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ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/kfly20

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To cite this article: Katharine L. Korunes, Russell B. Myers, Ryan Hardy & Mohamed A. F. Noor (2021) PseudoBase: a genomic visualization and exploration resource for the *Drosophila pseudoobscura* subgroup, Fly, 15:1, 38-44, DOI: 10.1080/19336934.2020.1864201

To link to this article: https://doi.org/10.1080/19336934.2020.1864201

Published online: 11 Jan 2021.

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METHODS AND TECHNICAL ADVANCES

PseudoBase: a genomic visualization and exploration resource for the *Drosophila pseudoobscura* subgroup

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ABSTRACT
Drosophila pseudoobscura is a classic model system for the study of evolutionary genetics and genomics. Given this long-standing interest, many genome sequences have accumulated for *D. pseudoobscura* and closely related species *D. persimilis, D. miranda*, and *D. lowei*. To facilitate the exploration of genetic variation within species and comparative genomics across species, we present PseudoBase, a database that couples extensive publicly available genomic data with simple visualization and query tools via an intuitive graphical interface, amenable for use in both research and educational settings. All genetic variation (SNPs and indels) within the database is derived from the same workflow, so variants are easily comparable across data sets. Features include an embedded JBrowse interface, ability to pull out alignments of individual genes/regions, and batch access for gene lists. Here, we introduce PseudoBase, and we demonstrate how this resource facilitates use of extensive genomic data from flies of the *Drosophila pseudoobscura* subgroup.

ARTICLE HISTORY
Received 15 September 2020
Revised 8 December 2020
Accepted 10 December 2020
Published online 8 January 2021

KEYWORDS
database; comparative genomics; genome browser; Drosophila

Introduction

Flies of the *Drosophila pseudoobscura* subgroup have a long and rich history as model systems in evolutionary genetics and genomics. The *pseudoobscura* subgroup is particularly recognized for its roles in the study of the population genetics of chromosomal inversions and the evolutionary genetics of species barriers. In the early 1900s, the development of cytogenetic methods that visualized karyotypes of polytene chromosomes enabled analysis of variation in *Drosophila* chromosome structure. These cytogenetic approaches enabled a wide range of genetic analyses, including early genetics-based species phylogenies based on inversion differences between closely related species of the *pseudoobscura* subgroup [1]. Surveys of natural populations of the *pseudoobscura* subgroup quantified extensive variation within and between populations as well as fixed structural differences between species [2–4]. In early analyses, inversion differences were often thought to be neutrally evolving, based on the logic that inversions can change gene order without disrupting gene content. With the rapid expansion of DNA sequencing technology in the early 2000s, the *pseudoobscura* subgroup played a key role in the resurgence of empirical and theoretical research on inversion polymorphisms [5]. Today, inversions are recognized for the diverse roles across various taxa in local adaptation, divergence, and speciation [6,7,8,9,10].

Flies of the *pseudoobscura* group remain prominent model systems for understanding the evolutionary genetics of differences between species. Thus, many genome sequences have accumulated both for *D. pseudoobscura* and for its close relative species. *D. pseudoobscura* and its naturally-hybridizing sister species, *D. persimilis*, provide a model for understanding how inversions shape speciation. Nearly all reproductive barriers between these species map to the fixed or nearly fixed inversions that differ between them [10,11]. A subspecies of *D. pseudoobscura, D. pseudoobscura bogotana*, also provides important insights in speciation research. There are two named subspecies of *D. pseudoobscura* – D. *pseudoobscura pseudoobscura* and *D. pseudoobscura bogotana*. Throughout this article, we use *D. pseudoobscura* to refer to both subspecies, and we specify D. *pseudoobscura pseudoobscura* or *D. pseudoobscura bogotana* when we are specifically referring to only one of the two

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subspecies. *D. pseudoobscura bogotana* is found near Bogota, Colombia, and does not exchange genes with allopatric *D. pseudoobscura pseudoobscura* or *D. persimilis*. Thus, *D. pseudoobscura bogotana* is an important point of genomic comparison for *D. pseudoobscura pseudoobscura* and *D. persimilis*, and has served as a model for the early stages of speciation [12–15]. Additional important points of comparison for *D. pseudoobscura* evolutionary genetics include *D. lowei* and *D. miranda*. *D. lowei* and *D. pseudoobscura* likely diverged 5–11 million years ago [16], and *D. lowei* often serves as an outgroup species for comparative genetics and genomics within the *pseudoobscura* subgroup [17–19]. *D. miranda* is also often leveraged as a point of comparison for *D. pseudoobscura* [19,20] and has become particularly known as a model for sex chromosome evolution [21,22].

