BeetleAtlas: An Ontogenetic and Tissue-specific Transcriptomic Atlas of the Red Flour Beetle *Tribolium castaneum*

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Abstract

The red flour beetle *Tribolium castaneum* has emerged as a powerful model in insect functional genomics. However, a major limitation in the field is the lack of a detailed spatio-temporal view of the genetic signatures underpinning the function of distinct tissues and life stages. Here, we present an ontogenetic and tissue-specific web-based resource for *Tribolium* transcriptomics: BeetleAtlas (https://www.beetleatlas.org). This web application provides access to a database populated with quantitative expression data for nine adult and seven larval tissues, as well as for four embryonic stages of *Tribolium*. BeetleAtlas allows one to search for individual *Tribolium* genes to obtain values of both total gene expression and enrichment in different tissues, together with data for individual isoforms. To facilitate cross-species studies, one can also use *Drosophila melanogaster* gene identifiers to search for related *Tribolium* genes. For retrieved genes there are options to identify and display the tissue expression of related *Tribolium* genes or homologous *Drosophila* genes. Five additional search modes are available to find genes conforming to any of the following criteria: exhibiting high expression in a particular tissue; showing significant differences in expression between larva and adult; having a peak of expression at a specific stage of embryonic development; belonging to a particular functional category; and displaying a pattern of tissue expression similar to that of a query gene. We illustrate how the different feaures of BeetleAtlas can be used to illuminate our understanding of the genetic mechanisms underpinning the biology of what is the largest animal group on earth.

Introduction

Insects constitute more than half of all extant metazoan species on earth.[1] Since the advent of the postgenomic era, the fruit fly *Drosophila melanogaster* has been regarded as the premiere model for understanding the molecular basis of animal biology.[2] However, work over the past decades has revealed that *Drosophila* biology is defined by many derived traits that make it a less than optimal representative of insect biodiversity.[3] Moreover, to gain a greater understanding of the genomics of living organisms in general, as well as those of insects in particular, it is necessary to develop a wider range of models that can support genetic and molecular studies, and thus act as complementary systems to *Drosophila*.[4,5] Such a role has been assumed by the red flour beetle *Tribolium castaneum*, a member of the largest Order of insects (*Coleoptera*). *Tribolium* has emerged as a powerful model in insect functional genomics,[6] where it is particularly favoured for studying developmental genetics[7] and population ecology.[8] Moreover, as a globally devastating agricultural pest, *Tribolium*

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has also recently attracted interest as a physiological model, given that the insights obtained are directly applicable to pest control strategies.[8–10] Studies in *Tribolium* are facilitated by a genomic assembly of high quality, as well as extensive genetic resources and established CRISPR/Cas9 protocols.[11] Further, the proteome of *Tribolium* is less diverged from vertebrates than that of *Drosophila*, and the biology is more representative of insects as a whole. Yet, in spite of the strategic arguments listed above, key resources promoting *Tribolium* as a model, such as a gene expression atlas, are still not available to the scientific community. Here we introduce BeetleAtlas, a tissue- and development-specific web-based application for *Tribolium* transcriptomics. BeetleAtlas is reciprocally integrated with the related *Drosophila* web application, FlyAtlas 2,[12,13] and links to information in iBeetleBase,[14] the *Tribolium* genomic facility, making it an invaluable addition to the rapidly expanding toolbox for *Tribolium* research.[15–17] Here, we illustrate the different facilities provided by BeetleAtlas to demonstrate how this resource will illuminate our understanding of the genetic mechanisms underpinning the biology of the largest animal group on earth.

Results

RNA-sequencing (RNA-seq) was performed to obtain quantitative transcriptomic data for a range of adult and larval tissues, and for developmental stages of the embryo of *Tribolium* (Materials and Methods). The transcriptomic data were then incorporated into the relational database, 'TriboliumDB', and a web interface to this, BeetleAtlas, was constructed to allow general access (https://www.beetleatlas.org). The different search options of BeetleAtlas will be described in turn, with pertinent examples to illustrate the utility of the resource.

