previous observations^{6,7}. As has been found in the two yeasts¹², no major conservation of gene order (synteny) could be detected between the two diatom genomes other than in a few examples of microclusters of up to eight genes (Supplementary Fig. 3). Furthermore, approximately two-thirds of intron positions are unique to each species (Supplementary Information). The widespread

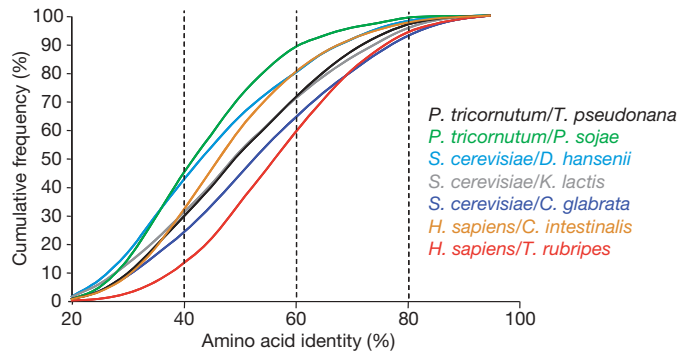


Figure 1 | Molecular divergence between *P. tricornutum* and *T. pseudonana*. Analysis of molecular divergence between the diatoms and other heterokonts, and comparison with selected hemiascomycetes and chordates. The diatom–oomycete pair displays the lowest amino acid identity (43.3%), in agreement with their proposed ancient separation, around 700 Myr ago¹⁰. The divergence between the pennate and centric diatom is similar to the fish–mammal divergence, which probably occurred in the Proterozoic era (550 Myr ago)¹¹. The centric–pennate divergence, on the other hand, has been dated to at least 90 Myr ago⁷. In the figure, we represent the cumulative frequencies of amino acid identity across each set of potential orthologous pairs shown in Table 2.

intron gain that has been reported in *T. pseudonana*¹³ was not found in *P. tricornutum* (Table 1), suggesting that it may be a recent event in the centric diatom.

Large-scale within-genome duplication events do not appear to have played a major role in driving the generation of diatom diversity (Supplementary Information), by contrast with what has been found in yeasts and metazoans^{14,15}. The observed high levels of diatom species diversity must therefore have been generated by other mechanisms. Whereas intron gain may be one factor in centric diatoms, the dramatic expansion of diatom-specific copia-retrotransposable elements may have contributed in the *P. tricornutum* genome (Table 1, Supplementary Figs 2, 4). These elements also appear to have expanded in other pennate diatoms (Supplementary Information), so they may have been a significant driving force in the generation of pennate diatom diversity through transpositional duplications and subsequent genome fragmentation.

Diatoms, and heterokonts in general, are believed to be derived from a secondary endosymbiotic process that took place around one billion years ago between a red alga and a heterotrophic eukaryote¹⁶. Diatom chloroplast genomes have fewer genes than red algal chloroplast genomes, indicating that a number of chloroplast genes were transferred to the nucleus after secondary endosymbiosis, and a few more genes appear to be in the process of transfer in one diatom species or the other⁵. It is generally thought that the diatom mitochondrion originated in the host, and the mitochondrial gene complement is almost identical to that of haptophytes and cryptophytes, which are other algal phyla that may have originated from the same secondary endosymbiotic event. We used a phylogenomic approach to search for genes of red algal origin in the two diatoms and the two sequenced oomycetes, *Phytophthora ramorum* and *Phytophthora sojae*⁹, using *Cyanidioschyzon merolae* as reference red algal genome¹⁷. We classified 171 genes as being of red algal origin, on

