

The genome of the extremophile crucifer *Thellungiella parvula*

Maheshi Dassanayake^{1,9}, Dong-Ha Oh^{1,9}, Jeffrey S Haas^{1,2}, Alvaro Hernandez³, Hyewon Hong^{1,4}, Shahjahan Ali⁵, Dae-Jin Yun⁴, Ray A Bressan^{4,6,7}, Jian-Kang Zhu^{6,7}, Hans J Bohnert^{1,4,7,8} & John M Cheeseman¹

***Thellungiella parvula*¹ is related to *Arabidopsis thaliana* and is endemic to saline, resource-poor habitats², making it a model for the evolution of plant adaptation to extreme environments. Here we present the draft genome for this extremophile species. Exclusively by next generation sequencing, we obtained the *de novo* assembled genome in 1,496 gap-free contigs, closely approximating the estimated genome size of 140 Mb. We anchored these contigs to seven pseudo chromosomes without the use of maps. We show that short reads can be assembled to a near-complete chromosome level for a eukaryotic species lacking prior genetic information. The sequence identifies a number of tandem duplications that, by the nature of the duplicated genes, suggest a possible basis for *T. parvula*'s extremophile lifestyle. Our results provide essential background for developing genomically influenced testable hypotheses for the evolution of environmental stress tolerance.**

According to phylogenetic studies based on fossil evidence³, the split between *A. thaliana* and the main Brassica group encompassing *T. parvula* in the subclade *Eutremeae* is thought to have occurred about 43 million years ago. Both *T. parvula* and *A. thaliana* have similar genome sizes, and their close taxonomic relationship provides unique opportunities for tracing evolutionary rearrangements between the two species.

The main goal of this project was to produce a *de novo*, scaffold-level, gap-free assembly of the *T. parvula* genome. To achieve this, we used second generation sequencing exclusively, including ROCHE-454 GS FLX Titanium sequencing for its read length advantage and Illumina GA2 sequencing for its higher quality reads. We included varying insert sizes of paired-end libraries in addition to single-end reads (Online Methods). In total, we obtained 7.8×10^9 high quality basepairs, equivalent to ~50-fold genome coverage. Of these, 85% came from the 454 sequencing (Supplementary Fig. 1 and Supplementary Table 1).

In the absence of genetic maps, with only limited physiological studies⁴, without prior genome information and with only very limited transcriptome sequences, we used an iterative hybrid approach to construct a draft genome (Online Methods). The result was a total

of 1,496 meta-contigs (scaffolds) of merged primary contigs, ranging in size from 1 kb to 13.08 Mb (Table 1). However, unlike typical scaffold sequences, these meta-contigs were free of gaps. Overall, 73% of the length of the *T. parvula* draft genome was represented in 20 contigs longer than 1.5 Mb, and 85% of the sequenced genome was represented by the largest 60 contigs, each 100 kb or greater in length. Based on flow cytometry of propidium-iodide-stained nuclei⁵, the *T. parvula* genome had previously been estimated to be about 160 Mb, or 15% larger than that of *A. thaliana*. The total size of the curated and assembled *T. parvula* genome sequence space, however, was 137.09 Mb. This discrepancy is similar to those for *A. thaliana* (estimated as ~150 Mb, or 25% longer than the sequenced genome⁶) and *Cucumis sativus* (estimated at 30% greater than the draft genome⁷).

Syntenic regions between *T. parvula* contigs and other Brassicaceae chromosomes were apparent after aligning *T. parvula* contigs with the *A. thaliana* genome (Fig. 1) and chromosome A3 of *Brassica rapa*⁸ (Supplementary Fig. 2). The 20 longest contigs covered all five *A. thaliana* chromosomes, with the exception of positions that approached and included centromeric regions. The largest *T. parvula* contig, c1 (13.08 Mb), aligned with the entire length of one arm of *A. thaliana* chromosome 1 (Fig. 1a).

For *T. parvula* contigs and *A. thaliana* chromosomes, we annotated repetitive elements (Online Methods). Overall, repetitive sequences amounted to 7.5% of the *T. parvula* genome based on similarity searches against genomic repeat databases and *de novo* clustering of repetitive sequences (Supplementary Tables 2,3). Figure 1a and b show repeat distributions in combination with overall sequence alignment comparisons using Circos plots.

Repetitive sequences were distributed unevenly in both species. Repeat-rich sequences were concentrated near the centromeric regions in *A. thaliana* chromosomes⁶, as reported for other plant genomes^{9,10}; these sequences were, however, enriched toward the ends of *T. parvula* contigs (Fig. 1a). As a result of established difficulties in assembling repetitive sequences¹¹, we found repeat-rich sequences more frequently among the smaller *T. parvula* contigs (Fig. 1b). Thus, the average repeat content in the largest 20 contigs was 5.5%, whereas the next 40 contigs, c21–c60, contained 17.5% repeat content.

¹Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ²Office of Networked Information Technology, School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ³Center for Comparative & Functional Genomics, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ⁴Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju, Korea. ⁵Bioscience Core Laboratory-Genomics, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia. ⁶Department of Horticulture & Landscape Architecture, Purdue University, West Lafayette, Indiana, USA. ⁷Center for Plant Stress Genomics and Biotechnology, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia. ⁸Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA. ⁹These authors contributed equally to this work. Correspondence should be addressed to M.D. (maheshi.dassanayake@gmail.com), D.-H.O. (ohdongha@gmail.com) or D.-J.Y. (dijun@gnu.ac.kr).

Received 21 March; accepted 24 June; published online 7 August 2011; doi:10.1038/ng.889

Table 1 Overview of the *T. parvula* draft genome sequence

Number of contigs	1,496
Total length of contigs	137.09 Mb
Number of contigs at least N50	8
Contig N50	5.29 Mb
Contig N90	53,201 bp
Minimum contig length	1,008 bp
Maximum contig length	13.08 Mb
Median contig length	5,657 bp
Mean contig length	91,635 bp
GC content	35.75%
Number of predicted genes ^a	30,419
Number of protein coding ORFs	28,901
Gene frequency	1 gene/4,507 bp
ORF frequency	1 ORF/4,705 bp
Mean gene length	2,110 bp
Mean predicted ORF length	1,252 bp
Longest gene	27,059 bp
Longest ORF	16,758 bp
Mean number of exons per gene	5.45
Mean exon length	230 bp
GC content in exons	53.10%
Mean intron length	191 bp
GC content in introns	22.47%
Mean intergenic region length	2,586 bp
GC content in intergenic regions	31.82%

N50 contig size is the value *X* such that at least half of the genome is contained in contigs of size *X* or larger. N90 is the equivalent value at which 90% of the genome is covered by contigs of size *X* or larger.

