# pATsi: Paralogs and Singleton Genes from *Arabidopsis thaliana*

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**ABSTRACT:** *Arabidopsis thaliana* is widely accepted as a model species in plant biology. Its genome, due to its small size and diploidy, was the first to be sequenced among plants, making this species also a reference for plant comparative genomics. Nevertheless, the evolutionary mechanisms that shaped the *Arabidopsis* genome are still controversial. Indeed, duplications, translocations, inversions, and gene loss events that contributed to the current organization are difficult to be traced. A reliable identification of paralogs and single-copy genes is essential to understand these mechanisms. Therefore, we implemented a dedicated pipeline to identify paralog genes and classify single-copy genes into opportune categories. PATsi, a web-accessible database, was organized to allow the straightforward access to the paralogs organized into networks and to the classification of single-copy genes. This permits to efficiently explore the gene collection of *Arabidopsis* for evolutionary investigations and comparative genomics.

**KEYWORDS:** *Arabidopsis thaliana*, paralogs, singletons

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## Introduction

*Arabidopsis thaliana*, belonging to the Brassicaceae family, is widely exploited as a reference in plant biology thanks to its short generation time, small size, that limited the requirement for growth facilities, prolific seed production through self-pollination, and its small diploid genome.[1,2] This is also the reason why it was the first plant with a genome completely sequenced in 2000. Since its first release, the *Arabidopsis* genome has been thoroughly investigated, providing a better assessment of the structure and the functionality of plant genomes[2–5] and creating the potential for a deeper understanding of the plant development and environmental responses. However, a deeper analysis of the genome sequence of *A. thaliana* also revealed a high complexity due to several events of whole-genome duplications, the occurrence of large-scale duplications, and reshufflings.[6] In particular, these studies showed evidence of at least three rounds of whole-genome duplications followed by diploidization events.[7–15] Moreover, the high frequency of gene reduction, ie, gene loss after each duplication event, translocations, inversions,[16,17] and probable chromosome losses,[18–20] further contributed to reshuffle the retained portions of the genome shaping the current organization.

Gene duplication plays a key role in the origin of novel gene functions.[21–25] A clear assessment of the duplicated gene content in a genome, accompanied by a suitable description of those genes that are in single copy, is fundamental for a reliable validation of gene expression,[26] of the complexity of gene regulatory networks[27] and also to support functional and evolutionary analyses in a species.[28]

Although several collections of ortholog genes are available today,[29–36] only one reference web-accessible database, Eukaryotic Paralog Group Database (EPGD),[37] is exclusively dedicated to describe paralogs in 26 available eukaryotic genomes. Indeed, EPGD is a web resource for integrating and displaying eukaryotic paralog information, in terms of paralog families and intragenome segmental duplications. However, the collections of *Arabidopsis* paralog genes can also be accessed from some of the collections available worldwide which are related to orthologs, such as Ensembl Compara[31] and NCBI Homologene,[30] which include both animals and plants, and Plaza,[38] which is exclusively dedicated to plants genomes. These databases, being mainly dedicated to comparative interspecies analyses, usually refer to the clusters of duplicated genes, where orthologs between species and paralogs are both included. These collections do not provide an overview of those genes that are associated with similarity relationships in a cluster, and therefore, it is rather difficult to identify subgroups in each cluster. Moreover, none of the existing collections enable a graphical visualization of *Arabidopsis* duplicated genes through a network view, which facilitates a direct and an easy exploitation of the information about paralog relationships.

PATsi, a database to organize *paralog and singleton gene in A. thaliana*, organizes the protein-coding gene collection of *A. thaliana* in sets of paralogs and singleton genes identified according to a dedicated pipeline described in Sangiovanni et al.[28] Gene association, mainly defined by protein similarity, is assigned using two different cutoffs. Similar genes at each cutoff are organized in the networks of paralogs, accessible as lists but also by graphs through a suitable web interface, with the aim of allow immediate access to genes that share direct similarity relationships in a network shaped at a given threshold. This supports further investigations on the structural and evolutionary relationships among genes.

A detailed classification and immediate download of genes that are not grouped into network of paralogs represent another novelty in this database. Moreover, collections of single-copy genes are not available for *A. thaliana*, though the wide interest in tracing gene reduction during diploidization events and/or in understanding speciation.

## Construction and Content

**Data source.** The entire *A. thaliana* genome, intergenic regions, and gene family information were downloaded from The *Arabidopsis* Information Resource (TAIR) web server.[39] *A. thaliana* expressed sequence tag (EST) sequences to confirm gene expression were downloaded from GenBank.

**Collection of paralogs and singletons.** A dedicated pipeline to identify paralogs and singleton genes was implemented and applied to the *Arabidopsis* protein-coding gene collection. Moreover, all the other nonprotein-coding genes available in the collection were filtered out from the subsequent investigations.[28] The analysis was based on an all-against-all protein sequence similarity search performed with the BLASTp software,[40] using two different cutoff settings: a more stringent expected value ($E \leq 10^{-10}$) was used to select the alignments with higher similarity, and a less stringent one was set at $E \leq 10^{-5}$.[26,41] Then, the two collections were filtered applying the Rost's formula,[42,43] to discriminate significant similarity relationships from less reliable sequence alignments. This formula asserts that all the alignments with an identity percentage lower than 30% and a length shorter than 150 amino acids should be discarded, due to the ambiguity of their relationships. The analysis resulted in 22,522 and 21,843 structurally related genes defined as paralogs and organized in two different datasets, a more stringent one (dataset A), with higher similarity levels between the genes, and a less stringent one (dataset B), including more genes sharing lower similarities.