Table 2 | Molecular divergence between different organism pairs

Pairwise comparison	Mean identity (%)	Number of compared pairs
<i>Phaeodactylum tricornutum</i> / <i>Thalassiosira pseudonana</i>	54.9	4,267
<i>Phaeodactylum tricornutum</i> / <i>Phytophthora sojae</i>	43.3	2,952
<i>Saccharomyces cerevisiae</i> / <i>Debaryomyces hansenii</i>	50.1	2,694
<i>Saccharomyces cerevisiae</i> / <i>Kluyveromyces lactis</i>	54.8	4,246
<i>Saccharomyces cerevisiae</i> / <i>Candida glabrata</i>	58.2	4,484
<i>Homo sapiens</i> / <i>Ciona intestinalis</i>	52.6	5,208
<i>Homo sapiens</i> / <i>Takifugu rubripes</i>	61.4	10,225

Summary of numbers of orthologous pairs (reciprocal best hits with an expected cut-off value of 10^{-10}) for each organism comparison and their mean percentage identities.

the basis of strong (>85%) bootstrap support for the red-alga-plus-heterokont clade (Supplementary Table 2). Of the 171 high-scoring genes, 108 were shared between the two diatoms and 74 (43%) were predicted to be plastid targeted. In addition, 11 of these genes were also present in oomycetes, as expected if the common ancestor of diatoms and oomycetes had a red algal plastid that was subsequently lost in the oomycetes⁹. The results of this survey support there being a red algal origin for the diatom plastid and many gene transfers from the red algal nucleus to the host nucleus before the former was lost.

A remarkably high number of *P. tricornutum* predicted genes appear to have been transferred between diatoms and bacteria (784; 7.5% of gene models). Specifically, by searching for orthologous genes in 739 prokaryotic genomes, followed by automated phylogenetic tree construction and manual curation, we confirmed that 587 putative *P. tricornutum* genes clustered with bacteria-only clades or formed a sister group to clades that included only bacterial genes (with or without other heterokonts). This finding indicates that horizontal gene transfer between bacteria and diatoms is pervasive and is much higher than has been found in other sequenced eukaryotes^{18,19}. Of the 587 identified sequences, 42% are only found in *P. tricornutum* whereas 56% are present in both diatoms (Fig. 2a), attesting to their ancient origin. Only 73 sequences are shared between *P. tricornutum* and *Phytophthora* spp. (Fig. 2a, Supplementary Table 3), 59 of which are also present in *T. pseudonana*, suggesting that the vast majority of gene transfers occurred after the divergence of photosynthetic heterokonts and oomycetes.

Many of the genes shared between diatoms and bacteria encode components that are likely to provide novel metabolic capacities, for example for organic carbon and nitrogen utilization²⁰ (xylanases and glucanases, prismatic, carbon-nitrogen hydrolase, amidohydrolase), functioning of the diatom urea cycle³ (carbamoyl transferase, carbamate kinase, ornithine cyclodeaminase) and polyamine metabolism related to diatom cell wall silicification²¹ (*S*-adenosylmethionine (SAM)-dependent decarboxylases and methyltransferases). Others are likely to encode novel cell wall components, and to provide non-orthodox mechanisms of DNA replication, repair and recombination for a eukaryotic cell (Supplementary Table 3).

Bacterial genes in diatoms do not appear to be derived from any one specific source, but from a range of origins including proteobacteria, cyanobacteria and archaea (Fig. 2a, b, Supplementary Table 3). Heterotrophic bacteria and cyanobacteria, especially diazotrophs and planctomycete bacteria, have been found in various close associations with diatoms^{22–24}, which may explain the unprecedented levels of horizontal gene transfer events that appear to have occurred. In *P. tricornutum*, bacterial genes are distributed throughout the

genome, although several clusters, as well as regions devoid of bacterial genes, can be observed (Supplementary Fig. 5). Some of these genes in diatoms share bacterial-specific gene fusions that support phylogenetic associations, such as assimilatory nitrite reductase B and D subunits; these are apparently of planctomycete origin (Fig. 2c).