'**Gene**' **search mode**

The overall appearance of BeetleAtlas is shown in Figure 1. At the top of each page a menu offers the six different search modes ('1'2') is the input for the chosen menu item—here illustrated with 'Gene'. This allows one to retrieve data for the tissue-specific expression of a particular gene, the results of which are shown below the input form and presented to the user in a format common to the six different searches. An anatomical map depicting the different tissues is available by clicking on 'Anatomical key' ('3') to help visualize the demarcation of each tissue as defined in BeetleAtlas. The results are tabulated for each adult and larval tissue ('4'), below which are shown the corresponding data for each transcript variant ('5'). Because of the possibility of several transcripts from a single gene, the data for the transcripts are presented in condensed form as a heat map, and genes and transcripts for a particular tissue can be visually correlated by the red highlighting that appears by clicking on a cell in the gene table ('6' and '7'). Full numerical results for the transcripts (and for the genes) can be downloaded ('8' and '9') in a tab-delimited form suitable for opening in a spreadsheet. For the embryo stages values for both genes and individual transcripts can be presented in a single table ('10'), and a graphic view of the total gene expression is also provided ('11') so that the user can see overall trends more easily. This table can be exported and saved as an SVG file for incorporation into a publication if needed.

An important feature of BeetleAtlas is the reciprocal integration with FlyAtlas 2 to allow comparison of the tissue-specific expression of the genes of *Tribolium* with that of the more extensively characterized genes of *Drosophila*. Thus, it can be seen from the input form in Figure 1 ('2') that a search for any corresponding *Tribolium* gene(s) can be made using a gene identifier for *Drosophila*. The corollary of this is a direct link to FlyAtlas 2 from the results of a *Tribolium* gene search ('12'), which invokes a new browser window showing the corresponding pattern of expression in related tissues for any *Drosophila* homologues. It should be emphasized that, through close co-operation with the curators of FlyAtlas 2, the results of searches for *Drosophila* genes with that web application also link to BeetleAtlas, allowing identification of *Tribolium* homologues. The mutual integration of the databases thus allows one to compare the spatial expression pattern of *Tribolium* and *Drosophila* homologues by a simple web search—a key feature for any researcher interested in the evolution of gene function.

An analogous link to that just mentioned allows one to view *Tribolium* paralogues ('13'), and we illustrate the power of using the homologue and paralogue links together by considering the putative gene for a member of the β-1,3-galactosyl transferase family—TC008954. This gene has three transcripts, each of which shows a distinct pattern of expression across different tissues (Figure 2A). All tissues express the transcript TC008954-RA, but only the rectal complex, the adult fat body and the male gonads express transcript TC008954-RC. A third transcript (TC008954-RB) is expressed weakly and appears to be confined to female gonads. Proceeding from this observation, one might ask how the pattern of expression compares with that of genes of the same family in *Drosophila* or in other members of this gene family in *Tribolium*. The paralogue link in BeetleAtlas for TC008954 retrieves nine paralogues (i.e. ten *Tribolium* genes in all); and by following the links of each of these to their *Drosophila* homologues a total of nine members of