Several filtering steps were also used to filter out genes with some similarity, lower than the threshold, with other protein-coding genes, or with nucleotide sequence similarity with any genic or intergenic region of the entire genome, permitting to collect genes that could be reliably classified as *true singletons* with respect to the whole-genome content. Therefore, all the genes are organized into two major groups: those that are in network at the less stringent threshold and those that are not in networks. Accordingly, this last group is classified considering several distinct features based on the pipeline described by Sangiovanni et al.[28] Therefore, all the *Arabidopsis* genes are organized in classes, which are summarized in Table 1 together with the number of associated genes.

**Networks construction.** Paralog genes were organized into two different sets of networks, depending on the expected value used. The less stringent cutoff ($E \leq 10^{-5}$) led to a set of 2,754 networks, including 22,522 paralog genes, while the more stringent cutoff ($E \leq 10^{-10}$) led to a set of 3,017 networks

**Table 1.** Details of the *Arabidopsis* gene collection and classification.

| CLASSIFICATION | GENE NUMBER | ANALYSIS |
|---|---|---|
| Nonprotein-coding genes | 6070 | miscRNAs, tRNAs, rRNAs, ncRNAs, pseudogenes, transposons and unknown genes |
| Paralogs classified into networks | 22522 | All-against-all BLASTp $E \leq 10^{-5}$ |
| Unassigned genes due to the Rost's formula | 405 | Filtering with Rost's formula |
| Unassigned genes due to the masking filter | 213 | All-against-all BLASTp $E \leq 10^{-5}$ without masking filter |
| Unassigned genes due to loose protein similarity | 440 | All-against-all BLASTp $E \leq 10^{-3}$ of protein-coding genes |
| Unassigned genes due to the ORF annotation error | 2 | Transcripts BLASTx $E \leq 10^{-5}$ versus proteins for ORF validation |
| Unassigned genes due to similarities with nonprotein-coding genes | 178 | Full genes BLASTn $E \leq 10^{-5}$ versus nonprotein-coding genes |
| Unassigned genes due to similarities with intergenic regions | 0 | Full genes BLASTn $E \leq 10^{-5}$ versus intergenic regions |
| Singletons not confirmed by ESTs (no EST trace) | 24 | Transcripts BLASTn (free *E*-value cutoff) versus ESTs |
| Singletons not confirmed by ESTs (discarded by E-value cutoff) | 688 | Filtering of BLASTn results by $E \leq 10^{-5}$ |
| Singletons not confirmed by ESTs (discarded by coverage and identity requirements) | 201 | Filtering of BLASTn versus EST results by coverage and identity |
| Singletons not confirmed by ESTs | 100 | Filtering by Delta $\geq$ 20 (EST length $\geq$ 20 nt than the transcript) |
| Singletons confirmed by ESTs | 9 | 0 < Delta < 20 (EST length greater than transcript but less than 20 nt) |
| Singleton confirmed by ESTs | 2387 | Delta $\leq$ 0 (Transcript longer than the EST) |

**Notes:** A summary of the classes of genes defined in pATsi. The analyses performed to obtain genes in each class are also reported.

containing 21,843 paralogs. Each gene (represented by a node) is connected by at least one paralogy relationship (represented by an edge) to at least another gene in the same network. The networks have various sizes depending on the gene content, ranging from 2 to 6,834 genes, this last number reflecting the maximum number of genes in a network and corresponding to the biggest network (Fig. 1) obtained with the less stringent threshold ($E \leq 10^{-5}$).

To keep track of the relationships between networks defined at different cutoffs, the network naming is assigned as follows: the networks at the less stringent threshold were named as NETxGy_z, where x indicates a number assigned when sorting the total amount of networks by decreasing network size; G stands for genes; *y* indicates the network size, ie, the number of included genes; and z is the number of networks and singletons in which the network is split when the more stringent cutoff is applied.

Results from the two cutoffs, both considered significant in similar approaches,[26,41] are provided here as they can be useful for an approximated estimate of stable or varying network organizations at the two settings.

**Database development.** The relational database used in this platform was designed under MySql v.5.5.31 and the InnoDb storage engine. For higher efficiency and lower execution time, all tables are indexed using a normal BTree indexing, based on individual and multiple index keys, according to the queries defined in the system. User interface and usage pATsi can be accessed at http://cab.unina.it/athparalog/main.html (Fig. 2A).