^aGene numbers were counted following the TAIR convention, that is, including the numbers of protein coding genes, transposable element (based on BLASTn with *A. thaliana* transposable elements) and noncoding RNA genes. ORF, open reading frame.

We predicted gene models using FGENESH++, GENSCAN and BLAST (see URLs) searches to minimize false positive predictions. We based annotations on sequence similarity identified using independent BLAST searches and the Blast2GO pipeline (Online Methods). We manually inspected predicted open reading frames (ORFs) whose length deviated more than 20% from the putative *A. thaliana* homologs for exon merging or splitting. *T. parvula* contained a total of 28,901 predicted protein-coding ORFs. This is about 7% more than *A. thaliana*, which contains 27,059 protein-coding complementary DNAs (cDNAs) (excluding chloroplast and mitochondrial genes and based on the TAIR9 release). We mapped Illumina short read sequences from the transcriptome of young

T. parvula plant tissues to 19,176 of these predicted ORFs (Online Methods and **Supplementary Table 4**).

The mean size of the predicted ORFs was 1,252 bp, with 71% of the ORFs between 201 bp and 1,500 bp in length (**Fig. 2a**). This distribution is similar to that of *A. thaliana* protein-coding cDNAs (**Supplementary Fig. 3**). The GC contents were substantially higher in exons than in introns and intergenic regions (**Table 1**). Based on sequence similarity searches to the NCBI nucleotide database, the primary matches for the *T. parvula* predicted ORFs were most frequently coding regions from *Arabidopsis lyrata* (53%), *A. thaliana* (29%) and *B. rapa* (5%) (**Supplementary Table 5**). BLASTn searches of *T. parvula* ORFs against *A. thaliana* cDNAs identified 25,783 (89%) hits (*e* value < 0.00001). Among these, 21,523 ORFs were of very similar lengths (80–120%) to their putative *A. thaliana* homologs (**Fig. 2b**). The arrangement of predicted ORFs in the *T. parvula* genome showed extensive macro-synteny with *A. thaliana* with infrequent rearrangements (**Supplementary Table 4**), mirroring the genome-wide alignments observed between *A. thaliana* chromosomes and *T. parvula* contigs. Each of the 20 largest *T. parvula* contigs consisted mostly of ORFs that shared sequence similarity with genes from a single *A. thaliana* chromosome, the exception being contig c3, which shared similarity with genes from three chromosomes (**Supplementary Table 6**).

A total of 3,118 predicted ORFs had no BLASTn hits to *A. thaliana* cDNAs even at lowered stringency levels (*e* value > 0.001). We have listed these as unidentified ORFs in **Supplementary Table 4**. Notably, these putative ORFs were enriched in regions containing larger numbers of repetitive sequences, possibly indicating *T. parvula*-specific transposable elements (for example, contigs c17 and c18 in **Supplementary Table 6** and the histograms in the outer circle of **Fig. 1a**). The draft genome also includes 86.6 kb of noncoding RNAs based on sequence searches against microRNA (miRNA) and other noncoding RNA databases (**Supplementary Tables 2,7**).

We assigned Gene Ontology (GO) terms for the *T. parvula* predicted ORFs using the Blast2GO pipeline¹² and compared them with the *A. thaliana* transcriptome (**Fig. 2** and **Supplementary Table 8**). In the GO class 'biological processes', subcategories of 'response to abiotic or biotic stimulus' and 'developmental processes' were enriched in *T. parvula*, whereas genes in the subcategory 'signal transduction' were underrepresented (**Fig. 2c**). In the GO class 'molecular function', we found the subcategories of 'transporter

Figure 1 Macro synteny between *T. parvula* contigs and *A. thaliana* chromosomes. Comparison of the 20 largest *T. parvula* contigs, c1–c20 (**a**) and the 40 next largest contigs, c21–c60 (**b**) with *A. thaliana* chromosomes. *A. thaliana* chromosomes 1–5 are depicted as red, green, yellow, purple and blue, respectively, with the centromeric regions indicated by black bands. *T. parvula* contigs are represented by gray blocks. Regions containing more than 75% similarity over a minimum of 2,000 bp with maximum gap allowance of 1,000 bp are connected with lines of colors matching those used for coloring the *A. thaliana* chromosomes. Ticks in each chromosome or contig block indicate lengths in 1 Mb. The distributions of protein coding regions and repetitive sequences are shown in the outer circles, with the percentage of protein coding genes, DNA transposons and retrotransposons shown in blue, yellow and orange, respectively, with a window size of 0.1 Mb. In the *T. parvula* contigs, predicted protein coding genes without BLASTn hits (*e* value < 0.0001) against the *A. thaliana* cDNA database are shown in green.

