

A stylized, high-contrast illustration of an insect head, likely a fly or similar dipteran, rendered in black, white, red, and teal. The illustration shows the compound eye, antennae, and various sensory structures. A teal horizontal band is overlaid on the upper portion of the head, containing the title text.

FUNCTIONAL CHARACTERIZATION OF INSECT CHEMORECEPTORS: RECEPTIVITY RANGE, EXPRESSION AND EVOLUTION

EDITED BY: William B. Walker, Emmanuelle Jacquin-Joly and Sharon R. Hill
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FUNCTIONAL CHARACTERIZATION OF INSECT CHEMORECEPTORS: RECEPTIVITY RANGE, EXPRESSION AND EVOLUTION

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Functional characterization of insect chemoreceptors, following expression in heterologous cell systems, exemplifies the level of sophistication that insect chemoreception research has attained. “Abstract” (2011) by Nathaniel P. Wilkerson symbolizes the core event of heterologous gene expression in the fly: once the foreign genetic material has been injected into an embryo (see paper by Gonzalez et al. 2016), a developmental program, steered by an elegant ectopic gene transcription system, drives the expression of single olfactory receptor genes in target olfactory neurons in sensilla on the fly antenna. “Abstract” illustrates the tremendous power and the potential residing in a developing fly embryo, and again reminds us of the wonder of life.

Olfaction and taste are of critical importance to insects and other animals, since vital behaviours, including mate, food and host seeking, as well as predator and toxin avoidance, are guided by chemosensory cues. Mate and habitat choice are to a large extent determined by chemical signals, and chemoreceptors contribute accordingly to pre-mating isolation barriers and speciation. In addition to fundamental physiological, ecological and evolutionary consideration, the knowledge of insect taste and especially olfaction is also of great importance to human economies, since it facilitates a more informed approach to the management of insect pests of agricultural crops and forests, and insect vectors of disease.

Chemoreceptors, which bind to external chemical signals and then transform and send the sensory information to the brain, are at the core of the peripheral olfactory and gustatory system and have thus been the focus of recent research in chemical ecology. Specifically, emphasis has been placed on functional characterization of olfactory receptor genes, which are derived from three large gene families, namely the odorant receptors, gustatory receptors and ionotropic receptors. Spatial expression patterns of olfactory receptors in diverse chemosensory tissues provide information on divergent functions, with regards to ecologically relevant behaviours. On the other hand, characterization of olfactory receptor activation profiles, or “deorphanization”, provides complimentary data on the molecular range of receptivity to the fundamental unit of the olfactory sense.

The aim of this Research Topic is to give an update on the breadth and depth of research currently in progress related to understanding the molecular mechanisms of insect chemoreception, with specific emphasis on the olfactory receptors.

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Molecular basis of peripheral olfactory plasticity in *Rhodnius prolixus*, a Chagas disease vector

Jose M. Latorre-Estivalis^{1*†}, Bonaventure A. Omondi^{2†‡}, Og DeSouza³, Ivana H. R. Oliveira¹, Rickard Ignell² and Marcelo G. Lorenzo¹

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Olfaction is fundamental for most animals and critical for different aspects of triatomine biology, including host-seeking, reproduction, avoidance of predators, and aggregation in shelters. Ethological and physiological aspects of these olfactory-mediated behaviors are well-understood, but their molecular bases are still largely unknown. Here we investigated changes in the molecular mechanisms at the peripheral olfactory level in response to different physiological and developmental conditions. For this, the antennal expression levels of the odorant (*Orco*) and ionotropic (*IR8a*, *IR25a*, and *IR76b*) coreceptor genes were determined in *Rhodnius prolixus* by means of quantitative real-time PCR (qRT-PCR) analysis. Gene expression changes were analyzed to test the effect of feeding and imaginal molt for both sexes. Moreover, we analyzed whether expression of these genes changed during the early life of adult bugs. Under these conditions bugs display distinct behavioral responses to diverse chemical stimuli. A significantly decreased expression was induced by blood feeding on all coreceptor genes. The expression of all genes was significantly increased following the imaginal molt. These results show that olfactory coreceptor genes have their expression altered as a response to physiological or developmental changes. Our study suggests that olfactory coreceptor genes confer adaptability to the peripheral olfactory function, probably underlying the known plasticity of triatomine olfactory-mediated behavior.