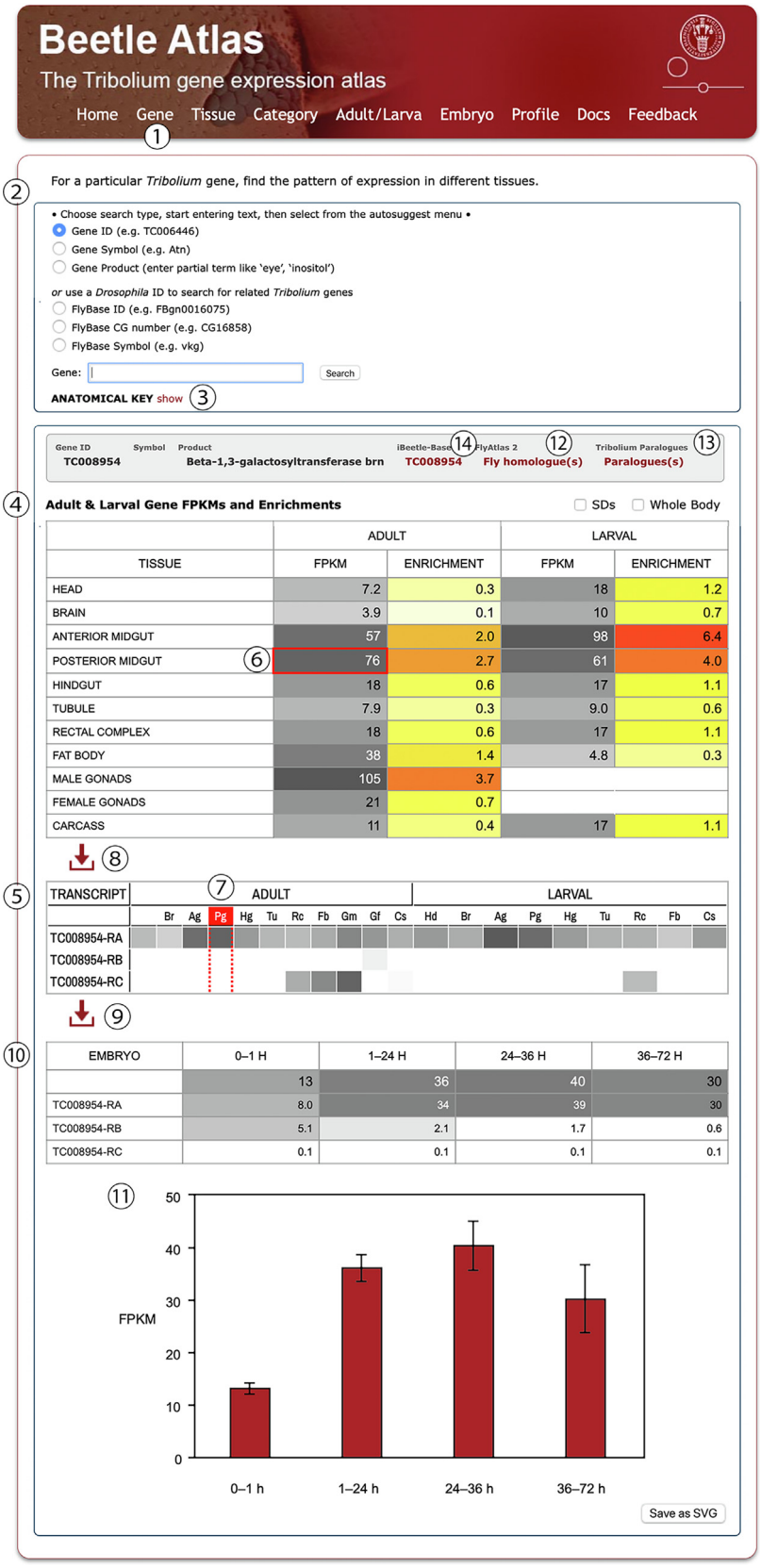

Figure 1. Web interface of BeetleAtlas and presentation of results. The illustration shows the input form for the gene search mode ('2'), chosen from the navigation menu ('1'), followed by the output of results. The red highlighting of the transcript tissue column ('7') is invoked by clicking on the cell ('6') for the abundance (FPKM) of transcripts in the same tissue. Other encircled numbers indicate features referred to in the text. Input forms for other types of searches are illustrated in Supplementary Figure S1.

