

HSDatabase—a database of highly similar duplicate genes from plants, animals, and algae

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Abstract

Gene duplication is an important evolutionary mechanism capable of providing new genetic material, which in some instances can help organisms adapt to various environmental conditions. Recent studies, for example, have indicated that highly similar duplicate genes (HSDs) are aiding adaptation to extreme conditions via gene dosage. However, for most eukaryotic genomes HSDs remain uncharacterized, partly because they can be hard to identify and categorize efficiently and effectively. Here, we collected and curated HSDs in nuclear genomes from various model animals, land plants and algae and indexed them in an online, open-access sequence repository called HSDatabase. Currently, this database contains 117 864 curated HSDs from 40 distinct genomes; it includes statistics on the total number of HSDs per genome as well as individual HSD copy numbers/lengths and provides sequence alignments of the duplicate gene copies. HSDatabase also allows users to download sequences of gene copies, access genome browsers, and link out to other databases, such as Pfam and Kyoto Encyclopedia of Genes and Genomes. What is more, a built-in Basic Local Alignment Search Tool option is available to conveniently explore potential homologous sequences of interest within and across species. HSDatabase has a user-friendly interface and provides easy access to the source data. It can be used on its own for comparative analyses of gene duplicates or in conjunction with HSDFinder, a newly developed bioinformatics tool for identifying, annotating, categorizing and visualizing HSDs.

Database URL: <http://hsdfinder.com/database/>

Introduction

Gene duplication is a near-ubiquitous phenomenon throughout the eukaryotic tree of life (1), one that can be advantageous or disadvantageous, depending on the circumstances. For example, under certain conditions, it can be detrimental for an organism to retain highly similar expressed genes (2). Thus, with notable exceptions, it is relatively rare for species to maintain duplicate genes encoding the same functions (3). Nevertheless, it is becoming more apparent that in some situations the generation and maintenance of highly similar duplicate genes (HSDs) is possible, particularly for genes encoding products that are in high demand, such as histones or ribosomal proteins (4). Indeed, there are many examples suggesting that genes involved in stress response, sensory functions, transport and/or metabolism are likely to be fixed as duplicated copies given specific environmental conditions (5).

Recently, Zhang *et al.* (6) revealed that hundreds of HSDs, involved in diverse cellular processes, are maintained in the psychrophilic Antarctic green alga *Chlamydomonas* sp. UWO241, which was recently renamed *Chlamydomonas*

priscuii (7). It is believed that these HSDs are aiding its survival via gene dosage (8). Unfortunately, the HSDs from most other eukaryotic genomes, particularly those of algae, remain uncharacterized. This is partly because the experimental methods for identifying HSDs are time-consuming and labor-intensive. Many of the available bioinformatics tools for characterizing homologs are limited by their designs (e.g. they only identify orthologs) or their specificity (e.g. they only identify retrocopies or co-localized duplicates) (9–13). Consequently, we recently developed a web-based tool called HSDFinder that can identify HSDs in eukaryotic genomes with high accuracy and reliability (14). For example, HSDFinder predicted 336 and 265 HSDs in the psychrophilic green algae UWO241 and *Chlamydomonas* sp. ICE-L (6), respectively, which is consistent with other experimental data (8). By applying HSDFinder to a variety of other species (15), we predicted and cataloged thousands of HSD candidates, which are now curated and documented in a new online repository called HSDatabase. Currently, it houses 117 864 HSDs from 40 eukaryotic species, with a focus on green algae, animals and land plants.

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Here, we briefly introduce the general features as well as the procedures and principles for collecting data from HSDatabase. In short, HSDatabase contains information on HSD number, gene copy number and gene copy length. Additionally, the protein functional domains and associated pathways of the HSDs can be retrieved from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and InterProScan (16). A built-in Basic Local Alignment Search Tool (BLAST)-search option is also provided, allowing users to conveniently explore potential homologous sequences of interest within and across species. HSDatabase also provides data on a range of other parameters about gene duplicates, such as the number of HSD per Mb, the most commonly conserved domains among HSDs and the functional categories of HSDs. It is our hope to build a comparative analysis framework across species, especially for best-assembled eukaryotic genomes from species living in extreme environments, to

better understand the role of gene duplication in adaptive evolution.

