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BeetleBase in 2010: revisions to provide comprehensive genomic information for *Tribolium castaneum*

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ABSTRACT

BeetleBase (http://www.beetlebase.org) has been updated to provide more comprehensive genomic information for the red flour beetle *Tribolium castaneum*. The database contains genomic sequence scaffolds mapped to 10 linkage groups (genome assembly release Tcas_3.0), genetic linkage maps, the official gene set, Reference Sequences from NCBI (RefSeq), predicted gene models, ESTs and whole-genome tiling array data representing several developmental stages. The database was reconstructed using the upgraded Generic Model Organism Database (GMOD) modules. The genomic data is stored in a PostgreSQL relatational database using the Chado schema and visualized as tracks in GBrowse. The updated genetic map is visualized using the comparative genetic map viewer CMAP. To enhance the database search capabilities, the BLAST and BLAT search tools have been integrated with the GMOD tools. BeetleBase serves as a long-term repository for Tribolium genomic data, and is compatible with other model organism databases.

INTRODUCTION

The red flour beetle, *Tribolium castaneum*, is a sophisticated genetic model organism for studies of insect development and pest biology as well as comparative genomics (1,2). A burgeoning wealth of genomic information, including a whole genome shotgun (WGS) assembly of the genome sequence, has become available in recent years (2).

BeetleBase was developed to provide a centralized database to serve the growing needs of the Tribolium research community. The first version of BeetleBase (3) was based on genome release Tcas_1.0. The database provided access to unmapped scaffolds, Fgenesh predicted genes, genetic markers, BAC-end sequences and ESTs. The database was implemented using an early version of the Chado schema, GBrowse, and CMAP developed by the Generic Model Organism Database (GMOD) project (http://www.gmod.org/).

Additional data has become available since the initial release of BeetleBase, including updates to the genome assembly and annotation, necessitating updates to the BeetleBase schema and data content. Here we present an updated version of BeetleBase (http://www.beetlebase.org), implemented using recent versions of Generic Model Organism Database (GMOD) tools, and integrated with BLAST (4) and BLAT (5) alignment tools. Data content has been expanded to include the latest version of the genome assembly (Tcas_3.0), a comprehensive collection of gene sets including the first unified release of the Tribolium Official Gene Set (OGS), EST alignments to the genome, and whole-genome transcriptional information from DNA tiling array experiments. These updates will provide a valuable resource for the Tribolium community, and serve as a foundation for integration of future datasets.

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Table 1. Genome sequence (Tcas_3.0) statistics

Chromosome	Length[a] (bases)	Number of contigs (bases)	Number of captured gaps (bases)	Number of uncaptured gaps[b] (bases)
ChLGX	10 877 635	338 (7 017 036)	299 (265 951)	12 (3 600 000)
ChLG2	20 218 415	393 (14 025 453)	338 (505 072)	19 (5 700 000)
ChLG3	38 791 480	1560 (27 070 658)	1355 (1 568 829)	34 (10 200 000)
ChLG4	13 894 384	331 (11 543 342)	299 (554 338)	6 (1 800 000)
ChLG5	19 135 781	388 (13 841 583)	335 (502 879)	16 (4 800 000)
ChLG6	13 176 827	667 (8 259 034)	549 (747 290)	14 (4 200 000)
ChLG7	20 532 854	445 (14 850 616)	401 (591 423)	17 (5 100 000)
ChLG8	18 021 898	676 (12 793 837)	570 (761 081)	15 (4 500 000)
ChLG9	21 459 655	695 (14 607 456)	598 (892 186)	20 (6 000 000)
ChLG10	11 386 040	585 (7 061 652)	495 (442 098)	13 (3 900 000)
Unknown[c]	41 251 169	3616 (20 543 639)	1254 (2 031 250)	1848 (18 480 000)

[a]Chromosome builds include scaffolds and contigs as well as uncaptured gaps between them. In GBrowse, scaffolds are not shown explicitly, but can be deduced from the contigs and captured gaps between uncaptured gaps. Since some contigs overlap, the total length of each chromosome build (column 2) is somewhat smaller than the combined total of columns 3, 4 and 5.
[b]The uncaptured gaps on chromosome builds X – 10 are blocks of 300 000 Ns that act as placeholders between the mapped scaffolds and contigs. The actually size of the uncaptured gaps is not known and may be considerably longer or shorter than 300 kb.
[c]Unknown is a linear compilation of scaffolds and contigs that have not been mapped to chromosomes.