Keywords: olfaction, olfactory coreceptors, triatomines, behavior, physiology

Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is transmitted to humans and other mammals by hematophagous insects of the subfamily Triatominae (Reduviidae). This zoonosis is endemic to 22 countries in Central and South America, where 90–100 million people live in endemic areas, 8 million people are estimated to be infected, and 12,000–14,000 deaths are reported annually (Senior, 2007; Coura and Viñas, 2010; Rassi et al., 2010; Schmunis and Yadon, 2010). *Rhodnius prolixus* Stål, 1859 (Hemiptera: Reduviidae) is the second most important vector of Chagas disease, and the main species transmitting *T. cruzi* to humans in Colombia and Venezuela (Fitzpatrick et al., 2008; Guhl et al., 2009; Rassi et al., 2010; Hashimoto and Schofield, 2012). In the absence of a vaccine and an effective drug treatment, vector control is central to prevent the disease

(Rassi et al., 2010). Due to insecticide resistance in triatomine populations in Bolivia (Lardeux et al., 2010), and parts of Argentina and Venezuela (Vassena et al., 2000; González Audino et al., 2004), there is a dire need for the improvement or development of vector control strategies for sustained control of Chagas disease.

The olfactory system plays an essential role in several aspects of the biology of triatomines, such as shelter location (Lorenzo and Lazzari, 1996), food search (Núñez, 1982; Barrozo and Lazzari, 2004a,b), reproduction (Pontes et al., 2008; Vitta et al., 2009; Zacharias et al., 2010; Pontes and Lorenzo, 2012), and avoidance of predators (Ward, 1981; Manrique et al., 2006). In addition, *R. prolixus* locate hosts mainly through olfactory cues; hence their sense of smell directly regulates disease transmission (Guerenstein and Lazzari, 2009). An increased understanding of the olfactory system is therefore crucial for sustainable control of this disease vector. In other insects, considerable progress has been made in the understanding of the molecular basis of olfaction, which has fostered the development of novel olfactory-based strategies against agricultural pests and disease vectors (Kain et al., 2013; Tauxe et al., 2013). Two molecular components have been shown to be central for the detection of odorant stimuli in insects: the odorant receptors (ORs) (Clyne et al., 1999; Vosshall et al., 2000) and the ionotropic receptors (IRs) (Benton et al., 2009). Genes encoding for these proteins are expressed in olfactory sensory neurons (OSNs), primarily on the insect antennae (Carey and Carlson, 2011). ORs and IRs function as heteromeric odor-gated ion channels composed of one, or in the case of IRs up to five, variable subunits and one, or in the case of ORs up to three, obligate coreceptors: *Orco* (Vosshall et al., 2000; Larsson et al., 2004; Vosshall and Hansson, 2011), and *IR8a*, *IR25a* and *IR76b*, respectively (Benton et al., 2009; Abuin et al., 2011). These coreceptor proteins are also required for the trafficking of the heteromeric OR and IR complexes to the cilia of the OSNs (Larsson et al., 2004; Benton et al., 2006; Abuin et al., 2011).

Changes in the behavioral responsiveness to host signals and reproductive mates have been reported for bugs of this subfamily and correlated with the ingestion of a blood meal and adult maturation (Bodin et al., 2009b; Vitta and Lorenzo, 2009). Similar changes in vector behavior have been found to be correlated with alterations in gene expression in mosquitoes (Rinker et al., 2013; Omondi et al., 2015a). Regulation of gene transcription tentatively underlies the observed functional changes of the peripheral (Jang, 1995; Siju et al., 2010; Saveer et al., 2012; Omondi et al., 2015a) and central olfactory systems (Anton et al., 2007; Barrozo et al., 2011). The main objective of this report was to analyze ontogenetic and blood-meal induced changes in the transcript levels of OR and IR coreceptor genes in both sexes of *R. prolixus*. Based on behavioral observations we hypothesize that coreceptor gene expression is decreased in recently fed bugs. Moreover, we hypothesize that imaginal molting induces an increase in gene expression. We observed that *RproOrco*, *RproIR8a*, *RproIR25a*, and *RproIR76b* transcript levels are altered, in ways correlated with the significantly decreased behavioral responsiveness known for fed insects, as well as the acquisition of sexual signal detection capabilities in adults.

