Insect Biochemistry and Molecular Biology 65 (2015) 28–34

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

Differential expression of the chemosensory transcriptome in two populations of the stemborer *Sesamia nonagrioides*

Nicolas Glaser [a, b], Aurore Gallot [a, c], Fabrice Legeai [c], Myriam Harry [b, d], Laure Kaiser [b], Bruno Le Ru [b, e], Paul-André Calatayud [b, e], Emmanuelle Jacquin-Joly [a, *]

[a] INRA, UMR 1392, Institut d'Ecologie et des Sciences de l'Environnement de Paris, Route de Saint-Cyr, F-78026 Versailles Cedex, France
[b] UMR Evolution, Génomes, Comportement et Ecologie, IRD, CNRS, Université Paris Sud, Campus CNRS, 91198 Gif-sur-Yvette Cedex, France
[c] IRISA, équipe GenScale, Campus universitaire de Beaulieu, 35042 Rennes Cedex, France
[d] Université Paris-Sud 11, 91405 Orsay Cedex, France
[e] UMR Evolution, Génomes, Comportement et Ecologie IRD, CNRS, Université Paris Sud, c/o icipe, NSBB Project, PO Box 30772-00100, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 7 February 2015
Received in revised form
30 June 2015
Accepted 8 July 2015
Available online 24 August 2015

Keywords:
Chemosensory genes
Adaptation
RNAseq
Sesamia nonagrioides

ABSTRACT

Among the proposed mechanisms of local adaptation to different ecological environments, transcriptional changes may play an important role. In this study, we investigated whether such variability occurred within the chemosensory organs of a herbivorous insect, for which chemosensation guides most of its host preferences. A European and an African population of the noctuid *Sesamia nonagrioides* that display significant differences in their ecological preferences were collected on *Zea mays* and *Typha domingensis*, respectively. RNAseq were used between the two populations for digital expression profiling of chemosensory organs from larval antennae and palps. Preliminary data on adult female antennae and ovipositors were also collected. We found 6,550 differentially expressed transcripts in larval antennae and palps. Gene ontology enrichment analyses suggested that transcriptional activity was overrepresented in the French population and that virus and defense activities were overrepresented in the Kenyan population. In addition, we found differential expression of a variety of cytochrome P450s, which may be linked to the different host-plant diets. Looking at olfactory genes, we observed differential expression of numerous candidate odorant-binding proteins, chemosensory proteins, and one olfactory receptor, suggesting that differences in olfactory sensitivity participate in insect adaptation.

1. Introduction

Local adaptation to different environments based on morphological, physiological and behavioral divergences is well documented (Schluter, 2001), but the underlying genetic mechanisms are poorly understood (Martin and Orgogozo, 2013). Herbivorous insects are good models for investigating these mechanisms since they present an important diversification via co-evolution with their host-plants. Mechanisms inducing variation in animal physiology and behavior include allelic variation, gene gain and loss, and transcriptional variation. Based on pioneer studies by (Britten and Davidson, 1969) and (King and Wilson, 1975), and more recently Carroll and colleague's work (Carroll, 2005; Prud'homme et al., 2007, Shirangi et al., 2009), it has been proposed that evolutionary changes are more often based on changes in the mechanisms controlling the gene expression than on sequence changes in proteins. This view is supported by recent studies suggesting that transcriptomic regulation participates in insect adaptation to host, with genes coding for ribosomal, digestive, metabolic and detoxifying proteins being the main gene families experiencing regulated expression (Alon et al., 2011; Bass et al., 2013; Celorio-Mancera et al., 2011, 2012, 2013; Dermauw et al., 2013; Smith et al., 2013). While most of these studies concentrated on the implication of defense and metabolic processes, only few studies investigated the modulation of olfactory gene expression (Li et al., 2013a). However, olfaction directly interfaces with the environment and thus plays a crucial role in ecological adaptation (Date et al., 2013); it allows the

Abbreviations: CSP, Chemosensory Protein; FG, Fold Change; IR, Ionotropic Receptor; GO, Gene Ontology; GST, Glutathion-S-Transferase; OR, Olfactory Receptor; OBP, Odorant-Binding Portein; ODE, Odorant-Degrading Enzyme; ORN, Olfactory Receptor Neuron; PBP, Pheromone-Binding Protein.

* Corresponding author.
E-mail address: emmanuelle.jacquin@versailles.inra.fr (E. Jacquin-Joly).

http://dx.doi.org/10.1016/j.ibmb.2015.07.008
0965-1748/

exploitation of a given resource and guides chemosensory preferences for food by larvae and adult stages, for mates by adults and for oviposition sites by mated females (Bruce and Pickett, 2011; De Bruyne and Baker, 2008; Linz et al., 2013).

At the molecular level, important olfactory protein families consist of: 1) secreted odorant-binding proteins (OBPs) and chemosensory proteins (CSPs), that are thought to bind and transport volatile ligands to the membrane of the olfactory receptor neurons (ORNs) (Leal, 2013; Pelosi et al., 2006), 2) membrane bound olfactory receptors (ORs) and ionotropic receptors (IRs), whose activation upon ligand binding leads to the generation of an electrical signal that is transmitted to the brain (Benton et al., 2009; Silbering et al., 2011; Touhara and Vosshall, 2009), 3) odorant-degrading enzymes (ODEs), that ensure a fast deactivation of the receptors and are thus important in the dynamic and the sensitivity of the olfactory response (Leal, 2013). ODEs from different families have been described, among which esterases (Ishida and Leal, 2005), cytochrome P450 (Maïbeche-Coisné et al., 2004) and glutathione-S-transferase (GST) (Rogers et al., 1999).

