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

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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.

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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.

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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

Table 1
Summary of RNAseq data.

Populations	France			Kenya			France	Kenya	France	Kenya
Tissues	Larvae n°1	Larvae n°2	Larvae n°3	Larvae n°1	Larvae n°2	Larvae n°3	Female antennae	Female antennae	Female ovipositors	Female ovipositors
Numbers of raw Illumina reads	42,747,969	43,358,859	20,611,819	27,503,668	34,908,485	21,567,094	45,097,179	36,430,026	58,581,441	58,579,062
Numbers of processed reads	42,745,643	43,357,076	20,605,140	27,499,605	34,904,719	21,561,270	45,094,117	34,087,925	55,686,597	55,688,903
Numbers of aligned reads	15,339,732	15,441,512	6,541,958	11,271,232	15,010,980	9,474,270	16,619,473	14,694,802	23,099,463	24,529,902
Numbers of contigs used in the analyses	42,962						43,298		42,215	
Numbers of regulated contigs (FDR < 0.01)	6,550						281		324	
Numbers of up-regulated contigs	4,786			1,764			125	156	156	168

imported in R version 2.11.0 (R Development Core Team, 2011). Non-expressed and low-expressed contigs were removed by filtering contigs represented by a number of reads inferior to the number of libraries analyzed. Overall, 42,962 contigs (larval libraries), 43,298 contigs (female antennae libraries) and 42,215 contigs (ovipositor libraries) were kept for further analysis. Count normalization was performed by estimating the normalization factor by the median of scaled counts. The data quality assessment was performed before differential expression testing in order to control if any sample suffered from an abnormality that renders the data detrimental to our analysis. For this purpose, Principal Composant Analysis (PCA) was performed. The objective of PCA analysis is to find the two directions (components) that maximize the variance in our dataset. The PCA on gene expression levels was performed using the packages ‘ggplot’ and ‘DESeq2’ in R, after filtering of low expressed genes and the normalization step. Differentially expressed contigs between the two populations and in the different organs were identified using the DESeq package v. 1.8.3 (Anders and Huber, 2010), implemented in R. DESeq provided *p*-values were next adjusted to *p*-values False Discovery Rates (FDR) for multiple testing with the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995). A cutoff of 0.01 was applied to consider the effects as significant. Annotation of the transcripts identified was performed as already described (Glaser et al., 2013). Briefly, BLASTX version 2.2.23 was used against the NCBI non redundant database with a $1e^{-8}$ value threshold, BLAST2GO was used for the Gene Ontology (GO) annotation (Conesa et al., 2005), contigs were translated to peptides using FrameDP 1.2.0 (Gouzy et al., 2009) and GO annotation was then completed with Interproscan annotation of translated peptides (Quevillon et al., 2005). 11,047 (26%) transcripts from the reference transcriptome could be associated with at least one GO term. Fisher's Exact test with multiple testing correction of FDR (Benjamini and Hochberg, 1995), available in BLAST2GO, was used to test GO term enrichment compared to the reference transcriptome. For larval samples, only transcripts specifically expressed in a given population were considered for the Fisher's exact test. All regulated transcripts were considered for the adult samples.

3. Results

3.1. Transcriptome differences between the two populations

To obtain an initial overview of gene expression pattern across the two populations, we performed a Principal Component Analysis (PCA) which clearly separates the data according to geographical origin of the population, which explained 91% of the variance (Fig. 1). The three biological replicates obtained over three independent samplings collected from 2011 to 2012 are highly grouped together, demonstrating an excellent reproducibility.

Although only one replicate could be obtained for the female antennae and the ovipositor samples, euclidian distances between data sets were calculated and revealed a clear clusterization between French and Kenyan samples (Supplementary information S1), indicating again a strong differentiation of transcriptome according to the population origin.

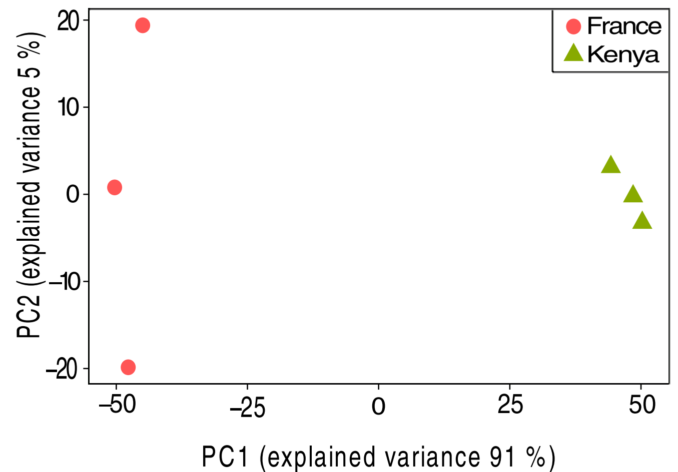

Fig. 1. Factorial map of the Principal Component Analysis (PCA) of messenger RNA expression level. Normalized RNAseq reads from three biological replicates of larvae antennae and palp across two populations (France and Kenya) were mapped onto consensus transcriptome. The proportion of the variance explained by the principal components is indicated in parentheses.