As is often the case with classic study systems, multiple laboratories have sequenced a variety of strains of *D. pseudoobscura* and closely related species. To facilitate the exploration of genetic variation within and among these species, we present PseudoBase as a resource that presents publicly available genomic data via an intuitive graphical interface, accessible to students or researchers without any prior experience in working with genomic data. Our intention is to provide a resource that maximizes the utility of sequence data by lowering barriers to working with these data. PseudoBase originated in 2012 as a resource primarily for the Noor laboratory at Duke University, for a handful of other laboratories, and for use as a classroom tool. The ongoing accumulation of new genomic data for these species as well as a desire for broader functionality recently prompted a redesign of the original interface. We released ‘PseudoBase 2.0 , http://pseudobase.biology.duke.edu/, to include a broader sampling of publicly available *pseudoobscura* subgroup genomes, update the underlying *D. pseudoobscura* reference genome, and provide a significantly improved user interface. The new user interface includes embedded JBrowse visualization tools, the ability to download FASTA formatted alignments of individual genes/regions, and batch access for gene lists. Here, we provide an overview of the underlying data within PseudoBase, we introduce its features and functionalities, and we illustrate how this public resource supports the use of flies of the *pseudoobscura* subgroup for biological discovery and education.

Materials and methods

Sequencing information and variant calling

PseudoBase aggregates whole genome paired-end Illumina experiments from multiple laboratory groups and experiments [17,19,23–25]. Raw data and associated details are available on the NCBI Short Read Archive under the sample accessions indicated on PseudoBase and listed in Table 1. When more than one whole genome experiment was available for a given strain (e.g., *D. pseudoobscura* Flagstaff 14), we included the one sequenced using the most recent technology (which happened to also provide the highest coverage). We did not include genomes resulting from crosses of multiple laboratory strains.

The pipeline used for genome alignment and variant calling is available on GitHub (https://github.com/kkorunes/PseudobaseScripts). We first used BWA-0.7.17 [26] to align all sequences to the *D. pseudoobscura* genome assembly (Dpse_3.04:

Table 1. Genomes represented in the PseudoBase 2.0 release.

Species	Genomes	NCBI Accessions (Strain details on PseudoBase)
D. pseudoobscura pseudoobscura	31	SRX091462, SRX091310, SRX091461, SRX091324, SRX091465, SRX091463, SRX091323, SRX091311, SRX7842600, SRX7842599, SRX7842598, SRX7842597, SRX7842596, SRX7842595, SRX7842594, SRX7842593, SRX7842591, SRX7842590, SRX7842589, SRX7842588, SRX7842587, SRX7842586, SRX7842585, SRX7842584, SRX7842583, SRX7842582, SRX7842581, SRX7842580, SRX7842579, SRX3430959, SRX3430958
D. pseudoobscura bogotana	5	SRX7260972, SRX7260973, SRX091468, SRX7260971, SRX7260970
D. persimilis	13	SRX104991 & SRX104992, SRX063440, SRX091471, SRX3430960, SRX3430961, plus 8 strains under SRA project PRJNA672098
D. miranda	11	SRX950183, SRX950187, SRX950188, SRX950189, SRX950190, SRX950211, SRX965452, SRX965455, SRX965460, SRX965461, SRX965462
D. lowei	1	SRX091467

GCA_000001765.2), obtained from FlyBase [27]. We next used Picard command line tools to mark adapters and duplicates (http://broadinstitute.github.io/picard/). Variants were called and filtered using GATK v4.1.1 [28,29]. We filtered SNPs and indels separately, according to the hard filtering recommendations provide by GATK. Specifically, we excluded SNPs with QualByDepth (QD) < 2.0, FisherStrand Bias (FS) > 60, and StrandOddsRatio (SOR) > 3.0, MQ < 40, MQRankSum < −12.5, ReadPosRankSum < −8. Indels were filtered to exclude variants with QualByDepth (QD) < 2.0, FisherStrand (FS) > 200, and StrandOddsRatio (SOR) > 10.0, ReadPosRankSum < 20.

Database architecture

PseudoBase server-side code is written in Python using the Django Framework. Sequence alignments are stored on the server as a series of indexed files (one indexed file per chromosome/strain), and indexes are created within each file for every reference sequence base position. This allows for fast retrieval of both aligned and unaligned sequences for any specifically requested chromosome region or gene searches. PseudoBase utilizes a mySQL database to store a) strain metadata, b) pointers to indexed sequence alignment file locations on the server for each chromosome/strain, and c) pointers to gene locations within sequences, to assist with optimizing search queries. Importantly, new strains can be readily added to PseudoBase as they become available. This is facilitated via an import mechanism which utilizes standard Variant Call Format (VCF) files as input. Each imported VCF file contains called indels and SNPs for each chromosome/chromosome group for a strain.