DATA ACQUISITION

Genome sequence

The initial assembly of the Tribolium genome (Tcas_1.0) was composed of 1107 unmapped genomic sequence scaffolds. For the second version of the assembly (Tcas_2.0), 70% of the genome sequence was mapped to 10 linkage groups corresponding to nine autosomes and the X chromosome (2,6). Subsequently, an additional forty-two of the largest unmapped scaffolds have been integrated into the genetic linkage map, and used by the Baylor College of Medicine to create the third version of the assembly (Tcas_3.0). The Tribolium genome sequence is assembled into 9686 contigs of 156 Mb in combined length. When these are assembled into scaffolds containing captured gaps of estimated length, the genome assembly is ~160 Mb. Scaffolds and contigs containing more than 90% of the sequenced genome have been assembled into 10 chromosome builds. The chromosome build statistics for Tcas_3.0 are summarized in Table 1. The GenBank accession numbers of the 9686 contigs that have been assembled into ten chromosome builds, 305 unmapped scaffolds and 1848 unmapped single contigs are AAJJ01000001–AAJJ01009708 (22 of which have been suppressed since their original submission), CM000276–CM000285 and DS497665–DS497969 and GG694051–GG695897, respectively. The genetic map has also been updated to include the additional 42 markers used to anchor scaffolds in the Tcas_3.0 assembly.

Gene models

Several gene prediction programs were previously used to annotate the Tcas_2.0 assembly, and were combined into a consensus GLEAN gene set (2,7). More than 2000 genes were manually curated by members of the Tribolium Genome Sequencing Consortium (2); however, new and updated gene models were not combined into a unified gene set. We generated the first Tribolium Official Gene Set (OGS) by merging the GLEAN gene set with the manually curated gene annotations. First, each manually curated gene was mapped to the Tcas_3.0 assembly, and the validity of the mRNA, CDS, and/or peptide sequence for each of the manually curated genes was checked to ensure that the peptide sequence represented the same exons/splice sites as the mRNA and CDS sequences. Incorrectly annotated exons were replaced with exon coordinates determined by Splign (8). In some cases, genes required manual curation to determine the correct or most likely gene structure. A non-redundant Official Gene Set was constructed by merging the GLEAN and manually curated gene sets, automatically replacing GLEAN models with overlapping, manually curated models. Finally, unique identifiers were assigned to each gene to facilitate communication of Tribolium genes in research publications. A total of 16 561 official genes were generated, assigned identifiers such as 'TC######', and submitted to NCBI (Table 2).

Since some researchers may benefit from access to other gene sets, we migrated the AUGUSTUS, Ensembl, Fgenesh, NCBI supported and ab initio gene models, and the combined GLEAN genes from the Genboree database hosted at Baylor College of Medicine (http://www.genboree.org), and converted their coordinates into the genome coordinates of Tcas_3.0 (Table 2). Only a few of the predicted genes were lost when the scaffolds were reorganized. In addition, the latest RefSeq annotation from NCBI (build 2.1, based on the same Tcas_3.0 assembly), which includes 3613 protein accessions that are new or changed from the original annotation run used for the GLEAN set, was imported into BeetleBase. Each gene set is available for viewing in a separate track in GBrowse (Figure 1).

