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SOFTWARE TOOL ARTICLE

# FlyOde - a platform for community curation and interactive visualization of dynamic gene regulatory networks in *Drosophila* eye development [version 1; referees: 3 approved]

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## Abstract

**Motivation:** Understanding the regulatory mechanisms governing eye development of the model organism *Drosophila melanogaster (D. m.)* requires structured knowledge of the involved genes and proteins, their interactions, and dynamic expression patterns. Especially the latter information is however to a large extent scattered throughout the literature.

**Results:** FlyOde is an online platform for the systematic assembly of data on *D. m.* eye development. It consists of data on eye development obtained from the literature, and a web interface for users to interactively display these data as a gene regulatory network. Our manual curation process provides high standard structured data, following a specifically designed ontology. Visualization of gene interactions provides an overview of network topology, and filtering according to user-defined expression patterns makes it a versatile tool for daily tasks, as demonstrated by usage examples. Users are encouraged to submit additional data via a simple online form.

## Open Peer Review

**Referee Status:** ☑☑☑

| | Invited Referees | | |
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| | **1** | **2** | **3** |
| version 1 published 21 Dec 2015 | ☑ report | ☑ report | ☑ report |

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**Competing interests:** No competing interests were disclosed.

## Introduction

Developmental biology is the study of processes that generate an entire organism from a single cell. A central question in this field is how differentiation produces specific cell types from pluripotent precursors. *Drosophila melanogaster (D. m.)* serves as a suitable and well-established model organism to address this question for numerous reasons including a short generation time, the multitude of available genetic methods, and its orthology shared with vertebrates (Reiter *et al.*, 2001). The *D. m.* eye allows the study of morphological rearrangements as well as differentiation of non-neuronal and neuronal cell types like photoreceptors (PRs) on the single cell level (Thomas & Wassarman, 1999).

Understanding cell differentiation requires knowledge of the involved genes, their temporally varying (dynamic) expression patterns, and interactions. Interaction data from different sources are accessible through e.g. Biogrid, Intact, String, and REDfly (Chatr-Aryamontri *et al.*, 2015; Gallo *et al.*, 2011; Orchard *et al.*, 2014; Szklarczyk *et al.*, 2015) and database collections, e.g. iRefindex or mentha (Calderone *et al.*, 2013) (Razick *et al.*, 2008). Expression data is mostly provided on embryonic development or organ systems, e.g. by FlyBase (dos Santos *et al.*, 2015) and the Berkeley Drosophila Genome Project (BDGP) (Hammonds *et al.*, 2013), but the coverage and precision of expression pattern annotation on the cellular level and the temporal resolution at later stages, e.g. larva and pupa, are limited (see Supplementary Material). On the other hand, a wealth of expression pattern data on these levels is contained in publications (e.g. (Potier *et al.*, 2014)). Systematic use of these data requires their structured assembly through an extensive curation effort. Since automated curation is prone to errors (Mao *et al.*, 2014), information must be extracted manually from the literature by experts, who can critically interpret the respective types of data like micrographs or expression profiles (Tomancak *et al.*, 2007) and convert these to machine-readable data for further computer-based analyses.

We have thus developed FlyOde, an online hub for the community-driven systematic assembly of data on *D. m.* eye development. FlyOde is a web interface for interactive exploration of gene and protein relationships by combining visualization of the curated gene regulatory network with filters specific to fly development. FlyOde is built on an ontology-driven curation process that stores data in a specifically formatted text file, which can easily be enriched and extended upon arrival of new data.