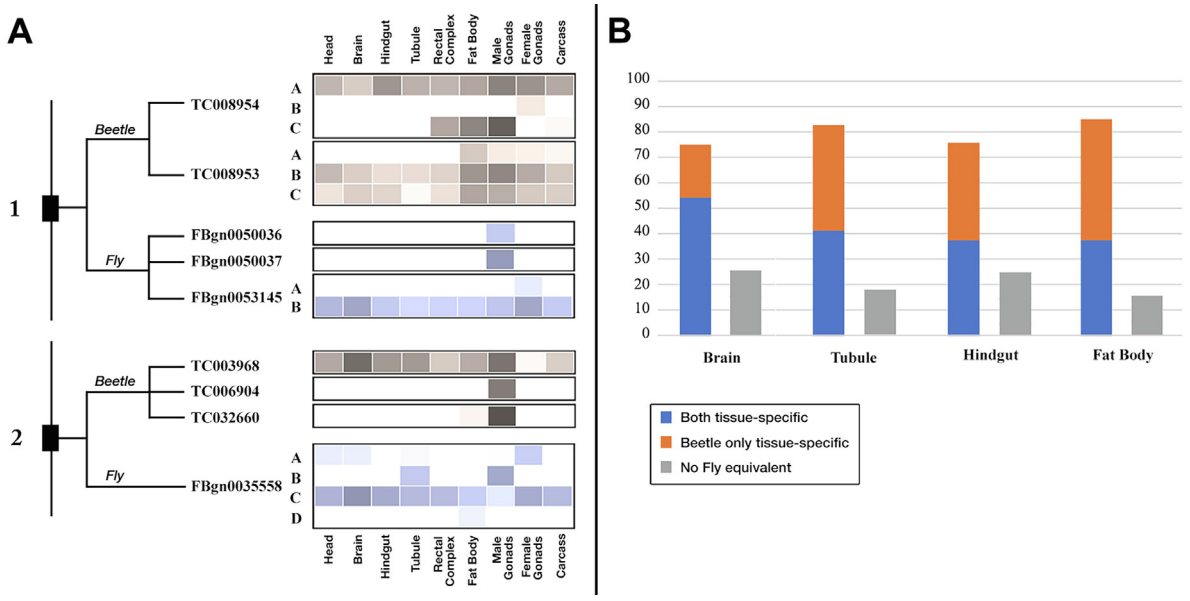

Figure 2. Comparison of genes in tissues of *Tribolium castaneum* and *Drosophila melanogaster*. (A) Expression of different isoforms of genes of the β-1,3-galactosyltransferase family. The evolutionary tree shows two of the six postulated ancestral galactosyltransferase paralogues ('1' and '2'), together with the *T. castaneum* and *D. melanogaster* orthologues. The heat maps are for common tissues, and have been taken from BeetleAtlas and FlyAtlas 2, rearranged and processed with different coloured photo filters to help distinguish them. (B) Conservation of tissue-specificity of gene expression. A 'Tissue' search (Supplementary Figure S1 (i)) was performed in BeetleAtlas to retrieve the genes the expression of which is most enriched in each of the tissues shown. The first 100 genes were selected that are expressed specifically in that tissue (i.e. >90% total expression). FlyAtlas 2 results for homologous genes in *Drosophila* were displayed (Figure 1—link at '12') and examined for specific expression in the same tissue. The criterion for specific expression here was less stringent: at least 50% of total expression. Where there were several *Drosophila* homologues, any one satisfying this criterion was accepted. In (A), with the exception of the gonad samples, the tissues from *Tribolium* are for mixed male and female insects, whereas those for *Drosophila* are for the male. In (B) the means of male and female values have been used for *Drosophila*.

the family were identified here. On this basis there are six groups of related β-1,3-galactosyltransferase genes among the two insects. The results of multiple sequence alignment using ClustalX[18] and pairwise comparison (https://www.ebi.ac.uk/jdispatcher/psa) confirmed this grouping, consistent with the last common ancestor of these insects having six distinct β-1,3-galactosyltransferase genes. For two of these six groups, the pattern of expression of transcripts in analogous tissues of *Tribolium* and *Drosophila* is presented in Figure 2. It is not our purpose here to discuss the similarities and differences between the nine *Tribolium* and eight *Drosophila* transcripts shown, but the general utility of BeetleAtlas in addressing problems of this type is clearly evident.