Materials and Methods

Insects

Experimental insects were obtained from the *R. prolixus* colony held at the Centro de Pesquisas René Rachou (CPqRR), which was established more than 20 years ago from a batch of domiciliary insects captured during field work in Honduras (donated by Dr. Carlos Ponce, Ministerio de Salud Pública, Honduras). Through the years, this colony has been kept as large as possible (ca. 20,000 insects) in order to preserve as much diversity as possible. Experimental insects were reared under controlled conditions at $26 \pm 1^\circ\text{C}$, $65 \pm 10\%$ relative humidity, and at a 12 h:12 h light/dark cycle provided by artificial lights (4 fluorescent lamps, cold white light, 6400 K, 40 W). All experiments were performed with 5th instar larvae or adults, and all tests were developed separately for female and male insects. For experiments with immature insects, a group of 4th instar larvae of similar age was sorted and fed *ad libitum* with citrated rabbit blood (2.5% buffered sodium citrate, provided by Centro de Criação de Animais de Laboratório-CECAL, FIOCRUZ), using an artificial membrane feeder. After molting to the 5th instar, half of these insects were kept unfed, while the remaining bugs were offered blood *ad libitum* at day 16 after ecdysis. To obtain adult bugs for the remaining experiments, 5th instar larvae of similar age were sorted by sex and offered an *ad libitum* blood meal to induce their imaginal molt. As in the case of larvae, the feeding procedure was performed 16 days after the ecdysis of adult bugs. Transcript abundances for *RproOrco* and each IR coreceptor genes were analyzed separately for male and female bugs as follows: (i) unfed 21 day-old 5th instar larvae; (ii) blood fed 21 day-old 5th instar larvae; (iii) unfed 1 day-old adult bugs; (iv) unfed 21 day-old adult bugs; and (v) blood fed 21 day-old adult bugs. All bug antennae were dissected between 10 am and 4 pm, and in the case of fed insects, antennae were cut 5 days after the ingestion of the blood meal. Each of the 5 treatments was replicated 6 times using pools of 60 antennae (i.e., 30 bugs) per sample.

Reference Genes and *R. prolixus* OR and IR Coreceptors

A set of candidate reference genes (Table 1) was selected because they were all previously used for qPCR normalization in triatomines (Majerowicz et al., 2011; Paim et al., 2012) and other insect species (Scharlaken et al., 2008; Lord et al., 2010; Ling and Salvaterra, 2011; Ponton et al., 2011). Table 2 lists all reference factors calculated as the geometric means of the most stable combinations of these genes (Omondi et al., 2015b), used to evaluate changes in gene expression in the antennae of *R. prolixus*. The sequences of reference and target genes (*RproOrco*, *RproIR8a*, *RproIR25a*, and *RproIR76b*) were identified in the *R. prolixus* genome (available on www.vectorbase.org/organisms/rhodnius-prolixus) using a local tBLASTn algorithm (Altschul et al., 1997). Orthologous sequences were obtained from the Swiss Institute of Bioinformatics (Table S1 in Supplementary Material). Primers were designed using Primer3 4.0.0 (<http://primer3.ut.ee/>) (Rozen and Skaletsky, 2000) and compatibilities tested

TABLE 1 | Reference and target genes, biological function, primer sequences, amplicon and intron lengths, squared correlation coefficient, and qRT-PCR efficiency.