3.2. Transcriptome profiling of the caterpillar chemosensory organs

Out of 42,962 transcripts used in the analysis, we identified 6,550 (15.2%) differentially expressed transcripts between the larval chemosensory organs of the French and the Kenyan populations of *S. nonagrioides* (Table 1, Supplementary information S2). In the French population, 4,786 transcripts (11.1%) were up-regulated, among which 796 were exclusively expressed in this population and 3,990 were overexpressed with fold change (FC) values from 2.9 to 3,648. In the Kenyan population, 1,764 transcripts (4.1%) were up-regulated, among which 485 were exclusively expressed in this population, and 1,279 were up-regulated (FC from 3.6 to 826) (Supplementary information S2). Among these 6,550 transcripts, 4,386 (67%) were putative non-coding transcripts (no hit in the protein database) or coding for orphan proteins (hit with hypothetical proteins or proteins of unknown function). Enrichment analyses revealed that the transcripts exclusively expressed in the French population presented an overrepresentation in GO terms related to transcription (polymerase activity, nucleic acid binding), whereas such activity is underrepresented in the Kenyan population (Fig. 2). A variety of transcripts identified as virus

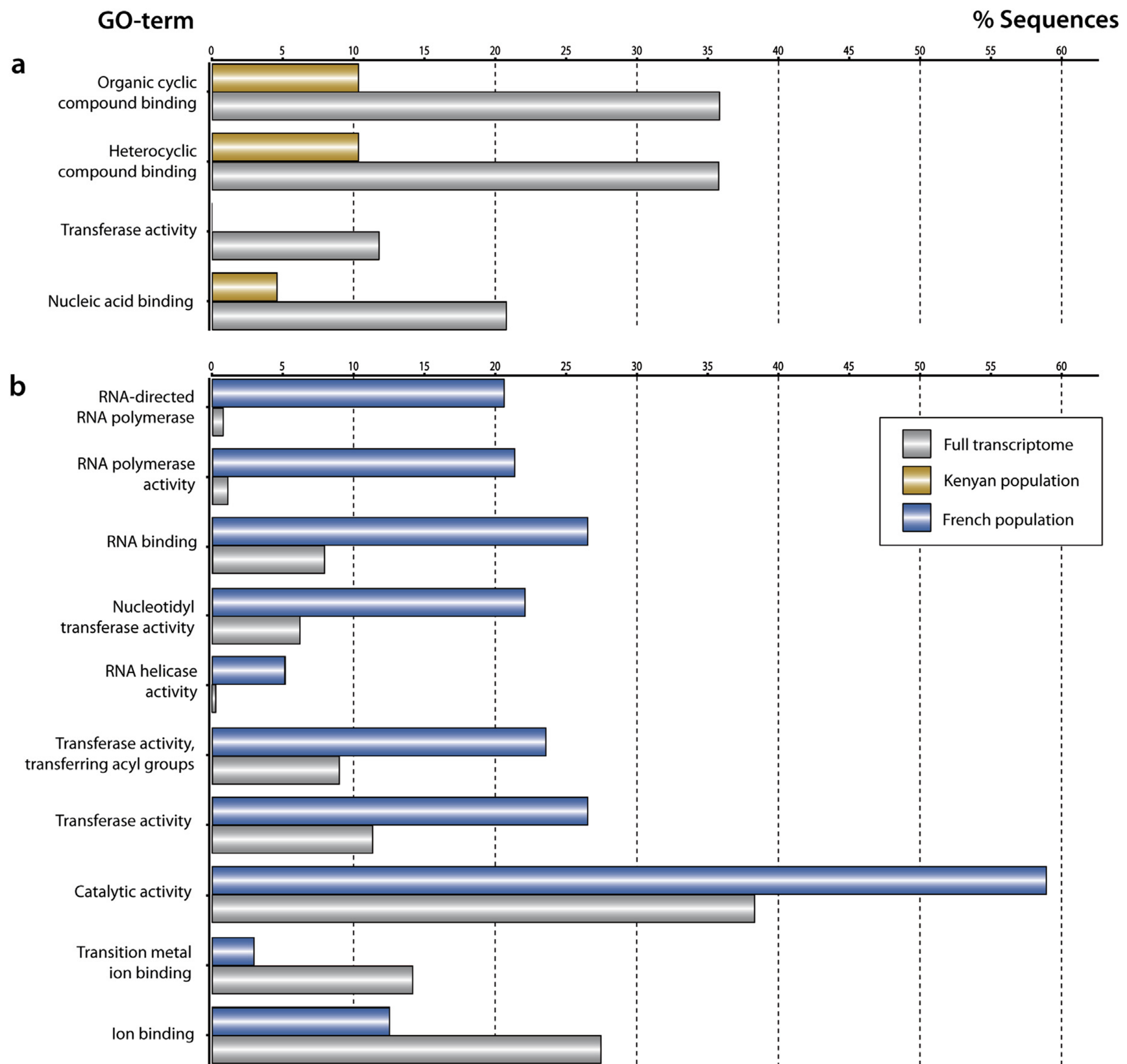

Fig. 2. Differential GO term distribution in the larvae chemosensory organs from two populations of *Sesamia nonagrioides* (BLAST2GO, Fisher's exact test with an FDR correction). a. Under represented GO terms in the transcripts specifically expressed in the Kenyan larval antennae and palps. Test set: transcripts specifically expressed in the Kenyan population. Reference set: *S. nonagrioides* full transcriptome. b. Over or under represented GO terms in the transcripts that are specifically expressed in the French larval antennae and palps. Test set: transcripts specifically expressed in the French population. Reference set: *S. nonagrioides* full transcriptome.