## Implementation

### Data

A directed gene interaction network representing eye development of *D. m.* from the third instar larva to the adult with focus on PR differentiation was constructed using Cytoscape (Shannon *et al.*, 2003), based on data extracted manually from 77 publications (Figure 1). Currently, the network contains 146 nodes representing genes/proteins, and 284 edges representing activating or inhibiting genetic, protein-protein, or protein-DNA interactions. The layout
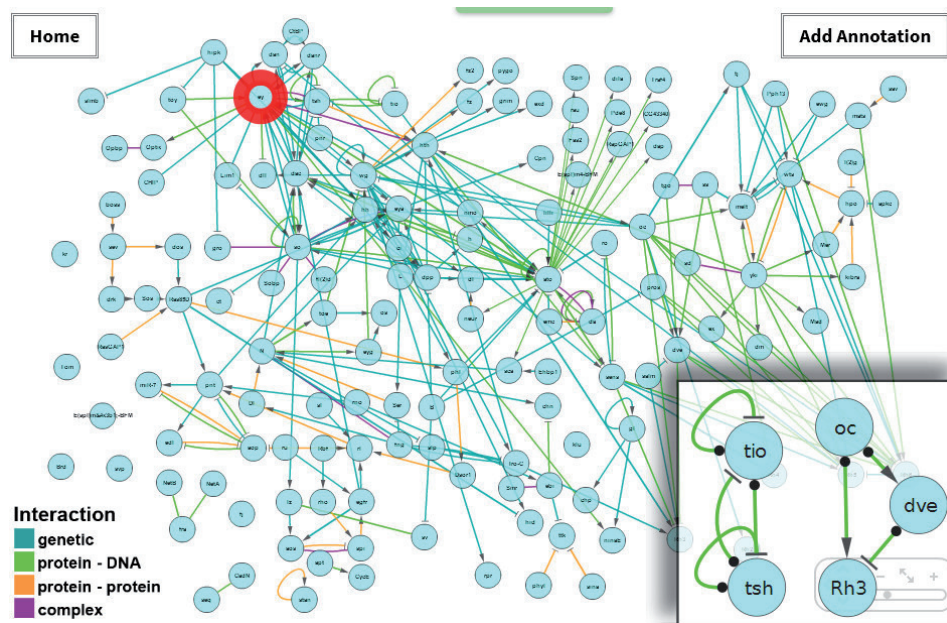


**Figure 1. The FlyOde interface displaying the eye developmental network.** The red circle indicates the *eyeless* gene (see Example 1). The inset shows selected interactions containing network motifs: negative autoregulation and feedback loop (left) and an incoherent feed forward loop (right).

is generated manually and organized to approximately represent developmental time along the horizontal axis, beginning with early third instar larval stage on the left, and network hierarchy along the vertical axis with master regulators (as defined by (Chan & Kyba, 2013)) placed towards the top. Nodes are associated with their FlyBase symbol, name, alternative names, FlyBase link, dynamic expression pattern, phenotypes, the terms for each of the three Gene Ontologies (GO) (Ashburner *et al.*, 2000), and the literature references. GO annotations were added using Cytoscape. Expression pattern annotation follows a specifically developed ontology which links developmental stage and cell type (Figure 2). We support and encourage annotation from the community to continually extend the dataset.

### Web application
The interactive JavaScript based web application renders the manually curated network, which is embedded using Cytoscape Web

(Lopes *et al.*, 2010). Genes are represented as nodes and gene (or protein) interactions are visualized as directed edges. Nodes and edges have been annotated during the curation process (see previous section), and this information is displayed when an item is selected. For the selection of nodes a module for searching gene symbols, names, alternative names, and GO-terms is provided. Additionally, the network can be filtered according to gene expression patterns using dropdown menus whose structure follows the FlyOde ontology (Figure 2). Filters can be combined with the Boolean operators AND, OR, and NOT. Extensions to the network can be submitted to us via a community curation form.