Bacterial histidine-kinase-based phosphorelay two-component systems, which are involved in environmental sensing, also appear to be highly developed in diatoms. For example, *P. tricornutum* contains a wide range of two-component signalling proteins sometimes organized in novel domain associations (Fig. 3). One of these proteins bears the classical features of bacterial phytochrome photoreceptors,

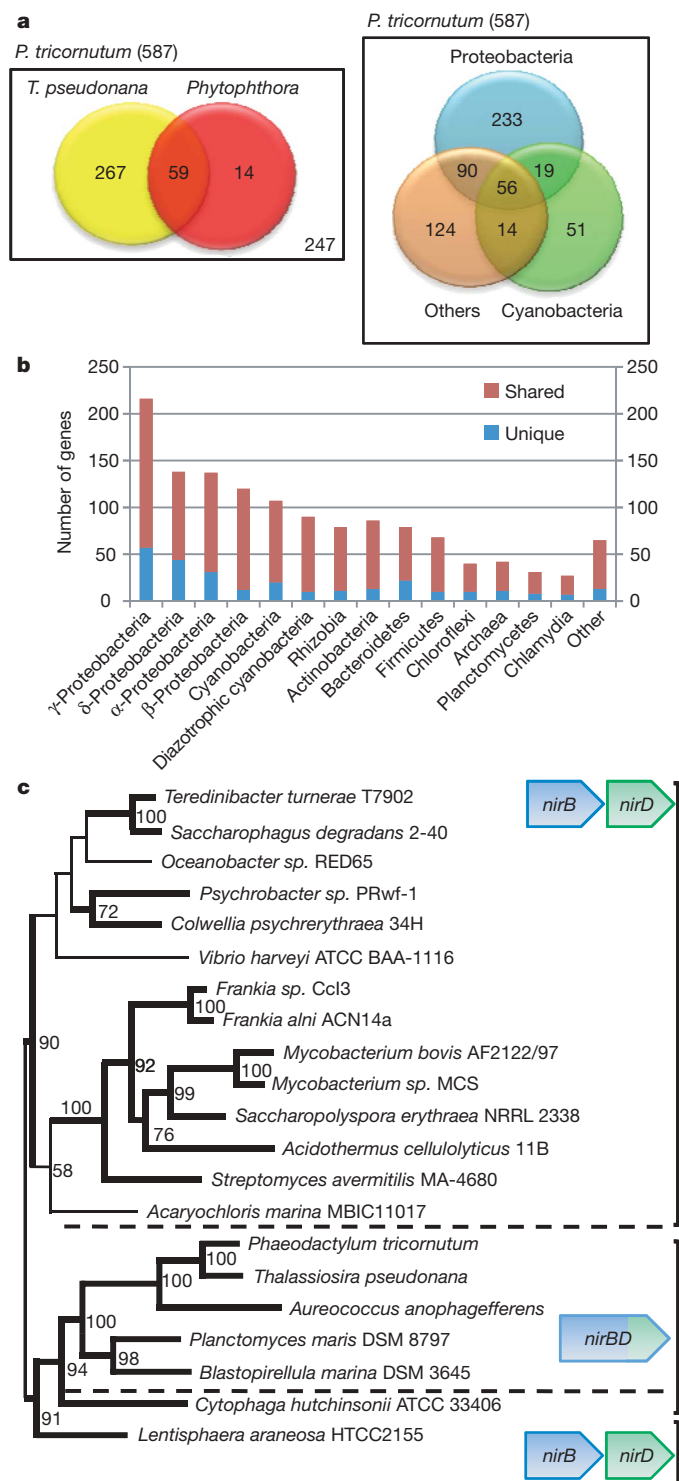


Figure 2 | Bacterial genes in diatoms. **a**, Venn diagrams showing how many of the bacterial genes identified in *P. tricornutum* are also found in other heterokonts (left panel), and which bacterial classes are most related phylogenetically (right panel). In each case, the Venn diagrams indicate the number of trees in which the designated taxa occur within the same clade or in a sister clade of *P. tricornutum*. **b**, Breakdown of different bacterial groups that occur in the same clade or in a sister clade of *P. tricornutum*. ‘Unique’ denotes a gene found only in a particular bacterial class; ‘shared’ denotes a gene that is most similar to a gene of that specific bacterial class but that is also present in other bacterial groups. **c**, PhyML maximum likelihood tree (log likelihood ratio, -22,358.321320) as inferred from the amino acid sequences of the large subunit of NAD(P)H assimilatory nitrite reductase (NirB). The choice of protein evolution model was WAG with gamma-distributed rates ($\alpha = 0.80$), as suggested by a ProtTest analysis of the alignment (see Supplementary Information for methods). Numbers above selected branches indicate maximum likelihood bootstrap