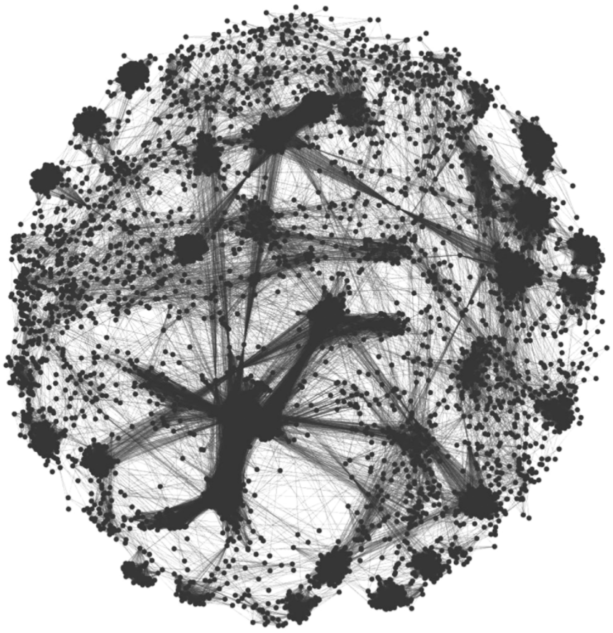


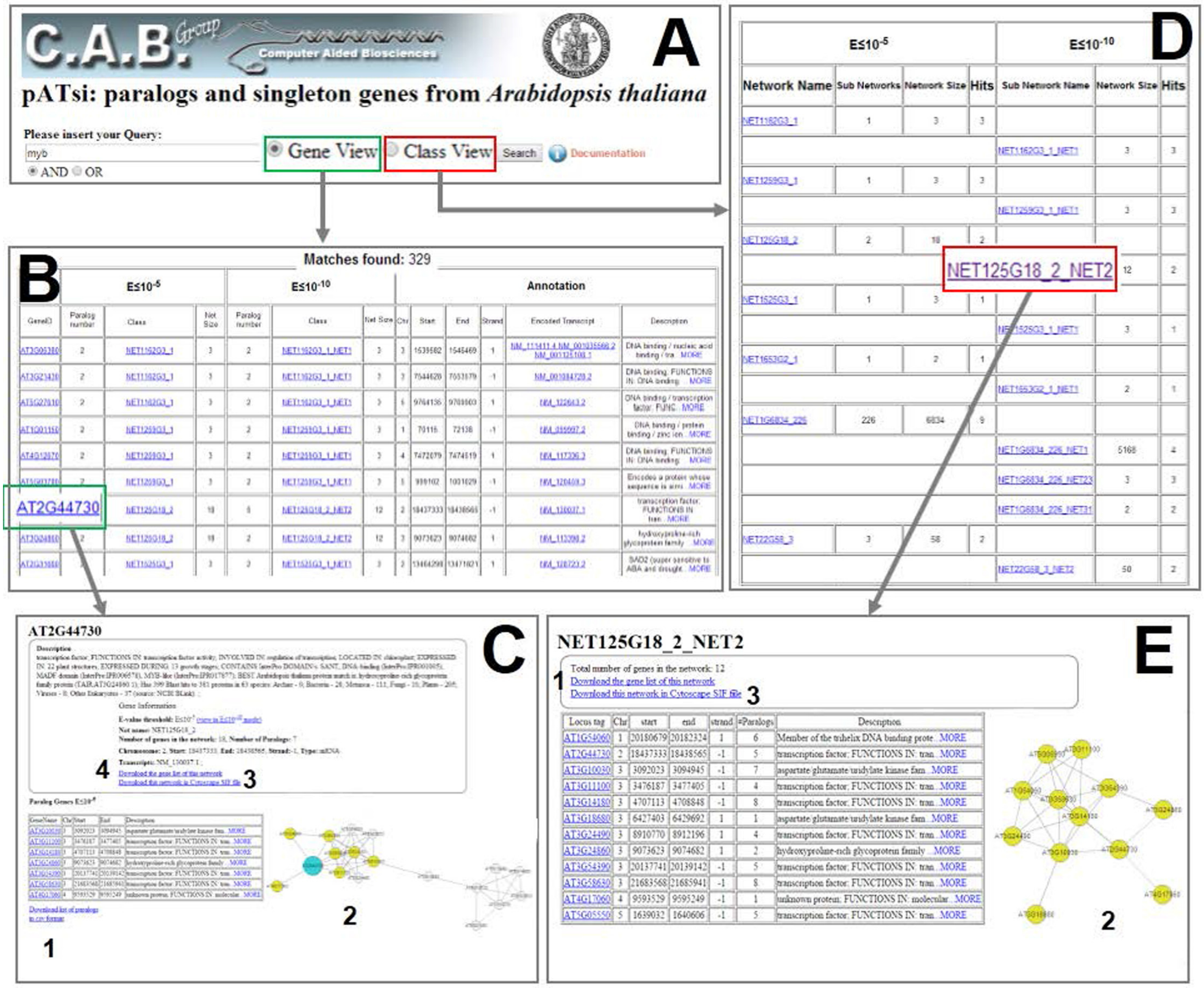
**Figure 1.** Example view of the largest network of paralogs. Network of paralogs consisting of 6,834 genes.
**Notes:** Each dot in dark gray represents a single gene, and each line in light gray represents a paralogy relationship between two genes.

All *A. thaliana* genes and networks can be browsed or searched using keywords. The keyword searching in pATsi can accept a gene locus ID, a network name, or every string to search the annotation content. Two different views are offered. The *gene view* (Fig. 2A, in green) results in the list of loci associated with the query (Fig. 2B). Each row of the list contains the following information:

- GeneID represents the official TAIR classification for *A. thaliana*. By clicking on the GeneID, it is possible to browse the GeneID-related page, containing information about the gene investigated and the two networks organization in which the gene may be included. In case the gene is a singleton, no network organization is shown.
- *Paralog number* shows the number of paralogs of the gene at the two different cutoffs or zero in the case of singletons or non-messenger RNA (mRNA) genes.
- *Class:* if the gene has paralogs at one of the e-value cutoffs, the network name is shown; for genes without paralogs, the name of the class is reported. In the case of unassigned genes, the classification field contains a brief explanation of the reason that led to that specific category.
- *Net size:* this field shows the number of all paralog genes contained into the network, zero if the corresponding gene is a *singleton* or a non-mRNA gene.
- *Chr:* the chromosome on which the locus maps.
- *Start/end:* starting/ending position of the locus on the chromosome.
- *Strand:* direction of the locus transcription.
- *Encoded transcript:* the RefSeq or the encoded transcript. Each RefSeq has a link to GenBank.
- *Description:* the TAIR functional annotation[39] for each of the RefSeq.