'Tissue' search mode

Gene-by-gene searches are likely to account for most of the traffic on BeetleAtlas. However, unexpected expression patterns can sometimes be identified by performing unbiased searches, i.e. asking what are the genes necessary to make a tissue function? The menu item 'Tissue' in Figure 1 links to a page where one can search for the most highly expressed genes in a particular tissue (*see* Supplementary Figure S1(i)). Up to 100 results are initially listed in a 'collapsed' form (Supplementary Figure S2), allowing one to scroll quickly through them, with buttons to display the full results table (as in Figure 1) for any or all members. As an example of its utility, we selected all genes from the expanded list that are only expressed (i.e. >90% of total) in the particular tissue used for the search. We did this for four tissues with counterparts in the *Drosophila* web application FlyAtlas 2, where a similar analysis was performed. For the tissues examined it emerged that approximately 80% of the tissue-specific genes in *Tribolium* have orthologues in *Drosophila.* Within that group, the proportion that are also tissue-specific in *Drosophila* varied between approx. 44% and 73% (Figure 2B).

'Category' search mode

It is not uncommon to wish to search for genes specifying proteins categorized as belonging to particular functional groups. The menu item

'Category' in Figure 1 links to such a facility. Unfortunately the gene ontology for *Tribolium* is not as extensive as in some other species, so we have been obliged to adopt a selective curated approach for this (Supplementary Figure S1ii). We have compiled a list of genes in 24 categories that can be used as search criteria, and which we hope to extend over time. The utility of the function may be illustrated by searches for genes, the products of which are classified as 'ion channel' and which are enriched in the tissues of the alimentary tract. Supplementary Table S1 shows how combining the results of such a search can provide a broad perspective of such a functional group, highlighting genes expressed throughout the alimentary tract, in contrast to those expressed in a more restricted part of it.

'**Adult/Larva**' **search mode**

The menu item 'Adult/Larva' in Figure 1 links to a page where one can identify genes that differ in expression between adult and larva for a particular tissue—either predominantly in adult or predominantly in larva (Supplementary Figure S1 (iii)). We illustrate the use of this facility in a search for genes expressed in the larval, but not the adult, rectal complex. This revealed an unanticipated association: in many cases the genes found were restricted to either larval head and larval rectal complex, or to larval head, larval rectal complex and larval carcass (Supplementary Table S2). In 85% of these cases there was expression in the embryo, restricted to between 36–72 hr. The function of many of the proteins specified by these genes is predicted to involve the cuticle or membrane, suggesting an explanation of the tissue specificity. The results would appear to allow one to differentiate between genes specifying proteins involved in the cuticular portions of all three larval tissues and those specific to head and rectal complex.

'**Embryo**' **search mode**

Data for the expression of a particular gene during embryonic development is presented in the results page (Figure 1), and the preceding section showed how this can provide useful context to searches focussed on adult and larval tissues. Although some genes may exhibit a pattern of temporal expression during embryogenesis that is too complex to lend itself to identification through a simple search, others show a peak of expression at a particular stage, and it is for these that a search is provided in BeetleAtlas (Supplementary Figure S1(iv)), accessed from menu item 'Embryo' in Figure 1.

One way we tested the general validity of the embryonic data was by examining transcripts with a peak during the first hour of embryogenesis. On the basis of previous work[19–21] one would expect such transcripts to represent predominantly maternal mRNAs originating in the ovary, together with some newly synthesized embryonic transcripts involved in the initiation of embryogenesis. Comparison of the transcripts from the embryo with those present in adult tissues showed that many were restricted to a single non-embryonic tissue, the adult female gonad (Supplementary Table S3i), consistent with their originating from the ovary. Using the option in the Embryo Search to restrict results to transcripts only expressed in the embryo, there was a decrease from approximately 300 to 20. Many of the latter are putative transcription factors or proteins involved in DNA metabolism (Supplementary Table S3ii).

'**Profile**' **search mode**

The Profile Search (menu item 'Profile' in Figure 1, entry form in Supplementary Figure S1 (v)) is rather different from the searches already discussed in that it is primarily a tool for identifying the function of genes or the possible physical association of their products. The search employs the pattern of expression of a query gene across different tissues and retrieves genes with a similar pattern of expression. The rationale is that although the function of the query gene may be unknown, that of target genes may have been elucidated. If the target genes are found to share a similar function, this may suggest the same or a related function for the query gene.