support (100 replicates). Gene fusions and distinct open reading frames are indicated adjacent to the appropriate clades. In most cases, the large (NirB) and small (NirD) subunits of NAD(P)H assimilatory nitrite reductase are encoded by distinct open reading frames, but in diatoms and planctomycetes the *nirD* and *nirB* open reading frames have been fused to encode a single gene product. A total of 587 trees show evidence for prokaryotic origins of diatom genes and are available in Supplementary Information.

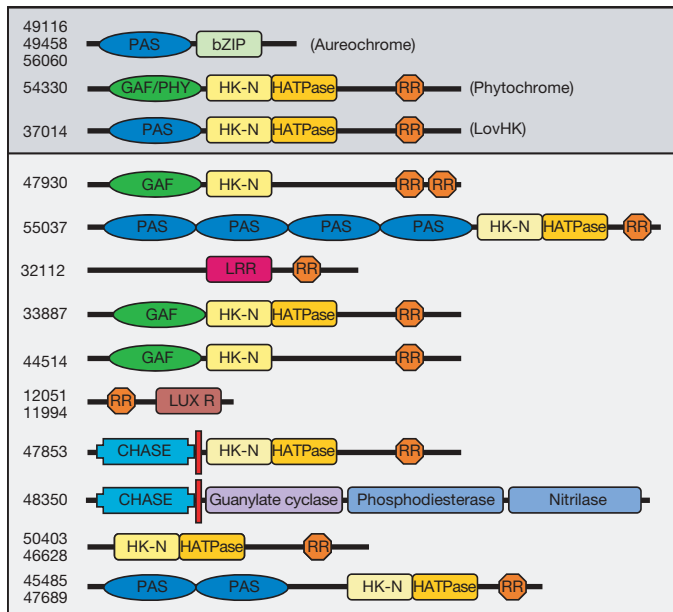


Figure 3 | Domain structures of two-component systems found in *P. tricornutum*. Domains likely to be involved in signalling are illustrated schematically and *P. tricornutum* protein identifiers are indicated on the left. Proteins corresponding to putative photoreceptors (aureochrome, phytochrome and LovHK) are indicated above (in grey, above the horizontal line). For further information about two-component systems, see Supplementary Information. Domain abbreviations are PAS, Per-Arnt-Sim; bZIP, basic region/leucine zipper; GAF, cGMP phosphodiesterase/adenylyl cyclase/Fh1A; PHY, phytochrome; HK-N, histidine kinase N-terminal domain; RR, response regulator; LRR, leucine-rich repeat; LUX R, LuxR transcriptional activator; CHASE, cyclases/histidine kinases associated sensory extracellular.

as previously noted in *T. pseudonana*^{3,4}. Another domain combination present in both diatoms resembles aureochrome blue-light photoreceptors²⁵, and *P. tricornutum* contains orthologues of LovHK and other light-dependent histidine kinases reported in bacteria^{26,27}.