By clicking on the GeneID in the results table, the user will be redirected to a new page (Fig. 2C). In the topmost part of the page, the GeneID, several details about the gene annotation and possible network information are reported. In the bottom left part of the page, the list of paralogs of the selected gene is shown. Each gene is also cross-linked to its specific description in the database. The list of paralogs can be downloaded (Fig. 2[C1]). In the bottom center part of the page, an interactive network graph is displayed (Fig. 2[C2]) using CytoscapeWeb, a web-based network visualization tool.[44] Users are enabled to interact with the displayed network by selecting nodes and edges and modeling the network view accordingly. For each network, it is possible to download a file (Fig. 2[C3]), which can be easily imported into Cytoscape, for onsite visualization or for managing more complex networks.[45] The list of genes that are included in the displayed network can also be downloaded (Fig. 2[C4]).

Selecting the *class view* (Fig. 2A in red), the query process organizes the genes associated with the query into classes,



pATsi: paralogs and singleton genes from *Arabidopsis thaliana*

Please insert your Query: myb
● AND ○ OR
● Gene View ○ Class View Search Documentation

Matches found: 329

| | E≤10^-5 | | | E≤10^-10 | | | Annotation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GeneID | Paralog number | Class | Net Size | Paralog number | Class | Net Size | Chr | Start | End | Strand | Encoded Transcript | Description |
| AT3G05380 | 2 | NET1162G3_1 | 3 | 2 | NET1162G3_1_NET1 | 3 | 3 | 1539582 | 1545469 | 1 | NM_111411.4 NM_001035566.2 NM_001125108.1 | DNA binding / nucleic acid binding / tra... MORE |
| AT3G23430 | 2 | NET1162G3_1 | 3 | 2 | NET1162G3_1_NET1 | 3 | 3 | 7544620 | 7553179 | -1 | NM_001084720.2 | DNA binding; FUNCTIONS IN: DNA binding ... MORE |
| AT5G27610 | 2 | NET1162G3_1 | 3 | 2 | NET1162G3_1_NET1 | 3 | 5 | 9764136 | 9769903 | 1 | NM_122643.2 | DNA binding / transcription factor; FUNC... MORE |
| AT1G01150 | 2 | NET1259G3_1 | 3 | 2 | NET1259G3_1_NET1 | 3 | 1 | 70115 | 72138 | -1 | NM_855997.2 | DNA binding / protein binding / zinc ion... MORE |
| AT4G12670 | 2 | NET1259G3_1 | 3 | 2 | NET1259G3_1_NET1 | 3 | 4 | 7472079 | 7474519 | 1 | NM_117336.3 | DNA binding; FUNCTIONS IN: DNA binding ... MORE |
| AT5G03780 | 2 | NET1259G3_1 | 3 | 2 | NET1259G3_1_NET1 | 3 | 5 | 999102 | 1001029 | -1 | NM_120469.3 | Encodes a protein whose sequence is simi... MORE |
| AT2G44730 | [illegible] | NET125G18_2 | 18 | 5 | NET125G18_2_NET2 | 12 | 2 | 18437333 | 18438565 | -1 | NM_130037.1 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G24860 | 2 | NET125G18_2 | 18 | 2 | NET125G18_2_NET2 | 12 | 3 | 9073623 | 9074682 | 1 | NM_113398.2 | hydroxyproline-rich glycoprotein family ... MORE |
| AT2G31080 | 2 | NET1525G3_1 | 3 | 2 | NET1525G3_1_NET1 | 3 | 2 | 13464298 | 13471821 | 1 | NM_128723.2 | SAD2 (super sensitive to ABA and drought... MORE |

AT2G44730

Description
transcription factor; FUNCTIONS IN: transcription factor activity; INVOLVED IN: regulation of transcription; LOCATED IN: chloroplast; EXPRESSED IN: 22 plant structures; EXPRESSED DURING: 13 growth stages; CONTAINS InterPro DOMAIN/s: SANT, DNA-binding (InterPro:IPR001005), MADF domain (InterPro:IPR006578), MYB-like (InterPro:IPR017877); BEST Arabidopsis thaliana protein match is: hydroxyproline-rich glycoprotein family protein (TAIR:AT3G24860.1); Has 399 Blast hits to 383 proteins in 63 species: Archae - 0; Bacteria - 20; Metazoa - 111; Fungi - 10; Plants - 205; Viruses - 0; Other Eukaryotes - 37 (source: NCBI BLink). ;

Gene Information

**E-value threshold:** E≤10^-5 (view in E≤10^-10 mode)
**Net name:** NET125G18_2
**Number of genes in the network:** 18, **Number of Paralogs:** 7
**Chromosome:** 2, **Start:** 18437333, **End:** 18438565, **Strand:** -1, **Type:** mRNA
**Transcripts:** NM_130037.1 ;
Download the gene list of this network
Download this network in Cytoscape SIF file

**Paralog Genes E≤10^-5**

| GeneName | Chr | Start | End | Description |
|---|---|---|---|---|
| AT3G10030 | 3 | 3092023 | 3094945 | aspartate/glutamate/uridylate kinase fam... MORE |
| AT3G11100 | 3 | 3476187 | 3477405 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G14180 | 3 | 4707113 | 4708848 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G24860 | 3 | 9073623 | 9074682 | hydroxyproline-rich glycoprotein family ... MORE |
| AT3G54390 | 3 | 20137741 | 20139142 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G58630 | 3 | 21683568 | 21685941 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT4G17060 | 4 | 9593529 | 9595249 | unknown protein; FUNCTIONS IN: molecular... MORE |