### Characterization
To characterize the content of the current FlyOde network GO enrichment analysis was performed with the ClueGO app in Cytoscape (Bindea *et al.*, 2009). It shows that terms correlated with development and morphogenesis, pattern formation and polarity,
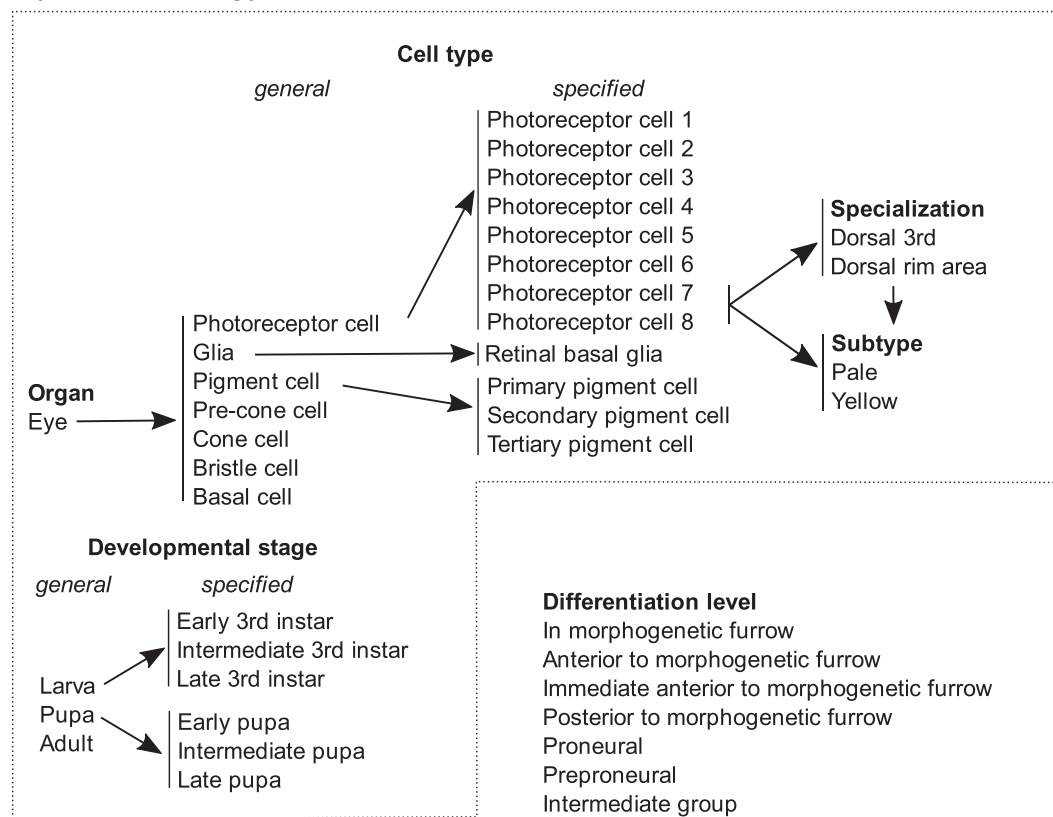


**Figure 2. The FlyOde eye-ontology.** Hierarchical representation of controlled vocabulary for the gene annotation with dynamic expression patterns is shown. In the annotation syntax terms for cell type and developmental stage are directly linked. Each term-combination represents expression in a specific cell type and developmental stage. As many comma-separated term combinations as needed to describe the expression pattern are given. During retinal development of *Drosophila,* cells at consecutive stages of differentiation coexist at the same developmental stage of the organism. The morphogenetic furrow marks the initiation of differentiation and moves across the eye precursor organ, the eye imaginal disc. Thus, the expression pattern is also defined by the localization relative to the morphogenetic furrow. This and additional terms that are common in the field are assigned to what we call "differentiation level".

apoptosis, mitosis, and regulation of transcription are highly over-represented, as expected (see Supplementary Materials and Supplementary Figure 1 and Supplementary Figure 2).

To evaluate the information that can be obtained from FlyOde the FlyOde filter function was compared with the FlyBase QueryBuilder. When queried for general terms like "larva and PR", FlyBase gave more gene hits than FlyOde, but when increasingly specifying the developmental stage and cell type, like "pupa and dorsal rim area R8 (photoreceptor cell 8)", more genes were found with FlyOde as compared to FlyBase. This shows that FlyOde already stands out in defining gene expression in a specific cell type and during a specific developmental stage of PR differentiation (see Supplementary Materials and Supplementary Figure 3).