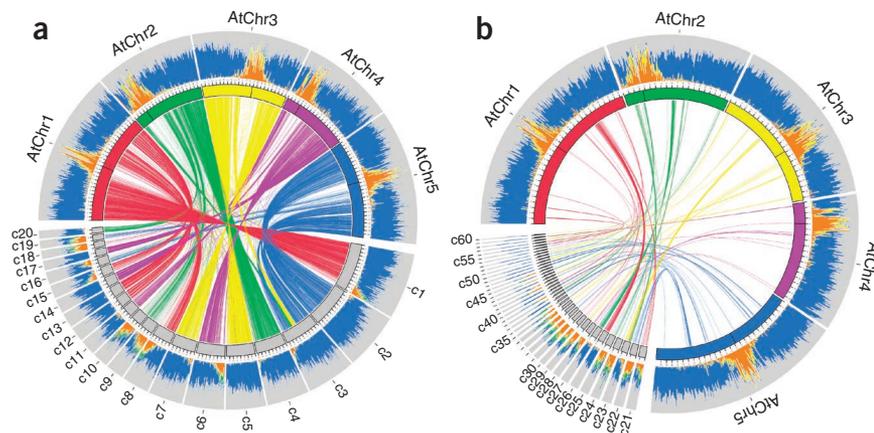
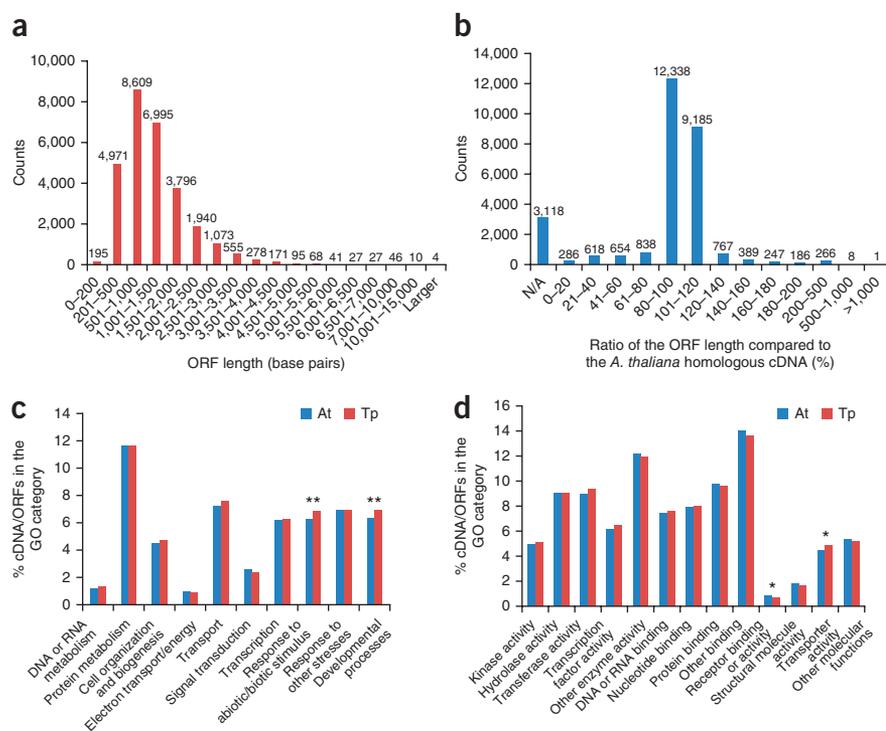


Figure 2 Prediction and annotation of ORFs in the *T. parvula* draft genome. **(a)** Length distribution of predicted *T. parvula* ORFs. **(b)** Comparison of *T. parvula* predicted ORFs with *A. thaliana* cDNAs showing the highest BLASTn hit score. The ratio of *T. parvula* ORF length to *A. thaliana* cDNA length is given as a percentage. In both **a** and **b**, the vertical axes and numbers above the bars are counts. Comparison of GO 'biological processes' **(c)** and GO 'molecular function' categories **(d)** between *A. thaliana* cDNAs (At) and *T. parvula* predicted ORFs (Tp). The GO categories are as defined in TAIR GOslim (see URLs). Categories with significant differences calculated using a χ^2 test, as described in the Online Methods, are indicated as * $P < 0.05$ or ** $P < 0.01$. In **c**, the GOslim categories 'other metabolic processes' (GO:0008152), 'other physiological processes' (GO:0007582) and 'other biological processes' (GO:0008150) are not shown. The complete list of cDNA and ORF numbers in each of the GO categories and their associated *P* values are listed in **Supplementary Table 8**.



activity' and 'receptor binding or activity' to be significantly different between the species (Fig. 2d). Among genes annotated as performing transporter activities, the numbers of ATPase and nucleotide, cation and sugar transporters were significantly higher in *T. parvula* than in *A. thaliana* (Table 2). These differences may reflect the different habitats and environmental pressures to which the species adapted. ATPase and nucleotide transporters with functions in pH homeostasis and cellular energy generation have, for example, been related to protection under salinity stress^{13,14}, whereas transport and accumulation of soluble sugars or polyols are considered key mechanisms that provide osmotic stress tolerance¹⁵. We found the most significant difference in gene copy numbers for transporters of cations other than Na⁺ and K⁺, perhaps reflecting the adaptation of *T. parvula* to soil not only containing saline but also imbalanced in other ions^{2,16}.

Gene copy number variation has also been proposed as a major mechanism of phenotypic differentiation and as reflecting evolutionary adaptation to the environment^{17–19}. The *T. parvula* genome included 1,842 more predicted ORFs than protein coding cDNAs in *A. thaliana* (Supplementary Fig. 3). Mirroring the observed differences in the GO subcategories, the *T. parvula* genome contained higher copy numbers of orthologous genes related to stress adaptation, for example, *AVP1*, *HKT1*, *NHX8* (ref. 20), *CBL10* (ref. 21) and *MYB47* (Supplementary Table 4).

Gene duplication as a vehicle for evolution has long been hypothesized²², and experimental evidence of this has recently been accumulating^{7,23–25}. In both *T. parvula* and *A. thaliana*, the major role in generating copy number variation has been played by tandem gene duplication (Fig. 3a) rather than by large gains or losses in segment composition following the *A. thaliana* and *T. parvula* divergence after the most recent whole-genome duplication³. We found a total of 1,278 and 1,113 tandem duplication events in the *T. parvula* and *A. thaliana* genomes, respectively. Only half of these were shared between the two species (Fig. 3b and Supplementary Table 9). Inspection of the GO class representation of tandem duplications revealed significantly different GO 'biological process' (Fig. 3c) and GO 'molecular function' (Fig. 3d) subcategories. Differences in gene numbers in the subcategories 'response to abiotic or biotic stimulus' and 'developmental

processes' (Fig. 2c) were most prominent among genes multiplied by tandem duplication (Fig. 3c), as supported by substantially lower *P* values (Supplementary Table 8).