Materials and methods

Database collection

HSDs were identified in 40 well-assembled nuclear genomes from diverse model species, including land plants (e.g. *Arabidopsis thaliana* and *Zea mays*), algae (e.g. *Chlamydomonas reinhardtii* and *Fragilariopsis cylindrus*) and animals (e.g. *Drosophila melanogaster*, *Homo sapiens* and *Mus musculus*) (Figure 1). We focused on model animal and plant genomes because of their high-quality assemblies and annotations. The genome sequences of the selected species are all retrievable from the National Center for Biotechnology Information (NCBI) (17) (Table 1). The HSDs, which are represented by gene copies with nearly identical lengths and

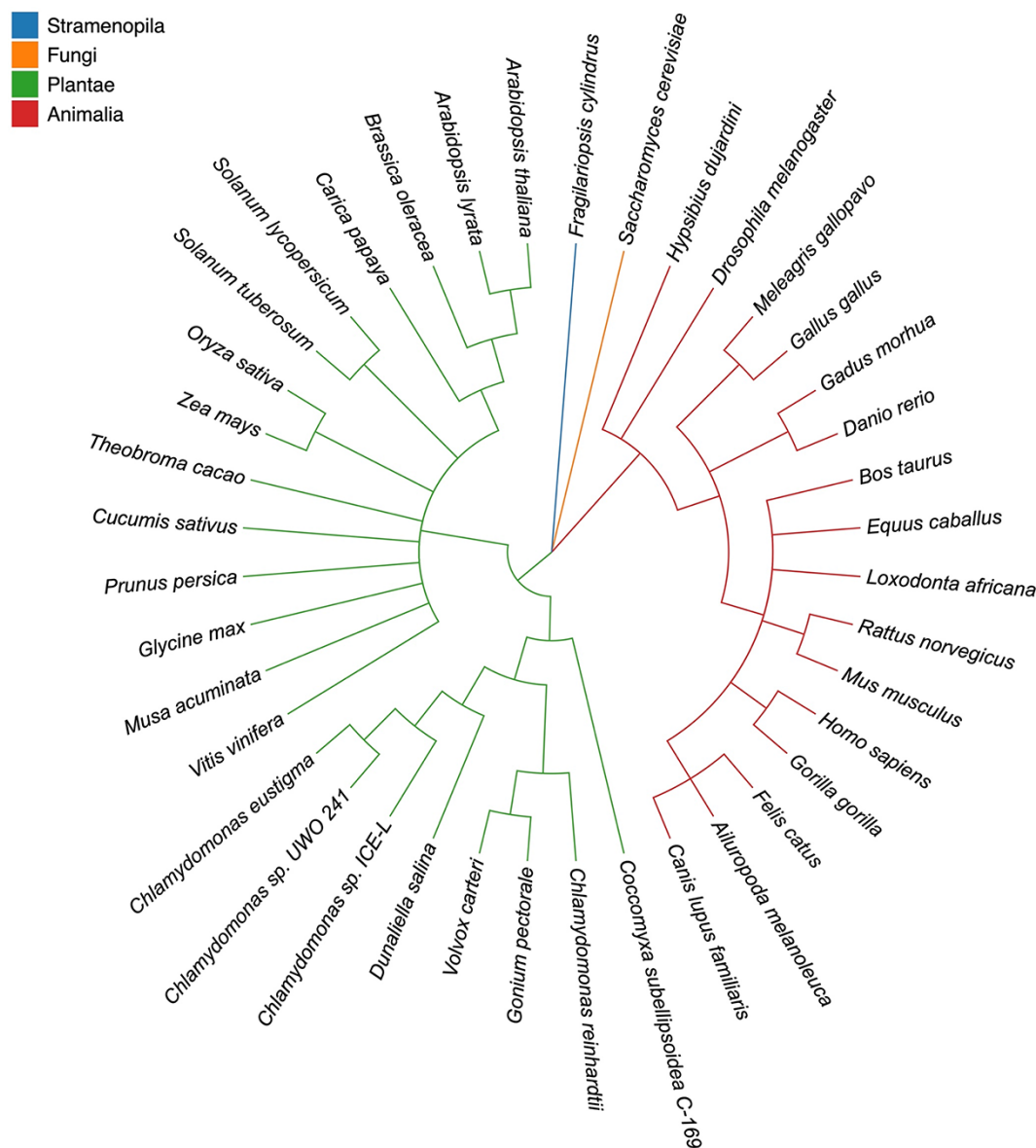


Figure 1. Taxonomic tree of 40 eukaryotic species in four highlighted categories. Stramenopila, Plantae, Fungi and Animalia are in blue, orange, green and red, respectively. The tree topologies were inferred by Taxonomy Common Tree from NCBI (<https://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi>).