Download list of paralogs in csv format

| E≤10^-5 | | | | E≤10^-10 | | |
|---|---|---|---|---|---|---|
| Network Name | Sub Networks | Network Size | Hits | Sub Network Name | Network Size | Hits |
| NET1162G3_1 | 1 | 3 | 3 | | | |
| | | | | NET1162G3_1_NET1 | 3 | 3 |
| NET1259G3_1 | 1 | 3 | 3 | | | |
| | | | | NET1259G3_1_NET1 | 3 | 3 |
| NET125G18_2 | 2 | 18 | 2 | | | |
| | | | | NET125G18_2_NET2 | 12 | 2 |
| NET1525G3_1 | 1 | 3 | 1 | | | |
| | | | | NET1525G3_1_NET1 | 3 | 1 |
| NET1653G2_1 | 1 | 2 | 1 | | | |
| | | | | NET1653G2_1_NET1 | 2 | 1 |
| NET1G6834_226 | 226 | 6834 | 9 | | | |
| | | | | NET1G6834_226_NET1 | 5168 | 4 |
| | | | | NET1G6834_226_NET23 | 3 | 3 |
| | | | | NET1G6834_226_NET31 | 2 | 2 |
| NET22G58_3 | 3 | 58 | 2 | | | |
| | | | | NET22G58_3_NET2 | 50 | 2 |

NET125G18_2_NET2

Total number of genes in the network: 12
Download the gene list of this network
Download this network in Cytoscape SIF file

| Locus tag | Chr | start | end | strand | #Paralogs | Description |
|---|---|---|---|---|---|---|
| AT1G54060 | 1 | 20180679 | 20182324 | 1 | 6 | Member of the trihelix DNA binding prote... MORE |
| AT2G44730 | 2 | 18437333 | 18438565 | -1 | 5 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G10030 | 3 | 3092023 | 3094945 | -1 | 7 | aspartate/glutamate/uridylate kinase fam... MORE |
| AT3G11100 | 3 | 3476187 | 3477405 | -1 | 4 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G14180 | 3 | 4707113 | 4708848 | -1 | 8 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G18680 | 3 | 6427403 | 6429692 | 1 | 1 | aspartate/glutamate/uridylate kinase fam... MORE |
| AT3G24490 | 3 | 8910770 | 8912196 | 1 | 4 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G24860 | 3 | 9073623 | 9074682 | 1 | 2 | hydroxyproline-rich glycoprotein family ... MORE |
| AT3G54390 | 3 | 20137741 | 20139142 | -1 | 5 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT3G58630 | 3 | 21683568 | 21685941 | -1 | 8 | transcription factor; FUNCTIONS IN: tran... MORE |
| AT4G17060 | 4 | 9593529 | 9595249 | -1 | 1 | unknown protein; FUNCTIONS IN: molecular... MORE |
| AT5G05550 | 5 | 1639032 | 1640606 | -1 | 5 | transcription factor; FUNCTIONS IN: tran... MORE |

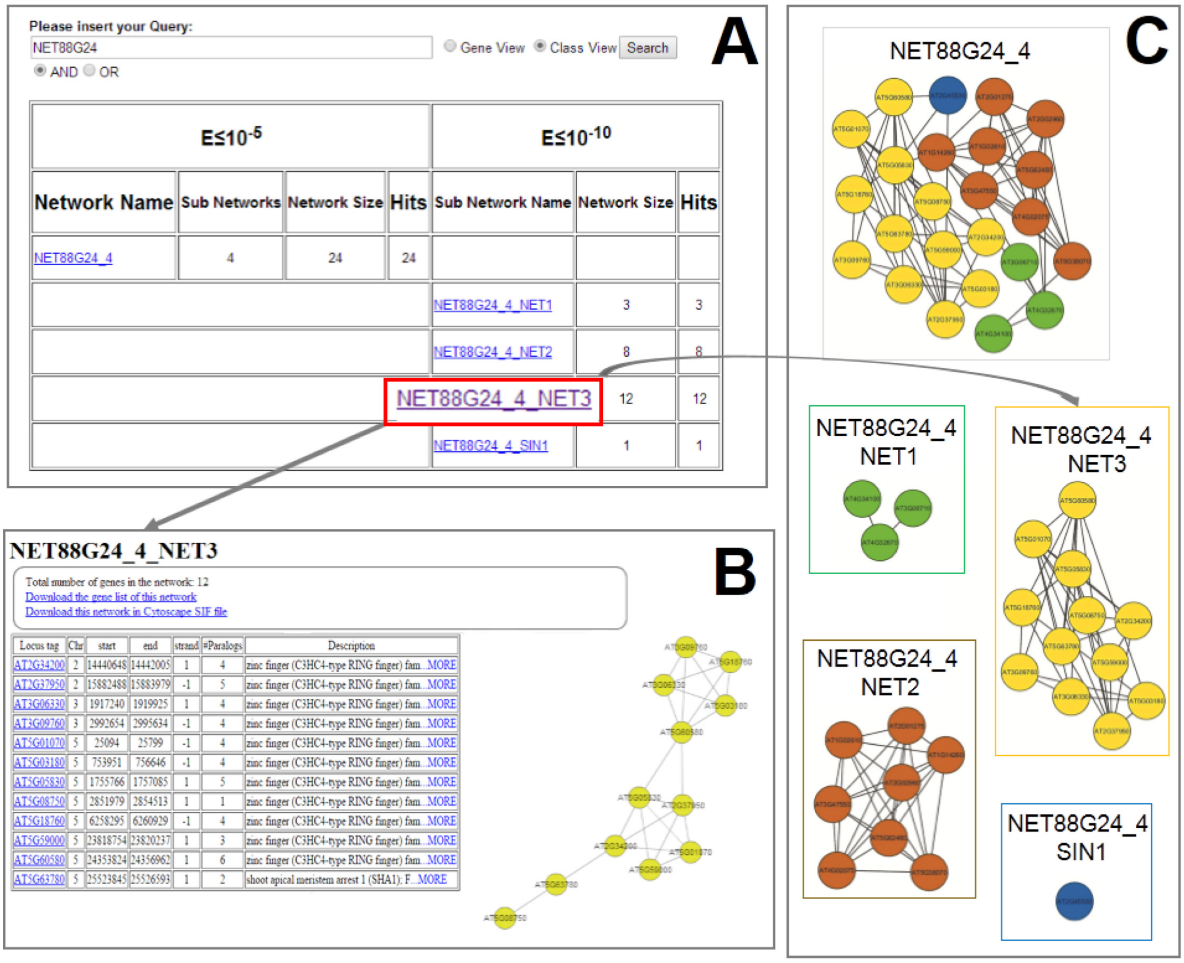
**Figure 2.** Possible queries workflow in pATsi web interface. (**A**) Main page of the pATsi database browser; for each query, the user can switch from the gene view (bordered in green) to the class view (bordered in red). (**B**) List of genes associated with a query. (**C**) Gene information page. In (C2), a network graph is shown; each circle is a GeneID, with the light blue-circled one representing the selected GeneID and the yellow-circled one(s) representing the paralog(s) of the selected GeneID; gray lines represent paralogies between genes. (**D**) List of networks associated with a query. (**E**) Network information page.