Finally, Figure 4 and Supplementary Table 10 show the assembly of the *T. parvula* contigs into the seven chromosomes that characterize

Table 2 Detailed comparison of GO categories with transporter activity or receptor binding or activity between *T. parvula* and *A. thaliana*

GO annotation	AT	TP
Total number of genes annotated as transporters^a	1,219	1,408
Amino acid transporters	65	80
Anion transporters	44	64
Aquaporins	39	46
ATPases	146 ^b	187 ^b
Cytochrome proteins	23	19
Fatty acid, lipid, lipoprotein and glycolipid transporters	41	44
K ⁺ , Na ⁺ ion transporters	93	104
Mitochondrial carrier protein	73	93
Nucleoside and nucleotide transporters	64 ^b	90 ^b
Oligopeptide transporters	71	79
Other cation (Zn ²⁺ , Fe ²⁺ , Ca ²⁺ , Mn ⁺ , Cu, Ni, Mo, Cd and NH ⁴⁺) transporters	104 ^b	195 ^b
Other transporters	295	327
Phosphate transporters	15	18
Phosphatidylinositol	29	37
Sugar transporters	120 ^b	174 ^b
Transporter, ABC superfamily	113	126
Total number of genes annotated as receptor binding or activity^a	232	201
Blue light receptors	4	9
Cytokinin receptors	3	4
Disease resistance-related	101 ^b	67 ^b
ER lumen protein retaining receptor	6	10
Osmosensors	4	7
Other receptors	66	64
Receptor kinases	37	54
Red and far-red receptors	4	4

^aThe total number of genes annotated as transporters or receptor binding or activity is not the sum of annotated genes in each subcategory. Some of the genes have multiple functions and are involved in multiple classes. ^bSubcategories showing significant differences (based on χ^2 tests, see Online Methods). The *P* values of individual categories are: ATPases, *P* = 0.098; nucleoside and nucleotide transporters, *P* = 0.091; other cation transporters, *P* = 0.0001; sugar transporters, *P* = 0.0095; and disease resistance-related, *P* = 0.0022.

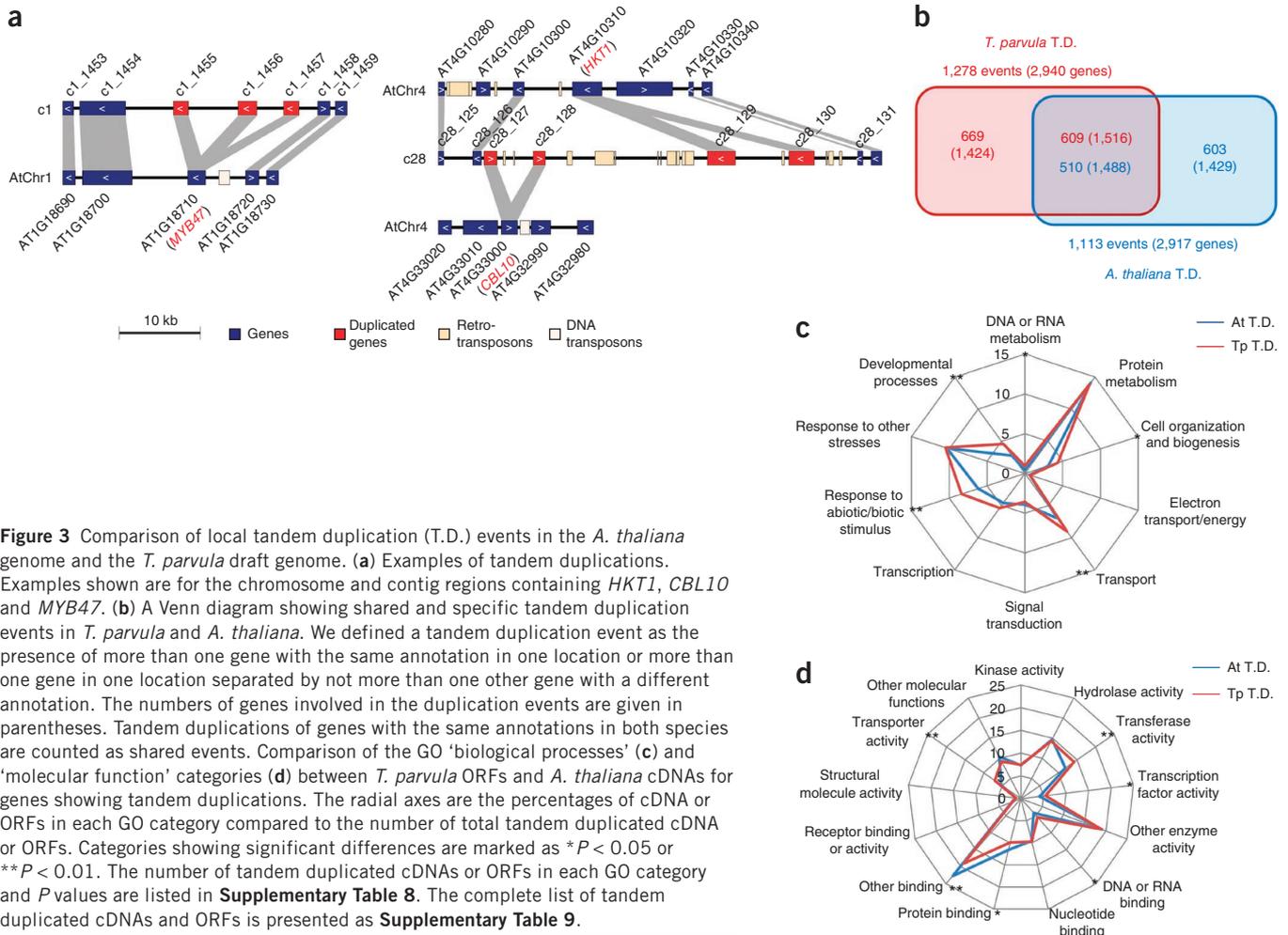


Figure 3 Comparison of local tandem duplication (T.D.) events in the *A. thaliana* genome and the *T. parvula* draft genome. **(a)** Examples of tandem duplications. Examples shown are for the chromosome and contig regions containing *HKT1*, *CBL10* and *MYB47*. **(b)** A Venn diagram showing shared and specific tandem duplication events in *T. parvula* and *A. thaliana*. We defined a tandem duplication event as the presence of more than one gene with the same annotation in one location or more than one gene in one location separated by not more than one other gene with a different annotation. The numbers of genes involved in the duplication events are given in parentheses. Tandem duplications of genes with the same annotations in both species are counted as shared events. Comparison of the GO 'biological processes' **(c)** and 'molecular function' categories **(d)** between *T. parvula* ORFs and *A. thaliana* cDNAs for genes showing tandem duplications. The radial axes are the percentages of cDNA or ORFs in each GO category compared to the number of total tandem duplicated cDNA or ORFs. Categories showing significant differences are marked as * $P < 0.05$ or ** $P < 0.01$. The number of tandem duplicated cDNAs or ORFs in each GO category and P values are listed in **Supplementary Table 8**. The complete list of tandem duplicated cDNAs and ORFs is presented as **Supplementary Table 9**.