Table 1. Summary statistics of the curated HSD groups in the selected genomes from HSDatabase

Species name (common name)	Classification	Curated HSD groups #	Genome size (Mb)	No. of considered genes	Gene copies	HSDs/ genes	HSDs/ Mb	2-group HSDs ^a	3-group HSDs	≥4-group HSDs	Genome assembly accession number ^b	Ref
<i>Alouatta palliata</i> (giant panda)	Animalia	2534	2371.8	22450	8091	0.113	1.068	1570	440	524	GCF_002007445.1	(23)
<i>Bos taurus</i> (cattle)	Animalia	2238	2667.85	21036	7399	0.106	0.839	1433	433	372	GCF_002263795.1	(24)
<i>Canis lupus familiaris</i> (dog)	Animalia	2372	2344.09	20945	7314	0.113	1.012	1541	434	397	GCF_014441545.1	(25)
<i>Danio rerio</i> (zebrafish)	Animalia	3799	1405.1	26439	11930	0.144	2.704	2263	689	847	GCF_000002035.6	(26)
<i>Drosophila melanogaster</i> (fruit fly)	Animalia	782	138.93	13739	2230	0.057	5.629	557	118	107	GCF_000001215.4	(27)
<i>Equus caballus</i> (horse)	Animalia	2403	2474.93	21126	7499	0.114	0.971	1509	468	426	GCF_002863925.1	(28)
<i>Felis catus</i> (domestic cat)	Animalia	2167	2493.14	20452	6691	0.106	0.869	1409	396	362	GCF_018350175.1	(29)
<i>Gadus morhua</i> (Atlantic cod)	Animalia	3269	627.04	23482	9757	0.139	5.213	1969	633	667	GCF_902167405.1	(30)
<i>Gallus gallus</i> (chicken)	Animalia	2026	1037.17	17797	6092	0.114	1.953	1323	374	329	GCF_016699485.2	(31)
<i>Gorilla gorilla</i> (western gorilla)	Animalia	2335	3063.36	20632	6683	0.113	0.762	1531	434	370	GCF_008122165.1	(32)
<i>Homo sapiens</i> (human)	Animalia	2178	2864.14	19531	6352	0.112	0.760	1442	395	341	GCF_000001405.39	(33)
<i>Hypsibius dujardini</i> (waterbear)	Animalia	2290	182.15	20853	5710	0.110	12.572	1618	366	306	GCA_002082055.1	(34)
<i>Loxodonta africana</i> (African savanna elephant)	Animalia	2031	3196.74	21094	7371	0.096	0.635	1327	360	344	GCF_000001905.1	(35)
<i>Meleagris gallopavo</i> (turkey)	Animalia	1486	1058.64	17974	3968	0.083	1.404	1061	240	185	GCF_000146605.3	(36)
<i>Mus musculus</i> (house mouse)	Animalia	2402	2588.62	30736	8855	0.108	0.928	1480	459	463	GCF_000001635.27	(37)
<i>Rattus norvegicus</i> (Norway rat)	Animalia	2434	2647.92	22219	8757	0.110	0.919	1478	471	485	GCF_015227675.2	(38)
<i>Saccharomyces cerevisiae</i> (yeast)	Fungi	397	11.83	6002	1006	0.073	33.559	331	41	25	GCA_003086655.1	(39)
<i>Arabidopsis lyrata</i>	Plantae	5302	202.97	29817	16901	0.178	26.122	3104	985	1213	GCF_000004255.2	(40)
<i>Arabidopsis thaliana</i> (thale cress)	Plantae	4428	119.75	27560	14225	0.161	36.977	2630	793	1005	GCF_000001735.4	(41)
<i>Brassica oleracea</i> (wild cabbage)	Plantae	8918	529.92	44382	30511	0.201	16.829	4565	1841	2512	GCF_000695525.1	(42)
<i>Carica papaya</i> (papaya)	Plantae	2094	360.63	18126	6311	0.116	5.807	1299	374	421	GCF_000150355.2	(43)

(continued)

Table 1. (Continued)