To identify additional novel features of the diatom gene repertoire, we compared the gene family content of the two diatoms with other eukaryotes (Fig. 4, Supplementary Figs 6, 7). Diatoms contain many species-specific multicopy gene families, as well as large numbers of species-specific single-copy genes (denoted orphans in Fig. 4a). The higher number of species-specific gene families in *P. tricornutum* may suggest that the more recent pennate diatoms possess more specialized functions, perhaps related to the heterogeneity of the benthic environments that they commonly inhabit. The centric diatom, by contrast, has retained more features found in other eukaryotes (Fig. 4b, Table 1), such as the flagellar apparatus²⁸. We found a similar number of diatom-specific gene families (1,011) and eukaryotic gene families not found in diatoms (1,062), revealing that the rates of gene gain and gene loss are very similar and consistent with the high diversification rates observed in diatoms. We also found that diatom-specific genes are evolving faster than other genes in diatom genomes (Fig. 4c), providing a further explanation for the rapid diatom divergence rates^{6,7}.

Of the gene families found in the diatoms, some contain higher numbers of genes in comparison with other eukaryotes (Supplementary Table 4, Supplementary Fig. 7); for example, genes involved in polyamine metabolism are over-represented. The expansion of polyamine-related components is of interest in consideration of the role of long-chain polyamines in silica nanofabrication²¹. Of the eight predicted spermine/spermidine synthase-like genes in *P. tricornutum*, three encode potentially bifunctional enzymes bearing both an aminopropyltransferase domain and a SAM decarboxylase domain. Interestingly, the only other organisms containing such

bifunctional proteins are *T. pseudonana* (four copies) and the bacteria *Bdellovibrio bacteriovorus* and *Delftia acidovorans*. Silaffins and silacidins are proteins/peptides believed to be involved in diatom silica formation^{21,29}. *P. tricornutum* contains only one silaffin-like protein, and no homologues of silacidin. Frustulin genes, encoding proteins that form organic constituents of the biosilica cell wall but