To illustrate this we selected a *Tribolium* gene, TC031168, to which no function had been assigned in iBeetleBase.[14] This gene has a *Drosophila* equivalent, FBgn0040931, which at the time of investigation was listed in release FB2023_01 of FlyBase[22] as also having no known function. The Profile search with TC031168 identified 39 genes having a similar pattern of expression, many of the products of which appeared to be components of the mitochondrial oxidative phosphorylation complexes. An analogous search on FlyAtlas 2 with FBgn0040931 identified 27 genes having a similar representation of products involved in oxidative phosphorylation. We refined the lists of genes so as to select only those with several 'neighbours', and visualized them using the software, BioLayout.[23] This presents the results as three-dimensional interactions between spheres (Figure 3). It can be seen that for both *Tribolium* and *Drosophila* the 'unknown' genes clustered with members of the mitochondrial complexes, in particular with those of complex I.

After having found this association we performed a more extensive literature search on FBgn0040931. It transpired that a few months before our investigation a three-dimensional structure of the mitochondrial respiratory complex I from *Drosophila* had been described, and mass-spectrometry showed that one of its subunits corresponded to the putative product of

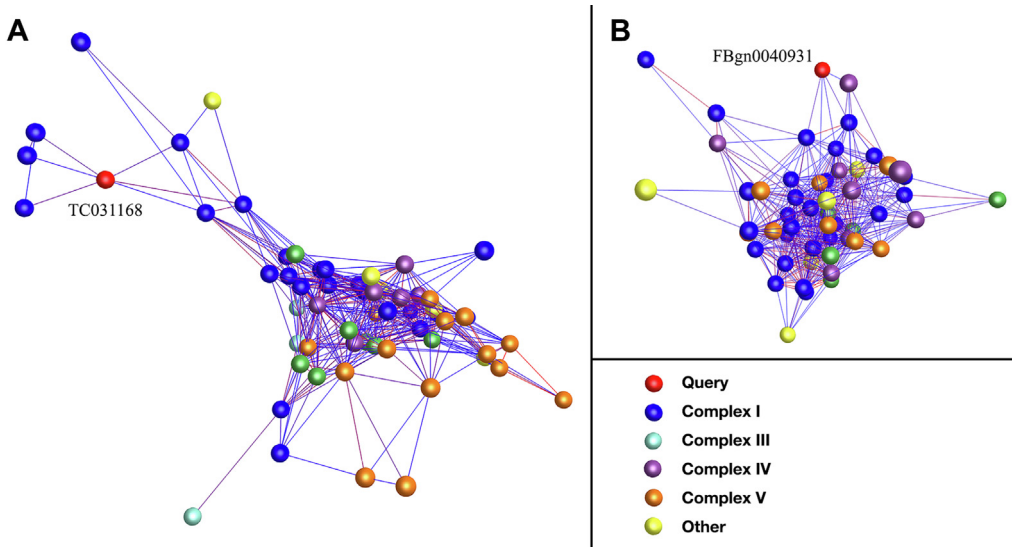

Figure 3. Three-dimensional clustering of two related insect genes of previously unknown function with those showing similar patterns of expression across different tissues. Profile searches were performed using as queries (A) *Tribolium* TC031168 in BeetleAtlas, and (B) *Drosophila* FBgn0040931 in FlyAtlas 2. The BioLayout application was then employed to cluster genes. The different colours refer to the constituents of the various mitochondrial respiratory complexes, as shown in the key.

FBgn0040931.[24] This protein occupies a position in the *Drosophila* complex 1 that in mammals is occupied by subunit NDUFA2, a protein that was known to have no *Drosophila* sequence homologue.

We regard this as a striking illustration of the potential of the 'Profile' search in BeetleAtlas.

Discussion

The purpose of this paper was to describe the web application, BeetleAtlas, rather than any particular scientific problem that it has been used to address. We have reported elsewhere how it has been employed in studying water retention in the rectal complex of *Tribolium*.[10] Some of the illustrations of its facilities presented here raise intriguing questions that justify further research, but we confine ourself in this Discussion to some general observations.