Species name (common name)	Classification	Curated HSD groups #	Genome size (Mb)	No. of considered genes	Gene copies	HSDs/ genes	HSDs/ Mb	2-group HSDs ^a	3-group HSDs	≥4-group HSDs	Genome assembly accession number ^b	Ref
<i>Chlamydomonas eustigma</i> (green alga)	Plantae	966	66.63	14 161	2199	0.068	14.498	778	109	79	GCA_002335675.1	(20)
<i>Chlamydomonas reinhardtii</i> (green alga)	Plantae	1129	111.11	19 870	3160	0.064	10.161	740	187	202	GCF_000002595.2	(44)
<i>Chlamydomonas</i> sp. ICE-L	Plantae	1540	541.86	17 731	3853	0.078	2.842	1139	224	177	GCA_013435795.1	(21)
<i>Chlamydomonas</i> sp. UWO 241 (green alga)	Plantae	1112	211.64	16 018	3282	0.068	5.254	741	139	232	GCA_016618255.1	(6)
<i>Coccomyxa subellipsoidea</i> C-169 (green alga)	Plantae	360	48.83	9839	1015	0.037	7.373	281	43	36	GCA_000258705.1	(45)
<i>Cucumis sativus</i> (cucumber)	Plantae	2891	240.99	20 038	9558	0.144	11.996	1655	532	704	GCF_000004075.3	(46)
<i>Dunaliella salina</i> (green alga)	Plantae	1589	343.7	18 740	3859	0.095	4.623	1227	194	168	GCA_002284615.2	(47)
<i>Fragilariopsis cylindrus</i> (diatom)	Stramenopila	1129	74.76	18 111	3192	0.062	15.102	766	172	191	GCA_001750085.1	(48)
<i>Glycine max</i> (soybean)	Plantae	11 107	995.27	47 064	38 274	0.236	11.160	6559	1295	3253	GCF_000004515.6	(49)
<i>Gonium pectorale</i> (green alga)	Plantae	1028	148.81	16 290	2669	0.063	6.908	719	143	166	GCA_001584585.1	(50)
<i>Musa acuminata</i> (dwarf banana)	Plantae	5489	461.54	22 177	20 934	0.179	11.893	2633	1083	1773	GCF_000313855.2	(51)
<i>Oryza sativa</i> (rice)	Plantae	4531	386.49	28 735	14 704	0.158	11.723	2641	812	1078	GCF_001433935.1	(52)
<i>Prunus persica</i> (peach)	Plantae	3454	220.9	23 133	11 210	0.149	15.636	2013	611	830	GCF_000346465.2	(53)
<i>Solanum lycopersicum</i> (tomato)	Plantae	4144	809.18	25 612	13 711	0.162	5.121	2345	768	1031	GCF_000188115.4	(54)
<i>Solanum tuberosum</i> (potato)	Plantae	4733	768.2	28 407	15 926	0.167	6.161	2647	880	1206	GCF_000226075.1	(55)
<i>Theobroma cacao</i> (cacao)	Plantae	3074	335.44	21 517	9933	0.143	9.164	1775	554	745	GCF_000208745.1	(56)
<i>Vitis vinifera</i> (wine grape)	Plantae	4039	427.21	25 830	13 613	0.156	9.454	2293	722	1024	GCF_000003745.3	(57)
<i>Volvox carteri</i> (green alga)	Plantae	863	137.68	14 436	2600	0.061	6.268	509	152	202	GCA_000143455.1	(58)
<i>Zea mays</i> (Maize)	Plantae	6801	2191.6	34 328	22 499	0.198	3.103	3910	1146	1745	GCF_902167145.1	(59)

^a2-group HSDs refers to the number of curated HSD groups with only two gene copies.^bAccession numbers are from the US NCBI GenBank assembly accession.

similar gene structures, were identified using HSDFinder (14). The identification method is based on all-against-all BLASTP analyses (18) carried out using uniform homology assessment metrics: E-value cut-off $\leq 1e-10$, amino acid pairwise identity $\geq 90\%$ and amino acid aligned length variance ≤ 10 . Note, the short form of these parameters is denoted as '90%_10aa'. Additionally, putative HSDs were expected to have similar structural information, such as matching protein family (Pfam) domains (19), corresponding InterPro annotations (16) and/or nearly identical conserved residues. The InterProScan tool (16), which is an integrated platform for protein signatures, was used to collect the structural information of the HSDs. The all-against-all BLAST and InterProScan results (tab-delimited files) were fed into HSDFinder to generate HSD candidates in an 8-column tab-delimited file (Figure 2A). These candidates were identified by parsing the BLAST all-against-all protein similarity search results with the homology metrics: amino acid pairwise identity and amino acid aligned length variance. To collect and curate the data in HSDatabase, we performed a series of combo thresholds for filtering putatively functional gene copies (described below at Database curation section).