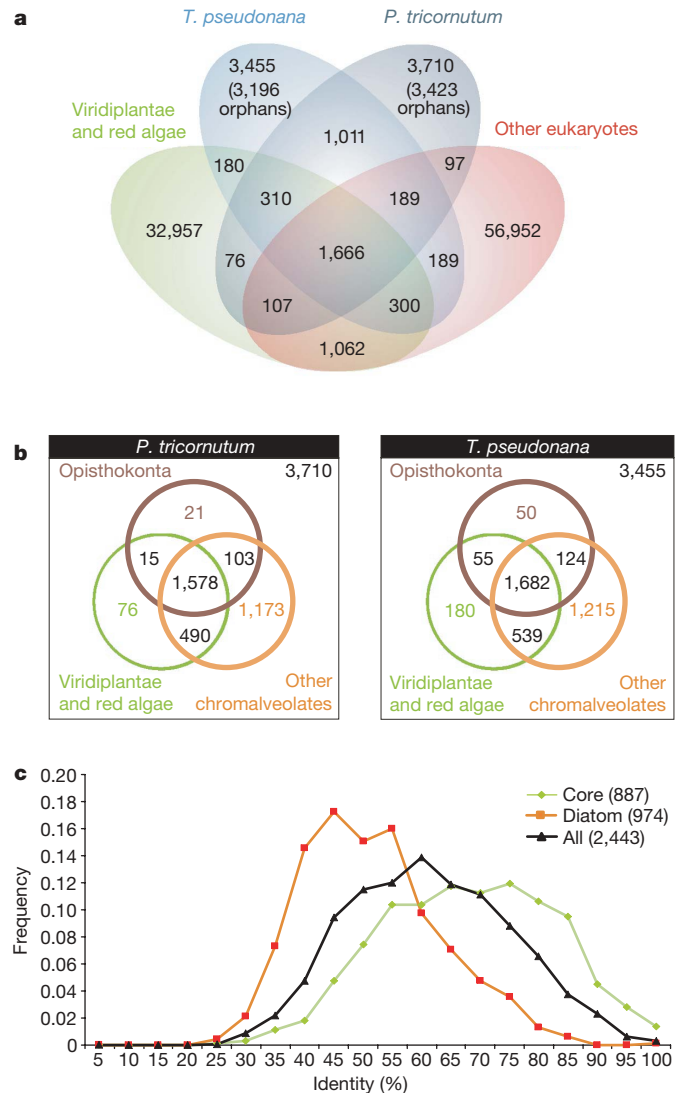


Figure 4 | Shared and unique gene families. **a**, Venn diagram representation of shared/unique gene families in *P. tricornutum*, *T. pseudonana*, Viridiplantae (plants and green algae) and red algae, and other eukaryotes (that is, other chromalveolates and Opisthokonta (fungi and metazoa)). In addition to the total number of gene families specific to *P. tricornutum* and *T. pseudonana*, the number of families consisting of a single gene (denoted 'orphans') is also indicated. For example, of the 3,710 gene families that are only found in *P. tricornutum*, 3,423 consist of single-copy genes whereas 287 gene families have at least two members. **b**, Venn diagram of the distribution of *P. tricornutum* (left panel) and *T. pseudonana* (right panel) gene families with homology to proteins from the Viridiplantae & red algae, Opisthokonta and other chromalveolates (including the other diatom). The numbers outside the circles indicate the number of *P. tricornutum* (left panel) or *T. pseudonana* (right panel) gene families with no homology to the examined proteomes. **c**, Percentage amino acid identity plot of orthologues (based on reciprocal best hits) of different classes of diatom genes identified in **a**. Numbers in parentheses indicate the numbers of orthologues per class. 'Diatom' corresponds to genes only found in *P. tricornutum* and *T. pseudonana* (members of the 1,011 gene families shown in **a**), 'core' corresponds to genes present in all eukaryotic groups (members of the 1,666 gene families shown in **a**), and 'all' corresponds to all orthologous gene pairs in *P. tricornutum* and *T. pseudonana*.

are not involved in silica formation, are present in large numbers and are highly expressed. Both diatoms contain a similar number of silicic acid transporters.

Other noteworthy diatom-specific expansions include histidine kinases (see above and Fig. 3), cyclins and heat-shock transcription factors. Cyclins are major regulators of the cell cycle in eukaryotes. In addition to members of each of the canonical families of cyclins, we found 10 and 42 diatom-specific cyclin genes in *P. tricornutum* and *T. pseudonana*, respectively. The dramatic expansion of this gene family may reflect the unusual characteristics of diatom life cycles due to the rigid nature of their cell walls, such as the control of cell size reduction, the activation of sexual reproduction at a critical size threshold, and life in rapidly changing and unpredictable environments⁷. Conversely, it may be significant that genes encoding RCC1 proteins (RCC, regulator of chromosome condensation), which are also involved in cell cycle control, have been expanded in both diatom genomes (Supplementary Table 4). For the putative heat-shock transcription factors, we found 69 copies in *P. tricornutum* and 89 copies in *T. pseudonana*⁴. These numbers represent almost 50% of the total number of transcription factors in the two sequenced diatoms. The significance of this expansion is unclear, but EST data indicates that the majority are expressed and that some are induced specifically in response to certain growth conditions (Supplementary Fig. 8).