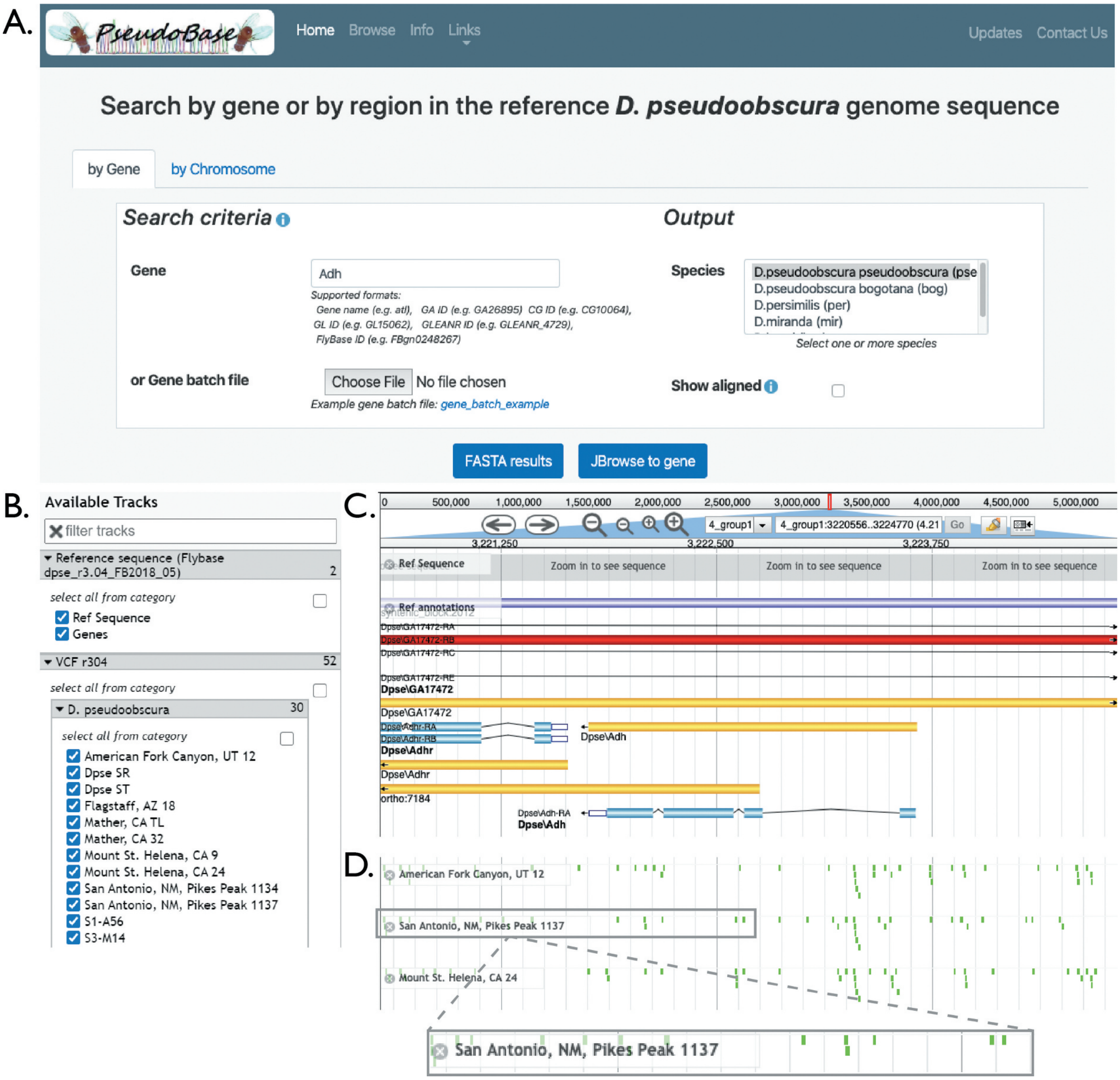
PseudoBase is served as a browser-based web app compatible with all major browsers. An embedded JBrowse instance is fully integrated within the PseudoBase application, allowing browsing to specific genes/regions [30]. All strains imported into PseudoBase are automatically made available for browsing within JBrowse utilizing 'HTMLVariant' JBrowse track types with store class of 'VCFTabix', while any other supplementary tracks/track types useful for analysis can also be uploaded directly to the JBrowse interface using standard JBrowse import mechanisms. The following JBrowse plugins are activated within PseudoBase: HierarchicalCheckBoxPugin, NeatHTMLFeatures and HideTrackLabels.