Database curation

Prior to uploading data into HSDatabase, we curated HSD candidates by filtering for redundancy and adding the newly curated HSDs (Figure 2B). For genes that have alternative protein products, we selected the longest gene isoform to reduce redundancy. Since highly similar gene copies are grouped

together as HSDs based on a simple transitive link between the remaining genes (14), it is possible for some highly duplicated genes to form mega HSD groups with varied gene copy lengths, especially those encoding histones, ribosomal proteins or retro-transcriptases. Moreover, some gene copies might appear multiple times causing redundancy among different HSD groups, which is because the BLAST algorithm limits the maximum target hits by default. In these cases, we manually curated the HSD groups, minimizing redundant gene copies.

Since the similarity of duplicate genes within and among genomes can vary significantly, we added newly curated HSDs to the database using a combination of thresholds to acquire a larger dataset of HSD candidates. We added the HSD candidates one after another at different homology assessment metrics (i.e. HSDs identified at more relaxed thresholds were treated more strictly than those found using more conservative thresholds) (Figure 2B). For example, HSDs identified at a threshold of 90%_30aa were added on to those identified at a threshold of 90%_10aa (denoted as '90%_30aa+90%_10aa'); any redundant HSDs candidates picked out at this combo threshold were removed if the more relaxed threshold (i.e. 90%_30aa) had the identical genes or contained the same gene copies from the stricter cut-off (i.e. 90%_10aa). Moreover, any HSD candidates pinpointed at the combo threshold (90%_30aa + 90%_10aa) were removed if the minimum gene copy length was less than half of the maximum gene copy length for each HSD or if HSD candidates had gene copies with incomplete conserved domains (i.e. a different number of Pfam domains).

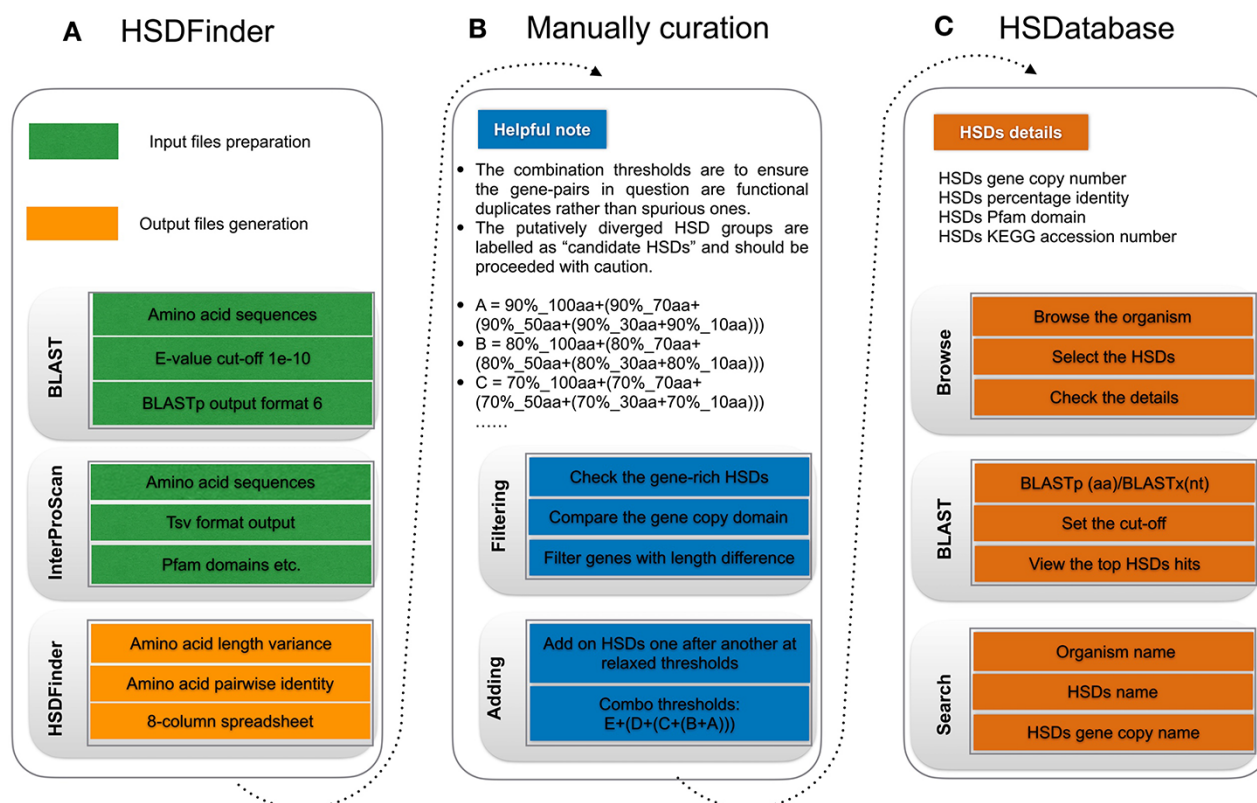


Figure 2. The workflow of HSDatabase. (A) Steps for using HSDFinder to collect candidate HSDs. (B) Manual curation of HSDs via filtering and adding new HSD candidates prior to being deposited into HSDatabase. (C) Steps of accessing HSD data in HSDatabase, including browsing via organism name, blasting query sequences against the database and searching through the HSD and gene copy IDs.