In conclusion, through our comparative analyses we have revealed diverse origins of diatom genes. Diatom-specific genes may have arisen from genome rearrangements and subsequent domain recombinations due to the action of diatom-specific transposable elements, from selective gene family expansions/contractions and from intron gain/loss. It was previously shown that diatoms have retained genes from both partners of the secondary endosymbiosis³, thus bringing together primary metabolic processes such as photosynthetic carbon fixation and organic nitrogen production by means of the urea cycle in a single organism³⁰. Our studies now suggest that genes acquired after secondary endosymbiosis by gene transfer from bacteria are pervasive in diatoms and represent at least 5% of their gene repertoires. This level of horizontal gene transfer is around one order of magnitude higher than has been found in other free-living eukaryotes, and is similar to the rates found between bacteria¹⁹. Although our analyses may be biased by the currently poor taxon sampling of whole genome sequences in eukaryotes (relative to that for prokaryotes), they are nonetheless supported by molecular phylogenies. We therefore propose that gene transfer from bacteria to diatoms, and perhaps vice versa, has been a common event in marine environments and has been a major driving force during diatom evolution. It has also brought together highly unorthodox combinations of genes permitting non-canonical management of carbon and nitrogen in primary metabolism and the sensing of external stimuli adapted to aquatic environments. The combination of mechanisms reported here may underlie the rapid diversification rates observed in diatoms and may explain why they have come to dominate contemporary marine ecosystems in a relatively short period of time.

METHODS SUMMARY

High-molecular-weight DNA was extracted from axenic cultures of *P. tricornutum* accession Pt1 8.6 (deposited as CCMP2561 in the Provasoli-Guillard National Center for Culture of Marine Phytoplankton) and used to construct replicate libraries containing inserts of 2–3 kb, 6–8 kb and 35–40 kb. Using the Joint Genome Institute (JGI) JAZZ assembler, approximately 556,000 reads involving 564 Mb of sequence were trimmed, filtered for short reads and assembled. All low-quality areas and gaps were identified and converted into targets for manual finishing. The draft genome sequence of *T. pseudonana*³ was finished in a similar way. Both diatom genomes were annotated using the JGI annotation pipeline, which combines several gene prediction, annotation and analysis tools. Complementary DNA libraries were constructed from messenger RNA extracted from *P. tricornutum* cultures grown under 16 different conditions. More than 130,000 ESTs were generated. Full information about all methods used for the analyses reported here is available in Supplementary Information.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions C.B. coordinated *Phaeodactylum* genome annotation and manuscript preparation. E.V.A. coordinated *Thalassiosira* genome annotation. D.S.R. and I.V.G. coordinated diatom genome sequencing and analysis at JGI. J.W. coordinated EST sequencing at Genoscope. A.E.A., J.H.B., J.G., K.J., A.K., U.M., C.M., F.M., R.P.O., E.R., A.S. and K.V. made equivalent and substantial contributions to the data presented, and should be considered joint second authors. B.B., A.G., M.H., M.K., T.M., K.V. and F.V. also made significant contributions. A.E.A., E.V.A.,

B.R.G., Y.V.d.P. and I.V.G. assisted in data interpretation and manuscript preparation. Other authors contributed as members of the *Phaeodactylum* genome sequencing consortium.

Author Information Assemblies and annotations of the *P. tricornutum* and *T. pseudonana* genomes are available through the JGI Genome Portal at <http://www.jgi.doe.gov/phaeodactylum> and <http://www.jgi.doe.gov/thalassiosira>. Genome assemblies together with predicted gene models and annotations have been deposited at DDBJ, EMBL and GenBank under the project accessions ABQD000000000 and AAFD000000000, respectively. The versions described in this paper are the first version, ABQD010000000, for *P. tricornutum*, which includes complete chromosomes 3 and 11 (CP001142 and CP001141), and the second version, AAFD020000000, for *T. pseudonana*, also including complete chromosomes 7 and 18 (CP001160 and CP001159). *P. tricornutum* EST expression profiles can be found at <http://www.biologie.ens.fr/diatomics/EST3>, which also provides links to gene models on the JGI genome browser. ESTs have been deposited at NCBI dbEST with GenBank accession numbers CD374840-CD384835, BI306757-BI307753, CT868744-CT950687 and CU695349-CU740080. Reprints and permissions information is available at www.nature.com/reprints. This paper is distributed under the terms of the Creative Commons Attribution-Non-Commercial-Share Alike licence, and is freely available to all readers at www.nature.com/nature. Correspondence and requests for materials should be addressed to C.B. (cbowler@biologie.ens.fr).