Zootoca vivipara as a model for testing evolutionary transition from oviparity to viviparity

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Introduction

The lizard Zootoca vivipara is one on the few example in Nature which shows, within the same species, populations with different reproductive modalities. Oviparous populations live in the southern part of its distributional range (the newly discovered Z. carnoliaca in Eastern-Iranian Al and Z. lousiti in the Pyrenees), while viviparous subspecies (e.g. Z. v. vivipara and Z. s. sachalinensis) are widely distributed from British Isles and central France to Scandinavia and north-eastern Asia [1][Fig.4]. This species is, therefore, particularly well suited for studying the evolutionary shift in reproductive mode.

Results and Discussion

Bioinformatic analysis performed using the pipeline software Stacks produced about 100.000 local alignments and about 260.000 Single Nucleotide Polymorphisms (SNPs). In order to minimize the total number of missing data, we selected 19.013 SNPs. These markers were used to describe the overall genetic variation between subspecies. The result of MDS (Fig.2) seems to confirm [according to [1]] the existence of two parapatric oviparous clades (Z. v. carniola and Z. lousiti) and one viviparous clade (represented by Z. v. vivipara and Z. v. sachalinensis).

Additionally, a Minor Allele Frequency Spectrum has been calculated dividing the whole dataset according to the phenotype (Viviparous/Oviparous). This analysis allows to underline polymorphisms that show low frequency in one phenotype and high frequency in the other, and vice versa. About 2.000 SNPs were selected according to MAFS. Furthermore, these polymorphisms were analyzed with TASSEL software [3] using GMM method to test for significant association between genotypes and phenotypes. After multiple-comparison correction, 289 SNPs showed a significant association with phenotype.

Genomic sequences (200-500 bp long, achieved with Illumina Paired-end protocol) physically linked to these markers were BLASTed against the whole NCBI Nucleotide Collection, with particular interest in looking for sequence similarities in Anolis Carolinesis genome (the only reptile genome available at the moment). Results are summarized in Figure 5 and Table 1.

Materials and Methods

A Next Generation Sequencing technique has been used to analyse 40 samples of Zootoca vivipara to cover the overall genetic variation of the species. RADtag sequencing (Fig.1) uses Illumina HiSeq technology to simultaneously discover and analyse thousands of SNPs at genome level [5]. Bioinformatic analyses have been conducted using the pipeline software Stacks v0.9995 [6].

Table 1. Some examples of genes with high values of sequence similarity with sequences selected in Zootoca vivipara according to TASSEL and MAFS

<table>
<thead>
<tr>
<th>Genes (Predicted protein)</th>
<th>Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toll-like receptor (immune system)</td>
<td>Anolis Carolinesis</td>
</tr>
<tr>
<td>Mitoferrin-1 like transporter</td>
<td>Anolis Carolinesis</td>
</tr>
<tr>
<td>Protein phosphatases</td>
<td>Anolis Carolinesis</td>
</tr>
<tr>
<td>Fibrinogen beta chain (coagulation)</td>
<td>Anolis Carolinesis</td>
</tr>
<tr>
<td>Aspartate beta-hydroxylase domain</td>
<td>Galáus gallus</td>
</tr>
<tr>
<td>Estrone-related receptor gamma</td>
<td>Homo sapiens</td>
</tr>
</tbody>
</table>

Figure 2 MultiDimensional Scaling based on genetic distances between individuals according to 19k SNPs

Figure 4 Zootoca vivipara European distribution and subspecific pattern (modified from [2])

Figure 5 Pie-chart that summarizes sequence categories obtained by BLASTing about 300 genomic sequences (250 of them were unknown)

Conclusions

Looking for genes and markers showing signals of selection is becoming relatively straightforward with the advent of NGS; RADtag, together with Paired-end sequencing, is a useful method to assemble millions of genomic reads into contigs which can be compared to known sequences in existing databases. However, not all genes can be identified, since non-model species may contain unknown genes or the closest reference genome may not be available.

In Zootoca vivipara, the matching proportion of contigs physically linked to SNPs which show signal of selection is around 14%. This value decreases to 6% when the entire dataset of contigs is analyzed.

However, about 20 conserved genes have been identified as possibly related to the two different reproductive modalities. In order to investigate this topic, previous studies have mainly focused on differential gene expression between mammals and viviparous squamate reptiles [7]. Evolution of viviparity poses a major immunological hurdle for mother and foetus. For instance, cytokines (interleukin-1a and -1b) are responsible of maternal-fetal tolerance) seem to play a similar role in mammals and viviparous squamates. Toll-like receptor, identified in this study, together with the interleukin-1 receptor forms a receptor superfamily, an important molecule in immune system. Moreover, genes like HBB and HSA15 may be involved in the placentation development of both mammals and reptiles. Hormone receptors have, for sure, an essential role in evolution of viviparity, in fact they may regulate follicular development and ovum maturation [7]. One example of this category has been identified in this study (Tab.1).

So far, only gene-by-gene or protein-by-protein approaches have been taken. This study is the first attempt to analyze the oviparous/viviparous transition at genomic level, with the consciousness that this is a very complex physiological process, probably mediated by thousands of genes.

Acknowledgments

We would like to thank Autonomous Province of Trento for funding the ACE-SAP project.

References