After filtering the combo threshold at (90%_30aa + 90%_10aa), we added on a more relaxed threshold 90%_50aa (i.e. 90%_50aa + (90%_30aa + 90%_10aa)) and then carried out the same HSD candidate removal/filtering process. To minimize redundancy and to acquire a larger dataset of HSD candidates, we processed each selected species with the following combination of thresholds: $E + (D + (C + (B + A)))$.

$$A = 90\%_{100aa} + (90\%_{70aa} + (90\%_{50aa} + 90\%_{30aa} + 90\%_{10aa}))$$

$$B = 80\%_{100aa} + (80\%_{70aa} + (80\%_{50aa} + (80\%_{30aa} + 80\%_{10aa})))$$

$$C = 70\%_{100aa} + (70\%_{70aa} + (70\%_{50aa} + (70\%_{30aa} + 70\%_{10aa})))$$

$$D = 60\%_{100aa} + (60\%_{70aa} + (60\%_{50aa} + (60\%_{30aa} + 60\%_{10aa})))$$

$$E = 50\%_{100aa} + (50\%_{70aa} + (50\%_{50aa} + (50\%_{30aa} + 50\%_{10aa})))$$

Database implementation

The database was built with the Django 3.0.5 web framework (<https://www.djangoproject.com/>), and all data were stored in an SQLite 3.36.0 database (<https://www.sqlite.org/index.html>) on an Amazon web server. Webpage templates used Bootstrap framework (<https://getbootstrap.com/>), D3.js (<https://d3js.org>), jQuery (<http://jquery.com>) and Bootstrap Table (<https://bootstrap-table.com/>) libraries to establish a user-friendly, front-end interface. On the browse page, NCBI's Sequence Viewer 3.44.0 (<https://www.ncbi.nlm.nih.gov/projects/sviewer/>) was employed to build a fast and scalable genome browser.

Results and discussion

Database content and analysis

HSDatabase was built using a relational database (MySQL) allowing the rapid retrieval of data and making resources easily maintainable. One entry corresponds to one eukaryote genome. The genomes can be accessed via the organism table or the taxonomic tree. The genome entry is then split into various subcategories of HSD entries. Database access is via a web interface based on python script and provides various ways to search for HSD entries, including species name, unique HSD IDs and gene copy IDs.

Using HSDfinder (15), we collected and curated 117 864 HSDs (representing 379 844 gene copies) from 40 well-assembled nuclear genomes of diverse model species (Table 1). Various green algae were included because of our specific interest in algal genomics and also because of their relatively modest genome sizes and penchant for gene duplications.

For example, the acidophilic green alga *Chlamydomonas eustigma* is known to have large numbers of gene duplicates in its nuclear genome, including 10 gene copies for arsenate reductase and 20 for glutaredoxin (20). Similarly, the psychrophilic green alga *Chlamydomonas* sp. ICE-L contains multiple copies of genes encoding carotene biosynthesis-related protein and Lhc-like protein (Lhl4) (21). These data are consistent with our identification of large numbers of HSDs in *C. eustigma* (276) and ICE-L (265) (Table 1), suggesting a potential adaptive role of gene duplication under different extreme environmental conditions.

Compared to algae, the investigated land plants had higher detected numbers of HSDs as well as larger ratios of HSDs/Mb and HSDs/genes (Table 1). For example, the HSDs/Mb values for *Arabidopsis lyrata* and *A. thaliana* are 26 and 37, respectively, whereas the average HSDs/Mb value among selected green algae is 8.2. Compared to algae and land plants, the HSDs/Mb values in animals are generally quite low with the exception of *Hypsibius dujardini* (13.6) and *D. melanogaster* (5.6). Two-group HSDs (i.e. HSDs containing two gene copies) represent the majority (>50%) of total HSDs for all explored species.