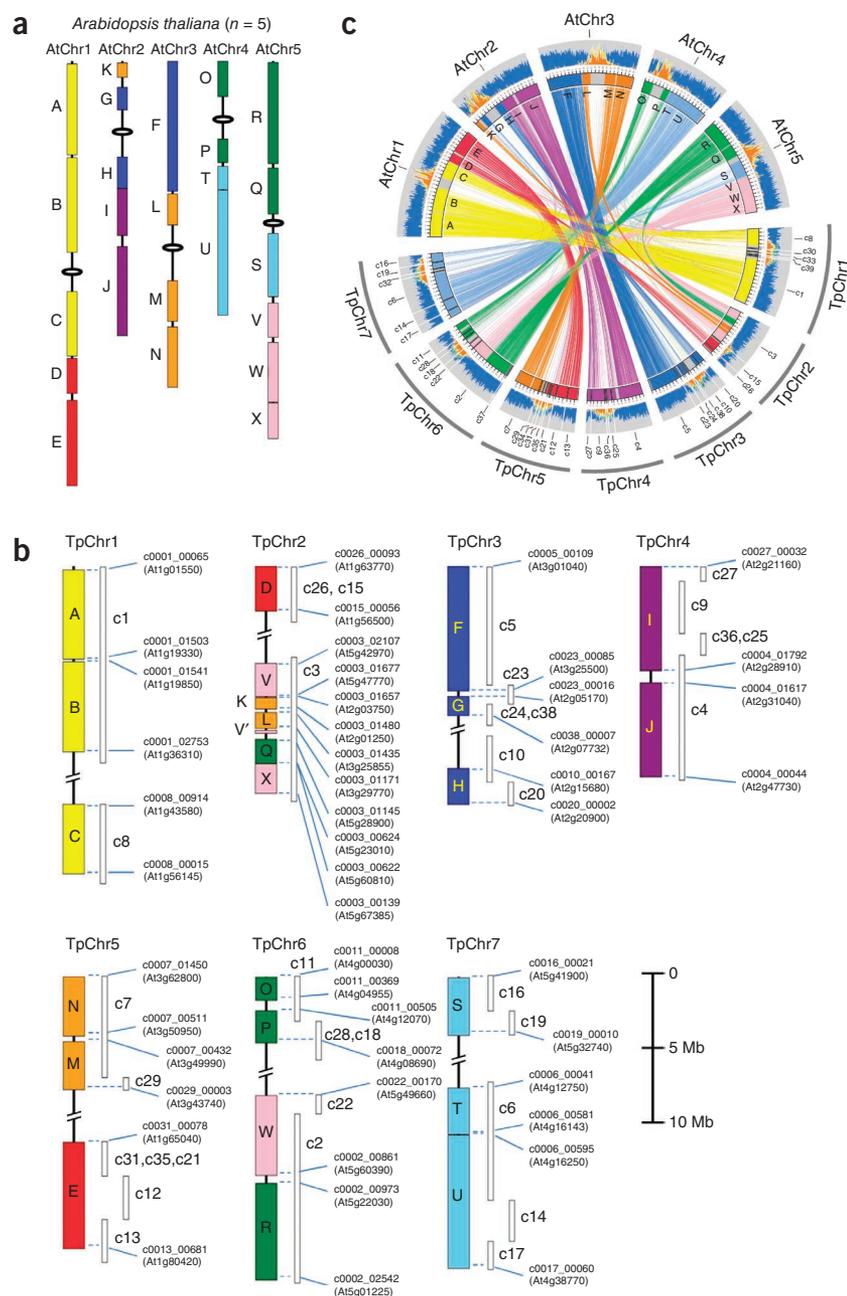
this species. The evolution of chromosome structures in *Brassicaceae* has previously been traced through comparative 'chromosome painting' techniques using BAC-size sequence probes from the *A. thaliana* genome²⁶. With these techniques, Lysak and colleagues²⁶ identified large genome segments, termed A to X, derived from an ancestral karyotype ($n = 8$). These ancestral karyotypes can be found in different assemblages in chromosome structures of different *Brassicaceae* clades²⁶, including *A. thaliana*²⁷ (**Fig. 4a**) and *Eutremaeae*²⁸ ($n = 7$). Using these as guides, the 40 largest *T. parvula* contigs could be unambiguously assembled into seven chromosomes²⁸ covering 114.39 Mb (83% of the draft genome) (**Fig. 4b** and **Supplementary Table 10**). Each of these has a distinct, repeat-rich region, signifying the centromere (**Fig. 4c**, outer histogram). That the five largest contigs (c1–c5) covered the entire lengths of single chromosome arms attests to the quality of the *de novo* assembly. It is further noteworthy that the genomic regions in *T. parvula* contigs c2 and c3, although showing extensive rearrangements compared to the *A. thaliana* genome sequence, matched distinct ancestral karyotype blocks (**Supplementary Fig. 4a**, ancestral karyotype blocks R and W for c2, and **Supplementary Fig. 4b**, ancestral karyotype blocks V, K, L, Q, V' and X for c3). Thus, our model for the *T. parvula* chromosomes provides sequence-based evidence for the Lysak model for crucifer species with $n = 7$, including the clade *Eutremaeae*²⁸. It also defines the boundaries of ancestral karyotype blocks more clearly and suggests more detailed structure than can be captured by chromosome

painting experiments alone. This is particularly clear with respect to ancestral karyotype block V, which, based on sequence information, was divided into the blocks V and V' in the *T. parvula* genome (**Fig. 4b** and **Supplementary Fig. 4b**). Also, ancestral karyotype block I extended to the pericentromeric region of *T. parvula* chromosome 4 (**Fig. 4b,c**) rather than falling entirely to one side of the centromere, as previously indicated by the chromosome painting experiments in various crucifer species with $n = 7$ (ref. 28).