Gene	Biological function	Primer sequence (5' to 3')	Amplicon length (bp)	Intron length (bp)	R ²	E (%)
REFERENCE GENES						
<i>Act</i>	Cytoskeletal protein	For—TGTCTCCCACACTGTACCCATCTA/ Rev—TCGGTAAGATCACGACCAGCCAA	87	338	0.992	88.2%
<i>eIF-1a</i>	Protein biosynthesis	For—TTGGAGGCCATGTGCTTTGAT/ Rev—AGGTTTCTTGCTTCATCTGGAGT	94	183	0.999	91.3%
<i>GAPDH</i>	Glycolytic protein	For—GACTGGCATGGCATTGAGATT/ Rev—CCCCATTAAAGTCCGATGACACC	182	1130	0.992	102.5%
<i>GST</i>	Metabolism	For—TACCCATCATTGGCGTGGACA/ Rev—CAAACCAATTGCCTCAGCGAT	177	Intron—Exon junction	0.987	103.2%
<i>G6PDH</i>	Metabolism	For—AGCCTGGAGAAGCGGTTTACGTTA/ Rev—GTGAGCCACAGAATACGTCGAGT	162	923	0.998	96.5%
<i>SDH</i>	Metabolism	For—TTGCCGGAGTAGATGTTACCAG/ Rev—CAGCTGCATAAAGTCCTTCCAC	147	1592	0.999	104.8%
<i>Sp</i>	Metabolism	For—AGGGACCATCTTTGACTGCTCTTC/ Rev—GAATCACCCCTGGCAAGCATCTTTT	157	Intron—Exon junction	0.996	98.8%
<i>Tub</i>	Structural subunit of microtubules	For—TGTGCCCAAGGATGTGAACG/ Rev—CACAGTGGGTGGTTGGTAGTTGAT	118	202	0.991	110.9%
TARGET GENES						
<i>RproOrco</i>	Odorant receptor coreceptor	For—GATCTGCACGTGTTGCTGCAC/ Rev—CCATGGATGCAGAACACAAA	157	Intron—Exon junction	0.996	102.6%
<i>RproIR8a</i>	Ionotropic receptor coreceptor	For—TGCAGTCCACAAGGTAGTCAC/ Rev—GCGTAATGCCTTCATCTTCGTCA	155	295	0.991	115.2%
<i>RproIR25a</i>	Ionotropic receptor coreceptor	For—AAGATGTGGCAGGCAATGAAGG/ Rev—CTGTTGCATACCAAGGAAAGC	118	732	0.994	104.6%
<i>RproIR76b</i>	Ionotropic receptor coreceptor	For—GCGTTTGCGTACCAAAATGGACA/ Rev—GCGTCCGGTAGATCCAAAGTGATT	113	1055	0.974	84.1%

R², squared correlation coefficient (calculated from the regression line of the standard curve); E, RT- qRT-PCR efficiency (calculated by the standard method).

TABLE 2 | Reference factors selected for each condition.

Condition	Normalizing factor for female antennae	Normalizing factor for male antennae
The effect of imaginal molt	<i>GST-Tub</i>	<i>SDH-GAPDH</i>
The effect of blood ingestion	<i>GST-Tub</i>	<i>SDH-GAPDH</i>
The effect of adult maturation	<i>GADPH-SDH</i>	All genes

with Oligoanalyzer (Integrated DNA Technologies, Inc. IA, USA) softwares. The melting temperature was set at 60°C. The specificity for each primer was tested *in silico* using BLASTn (Altschul et al., 1990) in the *R. prolixus* genome database.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from pools of 60 antennae with 500 μL of TRIzol[®] Reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Then, extracted RNA was resuspended in 30 μL of DEPC-treated water (Life Technologies), and its concentration was determined using a Qubit[®] 2.0 Fluorometer (Life Technologies). RNA integrity was analyzed by visualizing bands on agarose electrophoresis gels. Extraction of RNA was followed by a treatment using RQ1 RNase-Free DNase (Promega, Fitchburg, WI, USA). All treated RNA (11 μL per sample) was immediately used to

synthesize cDNA using SuperScript III Reverse Transcriptase (Life Technologies) and a 1:1 mix of Random Hexamer and 10 μM Oligo(dT)₂₀ primers in a final volume of 20 μL.

Quantitative Real-time PCR

For quantitative real time PCR (qPCR) analysis, 10 μL of SYBR Green PCR Master Mix[®] (Life Technologies) were used in the reaction mixture that also contained 0.8 μL of a 10 μM primer solution and 1 μL of cDNA sample diluted two-fold in a final volume of 20 μL. The reactions were conducted using an ABI PRISM 7500 Sequence Detection System (Life Technologies) under the following conditions: one 10 min cycle at 95°C, followed by 40 cycles of 15 s at 95°C, 20 s at 60°C, and 30 s at 72°C. Following the amplification step, a melting curve analysis and an agarose gel electrophoresis were performed to confirm the specificity of the reaction. In all qPCR experiments, no-template controls (NTC) were included in triplicate for each primer set to verify the absence of exogenous DNA. For each experimental condition, six biological replicates were made, with three technical replicates performed for each of them. The PCR efficiencies (E) and repeatability (R²) for each primer were determined using the slope of a linear regression model (Pfaffl, 2001). Information about primers, PCR amplicons and calibration curves is presented in **Table 1**. Besides, the output of

melt curve analysis for all primers is displayed in Figure S1 of Supplementary Material.