Results and discussion

Interface and key features

The PseudoBase site, http://pseudobase.biology.duke.edu/, is designed to be simple and intuitive. The landing page is divided into six tabs: 'Home', 'Browse', 'Info', 'Links', 'Updates', and 'Contact Us'. The homepage (Figure 1a) allows the user to select the species of interest and readily pull up genetic information by gene name or by genomic region. PseudoBase is currently configured to accept the following types of gene identifiers: *D. pseudoobscura* IDs prefixed with GA- (e.g., GA26895) and *D. persimilis* IDs prefixed with GL- (e.g., GL15062), *D. melanogaster* IDs prefixed with CG- or FBgn- (e.g., CG10064 or FBgn0035724), and gene name abbreviations when available (e.g., *atl* or *Adh*). *D. melanogaster* orthologs in other sequenced *Drosophila* genomes are reported by FlyBase as determined by OrthoDB, and PseudoBase uses this ortholog report to display the relevant orthologous *D. pseudoobscura* gene when a *D. melanogaster* gene identifier is entered [31, 27, FlyBase file 'dmel_orthologs_in_drosophila_species_fb_2020_04.tsv.gz']. We also use this ortholog report to look up gene identifiers of *D. persimilis*, by first determining the *D. melanogaster* ortholog, then looking up the *D. pseudoobscura* ortholog. We note that these search functions will be an important area for future PseudoBase updates, as more orthology predictions become available and as the maintenance of *D. pseudoobscura* annotations shifts from FlyBase to GenBank, as discussed further below.

Once the species of interest and the gene/region are indicated, the user has the option of either generating an alignment or navigating to the relevant JBrowse view. The 'FASTA results' option generates a FASTA formatted output (aligned or unaligned), which can be downloaded for downstream analyses. Alternatively, the 'JBrowse to gene' option allows the user to navigate to the region of interest within the JBrowse



Search by gene or by region in the reference *D. pseudoobscura* genome sequence

Search criteria

Gene: Adh

Supported formats: Gene name (e.g. atl), GA ID (e.g. GA26895) CG ID (e.g. CG10064), GL ID (e.g. GL15062), GLEANR ID (e.g. GLEANR_4729), FlyBase ID (e.g. FBgn0248267)

or Gene batch file: Choose File No file chosen

Example gene batch file: gene_batch_example

Output

Species: D.pseudoobscura pseudoobscura (pse / D.pseudoobscura bogotana (bog) / D.persimilis (per) / D.miranda (mir)

Select one or more species

Show aligned □

Available Tracks

filter tracks

Reference sequence (Flybase dpse_r3.04_FB2018_05) 2

select all from category □
- ☑ Ref Sequence
- ☑ Genes

VCF r304 52

select all from category □

D. pseudoobscura 30

select all from category □
- ☑ American Fork Canyon, UT 12
- ☑ Dpse SR
- ☑ Dpse ST
- ☑ Flagstaff, AZ 18
- ☑ Mather, CA TL
- ☑ Mather, CA 32
- ☑ Mount St. Helena, CA 9
- ☑ Mount St. Helena, CA 24
- ☑ San Antonio, NM, Pikes Peak 1134
- ☑ San Antonio, NM, Pikes Peak 1137
- ☑ S1-A56
- ☑ S3-M14

Figure 1. | Overview of the PseudoBase interface. (a) The PseudoBase homepage allows the user to query by gene (or genes if the user uploads a batch query) or by chromosomal region. In this example, the gene Adh (alcohol dehydrogenase) is entered. By selecting one or more species of interest, the user can either access a FASTA-formatted alignment or navigate to the JBrowse interface (snapshots in B-D) to explore the genomic region. (b) Track selection in the JBrowse interface enables the user to toggle tracks on or off to add or remove strains from the view. (c) An overview of the genomic region includes annotations from FlyBase. Clicking on any of these features brings up detailed information, including coordinates, the feature length, any aliases, the full nucleotide sequence, and the nucleotide sequence of each subfeature (e.g., introns). (d) JBrowse allows the user to visualize SNP and indels specific to each selected track. The zoomed view of a portion of the 'San Antonio, NM, Pikes Peak 1137' strain shows SNPs highlighted in green. Clicking on any of these SNPs brings up further details, such as the specific allele and its attributes (e.g., sequencing depth).

interface (Figure 1b-d). This interface can also be accessed from the 'Browse' tab. The user can select and deselect genomes of interest (Figure 1b), enabling comparative genomics between genomes of *D. pseudoobscura pseudoobscura*, *D. pseudoobscura bogotana*, *D. persimilis*, *D. miranda*, and *D. lowei*.