polypeptide encoding genes were found to be exclusively expressed or highly up-regulated (more than 1000x) in the chemosensory organs of larvae of the French population (Supplementary information S2). Among the transcripts exclusively expressed or enriched in the larval chemosensory organs of the French population, we also evidenced 36 transcripts encoding ribosomal proteins, 16 transcripts related to P450s, 10 GSTs, one esterase and three CSPs, one being specific for the French population (Supplementary information S2). Among the transcripts identified exclusively in the larval chemosensory organs of the Kenyan population, a gene encoding a putative diazepam-binding inhibitor was identified. A highly up-regulated sequence in the Kenyan population (626.9x) was identified as an antimicrobial peptide encoding gene (Supplementary information S2). Among other transcripts, we found 10 P450s, 10 GSTs and 4 esterases enriched in the Kenyan population. Focusing more precisely on chemoreception, SnonGOBP2, OBP3, CSP9, CSP11, CSP16 and esterase CXE5 were up-regulated in the larval chemosensory organs of the French population, whereas CSP2, CSP4 and CSP18 were up-regulated in the Kenyan population (Table 2).

3.3. *Preliminary data on transcriptome profiling of the female antennae and ovipositors*

The DESeq analyses were performed on only one replicate from each population for both female antennae and ovipositor samples. Thus, the following results are preliminary but deserve mentioning. We identified 281 (0.6%) and 324 (0.7%) differentially expressed transcripts between female antennae and ovipositors, respectively, from French and Kenyan populations (Table 1, Supplementary information S3, Supplementary information S4), some of them being unique to one population. The Fisher test revealed enrichment in GO terms related to transcription and olfaction (odorant binding) functional categories in the transcripts up-regulated in the antennae from French population, whereas defense and microbicidal activity GO terms were enriched in the transcripts up-regulated in the antennae from the Kenyan population (Supplementary information S5). In ovipositors, enrichment

Table 2
Sesamia nonagrioides chemosensory transcripts differentially expressed in the antennae and palps from larvae from two populations (France, Kenya). Regulated transcripts encoding enzymes supposed to be involved in pheromone biosynthesis are also indicated. "Over" indicates overexpression in the corresponding population. Snon: *Sesamia nonagrioides*; OR: olfactory receptor; GOBP: general-odorant binding protein; OBP: odorant-binding protein; CSP: chemosensory protein; CXE: esterase.