RT-PCR and Sequencing

Pure cDNA was used as a template for PCR reactions of the reference and target gene amplicons which were performed for 35 cycles (94°C for 30 s, 60°C for 30 s, and 72°C for 30 s) with 2 µL of cDNA, 2.2 µL of a 1 mM dNTP solution, 1.2 µL of a 10 µM primer solution and 1 U of Taq polymerase (Promega) in a final volume of 20 µL. The size of the resulting PCR products was visualized by means of electrophoresis in agarose gels. These PCR products were purified using the Wizard Genomic DNA Purification Kit (Promega). The sequencing reactions for the purified products were performed with both primers using an ABI Prism BigDye V 3.1 Terminator Cycle Sequencing kit and an ABI 3730 DNA sequencing system (Life Technologies). The consensus sequences were obtained using the Staden Package 2.0 (Staden et al., 2000) and verified by comparing with the *R. prolixus* genomic database, using the basic local alignment search tool (BLASTn).

Gene Expression and Statistical Analysis

Data treatment for quantification cycle (*C_q*) values obtained from technical replicates followed standard procedures for qPCR (Livak and Schmittgen, 2001; Sengul and Tu, 2008). Briefly, readings from each set of technical replicates were checked for consistency using GenEx software (MultiD Analyses AB, Sweden) and then used to calculate mean *C_q*-values for each biological replicate. To determine the gene expression measures, the *C_q*-values were normalized to those of reference genes, and then to mean *C_q*-values obtained with a control treatment (Livak and Schmittgen, 2001; Sengul and Tu, 2008). By using such procedures, we have prioritized a data processing method that allows comparison to most qPCR analyses available in the literature. All raw *C_q*-values are presented in Table S2 of Supplementary Material.

The relative expression of *RproOrco*, *RproIR8a*, *RproIR25a*, and *RproIR76b* in female and male antennae was calculated in GenEx software (MultiD Analyses AB, Sweden) using the 2^{-ΔΔC_t} method (Livak and Schmittgen, 2001). First, the expression levels of the four genes were normalized to the reference factors selected for each sex. Then, expression levels of each gene were normalized to the expression levels of unfed female and male larvae, respectively.

Fold-change values were subjected to statistical analysis to determine the effect of treatment on transcript abundance. All tests were performed separately for data obtained from female and male antennae and no comparisons were performed between sexes due to our experimental design. In order to inspect whether gene expression (y-var) was affected by developmental instar (larvae × adult) or feeding status (unfed × fed) (x-vars), data were subjected to Generalized Linear Modeling (GLM) under normal errors. Posterior residual analyses confirmed the choice of the error distribution and the suitability of the model. Modeling proceeded by building a full model, including all of the above parameters and their first order interactions and comparing this with a null model built without any of the above

factors. In finding significant differences between null and full models, model simplification was performed on the latter by backward term extraction, removing one term at a time. Terms returned to the model if their removal provoked a change of deviance with *P* < 0.05. The minimum adequate model was defined as the one holding only significant terms. The procedure above was applied independently for male and female bugs for each of the genes under study (*RproOrco*, *RproIR8a*, *RproIR25a*, and *RproIR76b*), each new test using a distinct subset of data. All tests were performed using R version 3.2.0 (R Core Team, 2015).

Results

Results from statistical analyses are summarized in **Table 3** and **Figure 1**. Regardless of gender, the expression of all studied genes was enhanced in adults compared to larvae. Similarly, feeding depressed the expression of all coreceptor genes in larvae and adults, irrespective of their gender.

In males, developmental stage (i.e., larvae × adults) and feeding status (i.e., unfed × fed) acted independently from each other on the expression of all genes, except for *RproOrco*. This could be confirmed by looking at the non-significant interaction terms (stage:feeding) for the three ionotropic coreceptors (*RproIR8a*, *RproIR76b*, and *RproIR25a*), and the significant interaction term for *RproOrco* (**Table 3**). That is, unfed larvae differed from fed larvae in the same proportion as unfed adults differed from fed adults for the three ionotropic coreceptors (**Table 3**). For *RproOrco* expression, however, the effect of feeding was different from larvae and adults, as revealed by the significant interaction term for this specific case (**Table 3**).