BeetleBase provides a detailed gene report page for each gene in the OGS, which can be accessed and managed through the web interface (Figure 2). The new

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Table 2. Gene model and EST statistics

Chromosome	Official Gene	Curated Gene	GLEAN	AUGUSTUS	Ensembl	Fgenesh	NCBI-*ab initio*	NCBI-sup	RefSeq	EST
ChLGX	798	81	799	691	1782	1196	700	507	544	2119
ChLG2	1661	287	1641	1225	3425	2524	1349	987	1018	4691
ChLG3	1998	220	1967	1654	4304	3444	1512	1137	1182	5062
ChLG4	1529	191	1530	1202	3029	2161	1293	868	954	3629
ChLG5	1806	254	1769	1441	3851	2530	1393	1088	1192	4442
ChLG6	1033	155	1021	908	2282	1281	904	660	695	2470
ChLG7	1887	293	1850	1555	3969	2805	1645	1255	1277	4653
ChLG8	1623	182	1618	1380	3391	2073	1394	976	1032	4735
ChLG9	1509	161	1522	1229	3138	2206	1239	920	972	3447
ChLG10	628	108	615	552	1432	896	515	375	398	2119
Unknown	2089	121	2086	1107	2358	2325	2018	653	N/A	13 006
Total	16 561	2053	16 418	12 944	32 961	23 441	13 962	9426	9264	50 277