Navigating to a particular region in the JBrowse interface allows the user to view any FlyBase annotations contained within the region (Figure 1c [27];. This interface also allows the user to visualize the genomic context of SNPs and indels in the genomes of interest (Figure 1d).

The other tabs on the homepage ('Info', 'Links', 'Updates', and 'Contact Us') provide documentation and help. The 'Info' tab provides users with general information about PseudoBase including an overview of PseudoBase, tool documentation, variant calling details, and a list of available strains and their accessions. The 'Links' tab consists of a dropdown menu with related external resources. 'Updates' serves as a location for Release Notes, and will summarize future changes to the site. Contact information for comments or assistance is located in the 'Contact Us' tab as well as the footer on each page.

Data content and data types

PseudoBase 2.0 includes a total of 61 sequenced genomes from *D. pseudoobscura*, *D. persimilis*, *D. miranda*, and *D. lowei* (Table 1). All genome alignment and variant calling was performed through a standardized workflow and uses a common genomic coordinate system based on the *D. pseudoobscura* genome assembly (initial version published in [32]). This structure contributes to the simplicity of the site and enables comparative genomics across species and strains (see Methods). Further, to our knowledge, this reference genome has received the most independent (not including reference-based assemblies to other species) assembly effort out of the *pseudoobscura* subgroup. The database was constructed to accommodate future additions of additional sequencing data and variant calls. The gene model annotations available through PseudoBase are pulled from FlyBase [27]. Importantly, the genome assembly and annotations obtained from FlyBase are static within PseudoBase, rather than being dynamically updated when FlyBase is updated. Recent releases of FlyBase are moving the focus of FlyBase away from non-melanogaster species (see Release Notes at flybase.org). As a result, FlyBase no longer maintains the *D. pseudoobscura* assembly and annotations. We plan to import future updates from GenBank, where the current assembly is maintained (GCA_000001765.2). However, we note that the static nature of assembly and annotations within PseudoBase gives us the flexibility to import future assemblies and annotations from other sources if they become available.

Applications

One of the key features of PseudoBase that sets it apart from other interactive tools for interfacing with *Drosophila* genomic data is its simplicity. We provide a simple user interface and a relatively focused dataset representing only genomic variation within the *pseudoobscura* subgroup. By lowering the barriers to working with genomic data, we provide a widely accessible tool particularly useful for pilot analyses, data checks, and educational purposes. Anyone can take advantage of this database without the burden of obtaining and downloading raw data, assembling genomes, or calling variants. For example, the 'FASTA results' option available on the homepage can generate a FASTA formatted alignment specifically for any gene of interest without requiring the user to handle full genomes. Such alignments can be readily downloaded for downstream analyses. The JBrowse feature further allows selection of specific strains as well as visualization of all polymorphisms or just SNPs or indels, thereby simplifying the process for rapid marker development.

While this database is useful for *Drosophila* researchers, it also offers educational opportunities. Simple bioinformatic exercises can be designed where students or trainees extract variation for genes of interest. Indeed, PseudoBase has already been leveraged extensively for many years by undergraduates in our research team as well as for an introductory-level college course laboratory exercise [33]. In sum, the accessibility of PseudoBase makes it useful to both the community of *Drosophila* researchers and to those who lack extensive computational or genomic analysis expertise but wish to do simple population genetic analyses or develop genetic markers.

Acknowledgments

The authors wish to thank past and present members of the Noor Lab whose contributions have facilitated the creation and improved the functionality of PseudoBase. Helpful feedback from two anonymous peer reviewers improved both the manuscript and the functionality of the site. The original version of PseudoBase was funded by NIH award GM086445 to MAFN. The current update was funded through NSF grants DEB-1754022 and DEB-1754439 to MAFN. KLK was supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. DGE 1644868.

Disclosure statement

The authors have no conflicts of interest to declare.

Funding

This work was supported by the National Institute of General Medical Sciences [GM086445]; National Science Foundation [DEB-1754439]; National Science Foundation [DGE 1644868]; National Science Foundation [DEB-1754022].

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