separating the genes that are not included in the network from those included in networks. The resulting page also provides the list of networks associated with the query (Fig. 2D), indicating the following information in each row of the list of networks:

- *Network*: the name assigned to the network at the lowest cutoff ($E \leq 10^{-5}$). By clicking on the network name, it is possible to browse the network ID-related page, containing information about the network investigated and the genes included in it. For each network, a list of one or more subnetworks is also shown.
- *Subnetworks*: it shows the number of subnetworks or singletons in which the network is split when the cutoff of $E \leq 10^{-10}$ is applied.
- *Network size*: ie, the number of genes included in the network.
- *Hits:* the number of matching genes with the user query.

By clicking on the network name in the resulting table, the user will be redirected to a new page (Fig. 2E). In the topmost part of the page, the network name and the number of genes are shown. In the left part of the page, the list of genes of the selected network is reported. It is also possible to download the list of genes (Fig. 2[E1]). In the right part of the page, the network graph is displayed (Fig. 2[E2]). The file of the network, in.sif format, can also be downloaded (Fig. 2[E3]).

As mentioned earlier, networks presented here are classified according to two different thresholds. The use of a more stringent threshold defines a lower number of paralogy relationships between genes, hence obtaining a larger number of networks in comparison with the ones obtained with the less stringent threshold. This is explained considering the effects of the less stringent cutoff that permits to also include genes in a network which are otherwise excluded when the more stringent threshold is used (Fig. 3).



Please insert your Query:
NET88G24
○ Gene View ● Class View Search
● AND ○ OR

| E≤$10^{-5}$ | | | | E≤$10^{-10}$ | | |
|---|---|---|---|---|---|---|
| Network Name | Sub Networks | Network Size | Hits | Sub Network Name | Network Size | Hits |
| NET88G24_4 | 4 | 24 | 24 | | | |
| | | | | NET88G24_4_NET1 | 3 | 3 |
| | | | | NET88G24_4_NET2 | 8 | 8 |
| | | | | NET88G24_4_NET3 | 12 | 12 |
| | | | | NET88G24_4_SIN1 | 1 | 1 |

NET88G24_4_NET3

Total number of genes in the network: 12
Download the gene list of this network
Download this network in Cytoscape SIF file

| Locus tag | Chr | start | end | strand | #Paralogs | Description |
|---|---|---|---|---|---|---|
| AT2G34200 | 2 | 14440648 | 14442005 | 1 | 4 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT2G37950 | 2 | 15882488 | 15883979 | -1 | 5 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT3G06330 | 3 | 1917240 | 1919925 | 1 | 4 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT3G09760 | 3 | 2992654 | 2995634 | -1 | 4 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G01070 | 5 | 25094 | 25799 | -1 | 4 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G03180 | 5 | 753951 | 756646 | -1 | 4 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G05830 | 5 | 1755766 | 1757085 | 1 | 5 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G08750 | 5 | 2851979 | 2854513 | 1 | 1 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G18760 | 5 | 6258295 | 6260929 | -1 | 4 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G59000 | 5 | 23818754 | 23820237 | 1 | 3 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G60580 | 5 | 24353824 | 24356962 | 1 | 6 | zinc finger (C3HC4-type RING finger) fam...MORE |
| AT5G63780 | 5 | 25523845 | 25526593 | 1 | 2 | shoot apical meristem arrest 1 (SHA1): F...MORE |

**Figure 3.** Network organization. (**A**) List of subnetworks associated with a network query. (**B**) Network information page. (**C**) Graphic representation of a network of 24 genes (NET88G24_4) splitted into three subnetworks (NET88G24_4 NET1-NET2-NET3) and one singleton (NET88G24_4 SIN1).