A number of angiosperm families include extremophile species, although fewer than 10% of all plant species may be classified this way. Extremophiles' presence in evolutionarily distinct lineages reveals genetic complexities that appear to have evolved from the common genetic makeup of all plants. In adaptation to various combinations of environmental stresses, these extremophiles show tolerance of stresses against which crop plants in particular have no defenses. Knowing how extremophiles operate can, however, instruct us about the underlying genetic requisites and mechanisms for successful stress defenses. In this report, we have now shown that it is possible to determine the genome sequence of extremophiles, as well as model glycophytes, exclusively relying on next-generation DNA sequencing tools and *de novo* assembly.

The availability of the *T. parvula* genome provides a unique view of chromosome structure, organization and gene complement. Of particular importance is the comparison of this genome with that of the related *A. thaliana*, which is unquestionably a stress-sensitive species. In our

Figure 4 Assembly of the seven chromosomes of *T. parvula*. **(a)** Outline of the ancestral karyotype segments determined by comparative chromosome painting techniques^{26,27} in *A. thaliana* chromosomes. The ancestral karyotype segments, denoted A to X, are drawn to scale based on the *A. thaliana* genome sequence. **(b)** *T. parvula* contigs aligned to the *Eutremaeae* ($n = 7$) karyotype schema^{26,28} and the ORFs defining the borders of the ancestral karyotype segments. *A. thaliana* locus IDs showing the highest homology with each ORF are given in parentheses. Shown are *T. parvula* contigs covering the ancestral karyotype segments. Complete chromosome assignment of the 40 largest contigs, including the contigs covering the centromeric regions, are presented in **Supplementary Table 10**. **(c)** Circos plot presenting the assembly of seven chromosomes. The 40 largest *T. parvula* contigs are shown. The links and histograms in the outer circles showing the distribution of protein coding genes and repetitive sequences were generated as in **Figure 1**. The ancestral karyotype segments in the *A. thaliana* chromosomes and *T. parvula* contigs and the links connecting them are depicted with colors as in **a** and **b**.



initial analysis, this halophyte, with a genome only ~15% larger than that of *A. thaliana*, shows striking differences in gene complement. The differences are partly because of tandem duplications in *T. parvula* of single copy genes in *A. thaliana* and preferential amplification of genes with known or assumed functions in stress defense responses. Within these differences, we expect, lie the unique solutions to understanding *T. parvula*'s particular lifestyle and adaptation to its demanding ecological niche. More detailed examination of genome structure, coding complexity, and gene structure and expression in stress response pathways in comparative studies will point the way toward correlating the *T. parvula* phenotype with its genetic makeup.

URLs. SeqAnswers online forum, <http://seqanswers.com/>; GENSCAN, <http://genes.mit.edu/GENSCAN.html>; BLAST, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>; FGENESH++, <http://linux1.softberry.com/berry.phtml?topic=fgene&shplus2>; TAIR GOslim, ftp://ftp.arabidopsis.org/home/tair/Ontologies/sff_extract program, http://bioinf.comav.upv.es/sff_extract/; Vmatch suite, <http://www.vmatch.de/>; AMOS pipeline, <http://sourceforge.net/apps/mediawiki/amos/index.php?title=AMOS>; Repbase, <http://www.girinst.org/repbase/>; Plant Repeat database, ftp://ftp.plantbiology.msu.edu/pub/data/TIGR_Plant_Repeats/; TAIR9 cDNA database, ftp://ftp.arabidopsis.org/home/tair/Sequences/blast_datasets/TAIR9_blastsets/TAIR9_cdna_20090619; miRBase, <http://www.mirbase.org/>; Rfam database, <http://rfam.sanger.ac.uk/>; GraphPad QuickCalcs, <http://www.graphpad.com/quickcalcs/contingency1.cfm>.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

Accession codes. The raw reads for this project are deposited in the NCBI SRA project under the accession number SRA026763. The Illumina reads can be accessed under SRX047632 and the 454 reads under SRX032604. The genome assembly is deposited with the NCBI Genome Project ID 63843, and the sequences are deposited with the GenBank ID AFAN000000001.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank M.P. D'Urzo (Purdue University, West Lafayette, Indiana, USA) for providing plant materials and J.-H. Mun (National Academy of Agricultural Science, Suwon, Korea) for providing the *B. rapa* chromosome sequence. We also gratefully acknowledge M. Vaughn (University of Texas, Austin, Texas, USA), S. Jackman, M. Krzywinski (Michael Smith Genome Sciences Center, Vancouver, British Columbia, Canada) and SeqAnswers online forum (see URLs) for advice on genome assembly and visualization. Funding has been provided by King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, by the World Class

University Program (R32-10148) at Gyeongsang National University, Republic of Korea and the Next-generation BioGreen21 Program (SSAC, PJ008025), Rural Development Administration, Republic of Korea.

AUTHOR CONTRIBUTIONS

M.D., D.-H.O., H.J.B. and J.M.C. designed, performed, analyzed experiments and wrote the paper; J.S.H. compiled programs and wrote custom scripts; A.H. and S.A. performed sequencing; H.H. prepared materials; D.-J.Y., R.A.B. and J.-K.Z. provided materials and intellectual feedback.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at <http://www.nature.com/naturegenetics/>.

Reprints and permissions information is available online at <http://www.nature.com/reprints/index/html>.