As for females, there was a distinct pattern: an interdependence of developmental stage and feeding status affected the expression of all genes except for *RproIR76b*, as revealed by the interaction terms (stage:feeding) in **Table 3**. That is, the proportion by which unfed larvae differed from fed larvae was distinct from the proportion by which unfed adults differed from fed ones for *RproOrco*, *RproIR8a*, and *RproIR25a* (**Table 3**). Conversely, such proportions did not differ for *RproIR76b* (**Table 3**). In summary, the effects of developmental stage and feeding status tended to affect gene expression independently in males, the opposite occurring in females.

The results obtained with unfed 1-day-old adults are presented in Figure S2 in order to allow their comparison to those of unfed 21-day-old larvae and unfed 21-day-old adults.

Discussion

The abundance of antennal transcripts of the olfactory coreceptor genes of *R. prolixus* changes in response to development and blood feeding, and can also be affected by interactions between these factors. The results of this study reveal that the expression of olfactory coreceptor genes is a plastic process, closely linked to the observed changes in olfactory-mediated behaviors in these insects. Proper olfactory function requires the obligatory presence of coreceptors in a hypothetical fixed stoichiometry together with olfactory receptors (Vosshall et al., 2000; Benton et al., 2009; Abuin et al., 2011). This would

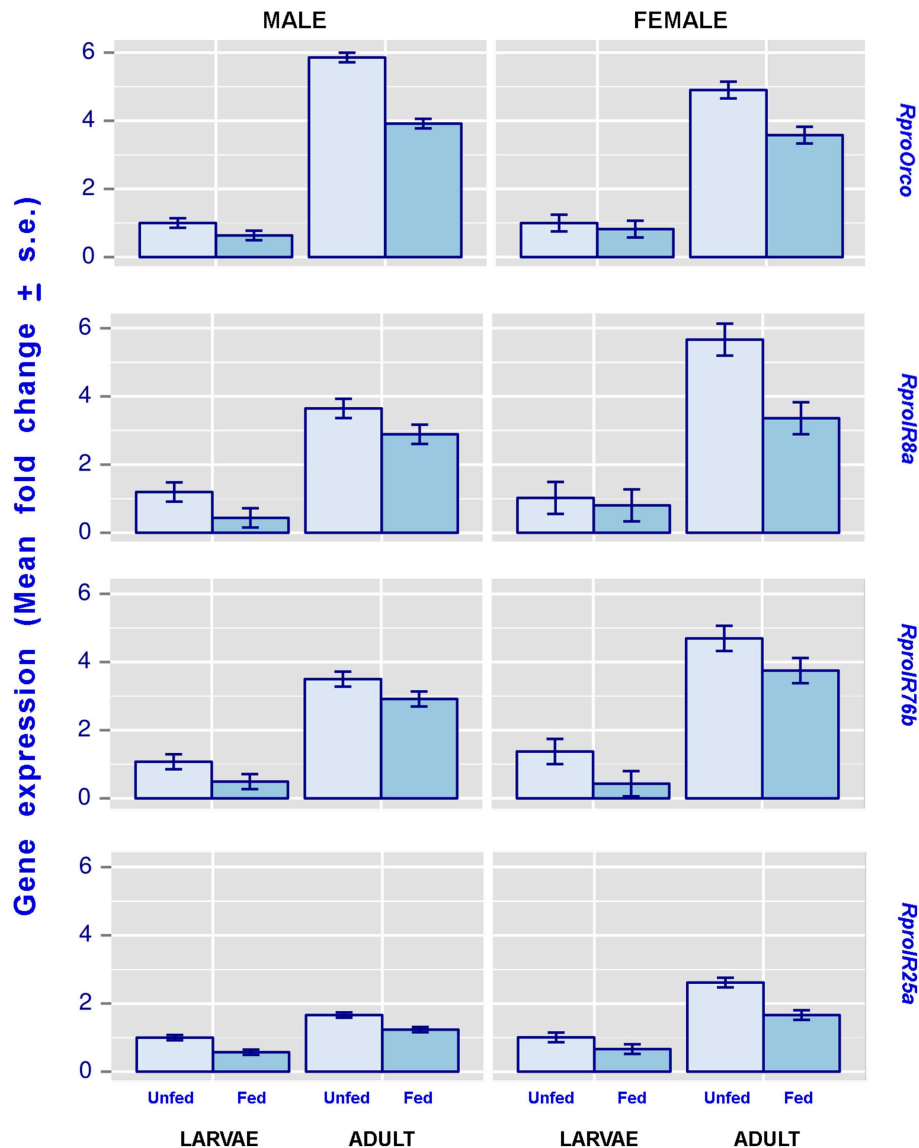


FIGURE 1 | The effect of imaginal molt and nutrition on male and female olfactory coreceptor gene expression levels, as seen in the antennae of 5th instar larvae and adults. Both 5th instar larvae and adults included in this figure were 21 day-old. Error bars represent the standard

deviation generated from 6 replicates per condition. All statistical comparisons, i.e., the effect of the imaginal molt and the effect of feeding, were significantly different (more details about these comparisons can be observed in **Table 3**).

mean that alterations in coreceptor expression levels may reflect changes in the sensitivity of the olfactory system. Nonetheless, variations in coreceptor abundance may not reflect specific states of particular ORs or IRs. In fact, the changes in coreceptor expression reported here may have been the overall outcome of up or down regulation, or even absence of alteration, in specific