Annotated chemosensory genes (Glaser et al., 2013)	Transcript ID http://www6.inra.fr/lepidodb/sesamia_nonagrioides	Larval antennae and palps	
		France	Kenya
Olfactory receptor:			
SnonOR14	Snon_454_Illumina_c12213	Over	
Odorant-binding proteins:			
SnonGOBP2	Snon_454_Illumina_rep_c335	Over	
SnonOBP3	Snon_454_Illumina_rep_c317	Over	
Chemosensory proteins:			
SnonCSP2	Snon_454_Illumina_rep_c10749		Over
SnonCSP4	Snon_454_Illumina_rep_c621		Over
SnonCSP9	Snon_454_Illumina_rep_c197	Over	
SnonCSP10	Snon_454_Illumina_rep_c2904		
SnonCSP11	Snon_454_Illumina_rep_c2018	Over	
SnonCSP16	Snon_454_Illumina_rep_c6958	Over	
SnonCSP18	Snon_454_Illumina_rep_c5066		over
Esterase:			
SnonCXE5	Snon_454_Illumina_rep_c2009	Over	
Pheromone biosynthesis:			
Snon_N-acetyltransferase 3	Snon_454_Illumina_rep_c28613	Over	
Snon_fatty-acyl CoA reductase 3	Snon_454_Illumina_rep_c6076	Over	
Snon_fatty-acyl CoA reductase b	Snon_454_Illumina_c15604		Over

analyses revealed that the transcripts up-regulated in the Kenyan population presented an overrepresentation in GO terms related to virus infection (Supplementary information S6). Focusing more precisely on olfactory genes, SnonPBP1 (pheromone-binding protein: OBP possibly involved in the binding of pheromone components), GOBP2 and OR14 appeared in our preliminary data as up-regulated in the female antennae of the French population, whereas SnonPBP2 and CSP2 were up-regulated in the Kenyan population (Supplementary information S7).

4. Discussion

In this study, we observed transcriptional differences in a variety of processes within the chemosensory organs in two populations of *S. nonagrioides* collected in different locations and on different host-plants. Since the two populations are not genetically separated - at least at the protein level - these changes in gene expression, possibly driven by changes in regulatory elements, could be the major force of their local adaptation, as supported by other studies (Carroll, 2005). However, since a high number of regulated transcripts were expressed uniquely in one or the other population, with no genomic data available on this species, we cannot determine whether the corresponding genes are indeed not expressed, present as pseudogenes in the genome of one population, or alternatively recently duplicated in the other population.

65% of the differentially expressed genes in the larval tissues were much more expressed in the French than in the Kenyan population, suggesting that the larvae from France had a higher transcriptional activity than the ones from Kenya. This was confirmed by the enrichment test that revealed over and under-representation of GO terms related to transcriptional activity in the French and the Kenyan populations, respectively. Although preliminary, the data we obtained on adult chemosensory tissues support enrichment of transcriptional activity in French population. Consistent with this hypothesis, we observed overexpression of many ribosomal protein transcripts in the larval tissues of the French population, such expression changes being proposed to reflect large-scale changes in transcription (Celorio-Mancera et al., 2012, 2013). Also, overexpression of ribosomal genes might help counteract the presence of ribosome-inactivating proteins (RIPs) in the food source (Celorio-Mancera et al., 2013). These RIPs inhibit protein synthesis in eukaryotes (Puri et al., 2012) and have been found to have insecticidal activity, in particular in noctuids (Zhou et al., 2000). In maize, two RIP protein isoforms, RIP1 and RIP2, have been identified, RIP1 being expressed throughout the plant (Bass et al., 2004) and RIP2 being induced by caterpillar feeding (Chuang et al., 2014). Thus, caterpillars from the French population that used to feed on maize before capture may constitutively overexpress ribosomal transcripts in response to RIP-induced stresses.

We also evidenced differential expression of a variety of virus proteins and cytochrome P450s in the larval sensing organs, some being enriched in one population, some others being enriched in the other population. Interestingly, the preliminary data we obtained on adult chemosensory tissues support differential expression of defense and microbe infection related transcripts. Although we cannot exclude that this differential expression arose from different reactions of the two populations to the laboratory conditions, this may reflect different virus contamination profiles according to the geographic area of origin, and different responses to xenobiotic expositions. P450s are involved in the metabolization of xenobiotic substances such as drugs (e.g. chemical pesticides) and other toxic chemicals produced by plants (Berenbaum et al., 1996; Dermauw et al., 2013). The differential regulation of P450 expression observed between the two *S. nonagrioides* populations may reflect adaptation to cultivated plants (enriched in insecticides/pesticides resulting in overexpression of P450s) and to host-plant species (necessity to express different P450s to detoxify different plant secondary compounds). Accordingly, a recent study conducted on the aphid *Myzus persicae* revealed that mutations leading to overexpression of a P450 were a prerequisite for host shift (Bass et al., 2013).