To get an idea of the basic mechanisms represented in FlyOde, the occurrence of network motifs was analysed manually in Cytoscape. Motifs commonly found during development of organisms, like autoregulation, feedback and feed forward loops were observed. This indicates that FlyOde at least partially displays the connectivity and level of detail to qualify for representation and detailed analysis of developmental processes (see Figure 1 and Supplementary Materials, Supplementary Figure 4 and Supplementary Table) (Alon, 2007; Davidson, 2010).

### Use cases
#### Example 1: Obtaining information on a specific gene
In this example we use the search functionality to explore a specific gene in the dataset. In order to do so, *Pax6* is entered into the search box. This gene is known under that name as an important regulator of development in many organisms (van Heyningen, 2002). However, the official name in *D. m.* is *eyeless*. Due to its annotation with alternative names it is found despite the search for the unofficial name *Pax6* and highlighted in the network (Figure 1). Its position on the left side of the graph indicates that it is mainly expressed early during eye development. In the web interface additional information is displayed in a content related text field below the graph, which we designate "report panel". A closer look at the expression pattern displayed there shows that it is expressed anterior to the morphogenetic furrow, in all photoreceptors in the early third instar larva, and in outer photoreceptors in the late pupa and adult. The top position in the hierarchy indicates that it is a master regulator. This is supported by its high number of interactions, and by the phenotypes, which are given in the report panel (Chan & Kyba, 2013). We also find that GO annotates *eyeless* as a transcription factor, and that it is involved in developmental processes of other organs. Finally, the literature references and the FlyBase link can be followed for further information.

#### Example 2: Which genes can be used as markers for PR R8 in the intermediate pupa?
Here we apply filter combinations to display genes that are expressed in R8, but not in any other PR in the intermediate pupa to obtain candidate markers for R8 in immunostaining and cell sorting experiments. In the dropdown menus we choose "pupa", "intermediate pupa", "photoreceptor cell", and "R8", respectively. We add

another filter line with the Boolean "NOT", and in the dropdown menus select "intermediate pupa", "photoreceptor cell", "R7", and another filter line with the Boolean "NOT", "intermediate pupa", and "at least one outer PR", which in combination with "NOT" means "no outer PR". All nodes are removed except for *scabrous* and *senseless*, which are therefore candidates for being R8 markers. The literature references for these two markers provide a starting point for future experimental studies.

More examples are provided in the tutorial at http://flyode.boun.edu.tr/quickguide.html.

### Discussion
Here we have presented FlyOde, which provides a platform for combining published data on gene regulatory networks (GRNs) of *Drosophila* organ development. As a starting point, we have equipped FlyOde with extensive GRN data for *D. m.* eye development, such that the web interface serves as a versatile tool for everyday tasks a fly researcher encounters.

FlyOde delivers high quality data standards by manual curation. We expect to achieve efficient data collection by distributing the annotation workload among community members with minimal effort for the individual contributors, who only need to submit a simple form to add new nodes, interactions, or annotation. In return, they directly profit from the improved tool by linking their data of interest with the shared knowledge.

We are constantly improving the ontology and web application, in parallel to ongoing data curation and dataset extension. FlyOde will be expanded to include other organs with the ultimate goal to compare their GRNs.

Future work will profit from the obtained data by constraining network inference from gene expression data (Hecker *et al.*, 2009). Another anticipated approach is to assign quantitative expression data to the established network to facilitate mathematical modelling (Graham *et al.*, 2010).

We envision FlyOde as a companion that guides researchers through developmental processes, for example while studying a paper, and believe that the community-curated dataset and its analysis will add significant knowledge to developmental biology.

### Data availability
FlyOde, including all network data, annotations and their corresponding references can be freely accessed via the web-application at http://flyode.boun.edu.tr/.

### Software availability
#### Software access
The source code for the web application can be downloaded from Github (https://github.com/begum-alaybeyoglu/FlyOde).