receptors co-expressed with them. The expression of some olfactory receptors might be expected to remain unchanged in triatomines, e.g., those responsible for alarm or aggregation pheromone detection, as bugs are responsive to these stimuli irrespective of their nutritional or developmental state (Figueiras and Lazzari, 2000). Alternatively, other receptors related to functions that are dependent on good nutritional status may be

anticipated to show an increase in expression, e.g., those that detect sexual pheromones (Vitta and Lorenzo, 2009).

The antennae of triatomines show a three-to-five-fold increase in the number of olfactory sensilla after their imaginal molt (Catalá, 1997; Gracco and Catalá, 2000; Akent'eva, 2008). Information about the ultrastructure of triatomines chemosensory sensilla is scarce, but available data suggest that triatomine trichoid sensilla may house up to 15 sensory neurons (Wigglesworth and Gillett, 1934). Rough estimates suggest that adult *R. prolixus* have approximately 1700 olfactory sensilla (Gracco and Catalá, 2000). Therefore, a concomitant increase in olfactory receptor expression would be expected when adult bug antennae are compared to those of fifth instar larvae.

TABLE 3 | Generalized Linear Modeling for olfactory coreceptor gene expression in male and female antennae of *Rhodnius prolixus*.

y-var	Model and Term	d.f.	F	P
MALE				
<i>RproOrco</i>	Full model	3;20	313.721	<0.001
	Stage	1;22	842.075	<0.001
	Feeding	1;21	67.549	<0.001
	Stage:Feeding	1;20	31.538	<0.001
<i>RproIr8a</i>	Full model	3;20	21.489	<0.001
	Stage	1;22	57.542	<0.001
	Feeding	1;21	5.514	<0.029
	Stage:Feeding	1;20	1.412	0.249
<i>RproIr25a</i>	Full model	3;20	24.408	<0.001
	Stage	1;22	51.691	<0.001
	Feeding	1;21	21.524	<0.001
	Stage:Feeding	1;20	0.01	0.922
<i>RproIr76b</i>	Full model	3;20	31.116	<0.001
	Stage	1;22	87.972	<0.001
	Feeding	1;21	5.079	<0.001
	Stage:Feeding	1;20	0.297	0.592
FEMALE				
<i>RproOrco</i>	Full model	3;20	66.202	<0.001
	Stage	1;22	183.831	<0.001
	Feeding	1;21	9.325	<0.001
	Stage:Feeding	1;20	5.449	0.03
<i>RproIr8a</i>	Full model	3;20	23.685	<0.001
	Stage	1;22	58.867	<0.001
	Feeding	1;21	7.237	0.014
	Stage:Feeding	1;20	4.95	0.038
<i>RproIr25a</i>	Full model	3;20	36.84	<0.001
	Stage	1;22	84.871	<0.001
	Feeding	1;21	21.001	<0.001
	Stage:Feeding	1;20	4.648	0.043
<i>RproIr76b</i>	Full model	3;20	25.096	<0.001
	Stage	1;22	66.74	<0.001
	Feeding	1;21	5.4	<0.001
	Stage:Feeding	1;20	3.148	0.091