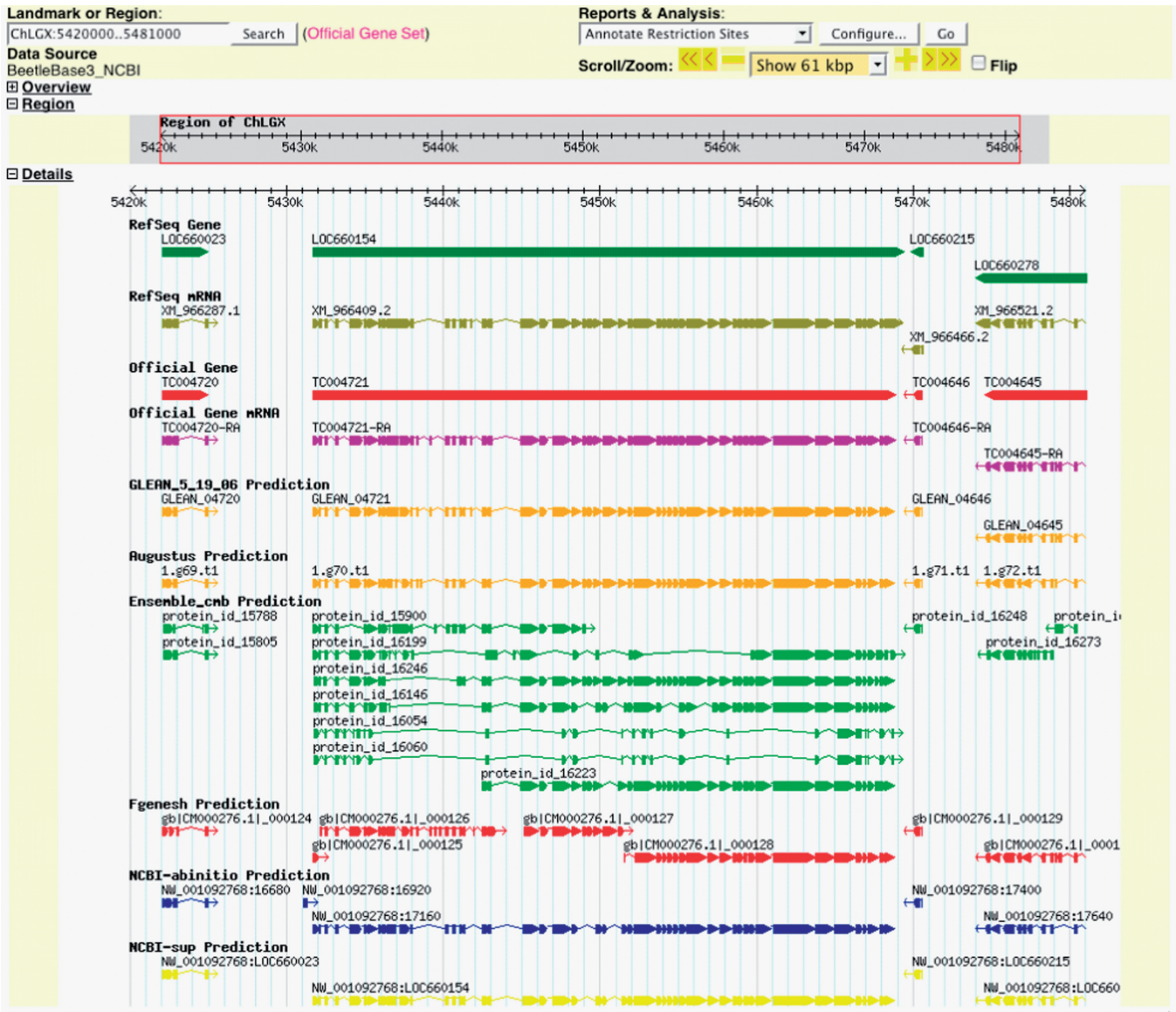

Figure 1. *Tribolium* gene models. Various gene models are shown in different tracks for easy comparison. By clicking one of the gene models, detailed information can be retrieved. The RefSeq gene models are linked to NCBI Entrez Gene report pages. The *Tribolium* official gene models and manually-curated gene models link to the BeetleBase gene report pages. Other gene models link to detailed pages provided by GBrowse.

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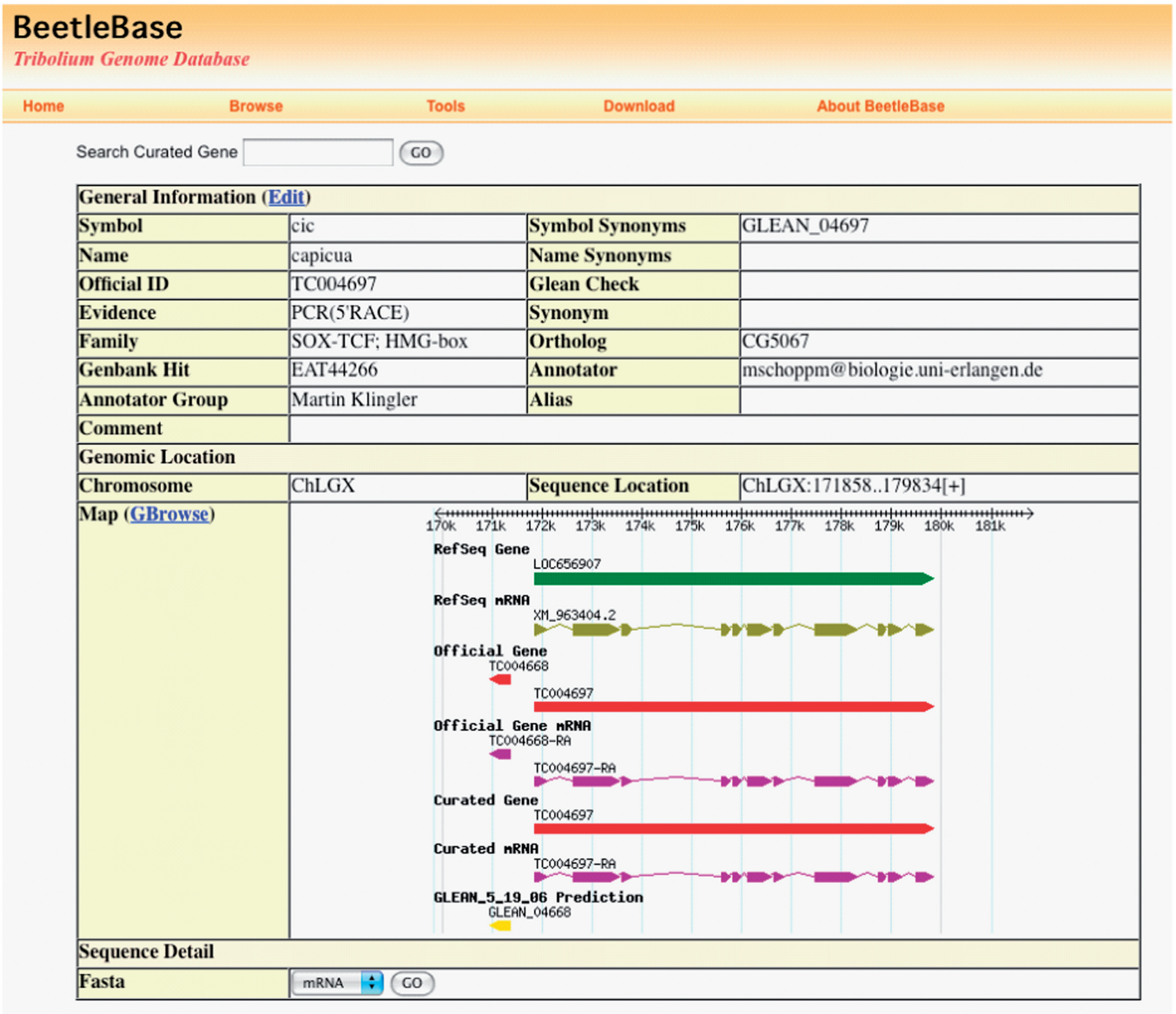

