The effect of anthocyanins intake on the evolution of redox genes: a comparative genomics approach using Drosophila

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Introduction

Conserved genes generally evolve slowly. However, when there are strong changes in processes and functions that regulate them in a species, how does these genes evolve? We investigated on this aspect studying evolution of conserved genes in Drosophila.
Drosophila suzukii is a disruptive crop pest coming from Asia and expanding in Europe and North America. It lays eggs in ripening small fruits, especially berries, and larvae grow up eating them. This behaviour is different from most of the other Drosophila species.[1]
Berries are rich in anthocyanins: blue/red pigments belonging to flavonoids class of particular interest due to their anti-cancer and anti-aging effects. These benefits are due to anthocyanins ability to remove reactive oxygen species (ROS) from the cell. It was seen that the effect of anthocyanins reduces cell natural activities to remove ROS. Genes involved in this activity are called redoxines. Also important is that ROS regulates the activity of other genes, which are the red ones in **Figure 3**. These genes and redoxines are called redox genes.

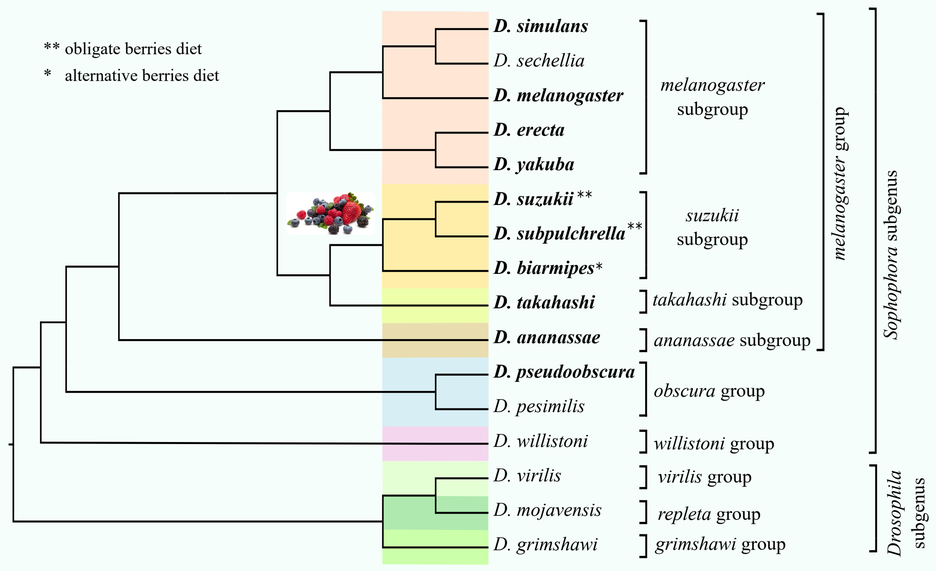

Figure 1. Species tree for Drosophila genus. In bold species considered in this project.

Redox genes are well conserved and *D. melanogaster* is the model organism to study them.[2]
Since *D. suzukii* in larvae stage has anthocyanins-rich diet, which most of Drosophila don't have: are these conserved genes evolved in a way to reduce their sensibility to ROS? We considered 10 species of Drosophila (**Figure 1**) to answer this question.

Figure 2. Drosophila suzukii on berry. Picture from https://plantgest.imagelinenetwork.com/it/news/2018/02/16/drosophila-suzukii-mantenere-alta-la-guardia/57351

Materials and Methods

We performed four analysis to get information on 32 selected conserved genes evolution in Drosophila.

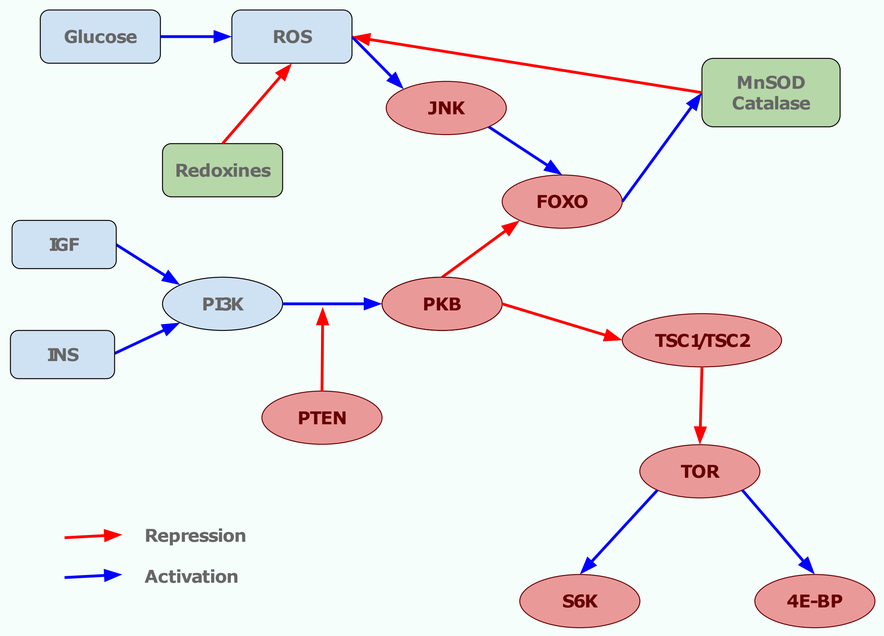

Figure 3. Pathway influenced by ROS. Red genes and redoxines were considered in this project.