- Al-Shehbaz, I.A. & O'Kane, S.L. Placement of *Arabidopsis parvula* in *Thellungiella* (Brassicaceae). *Novon* **5**, 309–310 (1995).
- Amtmann, A. Learning from evolution: *Thellungiella* generates new knowledge on essential and critical components of abiotic stress tolerance in plants. *Mol. Plant* **2**, 3–12 (2009).
- Beilstein, M.A. *et al.* Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **107**, 18724–18728 (2010).
- Orsini, F. *et al.* A comparative study of salt tolerance parameters in 11 wild relatives of *Arabidopsis thaliana*. *J. Exp. Bot.* **61**, 3787–3798 (2010).
- Oh, D.-H. *et al.* Genome structures and halophyte-specific gene expression of the extremophile *Thellungiella parvula* in comparison with *Thellungiella salsuginea* (*Thellungiella halophila*) and *Arabidopsis*. *Plant Physiol.* **154**, 1040–1052 (2010).
- Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**, 796–815 (2000).
- Huang, S. *et al.* The genome of the cucumber, *Cucumis sativus* L. *Nat. Genet.* **41**, 1275–1281 (2009).
- Mun, J.-H. *et al.* Sequence and structure of *Brassica rapa* chromosome A3. *Genome Biol.* **11**, R94 (2010).
- Schmutz, J. *et al.* Genome sequence of the palaeopolyploid soybean. *Nature* **463**, 178–183 (2010).
- International Brachypodium Initiative. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* **463**, 763–768 (2010).
- Alkan, C., Sajjadian, S. & Eichler, E.E. Limitations of next-generation genome sequence assembly. *Nat. Methods* **8**, 61–65 (2011).
- Conesa, A. & Götz, S. Blast2GO: A comprehensive suite for functional analysis in plant genomics. *Intl. J. Plant Genomics* **2008**, 619832 (2008).
- Oh, D.-H. *et al.* Intracellular consequences of SOS1 deficiency during salt stress. *J. Exp. Bot.* **61**, 1205–1213 (2010).
- Gao, F. *et al.* Cloning of an H⁺-PPase gene from *Thellungiella halophila* and its heterologous expression to improve tobacco salt tolerance. *J. Exp. Bot.* **57**, 3259–3270 (2006).
- Lugan, R. *et al.* Metabolome and water homeostasis analysis of *Thellungiella salsuginea* suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. *Plant J.* **64**, 215–229 (2010).
- Inan, G. *et al.* Salt cress. A halophyte and cryophyte *Arabidopsis* relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. *Plant Physiol.* **135**, 1718–1737 (2004).
- Sudmant, P.H. *et al.* Diversity of human copy number variation and multicopy genes. *Science* **330**, 641–646 (2010).
- Dassanayake, M. *et al.* Transcription strength and halophytic lifestyle. *Trends Plant Sci.* **16**, 1–3 (2011).
- Hastings, P.J. *et al.* Mechanisms of change in gene copy number. *Nat. Rev. Genet.* **10**, 551–564 (2009).
- Craig Plett, D. & Möller, I.S. Na⁺ transport in glycophytic plants: what we know and would like to know. *Plant Cell Environ.* **33**, 612–626 (2010).
- Quan, R. *et al.* SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect *Arabidopsis* shoots from salt stress. *Plant Cell* **19**, 1415–1431 (2007).
- Ohno, S. *Evolution by Gene Duplication* 160 (Springer, New York, New York, USA, 1970).
- Hanada, K. *et al.* Importance of lineage-specific expansion of plant tandem duplicates in the adaptive response to environmental stimuli. *Plant Physiol.* **148**, 993–1003 (2008).
- Cannon, S.B. *et al.* The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* **4**, 10 (2004).
- DeBolt, S. Copy number variation shapes genome diversity in *Arabidopsis* over immediate family generational scales. *Genome Biol. Evol.* **2**, 441–453 (2010).
- Lysak, M.A. & Koch, M.A. Phylogeny, genome, and karyotype evolution of crucifers (Brassicaceae). in *Genetics and Genomics of the Brassicaceae* (eds. Schmidt, R. & Bancroft, I.). 1–31 (Springer, New York, New York, USA, 2011).
- Lysak, M.A. *et al.* Mechanisms of chromosome number reduction in *Arabidopsis thaliana* and related Brassicaceae species. *Proc. Natl. Acad. Sci. USA* **103**, 5224–5229 (2006).
- Mandáková, T. & Lysak, M.A. Chromosomal phylogeny and karyotype evolution in $x = 7$ crucifer species (Brassicaceae). *Plant Cell* **20**, 2559–2570 (2008).

ONLINE METHODS

Plant material and DNA extraction. Total DNA was isolated from 10-day-old seedlings of *T. parvula*. The seeds were derived from a single plant propagated from single seeds over eight successive generations. The original accession was collected from a salt lake in Tuz Golu, central Turkey at an elevation of 905 m above sea level. At the collection site, the soil bulk density was 1.225 g/cm³ with 32.4% salts by weight. Genomic DNA was prepared using the Nucleon Phytopure Genomic DNA Extraction kit (GE Healthcare).

Strategy for a highly contiguous draft genome. Compared to Sanger sequencing, the shorter reads associated with either 454 or Illumina sequencing manifest decreased connectivity. As a result, considerably deeper coverage is required to generate contiguous assemblies. Deeper coverage alone, however, does not in itself solve the problem of fragmented assemblies; if reads are shorter than a repeat, gaps are unavoidable, and with deeper coverage, accumulated sequencing errors make assembly more computationally challenging. In assembling the *T. parvula* genome, the problem was mitigated by (i) using reads from different technologies, (ii) using paired reads with different insert lengths to span different repeat lengths and (iii) computationally selecting high quality reads.

Overview of sequencing, assembly and annotation. Library construction and sequencing were performed in the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. Random shotgun genomic libraries were constructed according to the manufacturer's recommendations for each of the two pyrosequencing platforms, GS FLX Titanium (454 Life Sciences) and Illumina GA2 (Illumina). Newbler (454-Roche), ABySS²⁹ and minimus2 (ref. 30) were used as the main assembly programs to generate the draft genome, and FGENESH++ (SoftBerry), GENSCAN, BLAST (see URLs) and Blast2GO¹² were used to predict and annotate gene models.

DNA library preparation and sequencing. For 454 pyrosequencing, both shotgun and paired-end libraries were constructed. Genomic DNA was randomly sheared by nebulization to fragments of 500–800 bp in length to construct two shotgun libraries. Additional DNA was processed to construct paired-end libraries with size spans of 3 kb (three libraries), 8 kb (two libraries) and 20 kb (two libraries). All libraries were constructed, clonally amplified and sequenced on the 454 Genome Sequencer FLX-Titanium according to the manufacturer's kits and protocols (454 Life Sciences). Signal processing and base calling were performed using the bundled GS FLX software version 2.0.01.

For Illumina sequencing, genomic DNA was nebulized, and fragments 200–500 bp in length were size selected to construct a shotgun library using the Illumina Genomic DNA Sample Prep kit (Illumina). The library was sequenced on three lanes of a flowcell from one end (single read) for 81 cycles on a Genome Analyzer IIx. The Illumina Pipeline 1.5 was used to generate fastq sequence files from the raw data.