The first is that BeetleAtlas fulfils both a specialized role in providing a resource for *Tribolium*, and—by its integration with the expression of the genes of *Drosophila* in FlyAtlas 2—a more general comparative role in studying tissue-specific gene expression in insects. Others have emphasized the large number of *Tribolium* genes that have no counterparts in *Drosophila*,[3] and our own results both confirm this and demonstrate that even in those cases where counterparts exist they may show different patterns of expression across tissues (Figure 2A). The gene ID on the results pages ('14' in Figure 1) provides a link to a page for the gene in iBeetleBase,[14] which in turn provides a specific link to OrthoDB (https://www.orthodb.org). This allows one to determine whether a particular gene of interest that lacks a homologue in *Drosophila* is present in other insect groups.

Our second observation is of a different kind. BeetleAtlas has been specifically designed to allow the user to recognize general trends in many large tables of results through the combined use of colour-coding, heat maps and bar graphs. This greatly facilitated scanning the 300 or more result sets mentioned in the example of an 'Embryo' search, and allowed us to identify the trends discussed previously. It also revealed the unanticipated associations described in the examples of the 'Adult/Larval' search. We hope that this aspect of BeetleAtlas will serve as a general aid in studies of the tissue-specific expression of the genes of insects and of other species.

In conclusion, we predict that BeetleAtlas will have a major impact on the insect functional genomics community by providing an extensive catalogue of gene expression across different tissues and life stages, and thus promote an ontogenetic and tissue-centred view of gene function. To this end we hope to extend BeetleAtlas to include more tissues, and encourage other members of the *Tribolium* community to contact us regarding the possibility of adding any data of their own that might enhance the facility.

Materials and methods

Data collection, processing and warehousing

Details of animals, tissues (Supplementary Figure S3), and extraction of total RNA were as previously described.[10] The RNA libraries were sequenced on a BGISEQ-500 machine using paired-end chemistry, and analysed using the Tuxedo pipeline[25] with version Tcas5.2 of the sequence of the *Tribolium* reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_000002335.3/) and version ogs3 of the gff gene annotations (https://ibeetle-base.uni-goettingen.de/downloads/OGS3.gff.gz).

The processed data, as FPKM (Fragments Per Kilobase of transcript per Million mapped reads), were used to populate a MySQL relational database, TriboliumDB (Supplementary Figure S4). This also includes tables of data from other sources. The table, 'FlyCorrelate', links homologous *Drosophila* and *Tribolium* genes, and was from Jürgen Dönitz and Gregor Bucher. It should be emphasized that inter-species homologues do not exist for all genes: 11,121 *Tribolium* genes (67%) have a *Drosophila* homologue, whereas 9779 *Drosophila* genes (56%) have a *Tribolium* homologue. The table, 'Paralogue', links paralogues of *Tribolium* genes and was generated by Karen McCluskey. It contains paralogues for 53% of the *Tribolium* genes.

The database contains the 16,593 distinct genes and 18,536 transcripts designated in the 2020 assembly specifying the *Tribolium* reference genome.[15] Individual tissues and embryonic stages expressed between 40% and 55% of these genes, of which 21% were not detected in any tissue or any embryonic stage studied (Supplementary Figure S5). Approximately half of the genes detected were common to adult, larval and embryonic stages, with the proportion of unique genes ranging from 1.6% in the embryo, 2.1% in the larva to 6% in the adult (Supplementary Figure S6). It has been shown previously that individual tissues in the database show distinct patterns of expression of gene transcripts (Figure 1B of reference[10]).

Web Application: Technical

The BeetleAtlas web application (https://www.beetleatlas.org) utilizes a Java servlet to generate web pages and communicate with the relational database, TriboliumDB. The technical details are similar to those previously described for FlyAtlas 2.[12–13] Scalable Vector Graphics were generated using the Apache™ Batik SVG Toolkit (https://xml-graphics.apache.org/batik/). BeetleAtlas runs on all standards-compliant desktop and mobile web browsers, and without the need for plugins or installation of Java on the client. No registration is required.

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CRediT authorship contribution statement

David P. Leader: Writing – review & editing, Writing – original draft, Software, Methodology, Data curation. **Muhammad T. Naseem:** Writing – review & editing, Methodology, Investigation. **Kenneth V. Halberg:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmb.2024.168520.

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