1. Gene phylogenetic trees analysis: for each gene a phylogeny tree was built, in order to achieve whether the evolution of that gene remarks the species evolution (**Figure 1** shows the Drosophila phylogeny tree). Maximum Likelihood method was used to build trees, with 100 bootstrap replications and GTR-GAMMA substitution model. MEGA was used to build trees.
2. Evolution analysis: PAML was used to build 4 models and to test whether genes underwent adaptive evolution and they have sites under positive selection.
3. Introns analysis: for each gene in each species quantity and length of introns were found. It was seen if in *D. suzukii* there were statistically significant differences using a t-test on introns length.
4. Gene expression analysis on *D. suzukii* against *D. melanogaster,* to know if putative genes found were differently expressed. Even if available RNA-seq datasets were from adults' ovipositors, we tried to see if adults have differences in these 32 conserved genes. However, best datasets for this study would have come from larvae caught in nature. For gene expression analysis, datasets were already trimmed with Trimmomatic. We used STAR as RNA-seq splice-aware reads aligner. Then, HTSeq-count to build the counts matrix. Finally, R package DESeq2 to performa exploratory analysis and find differentially expressed genes.

Results and Discussion

Phylogeny analysis:
Some genes have a peculiar phylogeny which does not recapitulate the species phyogeny. This may indicate a peculiar evolutionary hystory. One of the genes have a peculiar topology in *suzukii* subgroup (**Figure 4**).

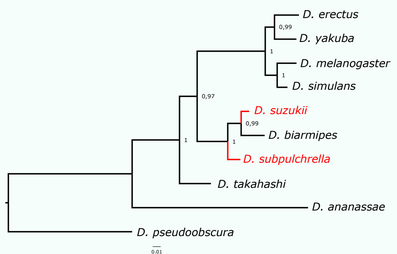

Figure 4. *tsc2* phylogeny tree.

Evolution analysis:
Some genes in some species underwent adaptive evolution or it has sites under positive selection (**Figure 5**). None of the genes have dN/dS > 1.

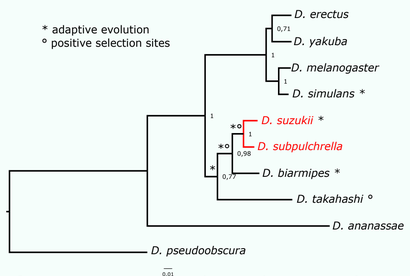

Figure 5. *tsc1* molecular evolution result.

Introns analysis:
Some genes have introns with different length in *D. suzukii* and/or other species in *suzukii* and *takahashi* subgroups (**Table 2**). Some genes have a different number of introns.

	01 intron	02 intron	03 intron
Dtak_g6pd	2441	59	1186
Dyak_g6pd	2554	62	86
Dere_g6pd	2576	62	85
Dbia_g6pd	2706	76	348
Dsuz_g6pd	2840	76	449
Dmel_g6pd	2863	62	85
Dsim_g6pd	2720	62	85
Dsub_g6pd	2563	79	424

Table 2. Introns analysis result for *g6pd*. Highlighted records are positive to t-test.

Three genes are all positive as can be seen in **Table 1**: *foxo*, *trxr2* and *tsc2*. However, since first two analyses were on cds sequences and the last on genes and transcripts sequences, also genes positive in one of the first two analyses and the last could be interesting: *g6pd*, *gclc*, *inr* and *trx2*. Following this approach, these 7 genes are candidate to have been evolved due to *D. suzukii* diet. The gene expression analysis didn't confirm any of these genes as differentially expressed in *D. suzukii*, problably because RNA-seq data came from adults, which have same diet as the other Drosophila species.

Gene name	4e-bp	bmm	dhd	fas	foxo	g6pd	gclc	gclm	grx	inr	jafrac1	jafrac2	jnk	myc	pkb	prx3	prx5	prx6	prx6-like	pten	puc	s6k	srx	tnf	tor	trx2	trxr1	trxr2	trxt	tsc1	tsc2	txl
Phylogeny analysis	yes	no	yes	yes	yes	no	yes	no	yes	no	yes	no	no	no	no	no	no	no	yes	no	no	no	yes	no	no	yes	no	yes	no	no	yes	no
Evolution analysis	no	no	no	yes	yes	yes	no	yes	yes	yes	no	no	no	no	no	no	no	no	no	yes	yes	yes	no	no	no	no	yes	yes	yes	yes	yes	no
Introns analysis	no	no	no	no	yes	yes	yes	no	no	yes	no	no	no	no	yes	no	yes	no	no	no	no	no	no	no	no	yes	no	yes	no	no	yes	no
Gene expression	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

Table 1. Resume of results obtained. Yes if there are particular events characterizing *D. suzukii*, no if not. Green for genes without positive results in analyses, yellow if gene is positive to one analysis, orange if gene is positive to two analyses and blue if gene is positive to three analyses.

Conclusion and Future Insight

Using these analyses we recognized genes which could be evolved due to the diverse diet characterizing *D. suzukii* in larvae stage. However, larvae gene expression analysis of *D. suzukii* against *D. melanogaster* and other Drosophila species could be helpful to confirm putative genes to have also a different expression. From these results we can say evolution of the new behaviour in *D. suzukii* did not influenced adults gene expression of related genes, even if they occured changements in cds and introns. Furthermore, with this approach it can be reduced the number of genes on which researchers have to work in wet lab whether putative genes evolved due to a behaviour are detected.

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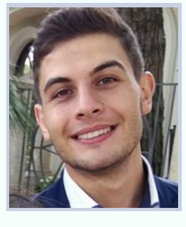

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