Hybrid genome assembly. A combined total of 7.8×10^9 bases resulted from sequencing using both platforms. Average read sizes were 355 bp and 80 bp for the 454 and Illumina sequences, respectively. Approximately 85% of all sequences were derived from the 454 sequencing. We followed an iterative approach for assembly starting from raw sequence reads assembled into primary contigs. We used two assembly programs and combined the primary contigs and paired-end data to build scaffolds in successive assemblies. Single and paired-end 454 sequences were assembled using the Roche GS assembler, Newbler (version 2.0.01.14), with a 40 bp minimum overlap and 90% identity. In both instances, reads were first assembled as single-end reads, after which the paired-end information was used to construct scaffolds.

To assemble Illumina reads, we tested both Velvet³¹ (v1.3) and ABySS²⁹ (v1.2) short-read assemblers using only reads that passed the Illumina chastity filter (base call values for chastity greater than 0.6 in the first 25 cycles); the k-mer size was set to 31 bp, and the coverage cutoff was set to 4. Both assemblers produced comparable results, but ABySS was much faster and was, therefore, chosen for further optimized short read assemblies. We also used Newbler contigs as single reads with the Illumina reads as the input in ABySS. We tested every odd-numbered length from 29 bp to 61 bp as the

k-mer size to find the optimal size, meaning that which yielded the longest N50 and the fewest total contigs while maintaining total contig length near the flow-cytometry-estimated genome size of 160–180 Mb.

Because ABySS can be very sensitive to sequencing errors such as short indels, when using raw 454 reads in ABySS, custom Perl scripts were used to remove any raw 454 read that had homopolymers exceeding 10 bases (all 454 homopolymer error reads cannot be removed but can be minimized). To enable the scaffold generating step in ABySS to proceed when 454 raw paired-ends reads were used, the program *sff_extract* (see URLs) was used to process the standard flowgram (*sff*) files generated by the GS FLX sequencer. Different k-mer sizes were selected based on the different paired-end libraries used for scaffolding, but in most instances, the optimum k-mer size found with ABySS was 41.

The collection of contigs and scaffolds created in the primary assemblies was an overlapping set with a high level of redundancy. To select a non-redundant set, we used *mktree* in the *Vmatch* suite (see URLs) to index the sequences by length; *Vmatch* was used to cluster sequences, including clusters with size of 1 (singlets). We matched for 100% identity and full coverage of the smaller sequences in pairs. Contigs longer than 1,000 bp were used for further processing. This set was further inspected with all-against-all BLAST searches and the aligner NUCmer in MUMmer 3.22 (ref. 32) to remove duplicate contigs that may have been assembled for the same region of the genome.

The meta-assembly of selected contigs from primary assemblies was carried out with the overlap-layout-consensus assembler, *Minimus2* in the AMOS pipeline (see URLs), using a minimum 40-bp overlap with 95% identity. The resulting contigs and singlets were combined and purged of further redundancy, contaminating DNA and mitochondrial and chloroplast DNA using BLAST searches. This resulted in 1,496 contigs with a total length of ~137 Mb.

Genome annotation. The *T. parvula* draft genome was masked for repetitive sequences by *RepeatMasker*³³ searching Repbase 14.01 and with BLASTn using the Plant Repeat database (see URLs). The masked contigs for known repetitive elements were further analyzed with NUCmer and custom scripts to search for long tandem repeats and for *T. parvula*-specific unclassified, non-exact, long repeats. Any sequences that were found more than five times were considered as repeats in this search.

FGENESH++ (SoftBerry) was used to predict protein coding ORFs in the *T. parvula* draft genome masked for repetitive sequences, with parameters optimized for dicot plants and protein sequences from the NCBI non-redundant (NR) database as reference. A total of 29,338 ORFs were predicted, of which 437 were further annotated as transposable elements based on BLASTn searches. Genomic regions that contained FGENESH++-predicted ORFs with lengths similar to their *Arabidopsis* homologs ($\pm 20\%$) were tested with another gene prediction program, GENSCAN. When the predictions from the two programs deviated for the same genomic region, the ORF closest in length to another known homologous cDNA was taken as the more likely prediction. All genomic contigs and predicted ORFs were searched against NCBI nucleotide and protein databases and TAIR9 cDNA database (see URLs) using BLASTn and BLASTx searches. The predicted proteins were further annotated with the Blast2GO pipeline¹² to assign GO and GOSlim-plant terms based on NCBI plant databases and InterProScan³⁴. To obtain experimental evidence for our *ab initio* predictions, we mapped the ORFs to high quality Illumina reads trimmed to 80 bp from a transcriptome sequence library generated from young seedlings. Using the program *Bowtie*³⁵ with 100% identity to a minimum length of 50 bp and with '-m' set to 1 to ensure unique mapping, we found that 73% of the high quality reads mapped uniquely to the predicted ORFs (Supplementary Table 4). The remaining reads are too repetitive in nature, map to multiple ORFs or contain low complexity regions and are therefore unusable in mapping.

BLAST searches were performed to identify miRNA genes and other RNA genes by searching against the miRBase database of plant miRNA collections (release 16) and the Rfam database (see URLs) (release 10) for other non-coding RNA families including rRNA and tRNA genes.

Statistical analyses. When comparing the distributions of GO subcategories between *A. thaliana* and *T. parvula* (Figs. 2 and 3), two-tailed χ^2 tests were used (see URLs). For each GO subcategory, a 2×2 contingency table was constructed

by recording the numbers of genes included or not included in a subcategory for each species and ranking the statistical significance of the differences.

29. Simpson, J.T. *et al.* ABySS: a parallel assembler for short read sequence data. *Genome Res.* **19**, 1117–1123 (2009).
30. Sommer, D.D. *et al.* Minimus: a fast, lightweight genome assembler. *BMC Bioinformatics* **8**, 64 (2007).
31. Zerbino, D.R. & Birney, E. Velvet: Algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* **18**, 821–829 (2008).
32. Kurtz, S. *et al.* Versatile and open software for comparing large genomes. *Genome Biol.* **5**, R12 (2004).
33. Jurka, J. *et al.* Repbase Update, a database of eukaryotic repetitive elements. *Cytogenet. Genome Res.* **110**, 462–467 (2005).
34. Zdobnov, E.M. & Apweiler, R. InterProScan—an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* **17**, 847–848 (2001).
35. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S.L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25 (2009).