Consistently our results showed that the antennal expression of all coreceptors studied presented a significant increase in 21-day-old adults (**Figure 1**). This indicates that both the OR and IR based olfactory subsystems (Silbering et al., 2011) seem to undergo a significant expansion in the adult phase of these hemimetabolous insects. Immature triatomines share several chemosensory mediated behaviors with adult bugs (Ward, 1981; Lorenzo Figueiras et al., 1994; Manrique et al., 2006; Guerenstein and Lazzari, 2009). These include the orientation to hosts, alarm and aggregation responses. Nevertheless, adult triatomines make use of sexual pheromones to find mates for reproduction (Pontes et al., 2008; Vitta et al., 2009; May-Concha et al., 2013) and the observed increase in coreceptor expression in adult antennae seems to support the hypothesis that a significant expansion is taking place on OR and IR subsystems to cope with sexual functions. Further experiments need to

be performed to determine whether this proposal is indeed correct.

The increase in coreceptor expression observed for adults could be hypothesized to be originated either during the imaginal molt or at the initial phase of adult life. Newly molted bugs do not respond to cues associated with their vertebrate hosts and recently molted adults show a low behavioral responsiveness toward mates, unlike older ones (Bodin et al., 2009b; Vitta and Lorenzo, 2009). The latter happens despite the fact that the antennae of triatomines show an increase in the number of olfactory sensilla after the imaginal molt (Catalá, 1997; Gracco and Catalá, 2000; Akent'eva, 2008). Combined, previous behavioral studies and our gene expression analyses suggest that the peripheral olfactory system of *R. prolixus* undergoes a post-eclosion maturation process in adult bugs (Figure S2 in Supplementary Material). Similar maturation has been reported in female mosquitoes (Omondi et al., 2015a), which at early imaginal life do not express proper host-seeking behavior, have a decreased neural sensitivity to host volatiles and a lower expression level of olfactory receptor genes (Davis, 1984; Grant and O'Connell, 2007; Bohbot et al., 2013). Since proper olfactory function requires the obligatory presence of coreceptors (Vosshall et al., 2000; Benton et al., 2009; Abuin et al., 2011), alterations in coreceptor expression levels may induce changes in the sensitivity of the olfactory system of *R. prolixus*, ultimately leading to an increased behavioral responsiveness toward vertebrate host volatiles and pheromones in mature adults.

Larval *R. prolixus* display reduced electrophysiological responses to ammonia after ingesting a blood meal (Reisenman, 2014). Moreover, engorged triatomine larvae are refractory to host odor stimulation for a prolonged time after feeding (Bodin et al., 2009a) and remain hidden in shelters for several days while their molting is completed. A similar refractory period has been observed in blood fed mosquitoes (Klowden and Lea, 1979; Takken et al., 2001). In both *R. prolixus* (our study) and the mosquito *Anopheles gambiae* (Rinker et al., 2013), blood feeding induces a reduction in chemosensory gene transcript production. Moreover, both *A. gambiae* and *Aedes aegypti* mosquitoes have reduced electrophysiological responses to host odors during the refractory period post-blood meal (Takken et al., 2001; Siju et al., 2010). We suggest that coreceptor down-regulation would represent a way to shut down the system and save energy. It is interesting to note that the decrease induced by the blood meal tended to be more significant for adult bug antennae, when compared to larval expression. This was the case for *RproOrco* (in both sexes), *RproIr8a* and *RproIr25a* (only for females). Further experiments would be necessary to clarify the functional bases of the observed differences.

Our results show that changes in olfactory coreceptor gene transcripts seem to be linked with the observed plasticity in behavioral responsiveness of larval and adult *R. prolixus* to host volatiles and mates. How these changes are reflected in the functional characteristics of the peripheral and central olfactory systems requires further analysis. This report is the first in line for understanding the molecular basis of neurophysiological modulation of triatomine olfactory driven behaviors.

Author Contributions

JML, Provided most experimental data, analyzed data and wrote the manuscript; BO, Advised with experimental procedures, analyzed data, wrote, and provided comments on the manuscript; OD, Analyzed data and wrote the manuscript; IO, Provided experimental data; RI, Wrote and provided comments on the manuscript; ML, Conceived the project, help and advised on experiments, wrote, and provided comments on the manuscript.

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Supplementary Material

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fevo.2015.00074>

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