As for the associated functions of the detected HSDs, three green algal species with relatively large values of HSDs/genes were compared previously. These algae can survive various extreme environmental conditions and include the Antarctic psychrophilic green algae UWO241 (0.068) and ICE-L (0.078) and the acidophilic *C. eustigma* (0.068) (Table 1). The identified duplicates are involved in a diversity of cellular pathways, including gene expression, cell growth, membrane transport and energy metabolism, but also include ribosomal proteins (6, 14). Although HSDs for protein translation, DNA packaging and photosynthesis are particularly prevalent, around 30% of the HSDs are hypothetical proteins without any Pfam domains.

Database composition and usage

Information about specific HSDs and their associated gene copies for a given species can be obtained through the 'Browse' and 'Search' tabs, which are located on the menu bar at the top of the page, or using nucleotide/amino acid sequences as queries to search against the database via BLAST (i.e. BLASTP or BLASTX). To categorize duplicated genes into their functional categories, KEGG pathway schematics are available for each species.

Browse

By selecting the 'Browse' option from the main menu, users are offered three ways to explore their species of interest. First, they can simply click the organism name on the taxonomic tree containing the 40 species. Secondly, users can select the 'Plantae and Stramenopila' or 'Animalia and Fungi' tabs (Figure 3A), which contain 23 and 17 species, respectively. Selecting a tab takes users to a summary table that contains the organism names, number of HSDs, species background information, GenBank accession links to genome assemblies, and reference links to PubMed.

Selecting a specific species from the browse page leads to the respective HSDs summary page (Figure 4A), which gives data on the total number of HSDs, unique HSD IDs, gene copy GenBank IDs and number of gene copies; it also provides access to the data download function. Choosing one of