#### Source code as at the time of publication
https://github.com/F1000Research/FlyOde

## Author contributions

S.A.K. established the concept, network, annotation and ontology, performed analysis and wrote the manuscript. B.A. generated the web application. C.X.W. performed analysis and provided input during manuscript preparation. A.C. coordinated the project. All authors contributed to proofreading the manuscript and have agreed to the final content of the manuscript.

## Supplementary Material

**Supplementary Figure 1.**

GO-term enrichment analysis with ClueGO.

Click here to access the data.

**Analysis and characterization of the FlyOde network.**

Includes Supplementary Figures 2, 3, 4.

Click here to access the data.

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# Open Peer Review

## Current Referee Status: ☑ ☑ ☑

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**Version 1**

Referee Report 15 February 2016

**doi:**10.5256/f1000research.8136.r11677

☑ **Fernando Casares**

CABD (Andalusian Centre for Developmental Biology), CSIC and Pablo de Olavide University, Seville, Spain

The Drosophila eye gene regulatory network ranks among the best textbook examples of organogenetic GRNs. FlyODE, by Koestler and colleagues is a welcomed attempt at integrating the many bits of expression, regulation, molecular interactions and functional analyses obtained to date, by providing a cytoscape-like tool in the form of a curated, directed graph network. FlyODE's also allows the expansion of this network through community-based contributions –which is a great philosophy. Therefore FlyODE is a valuable tool for those working on gene networks in general and on fly eyes in particular, and will help in expanding the network (and our knowledge of the system) further. FlyODE includes references to spatial gene expression patterns. It would be good if the authors tried to unify the somehow ambiguous terms used by different labs, such as "preproneural/precursors/anterior to the MF" or "proneural/immediately anterior to the MF", or perhaps provide some "equivalence" terms. When sorting genes according to their expression along third instar, I found that the network includes more genes at early L3 than at mid-L3, which shouldn't be the case –as the process unfolds more genes are called into action.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

*Competing Interests:* No competing interests were disclosed.

> Author Response 15 Feb 2016
>
> **Stefan A. Koestler**, Bogazici University, Turkey
>
> Thank you very much for your feedback!
> We are working on improving the ontology, e.g. by adjusting it to the Gene Ontology, and will incorporate your suggestions.
> The layout is currently done manually and is certainly to some extent biased depending on the references used for annotation. This can be expected to improve with an increasing number of annotations/references. In addition, we are planning an automatic layout to calculate the position from the annotations. In any case one has to keep in mind that it will only indicate a single (mean) value, while a gene might display a more complex expression pattern. Thus, currently one has to look at the report panel below the network for more detailed information. We are thinking about adding a graph displaying the expression profile when hovering over a node to make this information more intuitively accessible.

***Competing Interests:*** No competing interests were disclosed.

Referee Report 12 February 2016

**doi:**10.5256/f1000research.8136.r12188

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This is a very nice and useful tool to quickly and efficiently access information about fly retina development. The fact that it can be extended to any tissue and is an open platform for community-based annotation makes it all the more useful. I applaud the authors for their efforts and thank them for making such a cool and intuitive tool available. The video tutorial is very nice and helpful as well.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

***Competing Interests:*** No competing interests were disclosed.

Referee Report 11 February 2016

**doi:**10.5256/f1000research.8136.r12186

**Pelin Cayirlioglu Volkan**

Department of Biology, Duke University, Durham, NC, USA

FlyOde is a very nice resource for labs, which are interested in visualizing developmental dynamics of gene regulatory networks required for the development for many neuronal types in the Drosophila eye, in addition to finding lineage specific markers. It will be valuable in the future to expand this to other sensory systems such as the olfactory system.  The title and the abstract are appropriate. The video presentation is very useful.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

***Competing Interests:*** No competing interests were disclosed.

# Discuss this Article

**Version 1**

Author Response 19 Feb 2016

**Stefan A. Koestler**, Bogazici University, Turkey

We thank all referees for their feedback!

*Competing Interests:* No competing interests were disclosed.