Finally, differential expression of candidate chemosensory genes was observed. In particular, genes possibly involved in perireceptor events - namely the OBPs, CSPs - and one OR were regulated, as possible signatures of adaptation to different environments. This is in accordance with previous studies that revealed physiological and molecular differences as early as the olfactory peripheral organs between insect species or strains adapted to different hosts (Date et al., 2013; Li et al., 2013a; Linz et al., 2013; McBride et al., 2014;

Rinker et al., 2013). For instance, OBPs and antennal esterases are differentially expressed between two noctuid sibling species, *Helicoverpa assulta* and *Helicoverpa armigera*, that may affect host adaptation (Li et al., 2013a). In *Aedes aegypti*, evolution of preference for humans was linked to at least one OR (McBride et al., 2014). It has to be noticed that no functional data are yet available for any of the differentially expressed candidate chemosensory genes we identified in *S. nonagrioides* and their olfactory role is only speculative. Especially, the role of CSPs in chemoreception is still unclear (Pelosi et al., 2006). These secreted proteins are usually expressed in a diversity of tissues (Gong et al., 2007; Liu et al., 2010), and although some exhibit binding activity towards odorants and pheromones (Briand et al., 2002; Jacquin-Joly et al., 2001), they may participate in other physiological processes beyond chemoreception (Gonga et al., 2012; Kitabayashi et al., 1998). Interestingly, the three CSPs overexpressed in the French caterpillar chemosensory organs have all been previously quantified as greatly enriched in larvae compared to adult antennae, CSP11 being even larval specific (Glaser et al., 2013). Together with their differential expression between populations, this supports a specific role in caterpillar behavior. As for the other candidate chemosensory genes enriched in the French population, clear evidences have been accumulated to classify proteins from the Lepidoptera GOBP2 sub-family as olfactory proteins, binding pheromonal compounds and plant odors (Liu et al., 2015; Maibeche-Coisne et al., 1998; Zhou et al., 2009). SnonOBP3, overexpressed in the French caterpillar chemosensory organs, belongs to the "minus-C" OBP sub-family (Glaser et al., 2013), whose members have been previously shown to bind plant volatiles (Li et al., 2013b). SnonOBP3 demonstrated high expression in the female ovipositor-pheromone gland complex (Glaser et al., 2013), as its closest homologue, OBP5 from *Agrotis ipsilon* (73% amino acid identity) (Gu et al., 2013), suggesting a possible role in pheromone transport. SnonCXE5 has been previously reported to be highly expressed in the female ovipositor-pheromone gland complex of *S. nonagrioides* females and proposed to participate in pheromone biosynthesis by the generation of the alcohol component, Z11-16:OH, from its acetate precursor, Z11-16:OAc (Glaser et al., 2013). The presence of CXE5 in chemosensory organs may also suggest a function of the protein as an ODE, participating in the termination of the acetate pheromone component reception by its degradation in the corresponding alcohol. The single OR (SnonOR14) found to be differentially expressed is a candidate sex pheromone receptor (Glaser et al., 2013).

The expression in larvae chemosensory organs of genes whose products may be involved in pheromone transport, reception and degradation, is reminiscent of what has been previously evidenced in another noctuid, *Spodoptera littoralis*. In this latter species, larvae are attracted to the female sex pheromone, a signal that they may use to find food source (Poivet et al., 2012). This behavior has not been yet demonstrated in *S. nonagrioides* larvae, but enrichment of genes potentially involved in pheromone reception in the French population suggest a better detection and temporal resolution of the pheromone signal than in the Kenyan population. The ecological interpretation would deserve deeper studies but may be linked to the difficulties the French larvae encounter in identifying the corn host, a recently introduced crop in Europe to whom this population may not be yet completely adapted.

The genes highlighted as regulated in this study deserve special attention in future functional and genetic investigations in order to get a definitive proof of their involvement in selection and exploitation of new environments. Also, we cannot exclude the possibility that the transcriptional variation we observed in this study also reflect different responses of the two populations to an unfamiliar environment, namely the laboratory. Indeed we chose here to rear the two populations in the same conditions in the laboratory before tissue collection to avoid high expected variability between individuals when collected in the wild. As counterpart, the relative contribution of inherited transcriptome variation (ecological adaptation) and experimentally induced variation (difference between populations in their transcriptional response to lab conditions) still remain to be disentangled.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibmb.2015.07.008.

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