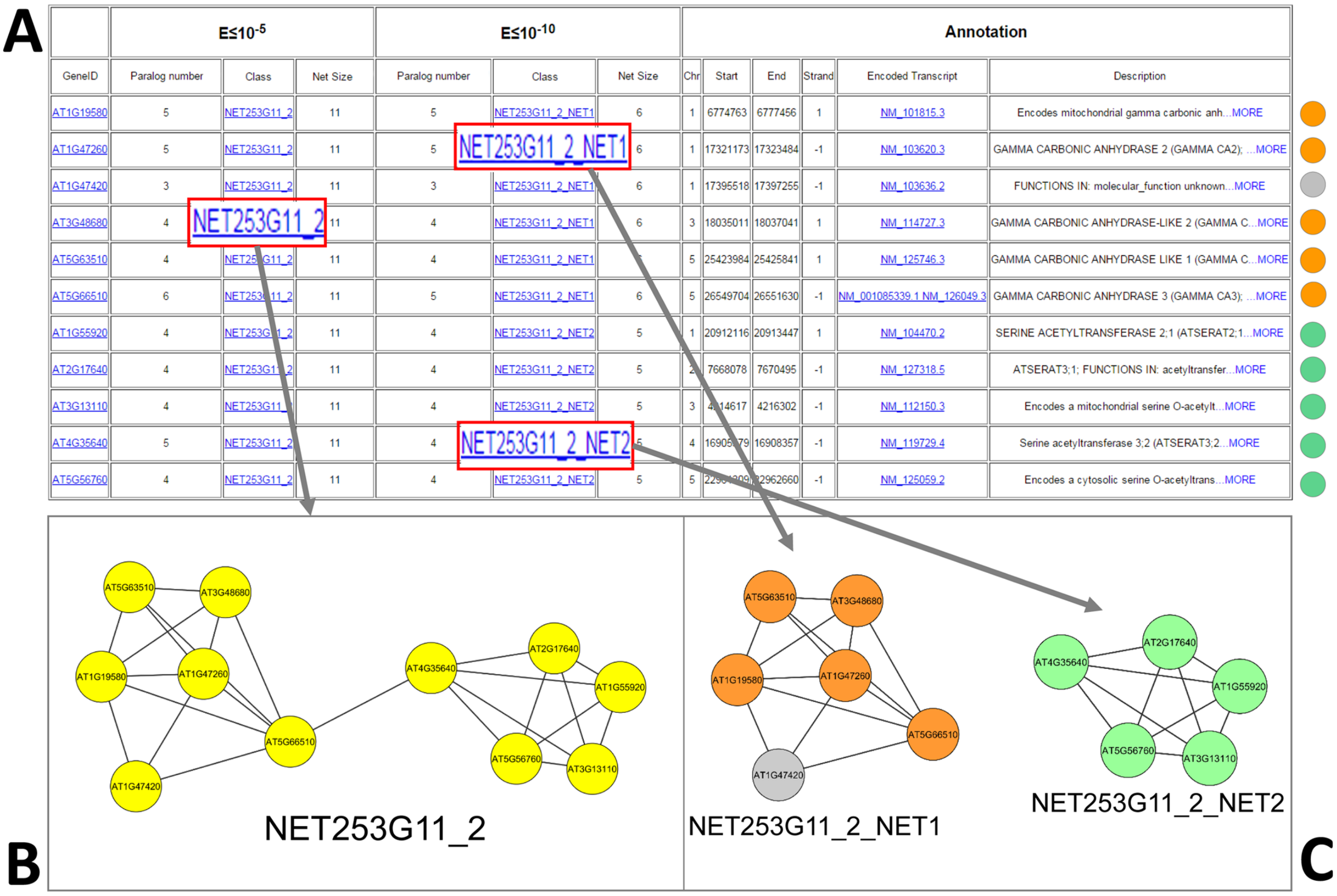
An example query in *pATsi* using *serine acetyltransferase* as keyword and selecting the *gene view* option resulted in six hits: one annotated as a pseudogene and five as protein-coding genes. These five genes are grouped into one network, NET253G11_2, together with other six genes (Fig. 4) not reflecting the same annotation.

The family of serine acetyltransferases catalyzes the limiting reaction in cysteine biosynthesis.[46–48] These enzymes are of great interest to the scientific community because of their active role in creating nutritionally essential sulfur amino acids.[49] Analyzing the network (NET253G11_2) organization at E5, we noted that the more stringent cutoff ($E \leq 10^{-10}$) identified two subnetworks. The first one (NET253G11_2_NET1) is formed by the five serine acetyltransferase enzymes previously discussed, while the other (NET253G11_2_NET2) is formed by five genes annotated as gamma carbonic anhydrases. Moreover, a gene with an unknown function falls in the second network (Fig. 4C). The function of the unknown gene can be more straightforward inferred thanks to the more stringent cutoff, since all the associated paralogs in the subnetwork have a similar function. Moreover, serine acetyltransferases and carbonic anhydrases belong to the same trimeric LpxA-like superfamily, a set of enzymes with trimeric repeats of hexapeptide motifs. This shows the reliability of their grouping at a less stringent threshold. Beyond the usage of the platform, this example also highlights that different thresholds can support the identification of not only well known but also unexpected relationships among genes in a network.[28]

## Conclusion

The collection described here is useful for promoting an efficient exploitation of the *Arabidopsis* gene collection in terms of intragenome similarities, contributing to the identification of structurally related genes, to their functional assignment, and to the classification of singleton genes within this reference genome.[28] In addition, pATsi provides a novel approach to the classification of protein-coding genes from *A. thaliana*, based on a similarity defined at both protein and genome level, focusing on paralogs to build gene networks and on an appropriate classification of singleton genes.