Changes in olfactory sensitivity can be driven by subtle mutations in key genes from these families (Leary et al., 2012; McBride et al., 2014), gene gains and losses (Gardiner et al., 2008; Guo and Kim, 2007; Vieira and Rozas, 2011), and/or variation in gene expression (Biessmann et al., 2005; Fox et al., 2001; McBride et al., 2014; Rinker et al., 2013). In this study, we investigated if local adaptation could be associated with transcriptional changes in the chemosensory organs. We took advantage of the occurrence of different populations of *Sesamia nonagrioides* (Lefebvre) (Noctuidae, Sesamiina) that exhibit different ecological preferences to perform a RNAseq approach on the chemosensory organs. *S. nonagrioides* is one of the major pests of cultivated maize, *Zea mays* L. (Monocot, Poaceae), in Mediterranean Europe (Anglade, 1972; Melamed-Madjar and Tam, 1980; Rousseau, 2009) whereas in East Africa, the species is rarely found on crops and it is not considered as a pest (Moyal et al., 2011; Nye, 1960; Ong'amo et al., 2013). The Palearctic population has been shown to originate from sub-Saharan Africa (Moyal et al., 2011) and previous works based on nuclear and mitochondrial sequences (Moyal et al., 2011) have shown that European and African populations are not genetically separated. The occurrence of different populations gives the opportunity to elucidate the mechanisms of host-plant adaptation, and specifically of crop adaptation, as a first step to understand the shift from natural ecosystems to anthroposystems. This study compared one population collected on maize (*Z. mays*) in France and one population collected on the southern cattail, *Typha domingensis* Pers. (Monocot, Typhaceae), in Kenya.

We have recently sequenced the chemosensory transcriptome of *S. nonagrioides* and have annotated numerous genes from all OBP, CSP, OR, IR and ODE families (Glaser et al., 2013). On this transcriptome, we mapped here Illumina libraries obtained from chemosensory organs from the two *S. nonagrioides* populations described above. The following tissues were sequenced: antennae and maxillary palps from larvae (driving feeding behavior), female adult antennae (driving female host preference) and female adult ovipositors. These latter are known to express some chemosensory genes (Widmayer et al., 2009) and thus possibly drive oviposition site preference. In the present study, we used these RNAseq data to perform a comparative analysis of the chemosensory transcriptome between the two populations, revealing uniquely expressed genes in each population and up- or down-regulated genes between the two populations, as possible molecular signatures associated with adaptation.

2. Material and methods

2.1. Sampling of natural populations

S. nonagrioides larvae (n ≈ 350) were collected on *T. domingensis* in Makindu (Eastern Kenya, 170 km from Nairobi, 1°29′S, 37°16′E, 990 m above sea level, henceforth referred to as the Kenyan population) several times a year in 2011–2012, and on maize in Rieumes (Southwest of France, 40 km from Toulouse, 43°22′N, 1°11′E, 300 m above sea level, henceforth referred to as the French population) during the winters of 2011 and 2012 (n ≈ 300).

2.2. Insect rearing

To eliminate local effects such as temperature, humidity, and possible stresses during collection and shipping that could be sources of inter-individual variability causing inter-populational differences, the two populations collected outdoor were reared in the laboratory for two generations before tissue collection. Insects were reared on an artificial diet according to Poitout & Bues (Poitout and Buès, 1974), containing agar, maize flour, wheat germ, dried yeast and a mixture of vitamins and antibiotics. The insects were kept in a controlled chamber at 24.4 ± 0.7 °C, 54.4 ± 5.8% relative humidity (means ± SD), and an L16:D8 reversed photoperiod.

2.3. Tissue collection and RNA preparation

For both populations, antennae and maxillary palps were dissected from 4th instar larvae in the middle of the scotophase, synchrony being necessary to avoid any circadian rhythm effects. Three biological replicates consisted of two larval tissue collections performed in 2011 (from ~350 larvae per replicate per population) and one in 2012 (from ~400 larvae per population). For both populations, adult antennae and ovipositors were dissected from approximately one hundred naïve, virgin, 1 to 2-day-old females, in the third hour of the scotophase. Due to problems encountered during insect pupation in 2011, only one biological replicate could be performed, consisting of adult tissue collected in 2012. The dissected organs were immediately frozen in liquid nitrogen and stored at −80 °C until extraction. Total RNAs were extracted from each tissue using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) (Glaser et al., 2013). Ten libraries from larval antennae and palps (three replicates for each population), female ovipositors (one replicate for each population) and female antennae (one replicate for each population) were independently used as templates for sequencing as described (Glaser et al., 2013) (one channel for the four adult libraries, one channel for the six larvae libraries, single read 51 bp length, HiSeq2000 Illumina; GATC Biotech). All RNAseq data have been deposited at the European Bioinformatics Institute (EMBL-EBI ENA: ERP004720) and have been used to create the reference transcriptome (Glaser et al., 2013).

2.4. Illumina reads alignment and statistical analysis

Illumina data were processed using FastQC v. 0.10.0 (www.bioinformatics.babraham.ac.uk/projects/fastqc), Cutadapt (Martin, 2011) and PRINSEQ v 0.17.3. (Schmieder and Edwards, 2011). Sequences shorter than 20 bp long were removed from all data sets. The 10 processed Illumina data sets were aligned on the *S. nonagrioides* transcriptome with Bowtie (Langmead, 2010). For each library, the number of reads aligned on each contig was counted with SAMtools (Li et al., 2009). Multiple aligned reads were excluded from further analyses. The final numbers of aligned reads for each sample are given in Table 1. The final counts table was