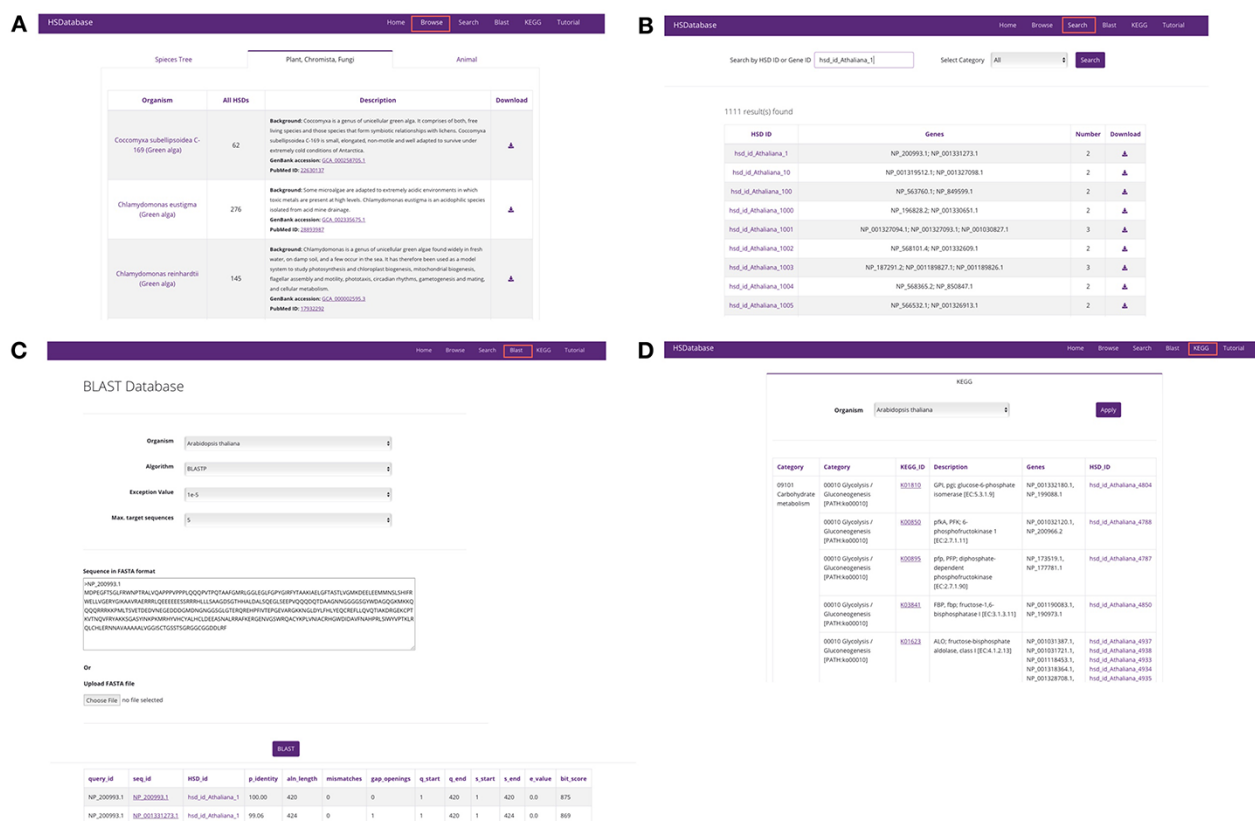


Figure 3. Screenshots of the HSDatabase interface. There are four main functions in the menu page: (A) Browse the database via species entries; (B) search the database via the HSDatabase unique ID (e.g. hsd_id_Athaliana_1) or gene ID (e.g. NP_200993.1); (C) use BLAST to search the database via amino acid sequence in FASTA format; and (D) categorize the gene copies and HSDs under the KEGG pathway functional categories.

the HSD ID entries, for example, brings up a page containing information and features of a detected HSD, including the associated gene copies for a unique HSD as well as the GenBank link, the sequence length, the Pfam domain ID/description and the InterPro database ID/description (Figure 4B). Clicking on the 'genome browser' tab allows for the visualization of a specific gene copy through the built-in NCBI genome browser (Figure 4C). The 'FASTA sequence' tab provides the option to download the sequence data (Figure 4D) and the 'alignments and identity%' tab brings up the gene copy alignment and percentage identity matrix created by the built-in Clustal v2.1 tool (22) (Figure 4E).

Search

Through the search option from the main menu users can search unique HSD IDs or gene copy IDs against the database (Figure 3B); they can also set the selection categories to limit the search results, which can improve search efficiency. After activation of the search button, 30 results per page are displayed (Figure 3B) in a four-column table, including HSD name, gene copy name, number of gene copies and the external download link to the output data (tab-delimited file). Users can navigate through the results page or download specific HSD entries. As described in the Browse section, the data file includes various summary statistics on the HSDs (Figure 4B).

BLAST

The BLAST tool bar allows users to input a nucleotide or amino acid sequence (in FASTA format) and carry out a

sequence similarity search using BLASTX or BLASTP. Users can specify the species against which the BLAST search will be performed. The *E*-value and maximum target sequence of results can also be adjusted, but all other parameters remain as default and cannot be changed (Figure 3C). The BLAST search output result is in the standard 13-column tabular format, including the linkable query sequence ID and HSD ID, percentage identity, aligned length and all other BLAST tabular output values. The most similar sequences are arranged at the top.

KEGG

The KEGG page contains details on the associated KEGG pathways of the HSD gene copies for the 40 species. To browse the data for a particular species, users can simply select the organism's name. The 6-column table lists the gene copies and HSDs under KEGG functional categories (Figure 3D). Gene copies involved in the same KEGG pathway are detailed with the first KEGG category (e.g. Carbohydrate metabolism), then the secondary category (e.g. Glycolysis/Gluconeogenesis) and finally the KEGG pathway function description (e.g. ENO, eno; enolase). The KEGG ID (e.g. K01689) is linked to the external KEGG database, providing more detailed information.

Future direction and limitation

Now that HSDatabase is publicly available, the next step is to analyze duplicate genes across a broader range of species,



With the decreasing cost of next-generation sequencing, biologists are dealing with ever larger amounts of data. However, many bioinformatics software suites require considerable knowledge of computer scripting and microprogramming. To facilitate the understanding and analysis of gene duplication in nuclear genomes, we developed HSDatabase, which currently contains 117 864 HSDs from 40 well-assembled eukaryotic genomes. In conjunction with HSDatabase, we designed HSDFinder, which can efficiently identify duplicated genes from unannotated genome sequences by integrating the results from InterProScan and KEGG. HSDatabase aims to become a useful platform for the identification and comprehensive analysis of HSDs in eukaryotic genomes, which could aid research into the mechanisms driving genome adaptation.

In the future, the database will be updated by incorporating advancements in the field of gene duplication.

Supplementary data

Supplementary data are available at Database Online.

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Conflict of interest

None declared.

Data availability

The datasets of eukaryotes supporting the conclusions of this article are available from Joint Genome Institute (JGI) (<https://phytozome.jgi.doe.gov/pz/portal.html>) or National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) database.

Author contributions

The study was conceptualized by X.Z. and D.R.S. The data were analyzed by X.Z., and Y.N.H. implemented the HSDatabase website. X.Z. and D.R.S. drafted the manuscript, and all authors commented to produce the manuscript for peer review.

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