Today, there are several collections available for paralog genes in plants[35,50] including data from *A. thaliana*. However, none of these manifold available resources allow to fully access descriptions of both the genes having at least one paralog and



| | $E\leq10^{-5}$ | | | $E\leq10^{-10}$ | | | Annotation | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GeneID | Paralog number | Class | Net Size | Paralog number | Class | Net Size | Chr | Start | End | Strand | Encoded Transcript | Description | |
| AT1G19580 | 5 | NET253G11_2 | 11 | 5 | NET253G11_2_NET1 | 6 | 1 | 6774763 | 6777456 | 1 | NM_101815.3 | Encodes mitochondrial gamma carbonic anh...MORE | ● |
| AT1G47260 | 5 | NET253G11_2 | 11 | 5 | NET253G11_2_NET1 | 6 | 1 | 17321173 | 17323484 | -1 | NM_103620.3 | GAMMA CARBONIC ANHYDRASE 2 (GAMMA CA2); ...MORE | ● |
| AT1G47420 | 3 | NET253G11_2 | 11 | 3 | NET253G11_2_NET1 | 6 | 1 | 17395518 | 17397255 | -1 | NM_103636.2 | FUNCTIONS IN: molecular_function unknown...MORE | ● |
| AT3G48680 | 4 | NET253G11_2 | 11 | 4 | NET253G11_2_NET1 | 6 | 3 | 18035011 | 18037041 | 1 | NM_114727.3 | GAMMA CARBONIC ANHYDRASE-LIKE 2 (GAMMA C...MORE | ● |
| AT5G63510 | 4 | NET253G11_2 | 11 | 4 | NET253G11_2_NET1 | 6 | 5 | 25423984 | 25425841 | 1 | NM_125746.3 | GAMMA CARBONIC ANHYDRASE LIKE 1 (GAMMA C...MORE | ● |
| AT5G66510 | 6 | NET253G11_2 | 11 | 5 | NET253G11_2_NET1 | 6 | 5 | 26549704 | 26551630 | -1 | NM_001085339.1 NM_126049.3 | GAMMA CARBONIC ANHYDRASE 3 (GAMMA CA3); ...MORE | ● |
| AT1G55920 | 4 | NET253G11_2 | 11 | 4 | NET253G11_2_NET2 | 5 | 1 | 20912116 | 20913447 | 1 | NM_104470.2 | SERINE ACETYLTRANSFERASE 2;1 (ATSERAT2;1...MORE | ● |
| AT2G17640 | 4 | NET253G11_2 | 11 | 4 | NET253G11_2_NET2 | 5 | 2 | 7668078 | 7670495 | -1 | NM_127318.5 | ATSERAT3;1; FUNCTIONS IN: acetyltransfer...MORE | ● |
| AT3G13110 | 4 | NET253G11_2 | 11 | 4 | NET253G11_2_NET2 | 5 | 3 | 4214617 | 4216302 | -1 | NM_112150.3 | Encodes a mitochondrial serine O-acetylt...MORE | ● |
| AT4G35640 | 5 | NET253G11_2 | 11 | 4 | NET253G11_2_NET2 | 5 | 4 | 16905[illegible]79 | 16908357 | -1 | NM_119729.4 | Serine acetyltransferase 3;2 (ATSERAT3;2...MORE | ● |
| AT5G56760 | 4 | NET253G11_2 | 11 | 4 | NET253G11_2_NET2 | 5 | 5 | 22961309 | 22962660 | -1 | NM_125059.2 | Encodes a cytosolic serine O-acetyltrans...MORE | ● |

**Figure 4.** Example of pATsi usage. (**A**) List of genes associated with the NET253G11_2 network. (**B**) Graph view of NET253G11_2 network. (**C**) Graph view of NET253G11_2_NET1 and NET253G11_2_NET12 subnetworks.

**Notes:** Orange circles represent genes annotated as gamma carbonic anhydrases; yellow circles represent genes annotated as serine acetyltransferases; gray circle represents a gene with an unknown function.

the ones being singletons in *A. thaliana*. Moreover, many of the currently available platforms are based on methods that are not sufficiently described and/or are not easily reproducible, defining often incomparable collections hard to be further exploited in associated efforts. Some independent studies also aimed to identify singleton genes from *A. thaliana*,[51] but no dataset is provided to the scientific community to support validations or further investigations, despite the importance of addressing the origin of single-copy genes in a highly duplicated genome, as they can be a trace of mechanisms of evolutions, of speciation events, or even annotation limits.[28,52]

One of the novelties of this database is to provide immediate access to gene classes and possible paralogy relationships, supported by graphical approaches, offering at the same time a detailed description of the methods and parameters used for each class.[28] Being fully reproducible, the database may provide a common reference and a shared framework for related efforts.

The database represents a permanent resource for studies that need a reference collection for gene family analysis and comparative genomics based on *A. thaliana* and may be used as an effective and powerful tool to address the process of deciphering the complexity of its genome.

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## Author Contributions

Wrote the manuscript and implemented the network visualization in the web pages: LA. Organized the database and implemented the query interfaces and the web-based resource: HB. Contributed to the web page implementation: PDS. Developed the pipeline and extracted the results collected in the database: MS, AV. Conceived the analysis and the system, supported, drove, and directed all the work, and wrote the manuscript: MLC. All authors reviewed and approved the final manuscript.

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