Figure 2. Official gene report page. The report page provides detailed information for an official gene. The information can be modified by clicking 'Edit' after obtaining a password from the BeetleBase webmaster. The report page links to GBrowse by clicking 'GBrowse'. The corresponding region will be highlighted in GBrowse.

BeetleBase database provides more comprehensive gene information than was previously available, including detailed information on the gene structure, nomenclature, and additional annotation data provided during the manual curation process. Links are also provided to overlapping RefSeq Gene and transcript records at NCBI (9,10) to facilitate use of data from both databases.

EST and BAC-end sequences

EST and BAC-end sequences were downloaded from the dbEST and GSS databases, respectively, at NCBI. 55 616 ESTs from five different tissue- and stage-specific cDNA libraries (11) were cleaned and polyA sequences removed using in-house software tools (http://bioinformatics.ksu.edu/ArthropodEST) and aligned to the genome using the Exonerate algorithm (12). A total of 50 277 EST-genome alignments were generated. BAC-end sequences from the *Tribolium* BAC library (constructed by Exelixis, Inc, South San Francisco, CA and archived for distribution by the Clemson University Genomics Institute (https://www.genome.clemson.edu/) were mapped to the genome and added to the database. Out of 28 788 BAC-end sequences, 27 810 were aligned to the genome using BLAST.

Tiling arrays

Using Roche NimbleGen HD2 whole genome DNA tiling arrays, whole genome expression data have been collected for several developmental stages including three embryonic, the last larval, three pupal and two adult stages.

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Figure 3. *Tribolium* genome tiling arrays. Tracks for 11 developmental time points are shown. Blue vertical bars of each track represent fluorescence intensity values for oligonucleotide probes tiled across a 54 kb region on the array. The official gene mRNA track (above time point tracks) contains purple gene structures. Note the different expression patterns of the annotated genes across development.

Briefly, fluorescently labeled cDNAs were hybridized to the custom-designed microarrays; GFF files were constructed using the signal intensities from each feature on the array that were quantified directly from the scanned array images without any data management such as background subtraction. This was done to provide immediate access to the data to help verify computed gene models and assist manual annotation efforts while the data are processed and further analyzed. Figure 3 shows empirically derived transcriptome tiling array data generated from several developmental stages for a portion of the *Tribolium* genome.

FTP site

Datasets that can be downloaded from BeetleBase (ftp://bioinformatics.ksu.edu/pub/BeetleBase/3.0/) include the contig sequences, GFF (.gff3) and assembly files (.agp) for Tcas_3.0. Sequences of GLEAN genes, GLEAN cDNAs and GLEAN peptides as well as the corresponding files for the OGS are also available.

IMPLEMENTATION

All the genomic information was compiled into Genetic Feature Format Version 3 (GFF3), which is the most common extension of GFF. The compiled information was implemented using GMOD tools (http://www.gmod.org) such as PostgreSQL-based Chado 1.0, GBrowse 1.68, and CMAP 1.0. To query sequences against the *Tribolium* genome, we also installed stand alone BLAST and BLAT servers. In this release of BeetleBase, we improved the usability by integrating these components.

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Conflict of interest statement. None declared.

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